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Current Status of Forage Crops Cultivation and Strategies for the Future in Turkey: A Review

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ABSTRACT
Forage crops cultivation area in Turkey is 2.312 million ha in 2020. The rate of forage cultivation in the total agricultural area is 6.1%, and its rate in the field land is 12.3%. Lucerne, silage corn, oat, vetch, and sainfoin are main forage crops in Turkey. The amount of produced is 16.8 million tons of hay and 48.8 thousand tons of seeds. The forage crop cultivation areas have increased by 206% since the beginning of the 2000s due to the government subsidies. However, shortage of quality roughage is still a big problem for animal husbandry of the country. For this reason, new strategies should be developed to increase forage cultivation areas by considering global warming, drought, and climate change. In order to meet the need for roughage, it is necessary to expand the cultivation area of forage crops as winter catch crops and second crops. In addition, some of the fallow fields should be utilized by growing drought-resistant forage species. The fields abandoned due to different problems should be used for the cultivation of suitable forage crops.

Keywords: Cultivation area, Hay and seed production, Organic production, New forage species, Animal husbandry

1. Introduction

Quality roughage is of great importance in feeding of livestock, since it ensures matching the nutrition requirements of animals for obtaining desired amounts of animal products such as milk, and meat. Rangelands, meadows and cultivated forage crop species in the arable lands are inevitable sources of quality roughage in Turkey (Sayar et al. 2010). In the mountainous regions where the winter season is harsh and long, the feed needed for feeding the livestock in the winter period is obtained from the forage crops cultivation areas and meadows. Therefore, for sustainable animal production, agricultural lands should be allocated for roughage production at a certain rate.

Besides using forage crop species for animal feeding, they are important for the agricultural ecosystem in many respects definitely oblige them to be included in different crop rotations systems (Acar et al. 2009). For instance, forage crops are sown more densely per unit area and harvested earlier than other product groups. In this way, they help the soil to rest and enrich its structure by leaving plenty of organic matter to the soil. Additionally, legume forage crops such as alfalfa, clover, and vetch fix the free nitrogen of the air to the soil and ensure that the next plants are more productive (Acikgoz 2001). Also, forage crops play an important role in reducing fallow lands, preventing soil and water erosion, and controlling diseases and pests (Tan 2018). Therefore, forage crops should be grown to improve soil conditions and control diseases and pests as well as produce forage.

Forage crop cultivation has been ongoing since the past because livestock is an important source of livelihood in Turkey. Moreover, forage cost constitutes 50-70% of operating input costs in animal production (Anonymous 2019a). Lucerne (*Medicago sativa* L.), sainfoin (*Onobrychis sativa* Lam.), common vetch (*Vicia sativa* L.) and bitter vetch (*Vicia ervilia* (L.) Willd.) are grown in Anatolia since the Hittites (Tarman 1972). Although bitter vetch cultivation has decreased, other forage crop species such as forage pea, silage corn, faba bean, and grass pea have begun cultivation in the last two decades. In addition to these forages, new species such as Italian ryegrass, forage turnip, Hungarian vetch, clovers, and some meadow grasses are becoming widespread all over the country. The product variety and the cultivation areas of forage crops are increasing in Turkey due to the government subsidises the cultivation of forage crops for animal production. Despite this, it is not enough to meet the quality roughage requirement of animal existence. Insufficient roughage production in Turkey is one of the major problems of animal production. For this reason, in this period when the effects of global warming are beginning to be felt, it is of great importance to determine the current situation of forage crop cultivation areas and to observe the change in forage cultivation areas in recent years, and develop new strategies to increase forage cultivation. Therefore, this article reveals the current situation of forage crop

cultivation areas within total agricultural areas in Turkey in 2020 and forages crop areas' change in recent years. In addition, with the help of this current data, the paper presents some approaches and new scenarios to solve the problems of forage cultivation by also considering global warming.

2. Agricultural Areas Use in Turkey

The agricultural area in Turkey is 37,753,000 ha in 2020 (Table 1, TUIK 2020). Meadow and rangeland areas are 14,617,000 ha, field crops cultivation areas are 18,788,000 ha and fruit and vegetable cultivation areas are 4,348,000 ha. The vast majority of crop cultivation lands are in dry farming areas and 3,173,000 ha of these areas left fallow every year. The ratio of fallow land to total agricultural land is 8.4%. On the other hand, 13.7% of the cultivated agricultural areas (field + garden) are left fallow.

The forage crop cultivation area in Turkey's agricultural lands is 2,312,000 hectares. The share of this area in total agricultural land and field land is 6.1% and 12.3%, respectively. The share of forage crops in total agricultural land was 2.9% in 2002 (Ozkan 2020) and 5% in 2007 (Yolcu & Tan 2008) in Turkey. Forage crop cultivation areas in Turkey have increased in the last two decades as a result of government subsidies, the shortage of quality roughage is however still a major problem for the country's livestock. Accordingly, many researchers emphasized that forage crops cultivation should be improved in order to minimise the deficit of quality roughage (Tan et al. 2002; Koc et al. 2012; Demiroglu Topcu & Ozkan 2017; Acar et al. 2020). As a matter of fact, the rate of forage crop cultivation areas in Turkey is lower than in some countries such as Germany, France and Italy (Acikgoz et al. 2005; FAO 2019).

Table 1- Agricultural areas in Turkey (TUIK 2020)

Agricultural Areas	*Area (1,000 ha)*	*Ratio (%)*
Cereal and other crops (Field area sown)	15,615	41.4
Fallow	3,173	8.4
Fruits, beverage and spice herbs	3,564	9.4
Vegetable gardens	779	2.1
Ornamental plants	5	0.01
Meadow-rangeland	14,617	38.7
Total	37,753	100
Forage Crops	2,312	6.1*

* This ratio is the ratio of the forage crops cultivation area to the total cultivated area. The ratio of forage crop cultivation area to field land is 12.3%

3. Forage Crops in Turkey

Forage production in Turkey was conducted in 2,268,660 hectares which produced a total of 16,833,009 tons of hay (Table 2). Lucerne has the highest share in cultivation areas with 662,888 ha. Lucerne is followed by corn for silage (520,589 ha), oat for forage (324,018 ha), common vetch (224,386 ha) and sainfoin (174,494 ha) respectively. Other forage crops have low cultivation areas. Corn for silage has the highest total hay yield with 8,156,085 tons in Turkey. It is followed by lucerne (4,822,630 tons), oat for forage (1,155,143 tons), common vetch (696,798 tons) and sainfoin (483,674 tons), respectively. Other forage crops have low total hay yields (Table 2). Corn for silage has the highest hay yield per unit area (15.67 tons ha^{-1}). This followed by Italian grass (11.51 tons ha^{-1}), sorghum (11.31 tons ha^{-1}), forage turnip (10.20 tons ha^{-1}) and lucerne (7.28 tons ha^{-1}) respectively. Other forage crops have low hay yields per unit area (Table 2).

Table 2- Cultivation areas, hay productions and yields of forage crops in Turkey (TUIK 2020)

Forage Crops	*Cultivation Area (ha)[1]*	*Total Hay Production (ton)[2]*	*Hay Yield (t ha^{-1})*
Lucerne	662,888	4,822,630	7.28
Sainfoin	174,494	483,674	2.77
Common Vetch	224,386	696,798	3.11
Hungarian Vetch	73,918	275,927	3.73
Vetches (Other)	77,639	163,015	2.10
Forage Pea	24,319	113,194	4.66
Bitter Vetch	2,294	3,641	1.59
Grass Pea	8,769	20,506	2.34
Clover	5	24	4.80
Corn (Silage)	520,589	8,156,085	15.67
Corn (for hay)	5,672	37,843	6.67
Sorghum	2,332	26,376	11.31
Italian Ryegrass	25,329	291,507	11.51
Oat	324,018	1,155,143	3.57
Wheat	17,866	104,651	5.86
Barley	31,319	161,120	5.14
Rye	6,851	29,459	4.30
Triticale	35,008	137,593	3.93
Meadow Grasses	44,637	97,949	2.19
Fodder Beet	1,670	8,376	5.02
Forage Turnip	4,657	47,498	10.20
Total	2,268,660	16,833,009	-

[a], Seed production areas (41,628 ha) and dried faba bean for feed (1,384 ha) are not included. [b], Hay productions are calculated from green material based on dry matter content of 30% in grasses, 25% in legumes, 20% in forage turnip and 10% in fodder beet.

Lucerne is the most cultivated in the Middle East Anatolia Region of Turkey (199,377 ha; Table 3). This is followed by the Northeastern Anatolia Region with 143,687 ha. The most cultivation regions of sainfoin are also Middle East Anatolia and Northeast Anatolia Regions (45,584 ha and 79,923 ha, respectively). It is noteworthy that lucerne and sainfoin cultivation areas are high in the eastern regions where animal husbandry is common and the winter period is long. Common vetch find more cultivation area in the Aegean and West Blacksea Regions (54,766 ha and 53,503 ha, respectively). These regions are followed by the Mediterranean region with 26,734 ha. 28.5% of silage corn cultivation areas (148,604 ha) is in the Aegean Region. West Marmara, East Marmara, West Anatolia, West Blacksea and Mediterranean regions are also the regions with the largest areas of corn for silage cultivation (Table 3).

Table 3- Regions of some forage crops cultivation for hay production in Turkey (TUIK 2020)

Statistical Regions	*Area (ha)*			
	Lucerne	*Sainfoin*	*Common Vetch*	*Silage Corn*
Mediterranean	19,933	4,696	26,734	47,123
West Anatolia	46,465	2,519	9,558	54,064
West Blacksea	41,149	8,680	53,503	50,476
West Marmara	17,272	285	7,009	70,357
East Blacksea	9,388	4,525	3,457	1,716
East Marmara	36,254	1,634	11,246	60,802
Aegean	58,680	3,142	54,766	148,604
Southeastern Anatolia	14,915	1,208	15,018	32,028
Northeast Anatolia	143,687	79,923	13,953	13,091
Middle Anatolia	75,568	22,300	16,237	33,713
Middle East Anatolia	199,377	45,584	12,901	7,522
Istanbul	200	-	6	1,093
Total	662,888	174,494	224,388	520,589

Researches reveals that it has not met the necessary roughage requirement for livestock in Turkey. Acar et al. (2020) reported that 86,880,000 tons of quality roughage was needed in the country, only 35.7% of this was met. Moreover, it was stated that the ratios of 29.8% (Demiroglu Topcu & Ozkan 2017) and 70.3% (Ozkan 2020) of the necessary roughage were met in Turkey. Studies made in the last two decades shown that the country's roughage deficit has been continuing for a long time (Yolcu & Tan 2008; Koc et al. 2012; Ozkan & Sahin Demirbag 2016; Acar et al. 2020). The amount of hay produced from 2,268,660 ha forage crop cultivation area in Turkey is a total of 16,833,009 tones, according to recent data (Table 2). On the other hand, there are 18,614,990 bovines and 55,063,391 ovine (TUIK 2020). Roughage production has increased; but the animal number is also increasing. Therefore, the forage deficit still exists in Turkey, and these deficits are met by using field crop residues having low nutritive value.

4. Change of Forage Crops Cultivation Areas According to Years in Turkey

Forage crops cultivation began to subsidy with 2000/467 Decree of The Ministry of Agriculture and Rural Affairs (About Decision Subsidy Livestock) in Turkey since 2000. The total forage cultivation area, which was 754,177 ha in 2000, reached 2,311,167 ha in 2020 with a substantial increase of 206% (Figure 1, TUIK 2020). The subsidy program for forage crop production is thought to be an important contribution for the increase in the production of forage crops after 2000 (Alas Eroglu et al. 2020). Lucerne cultivation areas, which were 250,800 hectares in 2000, reached 662,046 hectares in 2015 (Figure 1, TUIK 2020). However, no important increase was observed after 2015 for lucerne, sainfoin and vetch cultivation areas (Figure 1). On the other hand, there was an increase in silage corn, sorghum, Italian ryegrass and forage pea cultivation areas. The high yield potential of corn and the fact that sorghum and Italian ryegrass reached high yields by being harvested more than once a year in irrigated areas are the reasons why the cultivation of these plants has increases recently. There is a decrease in the cultivation areas of species such as fodder beet and forage turnips (TUIK 2020).

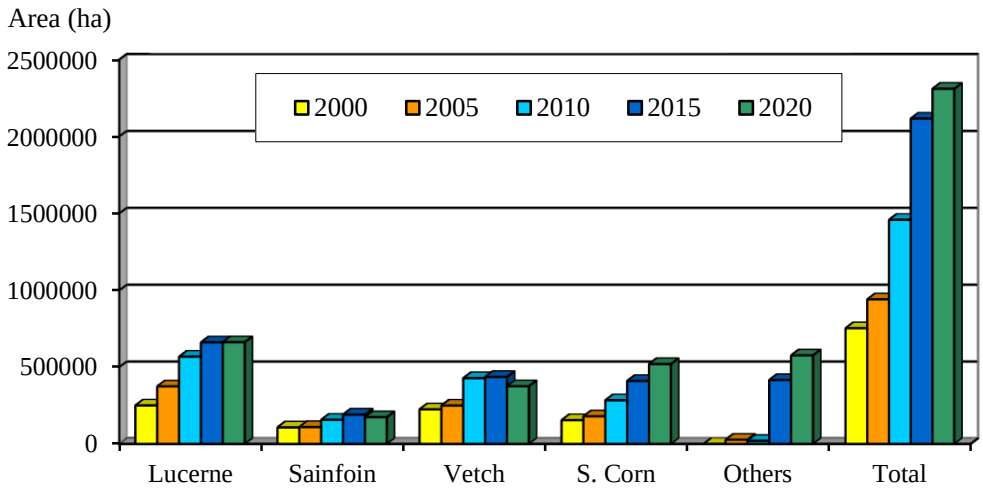

Figure 1- Changes of forage crops cultivation areas in Turkey, 2000-2020 period (TUIK 2020)

The cultivation area data of the small grain cereals as roughage is available since 2012 (Table 4). Although small grain cereals have some problems in terms of forage quality such as quick ripening and high lignin accumulation, they are an effective plant group in closing the roughage gap. The cultivation areas of small grain cereals for forage increase day by day due to the high adaptability, easy availability of seeds, and low labor requirements. Oat cultivation increased remarkably, especially after 2015. The fact that oat is a better roughage among small grain cereals, causes increase in its cultivation areas for hay.

Table 4- Cultivation areas of small grain cereals for forage in 2012-2020 in Turkey (TUIK 2020)

Years	*Area (ha)*				
	Oat	*Triticale*	*Wheat*	*Barley*	*Rye*
2012	82,551	5,208	22,388	2,509	615
2015	82,589	7,658	14,618	3,378	765
2020	324,018	35,009	17,866	31,319	6,851

5. New Types of Forage Crops in Turkey

Traditional forage plant species of Turkey's agriculture are lucerne, sainfoin, common vetch, and small grain cereals for forage. In addition to these, silage corn became widespread after 1980, and forage peas and fodder beet started to cultivate in the 2000s. In recent years, new forage crop species have started to be cultivated in Turkey. Some information about these species is given below.

Ryegrass (*Lolium multiflorum* L.): This type, which is a new plant for the agriculture of Turkey, has been grown in European countries for a long time. It is also known as Italian ryegrass or annual ryegrass in Europe, and it is recognized as milk grass in Turkey. This plant, which loves temperate climates, shows rapid and strong growth in a short time. It is usually grown alone or mixed with annual legumes to produce grass or to establish rotational pastures. It is also an ideal plant to conduct a short-term green area. Since it is an annual plant, it can easily find a place in crop rotation systems. In irrigation conditions with appropriate use of fertilizers, it gives more than one harvest and the hay yield rises above 10 ton ha^{-1} (Colak & Sancak 2016; Turk et al. 2019). Data of ryegrass cultivation areas are available in Turkey since 2014. The cultivation area was 483 ha in 2014, which reached 25,329 ha of cultivation area in 2020 (TUIK 2020).

Forage Turnip (*Brassica rapa* L.): Turnip plants are generally grown to produce tubers to be used in human nutrition. However, plants in this group are also used in animal feeding. Forage turnips have a life span of one or two years. As well as the varieties are grown for their tubers, there are also varieties that do not produce tubers and whose only above ground parts are used for animal feeding. There are many types of forage turnips registered in Turkey. These types have a delicious and succulent plant structure with a high green biomass yield as a forage plant. Animals consume as green forage as well as silage. Cultivation areas were 4,657 hectares in Turkey recently (TUIK 2020).

Hungarian Vetch (*Vicia pannonica* Crantz.): Hungarian vetch cultivation in Turkey began in the early 2000s. Its cultivation area is 73,918 ha and the seed cultivation area is 7,912 ha in Turkey. One of the most important advantages of Hungarian vetch is sowing in winter months in the Middle and Eastern Regions of Turkey. It can be grown as an alternative to common vetch in regions with cold winters due to its resistance to cold. Its resistance to drought is also high. Hungarian vetch is used as a roughage, but it is not suitable for use as a grain feed due to its small grains. The most important problem of Hungarian vetch cultivation is its excessive characteristic to lie down and the difficulty of seed production. For this reason, there is a shortage of seeds in the sowing season.

Other Species: Clovers (*Trifolium* sp.) with 5-6 ha of cultivation areas is one of the other species. The genus of clover includes about 300 species growing in the world. Some of them are used for animal feeding by mowing or grazing. The most important plant of this group, the red clover (*Trifolium pratense* L.), has been cultivated in the Eastern Anatolia Region like lucerne, but today its cultivation areas have come to the point of disappearing. In recent years, annual clovers have been grown in Turkey's agricultural lands. These cultivation areas are commonly spread around Bursa province.

Data of meadow grasses have been available in Turkey since 2020 as a new forage crop group. In this group, there are mostly grass forage plant species. The cultivation areas of these plants, which are also used in the establishment of artificial pastures, were 44,637 ha (TUIK 2020).

In addition to the new forage plant species grown in Turkey, there are also potential species suitable for cultivation. High yield potential elephant grass (*Miscanthus x giganteus*; Ozdogan & Geren 2019), different buckwheat types (*Fagophyrum esulentum* Moench., Kara & Yuksel 2014), heat-resistant summer grass (*Eragrostis teff* [Zucc.] Trotter.) and quinoa for salty soils (*Chenopodium quinoa* Willd., Tan & Temel 2018) are some of them. These species should be used for forage production in problem soils.

6. Organic Forage Crops Cultivation in Turkey

According to the data of the Ministry of Agriculture and Forestry for 2019, organic lucerne, organic sainfoin, organic vetch, organic forage pea, organic grass pea, and organic Hungarian vetch are cultivated as organic legumes forage crops in Turkey (Table 5; Anonymous 2019b). Corn for silage, Italian ryegrass, and millet were cultivated as organic forage grasses in Turkey. Meadow grass, forage turnip (hay), artificial meadow-rangeland, and fodder beet were cultivated as organic other forages (Table 5; Anonymous 2019b).

Table 5- Total hay yields of organic forage crops in Turkey (Anonymous 2019b)

Organic Forage Legumes	*Hay Production (ton)*
Lucerne	108,911.6
Sainfoin	59,202.5
Vetch	47,744.0
Forage Pea	560.9
Grass Pea	286.3
Hungarian Vetch	237.8
Bitter Vetch	140.5
Organic Forage Grasses	
Corn (For silage)	6,527.8
Italian Ryegrass	742.8
Millet/Sorghum	214.5
Organic Other Forages	
Meadow Grass	32,723.1
Forage Turnip (Hay)	406.5
Meadow-Rangeland (Artificial)	259.8
Fodder Beet	21.6

The fact that some areas of organic forage production in Turkey are away from the organic livestock areas and organic rangeland which is the cheapest source of organic forage is not available are the most important problems of Turkey organic livestock (Yolcu et al. 2014). It is very important to make organic forage crop production near organic livestock areas and organic livestock based on organic meadows and pastures in terms of economical organic meat and milk production.

7. Grain Feeds in Turkey

Grain or concentrate feeds are rich in terms of energy and protein content, and their digestion rate by animals is very high. Forage crop seeds are important concentrate feedstuffs. Exclusively corn, barley, oats, rye, triticale, sorghum, faba bean, common vetch, and bitter vetch species are grown for their grains in Turkey. Corn is the most grown and used grain feeds all over the world. Turkey produces 6 million tons of annual grain corn. But 7.8 million tons of grain corn are used domestically, 75% of this amount is used in concentrate feed sector (Anonymous 2020). Turkey's corn production is not sufficient for the domestic market, it has imported 1.5-2 million tons of corn each year (Anonymous 2019a). According to Basbag et al. (2021) ground corn seed was found superior than the other concentrate feedstuffs in terms of feed quality parameters. Wheat is used very little as a grain feed. Oats and barley whose seeds are covered with husk are more commonly used as grain feeds. Barley is one of the grain feeds mostly used by producers in animal feeding because of its cheapness and abundance. Rye is grown as grain feed in arid and infertile soils. The cultivation of triticale is widespread especially in Eastern Anatolia due to its high yield and durability as grain feed.

In the Aegean and Mediterranean Regions, faba bean is commonly cultivated as a grain legume feed. On the other hand, forage pea is grown as a grain feed in the Eastern Anatolia Region, and bitter vetch is cultivated in the Middle Anatolia, Mediterranean, and Southeastern Anatolia regions as a grain feed. Cultivation as a grain feed of common vetch spreads in all regions of Turkey. Forage pea is grown as a grain feed in all regions of Turkey recently, due to the newly developed forage pea varieties. Narbon vetch to be grown for winter in Middle Anatolia conditions also has a great potential as grain feed (Uzunmehmetoglu & Kendir 2006). Legume group grain feeds are important as the protein source of the rations. In addition, the seeds of some species in this group are used in poultry feeding.

Another importance of grain feeds is that they constitute the most important raw material for the concentrate feed industry. The concentrate feed industry uses cereals, oilseeds, and their by-products such as bran and pulps. Corn is mostly used as the raw material of concentrate feeds. Significant amounts of imports are also made in corn, feed oilseeds, and soybean for animal feed and soybean pulp is the leading one (Anonymous 2019a). The enhancement of feed grains cultivation as raw materials for the feed industry in addition to increasing the forage production in Turkey is of great importance for the development of the livestock sector.

8. Forage Crops Seed Production in Turkey

Lucerne, sainfoin, common vetch, Hungarian vetch, and other vetches are cultivated in order to get seeds in Turkey (Table 6). Lucerne in 2,885 ha, sainfoin in 452 ha, common vetch in 27,740 ha, and Hungarian vetch in 7,912 ha for seed production are cultivated in Turkey (TUIK 2020). Total seed production area is 41,628 ha that produced 48,799 tons of seeds.

Table 6- Forage crops areas sown for seed, seed production, and seed yields (TUIK 2020)

Species	*Area (ha)*	*Seed Production (ton)*	*Yield (t ha^{-1})*
Lucerne	2,885	1,695	0.58
Sainfoin	452	248	0.55
Common Vetch	27,740	33,031	1.19
Hungarian Vetch	7,912	10,158	1.28
Vetches (Other)	2,638	3,667	1.39
Total	41,628	48,799	-

Many crops for seed production are mostly cultivated in Western and Middle Anatolia Regions. The highest lucerne for seed production is cultivated in Middle Anatolia, Southeastern Anatolia and Western Anatolia Regions as 1,394 ha, 984 ha, and 260 ha, respectively. Almost all of the sainfoin seed cultivation (450 ha) is in Western Anatolia. Seed production of common vetch spread throughout the country. There are 15,022 ha of vetch cultivation areas for seed production in the Western Blacksea, 4,259 ha in Middle Anatolia, 3,543 ha in Western Anatolia, 1,769 ha in Southeastern Anatolia, and 1,653 ha in the Aegean Region (TUIK 2020).

The amount of seed produced in Turkey is not enough to meet the needs of the domestic market. According to the Records of the Ministry of Agriculture and Forestry, 2,269 tons of forage plant seeds and 7,089 tons of grass and meadow grass seeds were imported, and 519 tons of forage crops and 209 tons of grass-meadow grass were exported in 2019 (Anonymous 2019c).

9. Scenarios and Strategies in Forage Crops Cultivation of Turkey

Forage crops are cultivated in 2,268,660 ha that produced a total of 16,833,009 tons of hay from the field (Table 2). However, the calculations show that total roughage production together with the hay produced from meadows and pastures are not enough to meet the requirements of the animal existence. Therefore, ways to increase both hay and grain feed production should be investigated. Agricultural supports for forage crop cultivation have made a great contribution to the increase in cultivation areas of the forage since the early 2000s. However, data show that the increase in cultivation areas of important species such as lucerne, sainfoin, and vetch slowed down after 2015. Therefore, new subsidy programs are needed. The basin and product-based subsidy should be brought to the fore instead of the subsidy made according to the cultivation areas.

Forage crops share in total agricultural land in Turkey is 6.1%. This share is 12.3% in the total field areas (Table 1). This share is not sufficient to meet the forage requirement of total animal existence and there are some difficulties to increase this share much higher. Food prices are increasing as a result of the increasing demand for food products worldwide. This situation causes producers to cultivate strategic products such as wheat, corn, rice, and potatoes that are directly consumed as human food. Moreover, there are difficulties in increasing forage crop farming in irrigated areas, due to the globally increasing drought risk. Rainfed agriculture is obligatory in most of Turkey's agricultural lands. It is inevitable that forage crop cultivation areas will be shifted to rainfed areas from irrigated agriculture areas. Forages that need less water such as sainfoin, vetch, smooth bromegrass, and sorghums should be cultivated in dry farming areas of Turkey.

It is left to 3,173,000 ha area of fallow in Turkey each year. Since it is not possible to remove the fallow completely without irrigation in agricultural systems, but it is possible to reduce fallow areas by cultivating drought-resistant forage plants in the fallow areas (Kusvuran et al. 2011). In addition, reduced and minimum tillage methods in rainfed agricultural areas should be used as soon as possible to produce forage. Winter forages and winter cereals for forage should be cultivated to take advantage of rain and snow water against the drought risk in this season. While lucerne and corn for silage are cultivated in irrigated areas in the summer season, triticale, Hungarian vetch, and forage peas should be sown as winter in rainfed areas. Besides, efforts should be made to develop cold-resistant varieties for winter.

Forage crops cultivation as both second crops and catch crops should be increased so that forage crops cultivation are more involved in crop rotation systems. Growing forage crops in the early years of orchard facilities can contribute to roughage production (Hatipoglu et al. 2020).

The problem of seeds in forage crops and of the insufficient number of forage varieties are still not solved in Turkey. The development of drought and cold resistant varieties should be focused on, and new varieties should be distributed all over the country. In response to the adaptation problem of foreign seeds, new varieties should be developed from local gene sources or hybrid seeds. New variety development programs should be implemented mainly for sainfoin. Developed sainfoin varieties should be produced in high amounts with the contracted farmer model, and should be distributed across the country. It is required to increase grain feed crops cultivation area for animal husbandry. The products needed by the feed industry should be supplied domestically. It should be ensured that soybean agriculture, which is imported from a foreign market to a large extent, becomes widespread in Turkey. Seed productions of grain corn, soybean, cereal for forage, and annual legumes (such as vetch, faba bean and forage pea) should be subsidized on a basin basis. The use of straw and field crop residues as roughage will continue from now on as before. These are one of the remedies to close the roughage gap. However, the producers should be made aware of the use of straw in feed rations rather than using it alone. Any alternative material with feed value other than cereal straw should be utilized. It should be ensured that legume straw, beet leaf, beet pulp, fruit pulp, and similar wastes are used for feeding animals.

10. Conclusions

Forage crop cultivation has made great improvements in the last two decades in Turkey. The forage crops cultivation area in Turkey is 2,312,000 ha in 2020. The rate of this area in the total agricultural area is 6.1%, and its rate in the field land is 12.3%. However, the amount of produced forage is not sufficient to meet the needs of animal husbandry. Forage crops cultivation areas should be increased not only as the main product but also as the catch crop or second crop. Moreover, it should be also focused on the implementation of measures that provide more crops per unit area due to possible difficulties in further increasing the cultivation areas. Furthermore, in a part of 3,173,000 ha of fallow land, drought-resistant forage species should be grown to increase forage production. The increases in forage production can reduce the pressure on the rangelands as well as increase animal production and help protect natural resources. The development of forage crop farming will also contribute to the solution of erosion, inefficiency, disease, pest, and weed problems. In addition, forage crop farming should continue to be subsidized by the government. However, this subsidy should be done according to the amount of product, not the cultivation area. Special subsidy policies are needed to expand cultivation areas of drought-resistant forage crops and to increase their seed production and grain feed productions.

References

Acar Z, Ayan I & Gunaydın G (2009). Crop Rotation. OMU Faculty of Agriculture Textbook (In Turkish) No: 62.2

Acar Z, Tan M, Ayan I, Ugur O, Basaran U, Can E & Kaymak G (2020). Status and development possibilities of forage crops cultivation in Turkey. Turkey Agricultural Engineering IX. Technical Congress Proceedings Book (In Turkish), Ankara, pp. 529-553.

Acikgoz E (2001). Forage Crops (In Turkish). Uludağ University Improvement Foundation Publication No: 182. Vipaş Inc. Com. Publication No: 58, Bursa, 584 p

Acikgoz E, Hatipoglu R, Altınok S, Sancak C, Tan A & Uraz D (2005). Forage production and problems (In Turkish). Turkey Agricultural Engineering VI. Technical Congress, January 3-7, 2005, Ankara, pp: 503-518

Alas Eroglu N, Bozoglu M & Bilgic A (2020). The impact of livestock supports on production and income of the beef cattle farms: A case of Samsun province, Turkey. Journal of Agricultural Sciences 26(1): 117-129

Anonymous (2019a). Mixed Feed Industry Report-2019. Turkey Feed Industry Association. Poyraz Ofset Printing, Ankara, 64 p

Anonymous (2019b). Republic of Turkey Ministry of Agriculture and Forestry, 2019 Organic Farming Statistics

Anonymous (2019c). Seeds in 2019 Sector Report. Ministry of Agriculture and Forestry, General Directorate of Agricultural Enterprises, Ankara

Anonymous (2020). Corn Report 2020. TMMOB the Chamber of Agricultural Engineers, April 3, 2020, Ankara

Basbag M, Sayar M S, Cacan E & Karan H (2021). Determining quality traits of some concentrate feedstuffs and assessments on relations between the feeds and the traits using biplot analysis. Fresenius Environmental Bulletin 30(2A): 1627-1635

Colak E & Sancak C (2016). The effects of different nitrogen fertilizer doses on yield and some agricultural traits of Italian ryegrass (*Lolium italicum* L.) cultivars (In Turkish). Journal of Field Crops Central Research Institute 25(1): 58-66 DOI: 10.21566/tbmaed.55211

Demiroglu Topcu G & Ozkan Ş S (2017). General view to meadow-rangelands and forage crops cultivation of Aegean Region and Turkey (In Turkish). COMU Journal of Agriculture Faculty 5 (1): 21-28

FAO (2019). Food and Agricultural Organization, http://www.fao.org (Access date: 15.03.2021)

Hatipoglu R, Serbester U, Avcı M & Dönmez B (2020). Present status and development possibilities of forage production in Adana province (In Turkish). Turkish Journal of Agriculture-Food Science and Technology 8 (7): 1497-1501 DOI: 10.24925/turjaf.v8i7.1497

Kara N & Yuksel O (2014). Can we use buckwheat as animal feed? (In Turkish) Turkish Journal of Agricultural and Natural Sciences 1(3): 295-300

Koc A, Tan M & Erkovan H I (2012). An overview of fodder resources and animal production in Turkey. New Approaches for grassland research in a context of climate and socio-economic changes. Ciheam Mediterranean Seminars No: 102, October 3-6, 2012, Samsun, Turkey, pp. 15-22

Kusvuran A, Nazlı R I & Tansı V (2011). Current situation of meadow-rangelands, animal existence and cultivation for forage crops in Turkey and East Black Sea Region (In Turkish). Journal of Agricultural Faculty of Gaziosmanpasa University 28(2): 21-32

Ozdogan T & Geren H (2019). A preliminary study on the effect of different nitrogen levels on the biomass yield and some yield characteristics of elephantgrass (*Miscanthus x giganteus*) used as an energy crops (In Turkish). Journal of Ege University Faculty of Agriculture 56(2): 257- 262 DOI: 10.20289/zfdergi.481848

Ozkan U (2020). Comparative overview and evaluation of Turkey's forage crops agriculture (In Turkish). Turkish Journal of Agricultural Engineering Research 1(1): 29-43

Ozkan U & Sahin Demirbag N (2016). Status quo of quality roughage resources in Turkey (In Turkish). Turkish Journal of Scientific Reviews 9(1): 23-27

Sayar MS, Anlarsal AE & Basbag M (2010). Current situation, problems and solutions for cultivation of forage crops in the Southeastern Anatolian Region (In Turkish). Harran Journal of Agricultural and Food Science 14: 59-67

Tan M (2018). Legume and Grass Forage Crops (In Turkish). Ataturk University Faculty of Agriculture Textbook No: 256, Erzurum, 356 p

Tan M & Temel S (2018). Performance of some quinoa (*Chenopodium quinoa* Willd.) genotypes grown in different climate conditions. Turkish Journal of Field Crops 23(2): 180-186 DOI: 10.17557/tjfc.485617

Tan E, Avcioglu R, Munzur M & Demiroglu G (2002). Grassland and forage crop cultivation in Turkish agriculture. Anadolu, Journal of AARI 12(2): 100-109

Tarman O (1972). Forage Crops, Meadow-Pasture Culture (In Turkish). Ankara University Faculty of Agriculture Publications No: 464, Textbook No: 157, Ankara

TUIK (2020). Crop production statistics. Data Portal for Turkey Statistics, Retrieved in 15, March, 2021 https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1 (Access date: 15.03.2021)

Turk M, Pak M & Bicakci E (2019). The effects of different nitrogen fertilizer doses on the yield and quality of some annual ryegrass (*Lolium multiflorum* L.) cultivars (In Turkish). Journal of Isparta Applied Sciences University Faculty of Agriculture 14(2): 219-225.

Uzunmehmetoglu B & Kendir H (2006). Effects of winter and spring sowings on grain yields of narbon vetch (*Vicia narbonensis* L.) (In Turkish). Journal of Agricultural Sciences 12(3): 294-300. DOI: 10.1501/Tarimbil_0000000457

Yolcu H & Tan M (2008). General view to Turkey forage crops cultivation (In Turkish). Journal of Agricultural Sciences 14(3): 303-312. DOI:10.1501/Tarimbil_0000001045

Yolcu H, Okcu M & Tan M (2014). Current situation of organic roughage production in Turkey (In Turkish). Yuzuncu Yil University Journal of Agricultural Sciences 24: 201-209

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Exogenously Applied GA_3 Promotes Plant Growth in Onion by Reducing Oxidative Stress Under Saline Conditions

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ABSTRACT

Onion (*Allium cepa* L.) is a biennial crop of high commercial value in Pakistan. Onion is considered as salt sensitive plant species. The present investigation was carried out to investigate the effect of salinity on onion and its alleviation through exogenously applied gibberellic acid (GA_3; 100 mg L^{-1}). Foliar application of GA_3 (100 mg L^{-1}) was applied on onion seedlings grown under three levels (0, 2 or 4 dS m^{-1}) of salinity after 45 days of sowing. Results revealed that growth parameters and total soluble protein (TSP) contents declined with increase in soil salinity level. While, antioxidant enzyme activities (CAT, SOD and POD) were increased with salinity. However, exogenously applied GA_3 significantly enhanced the plant growth and TSP in onion seedlings. Interestingly, CAT, SOD and POD concentration decreased with GA_3 application which depicts stress alleviation in saline stressed onion plants due to GA_3. It was concluded that the growth of onion could be enhanced to some extent by the application of GA_3 under salinity stress.

Keywords: *Allium cepa*, PGRs, Saline stress, Antioxidative response, Yield

1. Introduction

Onion (*Allium cepa* L.) is a biennial crop, and it belongs to *Alliacea* family (Adamicki & Kepka 1974). It is the one of most popular vegetables in the daily diet. Onion is an important commercial crop for the economy of Pakistan (FAO 2012). Salinity has adverse effect on growth and production of agricultural crops (Munns & Tester 2008). Studies showed that due to the saline toxicity every aspect of physiological and biochemical of plant is effect. (Khan & Panda 2008). Plants face two basic problems under saline conditions. Firstly, excessive salt lower down the osmotic potential in soil solution which reduces uptake of water in plant. Secondly, maximum Na^+ and Cl^- ions uptake diverts the absorption rate of essential minerals and ascribe toxicity to plants (Tester & Davenport 2003). Specific ion toxicities damaged the tissues of transpiring leaves which occur due to boron, sodium, and chloride accumulation. This accumulation of adverse ions may reduce protein synthesis, photosynthesis, and inactivate enzymes, and also damaged chloroplasts as well as other plant organelles (Taiz & Zeiger 2002).

Onion lacks tap root system and root hairs. Most of the root system is confined within top 20-25 cm of soil. Plant growth rate is half of other vegetables such as cauliflower and cabbage (Brewster 1994). Onion is more vulnerable to salinity than other vegetables, particularly the seedling emergence stage (Brewster 1994). Threshold electrical conductivity (EC) level for onions is 1.2 dS m^{-1} at 25 °C and each unit change in EC cause about 16% reduction in yield (Allen et al. 1998).

Gibberellic acid (GA_3) affects production by increasing the stem length and internodes of onion plant. It restricts senescence by change in lipid peroxidation and adjusts high level of cellular antioxidants like superoxide dismutase and catalase (Dhindsa et al. 1982). It also promotes the growth of the plant, promote cell division and cell extension (Olszewski et al. 2002; Ubeda et al. 2009). Sarkar et al. (2018) reported that GA_3 at 60 mg L^{-1} increased bulb weight over the control under normal conditions. GA enhances the water use efficiency of tomato plants at low salinity level by reducing stomatal resistance (Maggio et al. 2010). So, in the present study, it was hypothesized that GA_3 can alleviate salt stress in onion. Therefore, the present work was taken up under field conditions to determine the impact of different level of salinity on growth of onion and alleviation of stress by GA_3.

2. Material and Methods

The Phulkara genotype which is local variety was selected for this experimental study. Seeds were obtained from Punjab Food Corporation Faisalabad, Pakistan. The experiment was carried out in the vegetable experimental area of Institute of Horticultural Sciences (IHS), University of Agriculture Faisalabad, Pakistan in the year 2018 and 2019.

Seed sowing was done in soil pots containing 10 kg soil. Before the sowing of onion seeds, the electrical conductivity (EC) of the soil from representative area was calculated and compared to the results of real EC dS m^{-1} of soil. Then artificially salinity was caused by adding the measured quantity of salt (NaCl) in soil and mixed well according to get the desired salinity dS m^{-1}. The calculations were calculated according to the Rayment & Higginson (1992) method. Measured quantity of salt was added to the soil and mixed well by the soil mixer to get the homogeneous mixture.

Then the experimental area was cleaned and layered with polythene tunnel sheet to avoid the leaching of salt in other soil. Inorganic fertilizers were supplied properly at the rate of 50 kg ha^{-1} K as potassium sulphate, 50 kg ha^{-1} of N as urea and 80 kg ha^{-1} of P as di-ammonium phosphate. The six treatments were maintained as; (T_0) control; (T_1) GA_3: $GA_3$100 mg L^{-1}; (T_2) salinity level: 2 dS m^{-1}; (T_3) salinity level: 2dS m^{-1} + Gibberellic acid: $GA_3$100 mg L^{-1}; (T_4) salinity level: 4 dS m^{-1}; (T_5) salinity level: 4 dS m^{-1} + Gibberellic acid: GA_3 100 mg L^{-1}. The foliar spray of GA_3 (100 mg L^{-1}) was applied after 45 days of sowing days. Onion plants were harvested after 135 days of GA_3 application and different growth parameters were analyzed. Samples were saved in -20 °C for biochemical analysis.

2.1. Growth parameters

The plant height was calculated by the scale from the tip of that plant to the base on ground. The length of the leaf blade was measured from the base of leaf of observing plant to the tip. It was done by using a scale meter. Evacuated plants were washed with clean water, straightened and after that its root length was estimating by utilizing a tape meter in centimeters and the average was taken for each replicate. The diameter of onion bulb was calculated at right angles to longitudinal axis at the widest form of the bulb of arbitrarily chosen plants in each plot by using veneer caliper (model 141) (Demisie & Tolessa 2017).

Plants chosen from each treatment were harvested at the end of the experiment. Roots were removed from that plants then they were washed with water to expel the dirt and soil. Then root weight was measured by using weighing balance. Root dry weight was estimated by putting the sample in oven at 65 ºC for 72 hr to dry the samples. At that point, weight was ascertained by adjust and the normal mean for each sample was figured.

2.2. Germination percentage

Data was recorded for germination on daily basis after one week of sowing seeds till 14 days. Germination percentage was then calculated by using the following formula:

$$\text{Germination percentage} = \frac{\text{no.of germinated seeds}}{\text{Total no of seeds sown}} \times 100$$

2.3. Biochemical analysis

Fresh samples were collected from the experimental area and stored at -20 °C. All the samples were crushed in 50 mL of 100 mM sodium phosphate (pH 7) buffer containing 0.5% (w/v) polyvinyl pyrrolidone and 1 mM ascorbic acid and homogenize mixture was prepared. After preparation of mixture sample were placed at 4 °C for 5 min. The collected mixture was filter by using filter paper and centrifuged it for 15 min at 5000 RPM and the supernatant was collected.

Peroxidase (POD) was determined by using the method of (Onsa et al. 2004). In this method 4-methylecatechol was used as a substrate. To check the activity of POD reaction mixture was prepared by using 4-methylcatechol, 10 mM sodium phosphate buffer, 5 mM H_2O_2 and 500 µL of total volume of 3 mL crude extract of the sample at room temperature. By using spectrophotometer absorption was measured at 420 nm.

Superoxide dismutase (SOD) activity was determined by using the method of (Kumar et al. 2012). Reaction mixture was prepared by using 0.2 mM EDTA, 12 mM L-methionine, 50 mM buffer of sodium phosphate (pH 7) .10 uM riboflavin, 50 µM NBT and 100 µL of final volume of 3 mL of the crude extracted sample. The reaction mixture was incubated into the white light for 15min at room temperature. After incubation of 15 min absorption was observed at 560 nm by using spectrophotometer.

Catalase (CAT) activity was measured by using the method of Aebi 1983. To calculate the CAT activity spectrophotometer was used. The reaction mixture was prepared by using 30 mM H_2O_2 100 mM sodium phosphate buffer (pH 7) and 100 uL crude extract of sample by volume of 3 mL. Absorption was calculated at 240nm at room temperature in spectrophotometer.

The soluble protein (TSP) was measured according to Coomassie Brilliant Blue G-250 Staining Method (Sedmak & Grossberg 1977).

2.4. Statistical analysis

All experiments were conducted in triplicate with two factorial randomized complete block design (RCBD) and all results were expressed as the average ± standard error of the measurements. Statistix 8.1 software was used for statistics.

3. Results and Discussion

Data about growth parameters (plant height, root fresh and dry weight, leaf blade length, root length, bulb diameter and germination percentage) of treated and untreated plants of onion seedlings are shown in Table 1. The foliar spray of GA_3 100 mg L^{-1} showed significant effect on plant height, root fresh and dry weight, leaf blade length, root length, bulb diameter and germination percentage under salinity (Table 1). Previously, Ali et al. (2015) also found that application of GA_3 has positive impact on growth and yield of onion. However, salinity stress reduced the growth parameters as compared to the control conditions (El-Shaieny 2015; Nasri et al. 2017). As the salinity increased, there was a gradual decline in all growth parameters of onion (Stia-Baba et al. 2010). Similarly, results showed that when salinity level increased the plant growth declined as compared to control (Table 1). The decrease in plant growth in saline stress might be as a result of that salinity removes the potassium ions via plant roots, which generates physiological discrepancy because potassium ion is essential for the synthesis of proteins and metabolism (Chen et al. 2007). However, exogenously applied GA_3 enhanced all the growth parameters under salinity stress as compared to their respective controls (Table 1). Similarly, Chauhan et al. (2019) reported that GA_3 enhanced the plant growth under the salinity condition. It might be due to the effect of GA_3 which partially diminishes the toxic effect of salinity by increasing anti-oxidative, vigor, accumulation of osmolytes, and enzyme activities (Neelambari et al. 2018). Another study reported that decline in growth of plant under saline stress because of osmotic stress (Hakim et al. 2010).

Table 1- Effect of Gibberellic acid (GA_3, 100 mg L^{-1}) and salinity (0, 2, 4 ds m^{-1}) on plant height, leaf blade length, root length, bulb diameter, root fresh weight, root dry weight and germination percentage of onion (phulkara variety) plants

Treatments	*Plant height (cm)*	*Leaf blade length (cm)*	*Root length (cm)*	*Bulb diameter (cm)*	*Root fresh weight (g)*	*Root dry weight (g)*	*Germination percentage (GP%)*
Control	46.7± 1.10ab	38.6± 1.37b	12.5± o.77ab	3.8± 0.52b	56.6± 2.71b	6.43± o.41b	77.7± 3.2b
GA_3	50.4± 1.15a	47.7± 1.08a	14.3± 0.72a	5.5± 0.33a	68.1± 2.11a	8.33± 0.31a	88.8± 3.2a
2 dS m^{-1}	43.7± 0.92b	36.3± 1.21bc	11.6± 0.50b	3.5± 0.21b	41.5± 2.06d	4.03± 0.42c	66.6± 3.2c
2 dS m^{-1} + GA_3	45.1± 1.82b	37.9± 1.19bc	12.3± 0.70ab	3.7± 0.29b	46.9± 1.62c	4.8± 0.34c	83.3± 3.2ab
4 dS m^{-1}	34.7± 1.99c	33.6± 1.35c	7.4± 0.81c	2.5± 0.28c	33.6± 2.19e	2.9± 0.55d	44.4± 3.2d
4 dS m^{-1} + GA_3	44.8± 1.23b	36.7± 1.55bc	11.8± 0.40b	3.3± 0.08b	36.2± 2.97e	3.1± 0.34d	75.9± 4.8bc

Each data values are represented as mean and ±SD of three replications and different lower case letters are representing the significant difference between treatments and same lower-case letters represent the no significant difference by according to LSD test ($P \leq 0.05$)

Results of biochemical assays depict that salinity stress significantly have increased the activity of antioxidant enzymes (CAT, POD and SOD) as compared to control conditions (Figures 1-3). When the salinity was increased then the activity CAT, POD and SOD was increased maximum in onion. While foliar application of GA_3 further enhanced the activity of CAT, POD and SOD under salinity stress as compared to their respective control. The basic function of POD in plants is to break down the hydrogen peroxide (H_2O_2) which is very toxic and reactive element (Botella et al. 1994). Saline stress condition might be causing the univalent reduction in O_2^- which produce hydrogen peroxide. In the salt stress condition, the level of H_2O_2 was reduced which was the damage of plant defence system due to the higher concentration of POD. Sancho et al. (1996) also stated same consequences in his study and recognized this to variations in the mechanical characteristics of cell wall which in turn, might be connected to the salinity adjustment mechanism. The enhancement of POD with GA_3 under salinity stress (Figure 1) might be due to that GA_3 increases the gibberellins which stimulates the decrease in hydrolytic enzymes and sugars (Mathew & Murray 1968). GA_3 have competition with saline conditions via improving the membrane permeability of plant cell and adjust the level of nutrients in cell. Eventually this leads to increase the growth, and GA_3 also has induced physiochemical variations which are responsible for the influence of salt tolerance (Amal & Mohamed 2014).

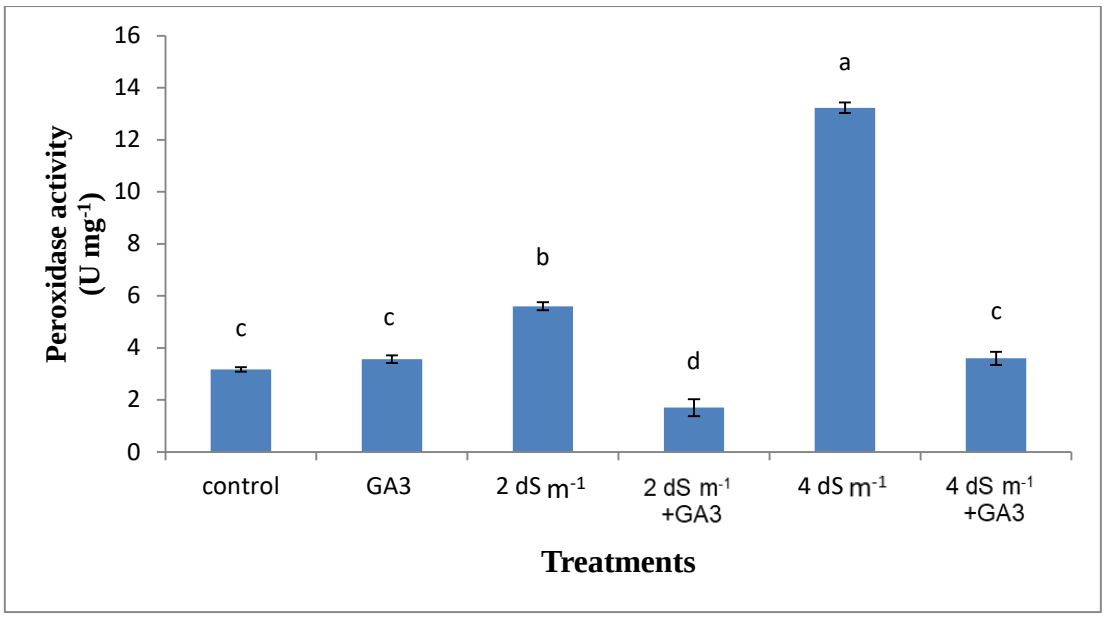

Figure 1- Effect of Gibberellic acid (GA_3, 100 mg L^{-1}) and salinity (0, 2, 4 dS m^{-1}) on peroxidase activity (POD) of onion seedlings. Each data values are represented as mean and ±SD of three replications and different lower-case letters are representing the significant difference between treatments and same lower-case letters represents the no significant difference by according to LSD test (P≤0.05)

Superoxide dismutase (SOD) is an important antioxidant which scavenges the reactive oxygen species (ROS) and it is activated under stress conditions. This is the reason that SOD enhanced in saline stress condition (Figure 2). It gives the initial line of protection against the noxious effects of stress. SOD eliminates superoxide radicals by catalyzing the dismutation of superoxides and reduces it to peroxide which is also oxidized by another antioxidant POD (Gill & Tuteja 2010). Foliar spray of GA_3 increased SOD activity to some extent because it increased the gibberellins which stimulate the decrease in hydrolytic enzymes and sugars (Mathew & Murray 1968). The SOD decreased in saline treated plants by foliar treatment of GA_3 (100 mg L^{-1}) as compared to control (Figure 2). It is due to that foliar application of GA_3 improves the membrane of plant cell and adjusts the level of nutrients in the cells (Chakrabarti & Mukharji 2003). Eventually this leads to decrease in SOD, and GA_3 induces physiochemical variations which are responsible for the influence of salt tolerance (Amal & Mohamed 2014).

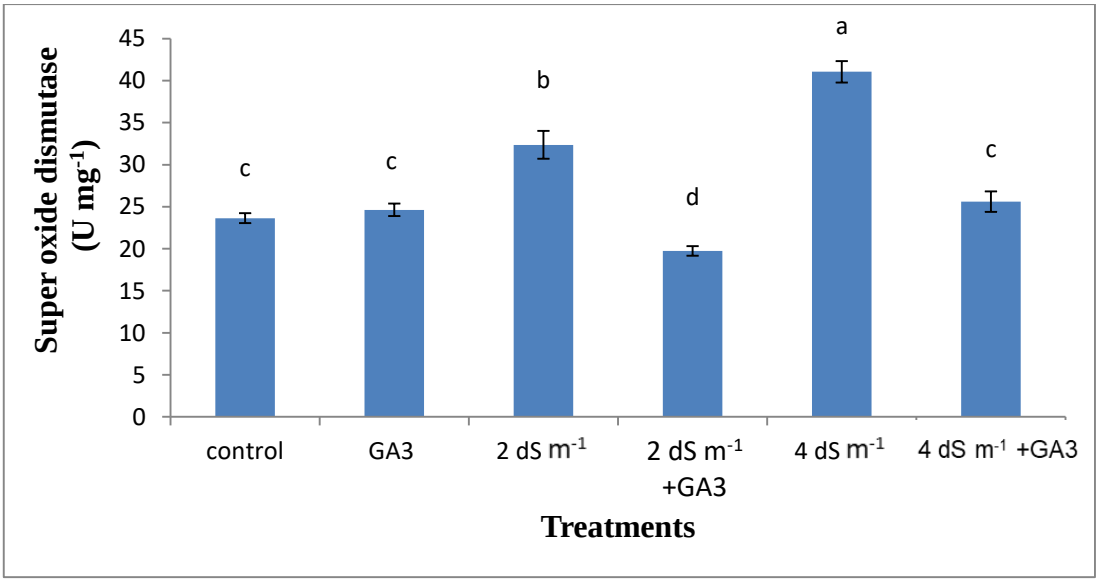

Figure 2- Effect of Gibberellic acid (GA_3, 100 mg L^{-1}) and salinity (0, 2, 4 dS m^{-1}) on superoxide dismutase activity (SOD) of onion seedlings. Each data values are represented as mean and ±SD of three replications and different lower-case letters are representing the significant difference between treatments and same lower-case letters represents the no significant difference by according to LSD test (P≤0.05)

Catalases (CAT) also plays important role in plant during the saline stress condition. During the saline stress condition, the CAT level increased as compared to control (Figure 3). Eyidogan & Oz (2007) observed that CAT level was increased in the

leaves of *C. arietinum* under saline conditions. In stress conditions, isoforms of CAT presents on different chromosomes which regulate independently (Scandalias 1990). CAT helps the plants to fight against oxidative stress that improves the plant growth (Polidoros & Scandalios 1999). Exogenous application of GA_3 improved the membrane permeability of plant cell and adjusts the level of nutrients in cell. This leads to decrease in CAT and also GA_3 induce physiochemical variations which are responsible for the influence of salt tolerance (Chakrabarti & Mukharji 2003; Amal & Mohamed 2014).

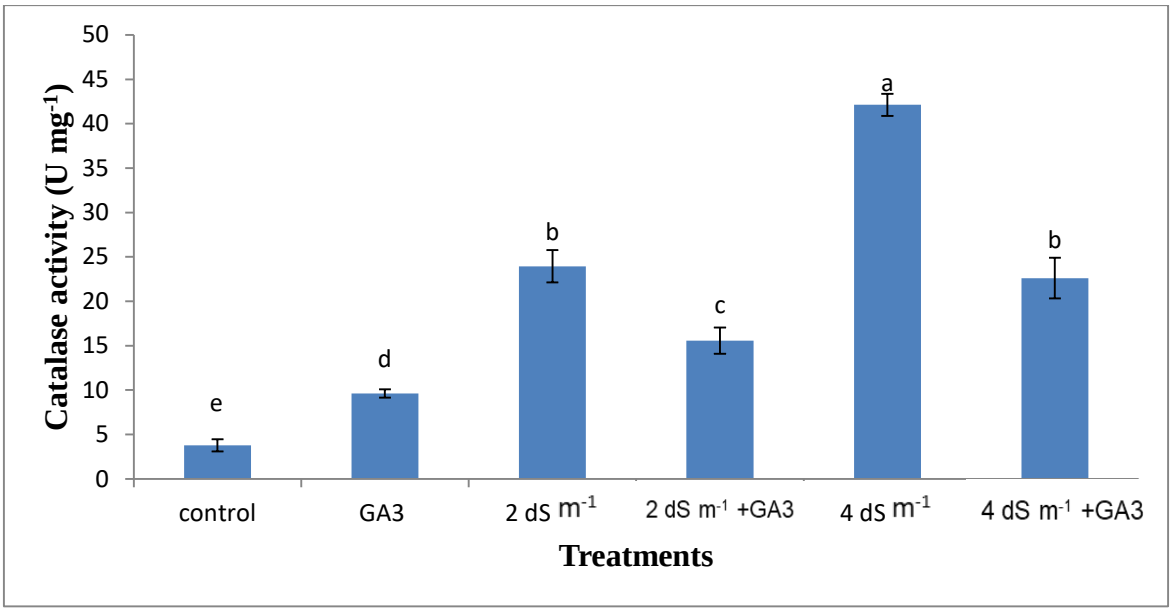

Figure 3- Effect of Gibberellic acid (GA_3, 100 mg L^{-1}) and salinity (0, 2, 4 dS m^{-1}) on catalase activity (CAT) of onion seedlings. Each data values are represented as mean and ±SD of three replications and different lower-case letters are representing the significant difference between treatments and same lower-case letters represents the no significant difference by according to LSD test (P≤0.05)

Total soluble proteins (TSP) has been observed lower when the plants are grown under saline toxic soils (Zhang et al. 2009), some proteins which have defensive mechanism, stimulated the plant growth under salinity and tolerate against salt stress (Aghaei et al. 2008). Significant effects of GA_3 and NaCl and its interaction was observed on TSP contents in onion (Figure 4). TSP contents firstly increased at low salinity levels with GA_3 and then the contents of TSP decreased at high concentrations of salinity level (Figure 4). The similar results were also found by Jiao et al. (2019) in caster bean as the concentration of GA_3 increased, the TSP content first increased and then decreased.

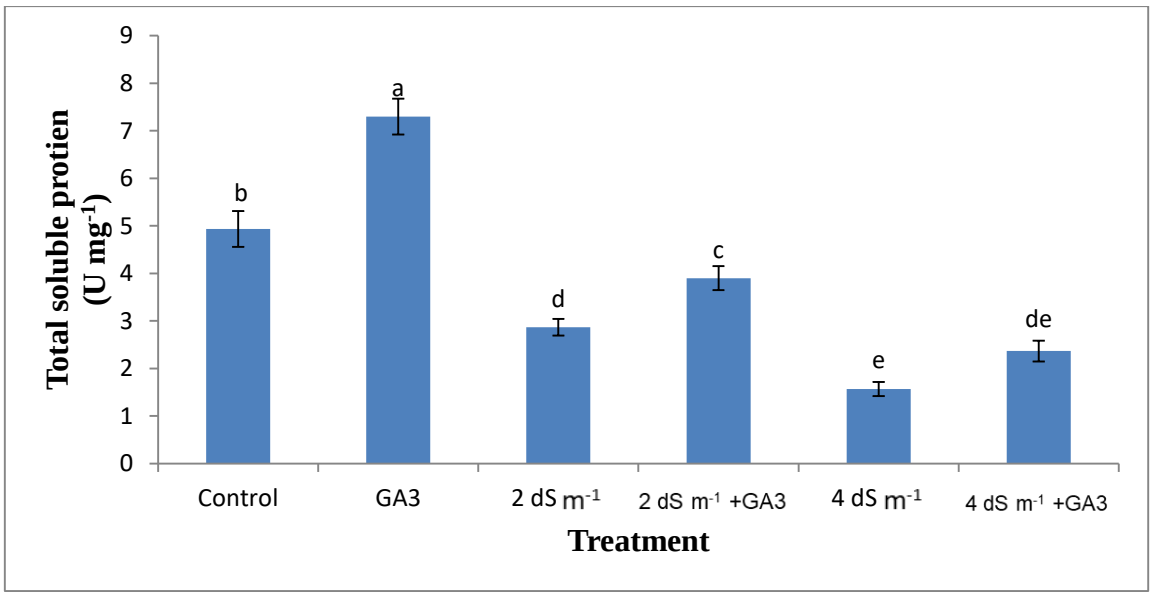

Figure 4- Effect of Gibberellic acid (GA_3, 100 mg L^{-1}) and salinity (0, 2, 4 dS m^{-1}) on SOD, POD, CAT, soluble protein, and proline content of onion seedlings. Each data values are represented as mean and ±SD of three replications and different lower-case letters are representing the significant difference between treatments and same lower-case letters represents the no significant difference by according to LSD test (P≤0.05)

4. Conclusions

Our findings revealed that the salinity stress significantly reduced plant growth parameters in onion seedlings. However, exogenously applied GA_3 significantly enhanced the plant growth and by reducing oxidative stress. Interestingly, a decline was observed in antioxidant enzyme activities (SOD, POD and CAT) with the application of GA_3 in saline stress conditions. Although these activities were increased to maximum level at salinity stress alone treatment. It is concluded from our study that we can enhance the growth of onion plant in saline stress with the foliar application of GA_3. Less is known about the mechanism of GA_3 in onion under salinity stress. Hence, to check the mechanism and role of GA_3 under saline conditions in different plant species more study is required.

References

Adamicki F & Kepka A K (1974). Storage of onions in controlled atmospheres. *Acta Horti Culturae* 38: 53-74 https://doi.org/10.17660/ActaHortic.1974.38.5

Aebi H E (1983). Catalase. In: Bergmeyer H U (Ed.), Methods of enzymatic analysis, Verlag Chemie, Weinhem, pp. 273-286

Aghaei K, Ehsanpour A A, Balali G & Mostajeran A (2008). In vitro screening of potato (*Solanum tuberosum* L.) cultivars for salt tolerance using physiological parameters and RAPD analysis. *American-Eurasian Journal of Agricultural and Environmental Sciences* 3: 159-164

Allen R G, Pereira L S, Raes D & Smith M (1998). Crop evapotranspiration-guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. Food and Agriculture Organization, Rome

Ali M A, Hossain M M, Zakaria M, Hossain T, Naznin A & Islam M M (2015). Effect of GA_3 on quality seed production of onion in Bangladesh. *Eco-Friendly Agriculture Journal* 8(03): 47-50

Amal M E & Mohamed A H H (2014). The effect of the exogenous gibberellic acid on two salt stressed barely cultivars. *European Scientific Journal* 10: 228-245

Botella M A, Quesada M A, Kononowicz A K, Bressan, R A, Pligo F, Hasegawa P M & Valpuesta V (1994). Characterization and in situ localization of a salt-induced tomato peroxidase mRNA. *Plant Molecular Biology* 25: 105-114 https://doi.org/10.1007/BF00024202

Brewster J L (1994). Onions and other vegetable alliums. Wallingford, Oxfordshire, CAB International, 236 pp UK

Chakrabarti N & Mukharji S (2003). Alleviation of NaCl stress by pretreatment of phytohormones in *Vigna radiata*. *Biologia Plantarum* 46: 589-594 https://doi.org/10.1023/A:1024827931134

Chauhan A, Abu-Amarah B A, Kumar A, Verma J S, Ghramh H A, Khan K A & Ansari M J (2019). Influence of gibberellic acid and different salt concentrations on germination percentage and physiological parameters of oat cultivars. *Saudi Journal of Biological Sciences* 26: 1298-1304 DOI: 10.1016/j.sjbs.2019.04.014

Chen Z C, Tracey A, Meixue Z, Amanda T, Bodapati N & Sergey S (2007). Compatible solute accumulation and stress-mitigating effects in barley genotypes contrasting in their salt tolerance. *Journal of Experimental Botany* 58: 4245-4255 DOI: 10.1093/jxb/erm284

Demisie R & Tolessa K (2017). Growth and bulb yield of onion (*Allium cepa* L.) in response to plant density and variety in jimma, south western Ethiopia. *Advances in Crop Science and Technology* 6: 357 DOI: 10.4172/2329-8863.1000357

Dhindsa R S, Plumb-Dhindsa P L & Reid D M (1982). Leaf senescence and lipid peroxidation: effects of some phytohormones, and scavengers of free radicals and singlet oxygen. *Physiologia Plantarum* 56: 453-457 https://doi.org/10.1111/j.1399-3054.1982.tb04539.x

El-Shaieny A H (2015). Seed germination percentage and early seedling establishment of five (*Vigna unguiculata* L. (Walp) genotypes under salt stress. *European Journal of Experimental Biology* 5: 22-32

Eyidogan F & OZ M T (2007). Effect of salinity on antioxidant responses of chickpea seedlings. *Acta Physiologiae Plantarum* 29: 485-493

FAO (2012). The State of Food and Agriculture. Retrieved in 2012 from http://www.fao.org/publications/sofa/2012/en/

Gill S S & Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* 48: 909-930 DOI: 10.1016/j.plaphy.2010.08.016

Hakim M A, Juraimi A S, Begum M, Hanafi M M, Ismail M R & Selamat A (2010). Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). *African Journal of Biotechnology* 9: 911-1918 DOI: 10.5897/AJB09.1526

Jiao X, Zhi W, Liu G, Zhu G, Feng G, Nimir N E A, Ahmad I & Zhou G (2019). Responses of foreign ga3 application on seedling growth of castor bean (*Ricinus communis* L.) under salinity stress conditions. Agronomy 9: 274 https://doi.org/10.3390/agronomy9060274

Khan M H & Panda S K (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiologiae Plantarum* 30: 91-89 https://doi.org/10.1007/s11738-007-0093-7

Kumar A, Dutt S, Bagler G, Ahuja P S & Kumar S (2012). Engineering a thermo-stable superoxide dismutase functional at subzero to 50°C, which also tolerates autoclaving. *Scientific Reports* 2: 387 https://doi.org/10.1038/srep00387

Maggio A, Barbieri G, Raimondi G & Pascale S (2010). Contrasting effects of GA3 treatments on tomato plants exposed to increasing salinity. *Journal of Plant Growth Regulation* 29:63-72 https://doi.org/10.1007/s00344-009-9114-7

Mathew A H & Murray A M (1968). The effect of Gibberellic acid on peroxidase levels in barley. *Planta* 83: 387-389 https://doi.org/10.1007/BF00387618

Munns R & Tester M (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651-681 https://doi.org/10.1146/annurev.arplant.59.032607.092911

Nasri N, Maatallah S, Saidi L & Lachaal M (2017). Influence of salinity on germination, seedling growth, ion content and acid phosphatase activities of *Linum usitatissimum* L. *Journal of Animal and Plant Sciences* 27(2): 517-521

Neelambari, Mandavia C & Ganesh S S (2018). Curative Effect of Ascorbic Acid and Gibberellic Acid on Wheat (*Triticum astivum* L.) Metabolism under Salinity Stress. *International Journal of Current Microbiology and Applied Sciences* 7(1): 522-533

Olszewski N, Sun T P & Gubler F (2002). Gibberellin signaling: biosynthesis, catabolism, and response pathways. *Plant Cell* 14: 61-80 DOI: 10.1105/tpc.010476

Onsa G H, Saari N, Selamat J & Bakar J (2004). Purification and characterization of membrane-bound peroxidases from metroxylon sagu. *Food Chemistry* 85: 365-376 https://doi.org/10.1016/j.foodchem.2003.07.013

Polidoros A N & Scandalios J G (1999). Role of hydrogen peroxide and different classes of antioxidants in the regulation of catalase and glutathione S-transferase gene expression in maize (*Zea mays* L.). *Physiologia Plantarum* 106:112-120 https://doi.org/10.1034/j.1399-3054.1999.106116.x

Rayment G E & Higginson F R (1992). Australian laboratory handbook of soil and water chemical methods, Melbourne, in kata press. pp. 330

Sancho M A, Forchettis M, Pliego F & Valpuesta M A (1996). Peroxidase activity and isoenzymes in culture medium of NaC1 adapted tomato suspension cells. *Plant Cell, Tissue and Organ Culture* 44: 161-166 https://doi.org/10.1007/BF00048195

Scandalias J G (1990). Response of plant antioxidant defense genes to environmental stress. *Advances in Genetics* 28: 1-41 DOI: 10.1016/s0065-2660(08)60522-2

Sedmak J J & Grossberg S E (1977). A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250. *Analytical Biochemistry* 79: 544-552 https://doi.org/10.1016/0003-2697(77)90428-6

Stia-Baba, Hachicha M, Mansour M, Nahdi H & Kheder M B (2010). Response of onion to salinity. *The African Journal of Plant Science and Biotechnology* 4(2): 7-12

Taiz L & Zeiger E (2002). Plant Physiology, 3rd ed. Publisher Sinauer, Sunderland, UK

Tester M & Devenport R (2003). Na+ tolerance Na+ transport in higher plants. *Annals of Botany* 91: 503-527 https://doi.org/10.1093/aob/mcg058

Ubeda T S, Federici F & Casimiro I (2009). Gibberellins signaling in the endodermis controls arabidopsis root meristem size. *Current Biology* 19: 1194-1199 https://doi.org/10.1016/j.cub.2009.06.023

Zhang X G, He Q Y, Li C J, Meng M L & Wang X D (2009). Change in osmoregulation substances in potato under mixed salt stress. *Chinese Potato Journal* 23: 129-132

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Reference Evapotranspiration Estimation With kNN and ANN Models Using Different Climate Input Combinations in the Semi-arid Environment

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ABSTRACT
The absolute prediction of reference evapotranspiration (ET_o) is an important issue for global water balance. Present study demonstrated the performance of k-Nearest Neighbour (kNN) and Artificial Neural Network (ANN) models for prediction of daily ET_o using four combinations of climatic data. The kNN and ANN models were studied four combinations of daily climate data during 1996-2015 in the Middle Anatolia region. The findings of ET_o estimation with kNN and ANN models were classed with the FAO Penman Monteith equation. The outcomes of ET_o values demonstrated that the kNN had higher performances than the ANN in all combinations. The statistical indicators of the kNN model showed ET_o values with MSE, RMSE, MAE, NSE and R^2 ranging from 0.541-0.031 mm day^{-1}, 0.735-0.175 mm day^{-1}, 0.547-0.124 mm day^{-1}, 0.937-0.997 and 0.900-0.994 in the testing subset. Thus, the kNN can be used for the prediction of reference evapotranspiration with full and limited input meteorological data.

Keywords: Evapotranspiration, Penman-Monteith, K-nearest neighbour, Artifical neural network

1. Introduction

Evapotranspiration (ET) can be described as water loss into the atmosphere via plant transpiration and soil evaporation (Landeras et al. 2008; Fan et al. 2018). Water resources are significantly reduced in semi-arid and arid environments due to the consequences of increasing climate change. In these regions where water shortage is a major problem, it is essential to estimate water loss by ET. Therefore, precise prediction of ET is an imperative step for managing water activities, especially in the area which faces water scarcity.

Numerous methods to estimate ET have been recommended but each method has benefits and limitations due to their activities. However, methods which are depending on measurement are high-cost and also have usage difficulties. Therefore, a more economical and practical alternative application to this method is developing tools which are depending on mathematical models using climate variables measured from meteorological stations.

The Penman-Monteith equation is frequently applied method due to recommendation of the Food and Agriculture Organization of the United Nations as a standard method (FAO PM) for reference evapotranspiration (ET_o) estimation. In literature (Lopez-Urrea et al. 2006; Ali & Shui 2009; Pereira et al. 2015), the method was evaluated under different time steps and environmental conditions. For calculation of ET_o, the method requires many climatic input parameters (Feng et al. 2017), which is a big disadvantage of this equation. Moreover, the prediction of ET is a complicated process dependent on a huge and good quality of climatic parameters; therefore, it is difficult to represent all these complicated processes in an empirical model. Especially in developing countries, the meteorological data are very limited. This problem brings another obstacle of using FAO PM method. Therefore, simplified empirical methods with less climatic input variables are getting interested for ET_o estimation (Trabert 1896; Priestley & Taylor 1972; Hargreaves & Samani 1985). However, these methods obtain less accurate results for daily ET_o estimation than on a weekly and monthly (Torres et al. 2011).

Interest in the machine learning method in ET_o estimation has increased over the last two decades (Kisi & Cimen 2009; Feng et al. 2016; Tangune & Escobedo 2018) because these non-parametric methods can work without specific knowledge about the variables that are used for the models (Gocić et al. 2015; Kişi 2015; Yamaç & Todorovic 2020). Among the machine learning methods for prediction of ET_o, one of the most common methods is the artificial neural network (ANN) model. Ferreira et al. (2019) investigated the ANN and support vector machine (SVM) to predict ET_o in Brazil, using different climatic variables. The findings showed that the ANN gives the best result for the temperature and relative humidity-based models. Antonopoulos &

Antonopoulos (2017) examined the prediction of ET_o comparing the ANN model and empirical equations in Greece. They pointed out that the performance metrics of the ANN model was higher than empirical equations. Landeras et al. (2008) studied the prediction of ET_o using empirical equations and the ANN in Spain. The ANN is better than the empirical equations. Traore et al. (2010) applied the ANN model for ET_o estimation in the Sudano-Sahelian zone. The model showed that the ANN can be used as an alternative model for prediction of ET_o. Khoob (2008) compared the Hargreaves-Samani (1985) equation and ANN to estimate ET_o in Iran. The result demonstrated that the ANN estimated better than the Hargreaves-Samani equation. Moreover, the recent study was done by Feng & Tian (2020). They compared nearest neighbor algorithms and empirical methods in China. The findings demonstrated that kNN method is more accurate than empirical methods. However, very few studies have used machine learning methods for estimation of ET in Turkey. Citakoglu et al. (2014) evaluated the estimation of monthly ET_o using adaptive network based fuzzy inference system (ANFIS) and ANN models in Turkey. The ANFIS estimated slightly higher performance than the ANN. Kisi (2016) investigated M5 Model Tree (M5Tree), multivariate adaptive regression splines (MARS) and least square support vector regression (LSSVR) methods in Turkey. The overall result indicates that the LSSVR observed the best results with local output and input variables while the MARS model performed the best results in estimating ET_o in the lack of local output and input data.

The goal of the study is to make a comparison of kNN and ANN models with a standard method of FAO PM using four combinations of meteorological data for the prediction of ET_o. In this way, the paper was purposed to understand the accurate modelling performance for prediction of ET_o in semi arid environment of Turkey comparing one recognized and widely used model (ANN) with recently used model (kNN) from first combination to fourth combination which is from less to more meteorological data.

2. Material and Methods

2.1. Study area and dataset description

The area under study is Konya in the Middle Anatolia region of Turkey. The meteorological station is placed at 1030 m altitude, 38° 14′ N latitude and 32° 40′ E longitude. The daily weather data was taken from the Turkish Meteorological Organization in Turkey. The climatic data was recorded from 1996 to 2015 (20 years). The collected data was maximum and minimum air temperature (°C), maximum and minimum relative humidity (%), solar radiation (MJ m^{-2}) and wind speed (m s^{-1}). Table 1 shows the statistical characteristics of the meteorological parameters. The climate of study region is a semi-arid (Kottek et al. 2006) and the average yearly precipitation is 548 mm. Figure 1 presents total annual precipitation variables for 20 years (1996-2015).

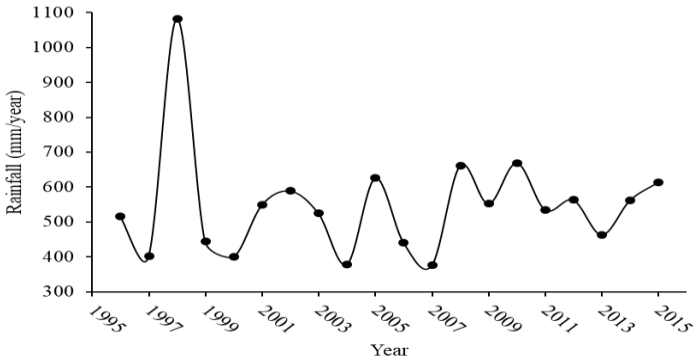

Figure 1- The total annual precipitations for 20 years (1996-2015)

Table 1- Statistical parameters of the used dataset

Variables	T_{min} (°C)	T_{max} (°C)	R_n (MJ m^{-2})	RH_{min} (%)	RH_{max} (%)	U_2 (m s^{-1})	ET_o (mm day^{-1})
Maximum	22.10	39.60	30.64	88.31	100.00	7.20	10.91
Minimum	-18.90	-9.20	0.10	9.83	24.83	0.10	0.37
Mean	6.64	18.85	16.57	37.63	74.08	2.56	3.77
Standard deviation	7.28	9.77	7.62	14.99	22.49	1.01	2.33
Skewness	-0.04	-0.16	-0.05	0.66	-0.16	0.28	0.44
Kurtosis	-1.04	-1.07	-1.12	0.22	-1.46	0.00	-0.89

(ET_o: reference evapotranspiration, T_{min}: minimum air temperature, T_{max}: maximum air temperature, R_n: solar radiation, RH_{min}: minimum air relative humidity, RH_{max}: maximum air relative humidity, U_2: wind speed).

2.2. FAO Penman-Monteith

The FAO PM equation (Allen et al. 1998) was used for prediction of daily ET_o;

$$ET_o = \frac{0.408\Delta(R_n-G)+\gamma\frac{900}{T+273}U_2(e_s-e_a)}{\Delta+\gamma(1+0.34\,U_2)} \quad (1)$$

Where; ET_o, is the reference evapotranspiration (mm day^{-1}), Rn is the net solar radiation (MJ m^{-2} day^{-1}); G, is the soil heat flux density (MJ m^{-2} day^{-1}), T, is the mean daily air temperature (°C); Δ, is the slope of the saturated vapour pressure curve (kPa $°C^{-1}$); γ , is the psychometric constant (0.066 kPa $°C^{-1}$), es is saturation vapour pressure (kPa)and ea is actual vapour pressure (kPa) and U_2 is the mean daily wind speed (m s^{-1}). T and U_2 was measured at 2m height.

The e_s was estimated as:

$$e_s = \frac{e^0(T_{max})+e^0(T_{min})}{2} \quad (2)$$

Where; e^0 (T), is the saturation vapour pressure (kPa), and T_{min} and T_{max} are minimum and maximum daily air temperature (°C), respectively. The $e^0(T)$ was calculated as:

$$e^0(T) = 0.6108\ exp\ \left[\frac{17.27\,T}{T+237.3}\right] \quad (3)$$

The e_a was calculated as:

$$e_a = \frac{RH_{mean}}{100}\left[\frac{e^0(Tmax)+e^0(Tmin)}{2}\right] \quad (4)$$

Where; $RH_{mean,}$ is the mean daily relative humidity.

2.3. k-Nearest neighbour

The kNN is the simple classification method, presented by Cover & Hart (1967), which is widely used machine learning methods (Wu et al. 2008). It is non-parametric which is easy to implement and which obtains efficient and competitive results. This advantage makes method much more significant than many other machine learning methods.

Figure 2 shows the kNN schematic illustration for 2 classes of k=1 and k=3. In Figure 1a, a known sample (-), nearest to the sample X, is used for categorization of sample X; in Figure 2b, three nearest (+) samples to X are employed for categorization. The present study was applied Euclidian distance equation (Equation 2) . It can be written as:

$$x(a,b) = \sqrt{\sum_{n=1}^{N}(a_i-b_i)^2} \quad (5)$$

Where; x, is the Euclidian distance, a and b are the data including to N dimensions. n is an index number.

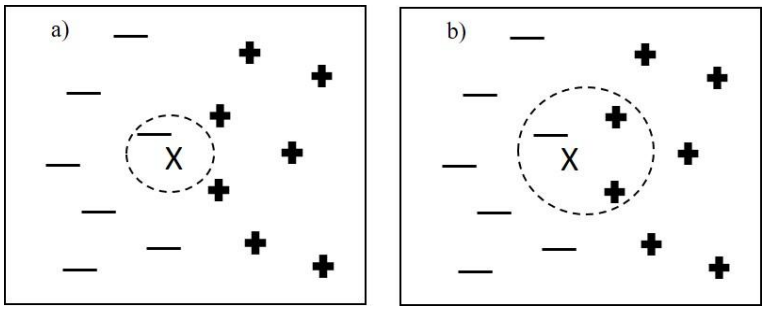

Figure 2- The k-nearest neighbour (kNN) schematic illustration

2.4. Artificial neural network

The ANN model based on numerical model that was developed and designed in order to analyse the performance of a biological neural system. The structure of ANN models is similar as biological brain with numerous layers of connected neurons. (Landeras et al. 2008). In recent decades, the ANN has been applied in hydrological and agricultural studies (Kumar et al. 2011). The general architecture of the ANN is shown in Figure 3. The model has the capability to learn, memorize and create relationships

between weighted neurons from a training dataset. When the testing data is implemented into the system, the model realises the relationships between neurons and assigns the data to the appropriate class. The well known structure of an ANN model is formed of an input layer, where the data is entered; hidden layer(s), where the data is processed; and output layer, where it gives the results (Yamaç et al. 2020).

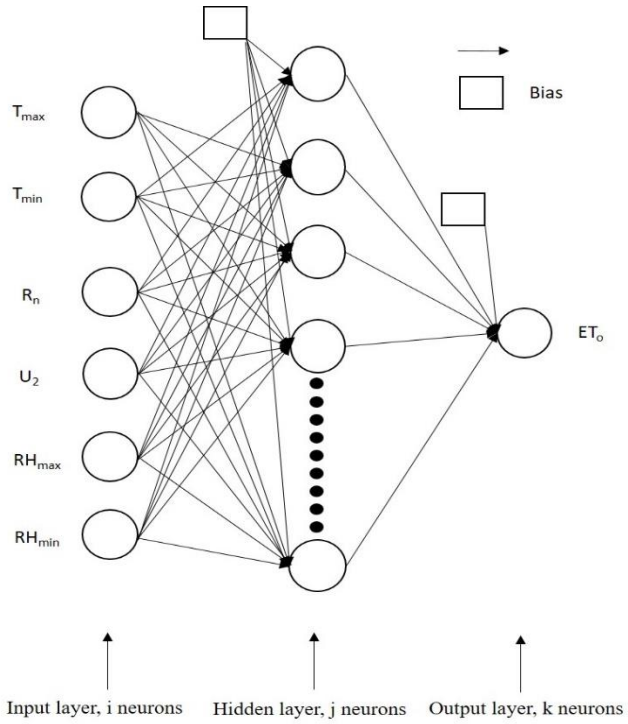

Figure 3- The general architecture of the artificial neural networks

2.5. Model development and performance evaluation

The kNN and ANN models were developed to simulate and estimate the daily ET_o in a semi-arid environment. To establish kNN and ANN models, six climatic variables (wind speed, solar radiation, minimum-maximum relative humidity and minimum-maximum air temperature) were employed as inputs, while ET_o was employed as the output variable. Correlations between these climatic variables and ET_o have been shown in Table 2. The reason of development of the correlation matrix was to understand which climatic variables have the best relations with ET_o. According to correlation matrix, the next nearest correlation was added for development of combinations. Table 3 shows different input combinations for the models.

Table 2- Correlation matrix between ET_o and climatic data

Variables	T_{min}	T_{max}	R_n	RH_{min}	RH_{max}	U_2	ET_o
T_{min}	1.000						
T_{max}	0.914	1.000					
R_n	0.687	0.839	1.000				
RH_{min}	-0.349	-0.691	-0.732	1.000			
RH_{max}	-0.700	-0.784	-0.665	0.580	1.000		
U_2	-0.031	-0.035	0.039	0.019	0.026	1.000	
ET_o	0.819	0.915	0.913	-0.680	-0.779	0.185	1.000

Before the models run, all the variables are standardized ranging between 0 to 1. The standardization equation is defined as:

$$z = \frac{x - \mu}{\sigma} \quad (6)$$

Where; σ is the standard deviation, μ is the mean value and x is the original data.

Table 3- Input combinations of the kNN and ANN models

Models		*Inputs combinations*
kNN	*ANN*	
kNN1	ANN1	T_{max}, T_{min}
kNN2	ANN2	T_{max}, $T_{min,}$ R_n
kNN3	ANN3	T_{max}, $T_{min,}$ $R_{n,}$ U_2
kNN4	ANN4	T_{max}, $T_{min,}$ $R_{n,}$ U_2, RH_{max}, RH_{min}

The total 20 years dataset (1996-2015), 70% of which was used for training and 30% for testing, was split randomly. For the training subset, k-fold cross-validation was applied to evaluate predictive models. The training dataset was separated into 10 folds. In this way, the kNN and ANN models were trained and tested 10 times and gave the results according to average of the 10 repetition.

The performance of kNN and ANN models were appraised using coefficient of determination (R^2), Nash-Sutcliffe model efficiency coefficient (NSE), mean absolute error (MAE), root means square error (RMSE) and the mean squared error (MSE) in the training and testing subsets. The good performance metrics of the models can be understood when MAE, RMSE and MSE values are smaller and NSE and R^2 are higher.

3. Results and Discussion

The kNN and ANN with four combinations of climatic input data were evaluate for training and testing subsets. The findings showed that the kNN and ANN models were able to describe the nonlinear relationships between meteorological variables to estimate daily ET_o values adequately. The performance metrics of the models, including MSE, RMSE, MAE and R^2 are presented in Tables 4 and 5 for the prediction of daily ET_o. As can be seen in Tables 4 and 5, all the applied kNN and ANN models presented accurate daily ET_o estimates during training and testing subsets. In general, the kNN4 showed the highest performance metrics. However, the ANN1 model has the lowest performance in the testing subset.

The best accuracy of the kNN under four climatic conditions to estimate daily ET_o over training and testing subsets was observed when the k was chosen as 5. Table 4 demonstrated the performance metrics of the kNN model to estimate daily ET_o during the training and testing subsets for four combinations of available climatic data. Employing four different climatic input combinations, the statistical indicators of daily ET_o using kNN presented that the lowest performance in the training and testing subsets was observed when ET_o was predicted only with maximum air temperature and minimum air temperature (kNN1). An appropriate improvement of model performance was observed for combination 2 with the reduction of statistical indicator in the testing subset. Among all combinations of kNN models, the highest performance was observed fourth combination (kNN4) in the testing subset.

Table 4- Performance metrics of the kNN models under four different climate input

	Training					*Testing*				
Model	*MSE*	*RMSE*	*MAE*	*NSE*	R^2	*MSE*	*RMSE*	*MAE*	*NSE*	R^2
	(mm day^{-1})	*(mm day^{-1})*	*(mm day^{-1})*			*(mm day^{-1})*	*(mm day^{-1})*	*(mm day^{-1})*		
kNN1	0.796	0.892	0.669	0.830	0.853	0.541	0.735	0.547	0.932	0.900
kNN2	0.350	0.591	0.429	0.920	0.936	0.232	0.482	0.349	0.961	0.957
kNN3	0.075	0.274	0.192	0.997	0.986	0.049	0.220	0.155	0.995	0.991
kNN4	0.048	0.220	0.154	0.981	0.991	0.031	0.175	0.124	0.997	0.994

For the ANN model, the 5 was identified for the number of neurons in the hidden layer. The best performance criteria was showed when ANN model has 2(3, 4, 6)-5-1 structure for daily ET_o estimation. This can be explained that the model occurs of 2 neurons for first, 3 neurons for second, 4 neurons for third and 6 neurons for fourth combinations in input layer, 1 in the output layer and 5 neurons in the hidden layer. For the activation function, the rectified linear unit function was employed for this study. Table 5 demonstrated the performance metrics of the ANN model to estimate daily ET_o during the training and testing subsets for four combinations of available climatic data. Among all ANN models, the ANN1 model demonstrated the lowest performance in training and testing subsets. From the first to the second combination, a relevant improvement of more than 30% ET_o estimate was observed for MSE, RMSE and MAE values when solar radiation is added together with minimum air temperature and maximum air temperature data (ANN2). It is noticeable that the ANN method had the highest performance for ANN4 model.

Table 5- Performance metrics of the ANN models under four different climate input

	Training					*Testing*				
Model	*MSE* (*mm day^{-1}*)	*RMSE* (*mm day^{-1}*)	*MAE* (*mm day^{-1}*)	*NSE*	R^2	*MSE* (*mm day^{-1}*)	*RMSE* (*mm day^{-1}*)	*MAE* (*mm day^{-1}*)	*NSE*	R^2
ANN1	0.724	0.851	0.653	0.883	0.867	0.695	0.834	0.635	0.893	0.872
ANN2	0.338	0.582	0.433	0.922	0.938	0.322	0.567	0.421	0.923	0.941
ANN3	0.097	0.311	0.225	0.989	0.982	0.106	0.325	0.237	0.968	0.981
ANN4	0.074	0.227	0.196	0.967	0.986	0.051	0.225	0.162	0.993	0.991

The scatter plot of predicted ET_o values by the kNN and ANN with four combinations of input climate variables, compared with the FAO PM equation during testing subset, are presented in Figures 4 and 5. In general, for all models, the fourth combination with maximum air temperature, minimum air temperature, solar radiation, maximum relative humidity, minimum relative humidity and wind speed correlated close to the line of 1:1. However, the first combination with maximum air temperature and minimum air temperature yielded more scattered ET_o values relative to the other climatic input combinations. The daily ET_o values estimated from kNN with first combination (kNN1) model were more close to the FAO PM equation values (Figure 4).

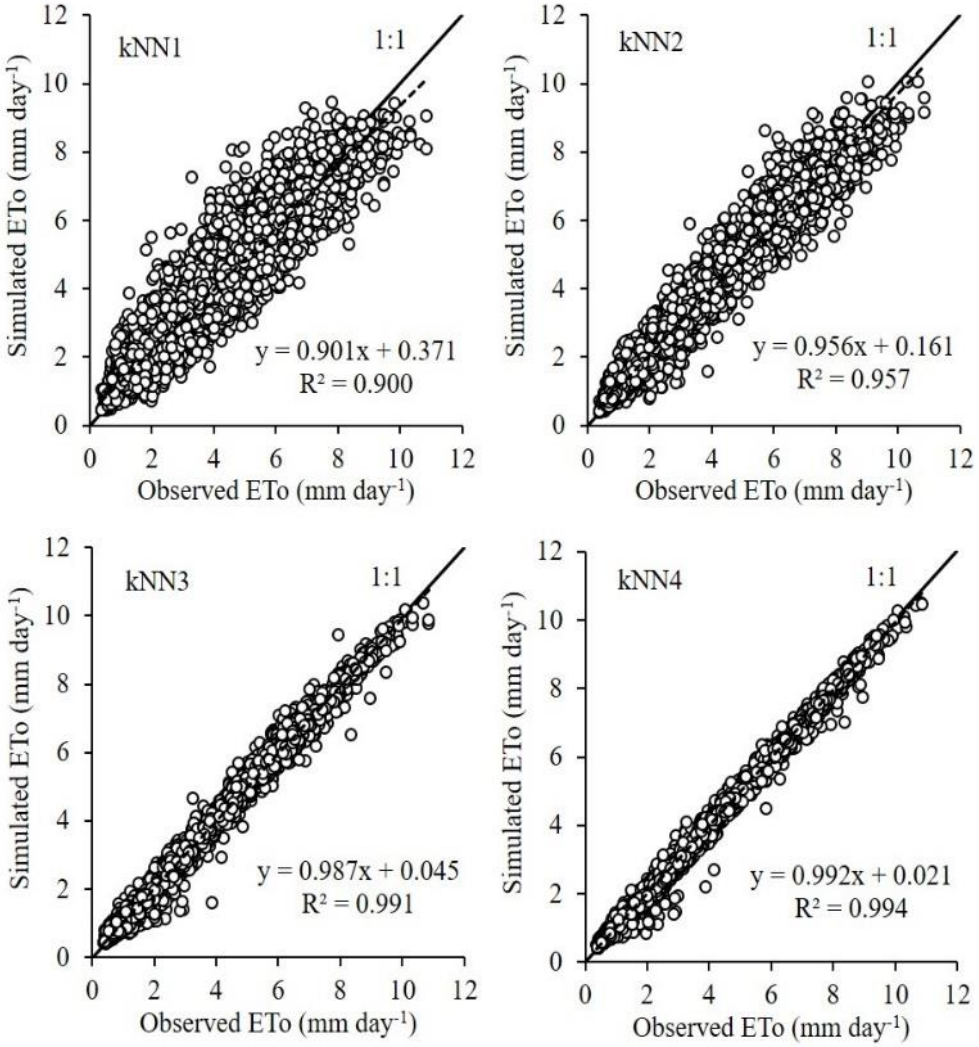

Figure 4- Scatter plots of the kNN models under four different climate input for testing subset

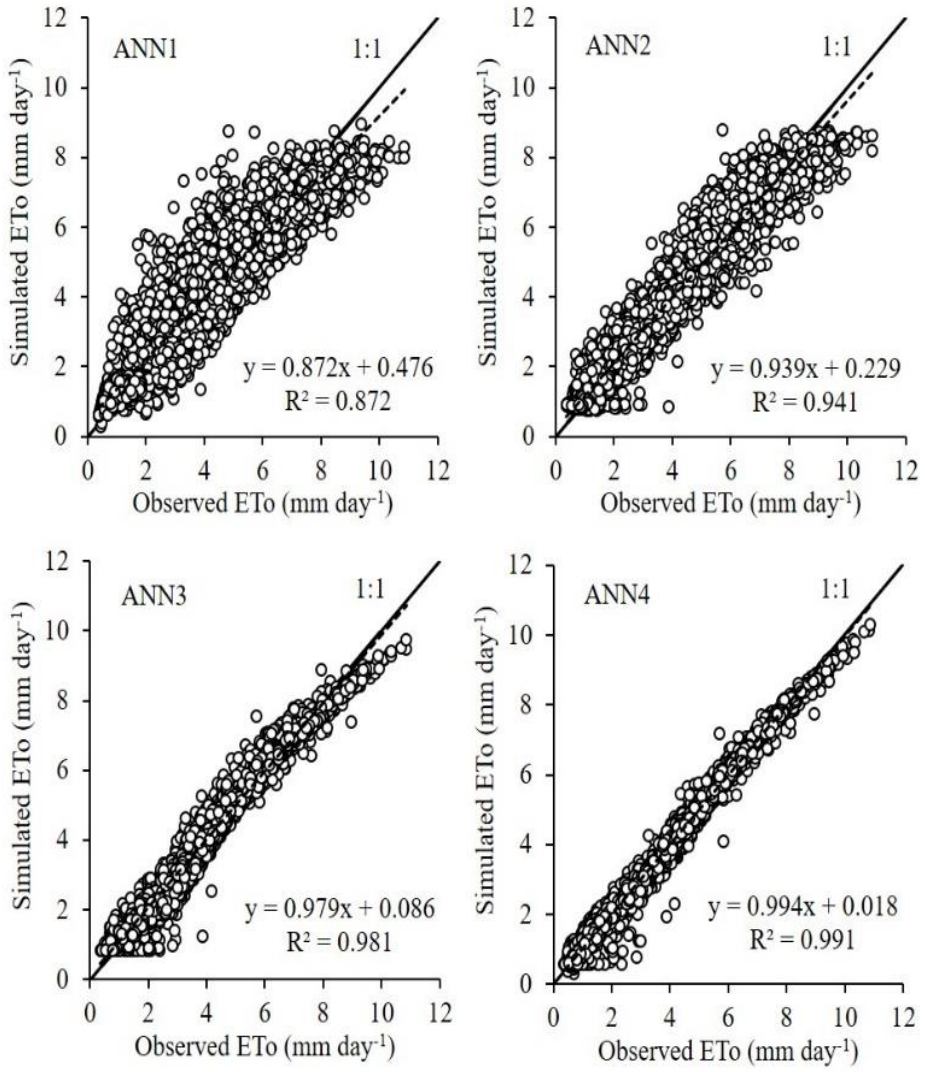

Figure 5- Scatter plots of the ANN models under four different climate input for testing subset

In general, the statistical indicators demonstrated that the fourth combination provides by far the best performance for kNN and ANN models with complete meteorological data while the poorest performance was obtained with the first combination fed with maximum and minimum temperature. In general, the findings are in agreement with literature (Torres et al. 2011; Tabari et al. 2012), concluding that more climatic input variables commonly increase modelling accuracy. This result is in accordance with Fan et al. (2018) who also indicated that machine learning models with temperature, relative humidity, wind speed and solar radiation inputs have the best performances comparing with the less meteorological variables in the semi-arid environment. Moreover, the findings showed that the kNN and ANN with maximum/minimum temperature, combined with solar radiation (second combination), have a better performance than the kNN and ANN models with minimum and maximum temperature in a semi-arid region. In that case, for testing subset, the kNN2 model, R^2 was 0.957, NSE was 0.961, MSE was 0.232, RMSE was 0.458 and MAE was 0.349. For ANN2 model, R^2 was 0.941, NSE was 0.923, MSE was 0.322, RMSE was 0.567 and MAE was 0.421 in the testing subset. These results demonstrated that the solar radiation input was more substantial than wind speed and relative humidity upon maximum/minimum temperatures in a semi-arid region. According to statistical indicators, with the kNN and ANN models based on solar radiation and maximum/minimum temperature (kNN2 and ANN2), meteorological input variables can also produce satisfactory ET_o estimates in the semi-arid environment of Turkey where other meteorological variables are not easily accessible.

Previous studies indicated that employing all meteorological input variables provided the best performances for predicting ET_o. Feng et al. (2017) predicted daily ET_o with random forests (RF) and generalized regression neural networks (GRNN) models using different meteorological variables concluding that the models with complete meteorological data is preferable than the combination which is added less meteorological variables. A similar result was pointed out also by Traore et al. (2010) when the ANN was used to predict daily ET_o variables in Sudano-Sahelian zone.

The kNN model showed the best performances in all combinations when compared to the ANN model. This could be explained by the fact that the kNN model concentrating on the characteristic of the nearest neighbours similar to the behaviour

of applied climatic variables and their correlation with the ET_o. Comparing result from previous study, larger RMSE and MAE were mentioned by (Feng & Tian 2020) using the kNN model. From this comparative analysis, it may be concluded that it is suitable to estimate ET_o employing kNN model in semi-arid environment of Turkey.

4. Conclusions

This paper presented an application of the kNN and ANN models for the accurate estimate of daily ET_o with full and limited meteorological data in a semi-arid environment of Turkey. To identify the optimal results to estimate daily ET_o in the mentioned semi-arid region, the kNN and ANN models with four different combinations of meteorological input variables were proposed. The recently used kNN model was implemented to estimate daily ET_o for analysing the performance metrics of different combinations of climatic input data and to compare with a well-known ANN model. This ANN was applied in many previous studies, therefore; it is used as a comparison model in order to evaluate the performance of kNN model in this study.

The statistical performance in the testing and training subsets was improved by adding one climatic parameter to each combination (from 1 to 4), which demonstrated positive correlations with the number of input variables to the kNN and ANN models. Among all the combinations, the kNN model offered better predictional accuracy and stability than the well-known ANN model. Therefore, the results advocated that the kNN has a high potential for ET_o prediction in the semi-arid region of Turkey, even possibly in other regions of the world with presenting similar environments. In addition, the overall results showed that less meteorological input combinations may be a suitable alternative solution where full meteorological data sets are not available. This finding is especially important for agricultural lands in developing countries, where meteorological data are missing to estimate ET_o.

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References

Ali M H & Shui L T (2009). Potential evapotranspiration model for Muda irrigation project, Malaysia. *Water Resources Management* 23: 57-69 DOI: 10.1007/s11269-008-9264-6

Allen R G, Pereira L S, Raes D & Smith M (1998). Crop Evapotranspiration: Guide Lines for Computing Crop Evapotranspiration. Rome, Italy: FAO Irrigation and Drainage Paper No. 56

Antonopoulos V S & Antonopoulos A V (2017). Daily reference evapotranspiration estimates by artificial neural networks technique and empirical equations using limited input climate. *Computers and Electronics in Agriculture* 132: 86-96 DOI: 10.1016/j.compag.2016.11.011

Citakoglu H, Cobaner M, Haktanir T & Kisi O (2014). Estimation of monthly mean reference evapotranspiration in Turkey. *Water Resources Management* 28: 99-113 DOI 10.1007/s11269-013-0474-1

Cover T M & Hart P E (1967). Nearest neighbor pattern classification. *IEEE Transactions on Information Theory* 13(1): 21-27

Fan J, Yue W, Wu L, Zhang F, Cai H & Wang X (2018). Evaluation of SVM, ELM and four tree-based ensemble models for predicting daily reference evapotranspiration using limited meteorological data in different climates of China. *Agricultural and Forest Meteorology* 263: 225-241 DOI: 10.1016/j.agrformet.2018.08.019

Feng F & Tian J (2020). Estimating potential evapotranspiration based on self-optimizing nearest neighbor algorithms: a case study in arid-semiarid environments, Northwest of China. *Environmental Science and Pollution Research* DOI: 10.1007/s11356-019-06597-7

Feng Y, Cui N, Zhoa L, Hu X & Gong D (2016). Comparison of ELM, GANN, WNN and empirical models for estimating reference evapotranspiration in humid region of Southwest China. *Journal of Hydrology* 536: 376-383 DOI: 10.1016/j.jhydrol.2016.02.053

Feng Y, Cui N, Gong D, Zhang Q & Zhoa L (2017). Evaluation of random forest and generalized regression neural networks for daily reference evapotranspiration modelling. *Agricultural Water Management* 193: 163-173 DOI: 10.1016/j.agwat.2017.08.003

Ferreira L B, da Cunha F F, de Oliveira R A & Fernandes Filho E I (2019). Estimation of reference evapotranspiration in Brazil with limited meteorological data using ANN and SVM – a new approach. *Journal of Hydrology* 572: 556-570 DOI: 10.1016/j.jhydrol.2019.03.028

Gocić M, Motamedi S, Shamshirband S, Petković D, Ch S, Hashim R & Arif M (2015). Soft computing approaches for forecasting reference evapotranspiration. *Computers and Electronics in Agriculture* 113:164-173 DOI: 10.1016/j.compag.2015.02.010

Hargreaves G H & Samani Z A (1985). Reference crop evapotranspiration from temperature. *Applied Engineering in Agriculture* 1: 96-99 DOI: 10.13031/2013.26773

Khoob A R (2008). Comparative study of Hargreaves's and artificial neural network's methodologies in estimating reference evapotranspiration in a semiarid environment. *Irrigation Science* 26: 253-289 DOI: 10.1007/s00271-007-0090-z

Kisi O (2016). Modelling reference evapotranspiration using three different heuristic regression approaches. *Agricultural Water Management* 169: 162-172 DOI: 10.1016/j.agwat.2016.02.026

Kisi O & Çimen M (2009). Evapotranspiration modelling using support vector machines. *Hydrological Sciences Journal* 54: 918-928 DOI: 10.1623/hysj.54.5.918

Kişi O (2015). Pan evaporation modeling using least square support vector machine, multivariate adaptive regression splines and M5 model tree. *Journal of Hydrology* 528: 312-320 DOI: 10.1016/j.jhydrol.2015.06.052

Kottek M, Grieser J, Beck C, Rudolf B & Rubel F (2006). World Map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift* 15: 259-263 DOI: 10.1127/0941-2948/2006/0130

Kumar M, Raghuwanshi N S & Singh R (2011). Artificial neural networks approach in evapotranspiration modeling: a review. *Irrigation Science* 29(1): 11-25 DOI: 10.1007/s00271-010-0230-8

Landeras G, Ortiz-Barredo A & López J J (2008). Comparison of artificial neural network models and empirical and semi-empirical equations for daily reference evapotranspiration estimation in the Basque Country (Northern Spain). *Agricultural Water Management* 95: 553-565 DOI: 10.1016/j.agwat.2007.12.011

Lopez-Urrea R, Martin de Santa Olalla F, Fabeiro C & Moratalla A (2006). Testing evapotranspiration equations using lysimeter observations in a semiarid climate. *Agricultural Water Management* 85: 15-26 DOI: 10.1016/j.agwat.2006.03.014

Pereira L S, Allen R G, Smith M & Raes D (2015). Crop evapotranspiration estimation with FAO56: Past and future. *Agricultural Water Management* 147: 4-20 DOI: 10.1016/j.agwat.2014.07.031

Priestley C H B & Taylor R J (1972). On the assessment of surface heat flux and evaporation using large-scale parameters. *Monthly Weather Review* 100: 81-92 DOI: 10.1175/1520-0493(1972)100<0081:OTAOSH>2.3.CO;2

Tabari H, Marofi S, Aeini A, Talaee P H & Mohammadi K (2012). Trend analysis of reference evapotranspiration in the western half of Iran. *Agricultural and Forest Meteorology* 151: 128-136 DOI: 10.1016/j.agrformet.2010.09.009

Tangune B F & Escobedo J F (2018). Reference evapotranspiration in São Paulo State: empirical methods and machine learning techniques. *Water Resources and Environmental Engineering* 10: 33-44 DOI: 10.5897/IJWREE2018.0772

Torres A F, Walker W R & McKee M (2011). Forecasting daily potential evapotranspiration using machine learning and limited climatic data. *Agricultural Water Management* 98: 553-562 DOI: 10.1016/j.agwat.2010.10.012

Trabert W (1896). Neue beobachtungen {ü}ber verdampfungsgeschwindigkeiten. *Meteorologische Zeitschrift* 13: 261-263

Traore S, Wang Y-M & Kerh T (2010). Artificial neural network for modelling reference evapotranspiration complex process in Sudano-Sahelian zone. *Agricultural Water Management* 97: 707-714 DOI: 10.1016/j.agwat.2010.01.002

Wu X, Kumar V, Ross Quinlan J, Ghosh J, Yang Q, Motoda H, McLachlan G J, Ng A, Liu B, Yu P S, Zhou Z H, Steinbach M, Hand D J & Steinberg D (2008). Top 10 Algorithms in Data Mining. *Knowledge and Information Systems* 14: 1-37 DOI: 10.1007/s10115-007-0114-2

Yamaç S S & Todorovic M (2020). Estimation of daily potato crop evapotranspiration using three different machine learning algorithms and four scenarios of available meteorological data. *Agricultural Water Management* 228: 105875 DOI: 10.1016/j.agwat.2019.105875

Yamaç S S, Şeker C & Negiş H (2020). Evaluation of machine learning methods to predict soil moisture constants with different combinations of soil input data for calcareous soils in a semi arid area. *Agricultural Water Management* 234: 106121 DOI: 10.1016/j.agwat.2020.106121

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Pedotransfer Functions for Estimation of Soil Moisture Constants from Penetration Resistance Measurements and Some Soil Properties

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ABSTRACT
Studies to prediction of soil moisture constants and other soil properties rather than direct measurements were never dwindle importance. Models were derived from other soil properties obtained easily. Therefore, in this study focused on the predictability of some moisture constants, whose determination was often difficult and time-consuming, from penetration resistance measurements. In the improvement of alternative models for the estimation of moisture constants; in addition to penetration resistance, textural fractions (sand, clay and silt), bulk density, $CaCO_3$ % and organic matter contents were included. The models were created according to soil groups with different textures (sandy, loamy, clay) for moisture constants at 0.1, 0.33, 0.5 and 15 bar. In the models for estimation of 0.1, 0.33, 0.5 and 15 bar moisture content, the highest differences in R^2 values (0.61, 0.60, 0.64 and 0.59) between the actual and the predicted data was obtained for loamy soils. For this group, the root means square error (RMSE) ranged between 1.32 and 1.90 %, and in addition, the mean error (ME) was determined to be in a range from 1.53 to 2.05 %. For the estimation of moisture content at different soil moisture tensions using organic matter, bulk density, clay and penetration resistance properties, the coefficient of determination ranged from 71 to 77 %. Therefore, it is concluded that the alternative models, developed using penetration resistance or by the addition of some other soil properties, could be used safely in the loamy texture soils.

Keywords: Penetration resistance, Moisture constants, Pedotransfer functions, Soil physics

1. Introduction

Pedotransfer functions (PTFs) explain the relationships within soil properties, and they have attracted the attention of researchers in recent years. In these studies, some soil properties are estimated indirectly by way of other easily measured soil properties, instead of laborious and time-consuming methods. Thus, it is possible to easily determine properties that are otherwise difficult to predict thanks to these functions. The field capacity and permanent wilting point moisture constants are great importance in determining the optimum amount of water to be used for plants. Bahtiyar (1978) stated that only part of the capillary water, which is associated with a soil moisture tension of 0.31 to 33 atmospheres in the soil can be used by the plants between the field capacity (0.33 atm) and the permanent wilting point (15 atm). Field capacity and permanent wilting point determination are very time consuming and laborious in field conditions. Studies where the field capacity and permanent wilting point properties could be estimated by other soil properties (Minasny 2009; Mohanty et al. 2015; Touil et al. 2016) have remained important for many years. When soil properties are examined, some soil features affect many other properties (Turgut et al. 2010; Negiş et al. 2016). Penetration resistance varies depending on many other properties, significant increases have been observed, especially for decreasing moisture content (Busscher 1990; Şeker 1997; Turgut et al. 2008). It was predicted that this property, which is greatly affected by soil moisture content, can be used to estimate soil moisture constants. In this study the focus was on models to estimate some soil properties by using data on other known properties of soil. The penetration resistance values in soils are significantly affected by moisture and the range of this property is wide; it is also easy to determine. The aim was to estimate moisture constants by using penetration resistance values. The use of digital penetrologger, which was a very important approach in determining soil moisture constant, would facilitate the use of the created pedotransfer functions, because the determination of moisture constants take a long time in the laboratory conditions. The penetration resistance value can be determined instantly in the field using digital penetrologger. However, determination of soil moisture constants (field capacity and permanent wilting point) takes between 3-5 days in the laboratory. The main purpose of this study is to determine the moisture constants such as field capacity, permanent wilting point instantly with the penetration resistance value that you determined during the field reading. In addition, the penetration resistance is highly influenced by the soil moisture content. So, this study was designed with the idea that penetration resistance can be used as a variable in the estimation of moisture constants. In this study, models were created according to soil groups with different textures (sandy, loamy, clay) for moisture constants (0.1, 0.33, 0.5 and 15 bar). Alternative models were produced using other

soil properties (sand, clay silt, bulk density, $CaCO_3$ equivalent and organic matter) in addition to the penetration resistance to investigate the effects of other soil properties on models.

2. Material and Methods

The study was carried out over an area of approximately 308,136 hectares of the Atabey, Keçiborlu, Gönen, Eğirdir, Gelendost, Yalvaç and Aksu districts of Isparta province in Turkey. Disturbed and undisturbed surface soil samples (0 to 20 cm) were taken in 107 different agricultural fields. At these locations, penetration resistance measurements (PR) were recorded at different moisture levels. Penetration resistance measurements were made in the field and then some soil samples were taken and weighed. Samples were placed in storage containers to determine moisture content and brought to the laboratory. Penetration resistance measurements were performed using penetrologger (Eijkelkamp) in the field. 1 cm^2 base area cone-shaped (Cone 60º, NEN 5140) tip was used for penetration measurements. A representative location in the field was selected and measurements were made there. The soil moisture content was measured across a range spanning from saturated to dry, and the penetration resistance was noted to determine the moisture-penetration resistance relationship in the field. The spring rains were expected to determine moisture levels close to saturation. The field part of the study was completed in 2 years. In the spring rains of the first year, sampling and measurements were made in 50 different field. The remaining 57 field soil measurements was made in the 2^{nd} years. After the excess water was drained in the soil, the penetration resistance measure and soil moisture determinations started and continued to dry after 3 days in the field conditions. After the first measurements, moisture-penetration resistance was monitored at representative location. In order to establish the relationship between moisture and penetration resistance measurements, sampling and measurements were performed 20-30 times at each point. Some physico-chemical analysis were carried out in the soil (disturbed and undisturbed) brought to the laboratory in the first sampling. The soils were divided into groups according to the principles of the Soil Survey Manual (1993) as sandy (S, LS), loamy (SL, L, SiL, Si, CL, SCL, SiCL) and clay (SC, SiC, C) soils. There were determined to be 21 sandy soils, 66 loamy soils and 20 clay soil groups. Soil moisture content at 0.1, 0.33, 0.5 and 15 bar, and some of their physical and chemical properties were investigated (Table 1). The moisture content was determined by gravimetric (M) and volumetric (Θ). The average moisture content (*am*), specific to a texture group, was found for each tension pressure (0.1, 0.33, 0.5 and 15 bars). The penetration resistance values (*PRam*), which correspond to the average moisture (*am*), for the soil in the group, were determined using the PR-moisture graph of the soil. Then, the *PRam* values of soils in the same group, and the actual moisture values (*M*) maintained at that pressure were associated (PR*am* − *M*) to obtain the prediction model. In order to express the penetration resistance measurements made at different moisture levels against a standard moisture level (*am*), the moisture-PR data were associated with all soils in each soil group. These relationships were used in the determination of the PR corresponding to the standard moisture (*am*), when implementing the estimation models. Then, moisture correction equations were included in the basic model to obtain the prediction models. In addition, the use of textural fractions (sand, silt, clay) and some other soil properties ($CaCO_3$, organic matter and bulk density) were also investigated for the prediction of moisture constants.

Table 1- Soil properties used in the models

Soil group		*BD* gr cm^{-3}	*Clay %*	*Silt %*	*Sand %*	*0.1 bar (%)*	*0.33 bar (%)*	*0.5 bar (%)*	*15 bar (%)*	*$CaCO_3$ %*	*OM %*	*pH*	*EC* µmhos cm^{-1}
Sandy	Min.	1.30	4.80	7.55	78.00	12.71	9.40	8.04	4.06	0.86	0.79	5.37	67
	Max.	1.58	10.71	16.65	86.74	17.74	14.53	11.88	7.35	3.95	1.85	7.86	696
	Mean	1.40	6.77	11.31	81.91	14.98	12.12	10.22	5.47	2.17	1.18	7.36	173
Loamy	Min.	1.21	5.47	4.90	10.87	14.52	11.17	9.65	6.04	1.29	0.87	6.63	101
	Max.	1.55	34.92	70.41	76.53	29.29	27.43	25.12	16.78	45.27	3.92	8.38	1236
	Mean	1.35	23.72	30.11	46.17	23.68	20.71	18.84	11.24	15.19	1.97	7.70	350
Clay	Min.	1.13	40.32	13.48	9.37	26.47	22.67	19.15	12.9	3.23	1.39	7.45	156
	Max.	1.49	49.49	43.39	38.8	32.84	28.99	27.65	18.77	44.88	3.5	8.3	725
	Mean	1.32	42.88	32.14	25.08	29.69	26.44	23.82	15.13	18	2.12	7.73	389.8

BD; Bulk Density, $CaCO_3$; Lime, OM; Organic matter, EC; Electrical conductivity

A mechanical analysis of the soils was performed using a hydrometer method and moisture content at 0.1, 0.33, 0.5 and 15 bar-determined by using Pressure Plate Apparatus (Demiralay 1993). The pH (Kacar 2009) and electrical conductivity (EC) (US Salinity Laboratory Staff 1954) were determined in a 1:1 soil-water suspension. The percentage of calcium carbonate equivalent ($CaCO_3$ %) was recorded using a Scheibler calcimeter, and the organic matter content measured by the Walkley Black method (Kacar 2009). The bulk density and moisture contents were determined according to Demiralay (1993). Minitab 16 statistical software was used to obtain models for moisture constants and to determine the significance levels. The normal distribution of the data was checked by the Kolmogorov-Smirnov test. Stepwise regression equations were used with all parameters (sand, silt, clay, $CaCO_3$, bulk density, organic matter and penetration resistance) as vary selection methods. In the

evaluation of the regression equations, the coefficient of determination (R^2), the root means square error (RMSE) and the mean error (ME) were used (as given Equation 1). These formulas are also widely used in the literatures (Qiao et al. 2018; Santra et al. 2018).

$$R^2 = 1 - \frac{\sum(yi-Y)^2}{\sum(yi-y)^2} \qquad RMSE = \sqrt{\frac{\sum (Xg-Xt)^2}{n}} \qquad ME = \sum_{i=1}^{n} \frac{(Xg-Xt)}{n} \tag{1}$$

The *yi* term used in the equation is the experimentally determined value, *Y* is the calculated value from the regression equation, *y* is the average of the experimental data, Xg is the actual value, Xt is the estimated value and n is the number of samples. The properties of the soil used in the testing phase of the models are given in Table 2.

Table 2- Properties of soil used in the models testing phase

Soil group		*BD* gr cm^{-3}	*Clay* %	*Silt* %	*Sand* %	*0.1 bar (%)*	*0.33 bar (%)*	*0.5 bar (%)*	*15 bar (%)*	*$CaCO_3$* %	*OM* %
Sandy	Minimum	1.21	5.20	7.80	78.10	11.60	8.29	5.95	4.35	2.55	1.05
	Maximum	1.62	10.10	16.70	84.80	16.20	13.84	12.20	6.81	9.20	2.81
	Mean	1.39	8.01	11.34	80.66	13.77	11.71	9.78	5.38	5.45	1.87
Loamy	Minimum	1.21	12.50	13.90	21.31	20.12	17.37	14.10	8.95	2.11	1.3
	Maximum	1.61	38.94	47.29	69.60	29.30	27.71	26.60	16.99	22.33	3.59
	Mean	1.42	26.46	31.67	41.86	25.22	22.53	20.33	12.54	8.44	2.11
Clay	Minimum	1.21	40.40	12.10	9.37	29.30	26.60	23.10	15.16	1.22	1.17
	Maximum	1.54	62.51	41.14	35.38	35.45	31.95	28.45	19.94	15.54	3.82
	Mean	1.36	52.71	28.54	18.75	32.41	29.39	26.54	17.62	6.47	1.97

BD; Bulk Density, OM; Organic matter, $CaCO_3$; Lime

3. Results and Discussion

The moisture constants estimation models obtained for different textured groups are given in Table 3. The coefficient of determination (R^{2**}), root mean square error (RMSE) and mean error (ME) values were used in the evaluation of the suitability of the models that are given in Table 3. The coefficient of determination ranged from 0.22 to 0.64 for different soil groups. The R^{2**} values were determined to be at low levels during the test phase of the models obtained for sandy soils. This situation, which is often encountered in different prediction models, is often related to the variability in effective soil characteristics. The lowest RMSE and ME values were determined for the 0.1 bar gravimetric moisture content (1.16, 0.85%) and 15 bar volumetric moisture content (1.23, 0.91%) prediction models. The RMSE and ME values were increased from 0.1 bar to 15 bar for the sandy soils. In the study by Silva et al. (2015), the R^2 values were found to be 0.41 to 0.73 for 0.33 bar, and 0.58 to 0.75 for the 15 bar moisture content estimation models. In another similar study, the R^2 value obtained was found to be between 0.23 and 0.85 for moisture constants (Mohanty et al. 2014), which is like in this study. In the comparison of the validity of the estimation models according to soil groups, the highest R^{2**} values (0.54-0.64) were obtained for the loamy soils (Table 3). The models obtained for the loamy soils could estimate the actual moisture content with about a 60% accuracy. In the study by Keshavarzi et al. (2010), the test phase of the model constructed to estimate the field capacity and wilting point, the R^2 values were 0.68 and 0.64, and the RMSE values were 4.46% and 5.21%, respectively. In a study conducted by Esmaeelnejad et al. (2015), the R^2 values were 0.79 and 0.87 for the 0.33 and 15 bar prediction models, respectively, and the R^2 values obtained in the test phase were 0.68 and 0.77, respectively. It was evident that the test phase R^2 values obtained in the present study approximately match the values in published literature. The ME values changed from 1.53 to 5.11% and RMSE from 1.23 to 3.08% for loamy soil groups. In this group, The R^2 was high, The RMSE and ME were low because the number of samples was higher than other groups. It is known that the soils in the region were medium structure. The relationships between the predicted and actual moisture content in the clay soils were determination ranged from 0.48 to 0.54 for the different moisture tensions. Gülser (2004) determined the R^2 of the prediction model for the field capacity to be 0.85, while the difference between the predicted and actual value was 0.92. However, in the study, different soil properties (sand, clay, organic matter, EC, porosity) were evaluated for the estimation of moisture constants, and predictability was determined by increasing the number of variables included in the model. In the clay soils, the changes in RMSE, ME and R^{2**} were close for all soil moisture tensions. When the gravimetric moisture contents generally were used, higher R^{2**} and lower RMSE and ME values were obtained in all soil groups and moisture tensions. Gravimetric moisture content determinations models were more stable than volumetric moisture content models, because soil bulk density could variable. Busscher (1990) used the moisture content gravimetrically in his models to estimate the penetration resistance by using the bulk density and water content of the saturation and soil moisture content properties. The similarity between the soils used in the testing phase and those used in the model was the reason for the high correlation between the estimated and actual values. Many researchers report that prediction models are not available for each soil group due to the different characteristics of soils (Mohawesh 2013; Abdelbaki 2018). It was determined that there was no consistent change in the models due to increased moisture tension for R^2 values.

Table 3- Moisture prediction models

Soil groups	*Moisture tension (bar)*	*Models*	*RMSE %*	*ME %*	R^{2**}
Sandy (M)	0.1	$Y: 16.494[Pr_x \exp((x-0.15)/0.112)]^{0.2671}$	1.16	0.85	0.46
	0.33	$Y: 12.362[Pr_x \exp((x-0.12)/0.112)]^{0.4511}$	2.22	1.88	0.32
	0.5	$Y: 9.5247[Pr_x \exp((x-0.10)/0.112)]^{0.4288}$	2.76	2.27	0.22
	15	$Y: 3.2231[Pr_x \exp((x-0.055)/0.112)]^{0.7353}$	2.74	2.45	0.42
Loamy (M)	0.1	$Y: 25.869[Pr_x \exp((x-0.24)/0.172)]^{0.2617}$	1.63	1.77	0.61
	0.33	$Y: 21.113[Pr_x \exp((x-0.21)/0.172)]^{0.3645}$	1.46	1.75	0.60
	0.5	$Y: 17.848[Pr_x \exp((x-0.19)/0.172)]^{0.411}$	1.32	1.53	0.64
	15	$Y: 7.5323[Pr_x \exp((x-0.11)/0.172)]^{0.4573}$	1.90	2.05	0.59
Clay (M)	0.1	$Y: 31.508[Pr_x \exp((x-0.30)/0.126)]^{0.125}$	1.75	1.50	0.54
	0.33	$Y: 27.256[Pr_x \exp((x-0.26)/0.126)]^{0.1545}$	1.78	1.55	0.50
	0.5	$Y: 23.67[Pr_x \exp((x-0.24)/0.126)]^{0.1748}$	1.65	1.47	0.52
	15	$Y: 11.769[Pr_x \exp((x-0.15)/0.126)]^{0.3178}$	1.59	1.44	0.54
Sandy (Ɵ)	0.1	$Y: 22.88[Pr_x \exp((x-0.21)/0.153)]^{0.2494}$	2.88	2.23	0.39
	0.33	$Y: 17.203[Pr_x \exp((x-0.17)/0.153)]^{0.4563}$	1.47	1.08	0.39
	0.5	$Y: 13.157[Pr_x \exp((x-0.14)/0.153)]^{0.4725}$	1.31	1.06	0.39
	15	$Y: 5.67737[Pr_x \exp((x-0.08)/0.153)]^{0.4028}$	1.23	0.91	0.39
Loamy (Ɵ)	0.1	$Y: 35.248[Pr_x \exp((x-0.32)/0.238)]^{0.248}$	3.49	5.11	0.54
	0.33	$Y: 28.5537[Pr_x \exp((x-0.28)/0.238)]^{0.3742}$	3.08	4.92	0.54
	0.5	$Y: 24.114[Pr_x \exp((x-0.26)/0.238)]^{0.4166}$	1.23	1.70	0.58
	15	$Y: 9.9342[Pr_x \exp((x-0.15)/0.238)]^{0.4858}$	3.49	5.11	0.54
Clay (Ɵ)	0.1	$Y: 42.571[Pr_x \exp((x-0.39)/0.178)]^{0.1665}$	2.36	1.99	0.52
	0.33	$Y: 36.387[Pr_x \exp((x-0.35)/0.178)]^{0.1948}$	2.97	2.53	0.48
	0.5	$Y: 31.203[Pr_x \exp((x-0.32)/0.178)]^{0.2292}$	2.15	1.81	0.51
	15	$Y: 16.04[Pr_x \exp((x-0.20)/0.178)]^{0.2846}$	1.53	1.53	0.54

Ɵ; Models to be evaluated in case of using volumetric moisture content, M; Models to be evaluated in the case of gravimetric moisture content, Y; Moisture content (%), Pr_x; Penetration resistance measured in the field (MPa), X; Moisture content of soil measured in the field (kg kg^{-1}) / (cm^3 cm^{-3}), **; coefficient of determination indicating the relationship between actual data and predictions

The multivariate regression equations obtained by using sand, silt, clay, organic matter, percentage of $CaCO_3$ and bulk density properties of soils are given in Table 4. It was found that the relationship between the models obtained for all moisture tensions was statistically significant ($P<0.05$). The values for R^{2*} were determination from 0.27 to 0.79, for the different soil moisture tensions and soil texture groups. The determination accuracy of this moisture tension model was 79% by using organic matter, penetration resistance properties, bulk density clay, sand and $CaCO_3$ for sandy soil 0.1 bar moisture tension. Gülser (2004) estimated the field capacity and wilting point by using sand, silt, clay, organic matter, EC and porosity properties, and the validity of the models were calculated to be 85% and 96%. In contrast to the present study, it was considered that the inclusion of EC and porosity properties in the model increased the accuracy of estimation. The loamy soil group demonstrated the highest predictability for all moisture tensions across the soil groups. The R^{2**}, RMSE and ME values for the test phase of the alternative models obtained are given in Table 4. In the general regression models for sandy soil group, the RMSE values were found to be between 0.78% and 3.55%. The ME value was 0.67 to 3.44% and the R^{2**} value was 0.60 to 0.75. The R^2 obtained during the model and the R^{2**} obtained in the test stage were found to be like each other. The relationship between the actual values and the predicted values were generally determined at a high level of accuracy by using general regression models for the sandy soils. For moisture contents of 0.33 and 15 bar, the R^{2**} values were quite close to each other, but RMSE and ME values were lower for the 15 bar estimation model. The R^{2*} values were determined to be 0.72, 0.77, 0.71 and 0.74 for the loamy soils, respectively. These estimation models, which were statistically significant ($P<0.01$), provided approximately 70% accuracy at all moisture tensions. In a study to estimate soil moisture constants Cemek et al. (2004) it was reported that the models generated using parameters such as particle size and bulk density would increase the prediction accuracy. In the general regression models for loamy soil groups, the RMSE values were found to be between 1.38 % and 2.44%. The ME value was 1.19 to 2.15% and the R^{2**} value was 0.10 to 0.82. While the R^{2**} value of the prediction models created for the moisture content of 15 bar was high, the R^{2**} value obtained in the test stage was determined at very low. Although there was a difference of about 2% in the RMSE and ME values, the R^{2**} was found to be so low. It shows that there was no regular variation between the predicted and actual values. The relationships between the estimated and actual values for the clay soils were found to be quite low in general regression models. In the context of regression equations obtained according the stepwise method are given Table 5. Increases in moisture content had a general tendency to decrease R^{2*} values for sandy soil. The penetration resistance and the $CaCO_3$ percentage were the main sources at low moisture tension, while the sand and silt percentage were found to predominantly affect moisture content at 15 bar. For moisture content

maintained at a soil moisture tension of 0.1 bar, the value of R^{2*} (0.62) obtained by using only penetration resistance, increased by about 11% with the inclusion of the $CaCO_3$ content. In dry soils, the adherence of the particles to each other is high. As the moisture content increased in the soil, the bond between the particles was weakened by the water layer, thus decreasing the penetration resistance due to the reduced friction. Aggregation is a factor that increases the water retention property of the soil (Zibilske & Bradford 2007). The increase in the $CaCO_3$ content affected aggregation (Yılmaz et al. 2005), this explained the relationship between water retention and $CaCO_3$. The estimation accuracy of the model was found to be 78% by using penetration resistance at 0.1 bar along with the percentage of $CaCO_3$ and silt. For moisture content maintained at a tension of 0.33 bar, the predictive accuracy of the models with penetration resistance was only 54%. An increase of 8% was observed by adding the $CaCO_3$ content to the model (Table 5). It was clear that the sand fraction was very effective in the sandy group. In the model where sand and silt were present at 15 bar, the R^{2*} value was determined to be 58%, and the $CaCO_3$ contribution was not found to be very effective. The RMSE, ME and R^{2**} values were determined to be between 0.91 to 4.16%, 0.78 to 4.06% and 0.01 to 0.51 for sandy soil, respectively. The accuracy of the 0.5 bar moisture content prediction models was determined at very low levels. It shows that there was no regular variation between the predicted and actual values. The minimum RMSE (0.91%) and ME (0.78%) determined at 15 bar. Because the wilting point contents of soils were narrow range. In the loamy soil group prediction models, the maximum R^{2*} values were determined to be 0.71, 0.77, 0.69 and 0.74 for soil moisture tensions of 0.1, 0.33, 0.5 and 15 bar, respectively (Table 5). The RMSE, ME and R^{2**} values were determined to be between 1.54 to 5.08%, 1.17 to 3.97% and 0.63 to 0.82 in the model testing phase, respectively. The R^{2*} value was found to be between 54 to 71% for all moisture tensions when using only the penetration resistance as the independent variable. Penetration resistance ensured accuracy levels of 65% and 71% for soil moisture tensions of 0.1 and 0.33 bar. In addition to penetration resistance, the inclusion of organic matter content in the models resulted in an increase of approximately 5%. With the further inclusion of silt, the accuracy of the models increased to 71% and 77%. Relationship between organic material and water retention (Yılmaz & Alagöz 2008) was increased the R^{2*}. An increase of 5% and 7% was determined in the R^{2*} values following the inclusion of the sand content for models at soil moisture tensions of 0.5 and 15 bar. The negative correlation between sand content and moisture content (Pan et al. 2012) has also shown an effect on models in this current study. It was observed that sand was more effective than organic material. The validity of the models for loamy soils were very high. As the number of samples was high, soil characteristics were different, and this affected the result. In the clay soil estimation models, the R^{2*} values were found to be 0.37, 0.33, 0.21 and 0.46 (Table 5). As the other soil groups, penetration resistance and bulk density were the most effective of the independent variables. In addition to penetration resistance, the bulk density added to the model resulted in an increase of about 8% (in R^{2*} value) for the 15 bar moisture content estimation model. Minasny (2009), determined the R^2 value to be 0.81 and 0.88 by using the sand content and bulk density properties of soil for the estimation of the field capacity. The RMSE, ME and R^{2**} values were determined to be between 1.57 to 1.96%, 1.29 to 1.59% and 0.32 to 0.51, respectively. The validity of the models was very low for clay soils. The distribution of soil samples is generally loamy texture. The clay soil texture groups are not very common in the region, the number of sampling was low. The more soil samples could increase the R^2. In addition, the number of loamy texture group sample was high. Their relationships were higher than the other groups.

Table 4- Regression equations for moisture constants according to soil groups

Moisture tension (bar)	*Sandy Soil*	R^{2*}	*RMSE*	*ME*	R^{2**}
0.1	= 19.4+0.21a-0.114b-0.107c+0.413d+0.626e+4.44f	0.79	3.5	3.44	0.75
0.33	= -3.6+4.20a-0.055b+0.044c+0.536d-0.24e+5.97f	0.66	2.76	2.61	0.64
0.5	= -7.9+5.8a+0.07b+0.046c+0.406d-0.33e+4.52f	0.59	2.64	2.17	0.46
15	= 29-0.63a-0.169b-0.274c+0.330d-0.423e+0.182f	0.64	0.78	0.67	0.60
	Loamy Soil				
0.1	=13.429+0.63a+0.0157b+0.0534c+0.0092d+0.789e+8.01f	0.72	2.44	2.03	0.45
0.33	= 11.4-0.89a+0.0290b+0.0580c-0.0151d+0.751e+6.94f	0.77	2.38	2.15	0.43
0.5	= 9.55-0.87a+0.0486b-0.0682c-0.0204d+0.721e+5.23f	0.71	1.38	1.19	0.82
15	= 3.78-0.17a+0.0631b+0.0757c-0.0031d+0.392e+1.29f	0.74	2.43	2.02	0.10
	Clay Soil				
0.1	= 28-4.11a+0.0036b+0.120c-0.0542d+0.018e+4.53f	0.48	4.22	3.72	0.13
0.33	= 26.6-1.95a+0.0105b-0.09c-0.0230d-0.558e+4.94f	0.37	2.65	2.20	0.36
0.5	= 25.6-4.67a+0.0112b+0.041c-0.0232d-0.580e+3.91f	0.27	1.86	1.62	0.33
15	=19.7-6.55a-0.0245b-0.002c-0.0186d+0.267e+2.12f	0.50	1.91	1.66	0.55

a: bulk density (g cm^{-3}) b: clay %. c: sand % d: $CaCO_3$ % e: organic matter % f:penetration resistance (MPa)

* coefficient of determination obtained during the creation of models, ** coefficient of determination indicating the relationship between actual data and predictions

Table 5- Stepwise regression equations for moisture constants according to soil groups

Moisture Tension (bar)	*Sandy Soil*				
		R^{2*}	*RMSE*	*ME*	R^{2**}
0.1	[1]=10.976 + 5.64a	0.62	4.16	4.06	0.51
	[2]=9.965 + 5.57a + 0.49b	0.74			
	[3]= 9.430 + 4.54a + 0.41b + 0.121c	0.78			
	a:penetration resistance (MPa), b: $CaCO_3$ %, c: silt %				
0.33	[1]=7.105 + 5.2a	0.54	2.98	2.78	0.44
	[2]= 6.075 + 5.2a + 0.47b	0.62			
	a:penetration resistance (MPa), b: $CaCO_3$ %				
0.5	[1]=6.246 + 3.38a	0.37	2.00	1.60	0.01
	[2]=-2.930 + 4.10a + 5.9b	0.51			
	[3]= - 4.032 + 4.17a + 6.1 b + 0.37c	0.58			
	a: penetration resistance (MPa), b:bulk density (g cm^{-3}), c: $CaCO_3$ %				
15	[1]=28.08 - 0.275a	0.48	0.91	0.78	0.43
	[2]=16.775 - 0.158a + 0.145b	0.58			
	[3]= 13.124 – 0.122a + 0.150b + 0.28c	0.59			
	a: sand %, b:silt %, c: $CaCO_3$%				
	Loamy Soil				
0.1	[1]=16.08 + 10.14a	0.65	1.70	1.25	0.76
	[2]=14.91+ 8.94a +1.06b	0.69			
	[3]=14.53+8.06a+0.82b+0.050c	0.71			
	a: penetration resistance (MPa), b:organic matter %, c:silt %				
0.33	[1]=12.28 +8.59a	0.71	1.60	1.44	0.76
	[2]=11.17 +7.60a+ 1.07b	0.75			
	[3]=10.75+6.88a+0.83b+0.052c	0.77			
	a: penetration resistance (MPa), b:organic matter %, c:silt %				
0.5	[1]=11+ 6.68a	0.62	1.54	1.17	0.82
	[2]=15.59+ 5.62a - 0.072b	0.68			
	[3]=13.76 + 5.25a - 0.057b + 0.78c	0.69			
	a: penetration resistance (MPa), b:sand %, c: organic matter %				
15	[1]=7.44+1.79 a	0.56	5.08	3.97	0.63
	[2]=11.595+1.36a - 0.080b	0.73			
	[3]=1.29+8.07a- 0.071b+ 0.41c	0.74			
	a: penetration resistance (MPa), b: sand %, c: organic matter %				
	Clay Soil				
0.1	[1]=26.44 + 5.1a	0.37	1.68	1.43	0.51
0.33	[1]=22.69 + 4.5a	0.33	1.96	1.59	0.32
0.5	[1]=20.03 + 3.6a	0.21	1.57	1.29	0.32
15	[1]=10.65 + 2.04a	0.38	1.57	1.29	0.32
	[2]=17.64 + 2.29a - 5.7b	0.46			
	a: penetration resistance (MPa), b: bulk density (g cm^{-3})				

* coefficient of determination obtained during the creation of models, ** coefficient of determination indicating the relationship between actual data and predictions

4. Conclusions

The main scope of this study was to predict moisture constants through penetration resistance. Then, in the regression models obtained using some other properties of the soils. The most accurate relationships predict moisture constants through penetration resistance were determined for the loamy soils at all soil moisture tensions. The 0.1 bar moisture content estimation

model of the sandy soils indicated a 75% accuracy in general linear regression. In the loamy soil group can be predicted at an accuracy of 63% to 82% in stepwise regression models. In the clay soils group, the highest R^{2**} and the lowest RMSE and ME values were found to be at 15 bar. In addition, it was found that the most effective parameter included in the model as an independent variable for all soil groups was the penetration resistance of the models formed using the stepwise method. As a result of testing alternative models, the recommendation for the estimation of field capacity was to use the 0.1 bar general regression model for sandy soils, and the 0.33 bar stepwise model for loamy soils. Consequently, there were differences in the reliability of the models according to the texture of the groups. More accurate relationships were obtained for the loamy soils. Moisture constants can be estimated by using penetration resistance, but more reliable models are produced by adding organic material, sand and silt to the models.

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References

Abdelbaki A M (2018). Evaluation of pedotransfer functions for predicting soil bulk density for US soils. *Ain Shams Engineering Journal* 9(4): 1611-1619 https://doi.org/10.1016/j.asej.2016.12.002

Bahtiyar M (1978). Soil water, water retention in the soil and moisture tension. *Atatürk University Journal of Agricultural Faculty* 9(4): 105-119 (In Turkish)

Busscher W J (1990). Adjustment of flat- tipped penetrometer resistance data to a common water content. Retrieved in March, 12, 2018 from http://naldc. nal.usda. gov/download/ 18014/PDF https://doi.org/10.13031/2013.31360

Cemek B, Meral R, Apan M & Merdun H (2004). Pedotransfer function fort the estimation of the field capacity and permanent wilting point. *Pakistan Journal of Biological Science* 7(4): 535-541 https://doi.org/10.3923/pjbs.2004.535.541

Demiralay M (1993). Soil Physical Analysis. Atatürk University Faculty of Agriculture Publications, No: 143 Turkey (In Turkish)

Esmaeelnejad L, Ramezanpour H, Seyedmohammadi J & Shabanpour M (2015). Selection of a suitable model for the prediction of soil water content in north of Iran. *Spanish Journal of Agricultural Research* 13(1):1202 https://doi.org/10.5424/sjar/2015131-6111

Gülser C (2004). Determination of field capacity and permanent wilting point with pedotransfer functions related to soil physical and chemical properties. *Journal of Faculty of Agriculture Ondokuz Mayıs University* 19(3): 19-23 (in Turkish with an abstract in English)

Kacar B (2009). Soil Analysis. Nobel Publication No: 1387 Ankara (In Turkish)

Keshavarzi A, Sarmadian F, Sadeghnejad M & Pezeshki P (2010). Developing pedotransfer functions for estimating some soil properties using artificial neural network and multivariate regression approaches. *Proenvironment Promediu* 3: 322-330

Minasny B (2009) Prediction of the water content at field capacity from disturbed soil samples. Retrieved in May, 12, 2017 from http://soil-research.com/sr/wpcontent/uploads/2010/05/fieldcapacity_ disturbed_ samples .pdf

Mohanty M, Sınha N K, Painuli D K, Bandyopadhyay K K, Hatı K, Reddy K S & Chaudhary R S (2014). Pedotransfer functions for estimating water content at field capacity and wilting point of Indian soils using particle size distribution and bulk density. *Journal of Agricultural Physics* 14(1): 1-9

Mohanty M, Nishan K, Sinha D K, Painuli K K, Bandyopadhyay K M, Hati K & Sammi Reddy Chaudhary R S (2015). Modelling soil water contents at field capacity and permanent wilting point using artificial neural network for Indian soils. *National Academy Science Letter* 38(5): 373-377 https://doi.org/10.1007/s40009-015-0358-4

Mohawesh O E (2013). Assessment of pedotransfer functions (ptfs) in predicting soil hydraulic properties under arid and semi arid environments. *Jordan Journal of Agricultural Sciences* 9(4): 475-491

Negiş H, Şeker C, Gümüş İ, Özaytekin H H, Atmaca E & Karaca Ü (2016). Determination of penetration resistance in sugar beet farming. *Nevsehir Journal of Science and Technology* pp. 272-279 (in Turkish with an abstract in English)

Pan W, Boyles R P, White J G & Heitman J L (2012) Characterizing soil physical properties for soil moisture monitoring with the north carolina environment and climate observing network. *Journal of Atmospheric and Oceanic Technology* 29(7): 933-943 https://doi.org/10.1175/jtech-d-11-00104.1

Qiao J, Zhu Y, Jia X, Huang L & Shao M A (2019) Pedotransfer functions for estimating the field capacity and permanent wilting point in the critical zone of the Loess Plateau, China. *Journal of Soils and Sediments* 19(1):140-147 https://doi.org/10.1007/s11368-018-2036-x

Santra P, Kumar M, Kumawat R N, Painuli D K, Hati K M, Heuvelink G B M & Batjes N H (2018) Pedotransfer functions to estimate soil water content at field capacity and permanent wilting point in hot Arid Western India. *Journal of Earth System Science* 127(3): 1-16 https://doi.org/10.1007/s12040-018-0937-0

Silva E D, Curi N, Ferreira M M, Volpato M M L, Santos W J R D & Silva S H G (2015). Pedotransfer functions for water retention in the main soils from the Brazilian coastal plains. *Ciência e Agrotecnologia* 39(4): 331-338 https://doi.org/10.1590/s1413-70542015000400003

Soil Survey Manual (1993). Soil survey division staff, United States department of agriculture

Şeker C (1997). The effect of water content on the penetration resistance of different soils, and regression models. *Turkish Journal of Agriculture and Forestry* 23(2): 467-471 (in Turkish with an abstract in English)

Touil S, Degre A & Chabaca M N (2016). Sensitivity analysis of point and parametric pedotransfer functions for estimating water retention of soils in Algeria. *Soil* 2(4):647 https://doi.org/10.5194/soil-2-647-2016

Turgut B, Aksakal E L, Öztaş T & Babagil G E (2008). Defining partial effect coefficients of soil properties affecting on soil penetration resistanceusing multiple regression analysis. *Journal of Faculty of Agriculture Atatürk University* 39(1): 115-121 (in Turkish with an abstract in English)

Turgut B, Öztaş T & Aksakal E L (2010). Defining direct and indirect effect of some soil properties on soil penetration resistance. *Journal of the Faculty of Agriculture Süleyman Demirel University* 5(2): 45-53 (in Turkish with an abstract in English)

U.S Salinity Laboratory Staff (1954). Diagnosis and Improvement of Salina and Alkali Soils. Agricultural Handbook 60, USDA

Yılmaz E, Alagöz Z & Öktüren F (2005). Aggregate formation and stability in soil. *Selcuk Journal of Agriculture and Food Sciences* 19(36): 78-86

Yılmaz E & Alagöz Z (2008). Relation between soil water and organic matter. *Turkish Journal of Scientific Reviews* 1(2): 15-21

Zibilske L M & Bradford J M (2007). Soil aggregation, aggregate carbon and nitrogen, and moisture retention induced by conservation tillage. *Soil Science Society of America Journal* 71(3): 793-802 https://doi.org/10.2136/sssaj2006.0217

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Determining the Most Stable Potato Genotypes Using AMMI Yield Stability Analysis Method

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ABSTRACT
Genotype-environment interaction (GEI) is very important for breeders. It is considered a complicated issue in breeding programs to obtain stable and high-yielding genotypes to release new genotypes. This study was conducted to achieve a stable high-yielding genotype that is adaptive to climatic conditions of potato-producing regions in Iran. A total of 20 potato breeding lines along with five commercial varieties (Savalan, Agria, Caesar, Luta and Satina) were evaluated in a randomized complete block design with three replicates in the Agricultural Research and Natural Resources Stations of five location (Ardabil, Razavi Khorasan, Karaj, Isfahan and Hamadan) in Iran, for two years (2016 and 2017). Combined ANOVA of yield data for studied genotypes and environments indicates significant differences among potato genotypes, environments, and GE interaction was significant. Thus, the AMMI method and its parameters were used to analyze yield stability. The results indicated that only four interaction principal components were significant ($P<0.01$), which accounted for 81.2% of the GEI sum of squares. Based on type 1 parameters ($SIPC_1$, FA_1, Za_1, Dz_1, EV_1, and Da_1), genotypes G7, G10, G14, G20 and G24 were identified as to be stable. Moreover, according to the results of type 2, 3 and 4 parameters, genotypes G2, G6, G7, G14, G15 and G20, as well as cultivars Agria (G24) and Luta (G23), were found to be stable. Genotypes G6, G7, G14, G15, G20, and G24 were stable according to the ASV parameter, and genotypes G6 and G7 were stable based on the MASV parameter. Amongst the stable genotypes identified by the AMMI parameters, while genotype G6 was high-yielding, G14 and G24 (Agria) were moderate-yielding.

Keywords: AMMI Parameters, Potato Genotypes, Stability, Tuber Yield

1. Introduction

Potato (*Solanum tuberosum* L.) is the fourth staple food after wheat, rice, and corn in terms of nutrition and production importance (Fufa 2013). Given the growing rate of population and its consequences, such as hungrier people and more demand for food, the Food and Agricultural Organization (FAO) has introduced potato as a food security plant (Devauxet et al. 2014). Thus, the need for expanding potato production is globally felt to manage the increase in food demands and food security (Hassanabadi et al. 2013). Achievement to high-yield, adaptive, and stable genotypes is one of the main goals of potato breeders. However, Genotype-environment interaction (GEI) renders breeding programs difficult and complex (Kadhem & Baktash 2016). GEI is a genotype's response to environmental changes (Crossa et al. 1991). It is important to understand the GEI structure and nature in breeding programs because a significant GEI can restrict efforts to select superior genotypes when introducing new varieties and cultivars in breeding programs (Shafii & Price 1998). Numerous statistical methods have been proposed to study GEI to determine stable genotypes (Sabaghnia et al. 2008). These methods can be divided into two categories-univariate and multivariate (Karimizadeh et al. 2012).

Among the multivariate methods, the additive main effect and multiplicative interaction (AMMI) model is more credible and widely used (Byarugaba et al. 2018). The AMMI model was first used by Gauch (1988) to analyze yield data. Then, Gauch and Zobel (1988) fully expanded and analyzed this model. In plant breeding, this method affects the accuracy of genotype yield estimation in multi-environment trials. Such an accurate estimation is obtained through evaluation and prediction via dissociating the data into modeling and validation data as well as comparing the values expected from the model with validation data (Safavi & Bahraminejad 2017). The AMMI is an integrated model of analysis of variance (ANOVA) and principal component analysis. This model first calculates the main effect of genotypes and environments using the ANOVA methods, and then, the genotype and environmental components of the interaction are computed for the matrix of deviation from incremental effect (Crossa et al.

1990). The first part of the AMMI model, the summable part, uses usual variance analysis, while the second part is multiplicative and utilizes the method of interaction principal component analysis to dissociate GEI into a range of 1to *n* principal components (Omrani et al. 2018). It is noteworthy to mention that in the AMMI method, the calculations are performed on the values of GEI, while the computation of principal components is performed on the differentials of main data from the total mean of entire data (Gauch 1988). The estimation accuracy of the AMMI method is similar to increasing the number of replicates. This method can be used to reduce replicates and related expenses, which, in turn, allows the use of more treatments to the experiment (Crossa et al. 1991).

Tarakanovas and Ruzgas (2006) introduced AMMI as an effective method to study GEI and stated that its bi-plots could determine the suitable varieties for cultivation in various environments as well as the varieties for cultivation in certain environmental conditions. The AMMI method is widely used to evaluate GEI and has been employed by various researchers for the selection of stable genotypes of potato (Byarugaba et al. 2018), sunflower (Khomari et al. 2016), oats (Safavi & Bahraminejad 2017), durum wheat (Karimizadeh et al. 2016), canola (Pourdad & Jamshid Moghaddam 2013) and lentil (Sabaghnia et al. 2008). Worku et al. (2018) used the multivariate method and index of the AMMI stability value and concluded that the clone CIP-396004.337 possessed the highest yield and stability. In a study conducted by Byarugaba et al. (2018) on 21 Dutch potato varieties from 2015 to 2016 in five regions, they utilized the multivariate methods, including the AMMI, and suggested eight varieties for the Uganda region.

To investigate the stability based on the AMMI model, various parameters have been proposed, such as Euclidean distance from the origin of significant Interaction Principal Component (IPC) axes (Da) (Annicchiarico 1997), distance of Interaction Principal Component (IPC) point with origin in space (Dz) (Zhang et al. 1998), absolute value of the relative contribution of IPCs to the interaction (Za) (Zali et al. 2012), stability statistic based on the first IPC axes in the fitted AMMI model (FA) (Raju 2002). Zobel (1994) introduced a parameter, i.e., averages of square eigenvector values (EV). Alternatively, another parameter, i.e., sums of the absolute value of the IPC scores (SIPC), was propounded by Sneller et al. (1997). Based on the two first Interaction Principal Component Analysis (IPCAs), AMMI stability value (ASV) (Purchase 1997) and modified AMMI stability value (MASV) (Zali et al. 2012) have been proposed.

The present study aims to identify and select stable high-yielding genotypes for potato-producing regions in Iran using the AMMI model and its parameters.

2. Material and Methods

2.1. Plant material and treatments

In this study, 20 potato genotypes (Table 1) and five standard varieties (Savalan, Agria, Caesar, Luta, and Satina) were evaluated in five locations (Ardabil, Razavi Khorasan, Karaj, Isfahan, and Hamadan) in Iran in two years (2016-2017). The climatic conditions and geographic location of the studied areas are presented in Table 2. The experimental design in all locations was randomized complete block with three replicates in both years. The chemical fertilizers, including ammonium phosphate, urea, and potassium sulfate whose dosages were calculated by the soil test, were incorporated into the soil (Table 3). All breeding lines and control varieties were cultivated in two six-meter-long rows with inter-row spacing of 75 cm and inter-plant spacing of 25 cm. Colorado beetles were controlled by applying 250 mL ha^{-1} Imidacloprid (Confidor). The plots were weeded in two stages within a 10- to 15-centimeter distance from the plants. The genotype yields were measured after the harvest.

Table 1-The list of the potato breeding lines and standard cultivars studied in this research

NO	*Line cod*	*Parents*		*NO*	*Line cod*	*Parents*		*NO*	*Standard cultivars*
		♀	♂			♀	♂		
G1	16	Luta	Caesar	G11	3	Luta	Caesar	G13	Caesar
G2	9	Luta	Caesar	G12	2	Luta	Caesar	G22	Satina
G3	15	Luta	Caesar	G14	21	Luta	Savalan	G23	Luta
G4	11	Luta	Caesar	G15	5	Luta	Savalan	G24	Agria
G5	13	Luta	Caesar	G16	1	Luta	Savalan	G25	Savalan
G6	5	Luta	Caesar	G17	2	Luta	Savalan		
G7	23	Luta	Caesar	G18	3	Luta	Savalan		
G8	56	Luta	Caesar	G19	16	Luta	Savalan		
G9	12	Luta	Caesar	G20	14	Luta	Savalan		
G10	4	Luta	Caesar	G21	13	Luta	Savalan		

Table 2- Climatic conditions and geographical position of studied regions

Location	*Latitude*	*Longitude*	*Altitude (m)*	*Temperature (^{0}C)*			*Precipitation (mm)*	*Relative humidity (%)*
				Average	*Minimum*	*Maximum*		
Ardabil	48° 18'E	38° 15'N	1351	9.90	4.10	15.80	277	68
Hamedan	48° 32'E	34° 48'N	1550	11.35	3.93	18.77	384	53
Karaj	51° 00'E	35° 48'N	1312	14.40	8.00	20.80	247	53
Razavi Khorasan	59° 23'E	35° 34'N	1600	14.10	7.10	21.10	225	55
Esfahan	51° 40'E	32° 37'N	1550	16.25	9.10	23.40	123	40

Table 3- Planting date and harvest of potato genotypes and Chemical fertilizers consumption in studied locations

Location	*Planting dates*	*Harvest dates*	*Chemical fertilizers*		
			Ammonium phosphate (kg ha^{-1})	*Urea (kg ha^{-1})*	*Potassium sulfate (kg ha^{-1})*
Ardabil	25-30 April	2-15 October	150	300	100
Hamedan	15-20 June	16-21 November	100	250	150
Karaj	25-30 June	19-21November	150	300	100
Razavi Khorasan	10-15 June	18-22 October	100	350	200
Esfahan	5-10 June	17-20 October	150	350	150

2.2. Statistical analysis

To determine the stability of the genotypes, the multivariate AMMI model whose statistical model is as follows was utilized:

$$Y_{IJ} = \mu + g_i + e_j + \sum_{n=1}^{N} \lambda_n \gamma_{in} \delta_{jn} + \rho_{ij} \quad (1)$$

Where; Y_{ij}, denotes the yield of *i*th genotype in *j*th environment; μ, is the grand mean; g_i and e_j , are the genotype and the environment deviations from the grand mean, respectively; λ_n , is the eigenvalue of the *n*th principal component axis; $\gamma_{in}$and δ_{jn} , are the eigenvectors of the genotype and environment for the axis *n*, respectively; and ρ_{ij} ,is the error term.

Several statistics of the AMMI model were employed to investigate the stability of the genotypes. Various parameters, including EV (Zobel 1994), SIPC (Sneller et al. 1997), Da (Annicchiarico 1997), Za (Zali et al. 2012), and FA (Raju 2002) were also calculated. Furthermore, the first tow IPCA was used for computing ASV (Purchase 1997) and MASV (Zali et al. 2012).

$$Zai = \sum_{n=1}^{N} |\gamma_{in} \partial_n| \quad (2)$$

$$DZi = \sqrt{\sum_{n=1}^{N} \gamma_{in}^2} \quad (3)$$

$$MASV = \sqrt{\sum_{K=1}^{N-1} (SSIPC_n / SSIPC_{n+1}) (IPCn)^2 + (IPCn + 1)^2} \quad (4)$$

$$ASV = \sqrt{(\mathrm{SSIP1}/\mathrm{SSIP2}) (\mathrm{IPC1})^2 + (\mathrm{IPC2})^2} \quad (5)$$

$$Dai = \sqrt{\sum_{\mathrm{n=1}}^{\mathrm{N}} (\lambda_{\mathrm{n}} \gamma_{\mathrm{in}})^2} \quad (6)$$

$$EV = \sum_{\mathrm{n=1}}^{\mathrm{N}} \gamma_{\mathrm{in}}^2 / \mathrm{n} \quad (7)$$

$$SIPC = \sum_{\mathrm{n=1}}^{\mathrm{N}} \lambda_{\mathrm{n}}^{0.5} \gamma_{\mathrm{in}} \quad (8)$$

$$FA = \sum_{n=1}^{N} \lambda_n^2 \gamma_{in}^2 \quad (9)$$

To draw the bi-plot, Minitab 16 software was used, and the entire analyses and calculations of the AMMI model were carried out with of Genstat Release 12.0.

3. Results and Discussion

The results of the combined analysis of variance of yield data showed that the effects of environment (each location and year was considered as different environment), genotype, and their interactions were significant (P<0.01; Table 4). The effect of genotype, environment, and GEI accounted for 7.2%, 35.5%, and 42.3% of the total sums of squares, respectively. The mean squares of $IPCA_1$ and $IPCA_2$ were found to be significant (P<0.01) and cumulatively captured 57.7% of GEI (Table 4) as $IPCA_1$ and $IPCA_2$ components accounted for 34% and 23.7% of GEI sum of the squares, respectively. The $IPCA_3$ and $IPCA_4$ explained 13.1% and 10.4% of the GEI variations, respectively. In total, the first four components accounted for 81.2% of the GEI variation. Thus, the remaining components of the model cover only 18.2% of the sum squares of GEI.

Table 4- Combined analysis of yield data of potato genotypes (lines and standard cultivars)

Source	*df*	*SS*	*MS*	*Proportion*	*Noise*
Environments	9	27981	68.51**	0.355a	0.007c
Genotypes	24	5676	10.33**	0.072a	0.097c
Genotype × Environment	216	33347	154.4**	0.423a	0.148c
IPCA 1	32	11334	354.2**	0.340b	–
IPCA 2	30	7893	263.1**	0.237b	–
IPCA 3	28	4390	156.8**	0.131b	–
IPCA 4	26	3477	133.7**	0.104b	–
Residuals	100	6252	62.5	–	0.423d
Error	480	10984	22.9	–	–
Total	749	78896	105.3	–	–

* and **, significant at 0.05 and 0.01 respectively; IPCA , Interaction Principal Component Analysis; a, Calculated by dividing on sum of (GEN, ENV, and GEN×ENV) SS; b, Calculated by dividing on ENV×GEN interaction SS; c, Calculated by [(df×MS Error)/SS]; d, The portion of residual SS from total GEN×ENV was calculated as SSE/(ENV×GEN SS)

To better understand the AMMI model, a bi-plot (Figure 1) was drawn. Genotypes G5, G9, G12, G15, G16, G17, G18, G21, and G23 (cv. Luta) and environments E2, E9 and E5 were found to have higher IPCA1 and, hence, the highest interactions. In Figure 1, the vertical line in the mid-section of the bi-plot indicates the grand mean value of the two experimental years. The genotypes and environments on the right had higher yields than mean. As the bi-plot illustrates, genotypes G1, G3, G5, G6, G8, G9, G17, G19, and G25 (cv. Savalan) possessed the highest mean yield. Among the environments, E1, E2, and E9 had the highest yield, while E3 had the lowest (Table 5).

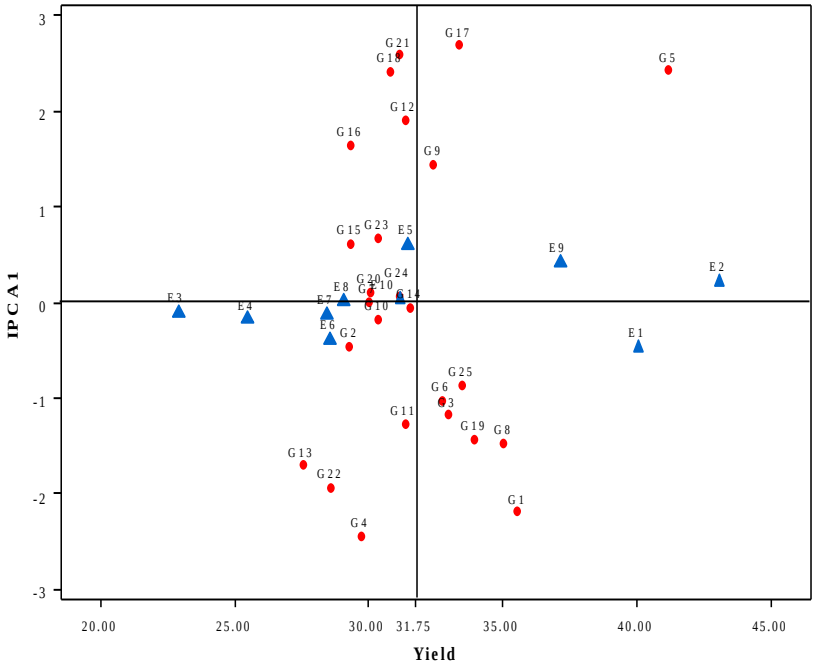

Figure 1- The bi-plot of mean yield and first principle component for potato genotypes and 10 environments

Table 5- The mean yield and interaction principle components (IPCAs) of yield for all environments

Environment	*Environment Code*	*Yield (t ha^{-1})*	*IPCA1*	*IPCA2*	*IPCA3*
Ardabil (2016)	E1	40.07	-3.677	-0.086	-2.61
Hamedan (2016)	E2	43.05	1.701	-5.278	0.761
Karaj (2016)	E3	22.88	-0.909	1.762	4.107
Isfahan (2016)	E4	25.45	-1.411	1.296	-0.272
Razavi Khorasan (2016)	E5	31.44	4.626	3.144	-1.497
Ardabil (2017)	E6	28.55	-3.05	-1.11	-1.027
Hamedan (2017)	E7	28.44	-0.98	2.385	-0.266
Karaj (2017)	E8	29.05	0.133	-0.795	-0.526
Isfahan (2017)	E9	37.12	3.23	-1.024	-1.509
Razavi Khorasan (2017)	E10	31.16	0.337	-0.301	2.833

To evaluate the stability of the genotypes and environments and to associate the genotypes to the various environments, another bi-plot was utilized (Figure 2). Figure 2 illustrates the bi-plot using IPCA1 and IPCA2 for studied genotypes and environments. Accordingly, stable genotypes can be introduced and various environments can be classified. The genotypes and environments in the center of the bi-plot, i.e., environments E3, E4, E7, E8, and E10 along with genotypes G7, G14, G20 and G24 (cv. Agria) had the minimum genotype × environment interaction. So, they were superior to the other genotypes and environments in terms of not having GEI. The genotypes adjacent to an environment were specifically adaptive to the related environment and the genotypes near the component axes were found to have more general adaptation. Therefore, genotypes G14 and G24 (cv. Agria) were specifically adapted to environments E10; genotypes G15 and G23 (cv. Luta) were specifically adapted to environment E5; and genotypes G7 and G20 were specifically adapted to environments E1, E3, and E6 (Figure 2). On the other hand, genotypes G6, G7, G10, G14, G16, G20, G21, G24 (cv. Agria), and G13 (cv. Caesar) exhibited general adaptation because they were closer to the axes of the principal components of the interactions (Figure 2). Since 57.5 percent of the variance – i.e. more than half of the entire variance – was captured by the first and second principal components, it was better to use the results of the AMMI model's statistics to determine stability.

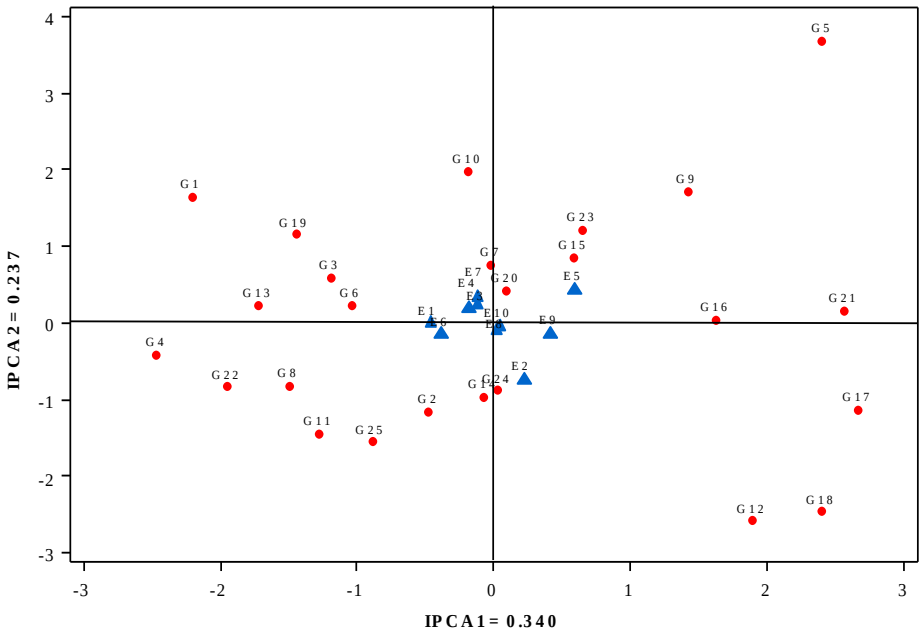

Figure 2- The bi-plot of the first two principal axis scores of potato breeding lines, standard cultivars, and environments

This study calculated other parameters of the AMMI method including $SIPC_1$, FA_1, Za_1, Dz_1, EV_1, and Da_1 (type 1, using the first IPCA); $SIPC_2$, FA_2, Za_2, Dz_2, EV_2, and Da_2 (type 2, based on the first and second IPCA); $SIPC_3$, FA_3, Za_3, Dz_3, EV_3, and Da_3 (type 3, using three principal components); and $SIPC_4$, FA_4, Za_4, Dz_4, EV_4, and Da_4 (type 4, based on the four components) (Tables 6, 7, and 8). Also, MASV and ASV were calculated based on the first two and four principal components and their sum of squares, respectively (Tables 6, 7, and 8).

Table 6- The SIPC and FA parameters of the AMMI model for tuber yields of 25 genotypes tested in 10 environments

Genotypes	*MTY*	*SIPC1*	*SIPC2*	*SIPC3*	*SIPC4*	STD_{SIPC}	FA_1	FA_2	FA_3	FA_4	STD_{FA}	*MASV*
G1	35.57	2.203	3.829	5.066	6.122	1.686	298.313	433.886	492.426	530.393	101.70	4.352
G2	29.30	0.47	1.652	1.807	2.449	0.825	13.601	85.230	86.145	100.167	39.06	2.166
G3	32.99	1.192	1.765	3.299	3.871	1.260	87.286	104.125	194.136	205.266	60.60	2.934
G4	29.71	2.462	2.892	3.201	3.814	0.569	372.493	381.965	385.624	398.401	10.72	3.133
G5	41.21	2.407	6.081	6.335	7.589	2.230	356.071	1048.471	1050.932	1104.501	356.89	6.914
G6	32.73	1.035	1.243	1.249	1.43	0.161	65.827	68.040	68.042	69.155	1.40	1.301
G7	30.01	0.016	0.768	1.399	2.017	0.857	0.015	29.022	44.248	57.270	24.62	1.693
G8	35.05	1.49	2.335	3.108	4.835	1.425	136.385	173.019	195.886	297.424	69.00	3.085
G9	32.44	1.427	3.139	4.593	6.495	2.153	125.142	275.428	356.250	479.412	148.55	4.419
G10	30.39	0.188	2.158	2.678	4.154	1.639	2.176	201.146	211.473	285.652	121.28	3.701
G11	31.36	1.278	2.739	4.891	5.685	2.011	100.377	209.869	387.106	408.541	147.34	4.407
G12	31.37	1.897	4.497	5.87	8.087	2.591	221.253	567.939	640.067	807.411	246.60	5.767
G13	27.55	1.717	1.932	2.915	3.504	0.840	181.196	183.563	220.562	232.384	25.93	2.626
G14	31.58	0.071	1.052	2.759	3.494	1.563	0.306	49.687	161.172	179.570	86.65	3.135
G15	29.35	0.596	1.434	1.762	3.676	1.302	21.822	57.838	61.949	186.627	72.00	2.526
G16	29.32	1.631	1.645	2.758	3.47	0.900	163.450	163.461	210.879	228.127	33.12	2.670
G17	33.38	2.673	3.819	5.798	6.568	1.787	439.307	506.661	656.524	676.716	115.42	4.836
G18	30.84	2.399	4.884	5.929	7.335	2.083	353.755	670.552	712.351	779.661	188.97	5.476
G19	33.96	1.443	2.582	2.897	4.443	1.237	127.907	194.422	198.228	279.612	62.10	3.039
G20	30.10	0.094	0.502	2.308	2.722	1.302	0.544	9.092	133.877	139.719	76.32	2.833
G21	31.18	2.572	2.708	3.277	4.864	1.051	406.450	407.400	419.787	505.526	47.55	3.578
G22	28.61	1.944	2.796	4.237	5.311	1.497	232.361	269.618	349.071	388.306	71.45	3.648
G23	30.37	0.659	1.862	2.728	2.734	0.981	26.657	100.915	129.600	129.601	48.61	2.524
G24	31.14	0.031	0.933	2.943	4.192	1.889	0.060	41.830	196.413	249.486	119.84	3.602
G25	33.52	0.886	2.462	4.336	4.593	1.738	48.241	175.582	309.945	312.189	126.19	4.011
Mean yield	31.75											

MTY, Mean Tuber Yield (t ha^{-1}); STD, Standard Deviation

Table 7- The Za and Dz parameters of the AMMI model for tuber yields of 25 genotypes tested in 10 environments

Genotypes	*MTY*	Za_1	Za_2	Za_3	Za_4	STD_{Za}	Dz_1	Dz_2	Dz_3	Dz_4	STD_{Dz}	*ASV*
G1	35.57	0.096	0.149	0.176	0.195	0.043	0.281	0.361	0.413	0.451	0.073	3.100
G2	29.30	0.020	0.060	0.063	0.074	0.023	0.060	0.176	0.177	0.209	0.065	1.309
G3	32.99	0.052	0.071	0.103	0.114	0.029	0.152	0.172	0.302	0.317	0.086	1.539
G4	29.71	0.107	0.121	0.128	0.139	0.013	0.314	0.320	0.324	0.340	0.011	2.981
G5	41.21	0.104	0.226	0.231	0.254	0.067	0.307	0.598	0.599	0.637	0.153	4.671
G6	32.73	0.045	0.052	0.052	0.055	0.004	0.132	0.135	0.135	0.139	0.003	1.258
G7	30.01	0.001	0.026	0.039	0.050	0.021	0.002	0.105	0.146	0.181	0.077	0.752
G8	35.05	0.065	0.093	0.109	0.140	0.031	0.190	0.224	0.256	0.391	0.088	1.975
G9	32.44	0.062	0.119	0.150	0.183	0.052	0.182	0.300	0.381	0.502	0.135	2.420
G10	30.39	0.008	0.073	0.084	0.111	0.044	0.024	0.276	0.289	0.384	0.154	1.983
G11	31.36	0.055	0.104	0.150	0.164	0.049	0.163	0.261	0.435	0.456	0.141	2.117
G12	31.37	0.082	0.168	0.198	0.237	0.066	0.242	0.436	0.490	0.620	0.157	3.454
G13	27.55	0.074	0.082	0.103	0.113	0.018	0.219	0.221	0.272	0.290	0.036	2.069
G14	31.58	0.003	0.036	0.072	0.085	0.037	0.009	0.137	0.308	0.333	0.153	0.985
G15	29.35	0.026	0.054	0.061	0.095	0.028	0.076	0.140	0.149	0.360	0.124	1.101
G16	29.32	0.071	0.071	0.095	0.108	0.018	0.208	0.208	0.275	0.301	0.047	1.954
G17	33.38	0.116	0.154	0.196	0.210	0.043	0.341	0.377	0.494	0.512	0.085	3.402
G18	30.84	0.104	0.186	0.209	0.234	0.056	0.306	0.463	0.493	0.548	0.104	3.800
G19	33.96	0.063	0.100	0.107	0.135	0.030	0.184	0.243	0.248	0.363	0.075	2.071
G20	30.10	0.004	0.018	0.056	0.064	0.029	0.012	0.058	0.298	0.306	0.155	0.423
G21	31.18	0.112	0.116	0.128	0.156	0.020	0.328	0.329	0.341	0.436	0.052	3.085
G22	28.61	0.084	0.113	0.143	0.162	0.034	0.248	0.275	0.360	0.405	0.073	2.480
G23	30.37	0.029	0.068	0.087	0.087	0.028	0.084	0.188	0.234	0.234	0.071	1.439
G24	31.14	0.001	0.031	0.074	0.096	0.043	0.004	0.126	0.349	0.409	0.190	0.903
G25	33.52	0.038	0.091	0.131	0.135	0.045	0.113	0.247	0.391	0.394	0.134	1.900

Table 8- The EV and Da parameters of the AMMI model for tuber yields of 25 genotypes tested in 10 environments

Genotypes	*MTY*	*EV_1*	*EV_2*	*EV_3*	*EV_4*	*STD_{EV}*	*Da_1*	*Da_2*	*Da_3*	*Da_4*	*STD_{Da}*	*ASV*
G1	35.57	0.079	0.065	0.057	0.051	0.012	17.272	20.830	22.191	23.030	2.540	3.100
G2	29.30	0.004	0.015	0.010	0.011	0.005	3.688	9.232	9.281	10.008	2.931	1.309
G3	32.99	0.023	0.015	0.030	0.025	0.006	9.343	10.204	13.933	14.327	2.545	1.539
G4	29.71	0.099	0.051	0.035	0.029	0.032	19.300	19.544	19.637	19.960	0.273	2.981
G5	41.21	0.094	0.179	0.120	0.101	0.038	18.870	32.380	32.418	33.234	6.915	4.671
G6	32.73	0.017	0.009	0.006	0.005	0.006	8.113	8.249	8.249	8.316	0.085	1.258
G7	30.01	0.000	0.006	0.007	0.008	0.004	0.123	5.387	6.652	7.568	3.329	0.752
G8	35.05	0.036	0.025	0.022	0.038	0.008	11.678	13.154	13.996	17.246	2.355	1.975
G9	32.44	0.033	0.045	0.048	0.063	0.012	11.187	16.596	18.875	21.895	4.523	2.420
G10	30.39	0.001	0.038	0.028	0.037	0.017	1.475	14.183	14.542	16.901	6.972	1.983
G11	31.36	0.027	0.034	0.063	0.052	0.017	10.019	14.487	19.675	20.212	4.805	2.117
G12	31.37	0.059	0.095	0.080	0.096	0.018	14.875	23.831	25.300	28.415	5.810	3.454
G13	27.55	0.048	0.024	0.025	0.021	0.012	13.461	13.549	14.851	15.244	0.906	2.069
G14	31.58	0.000	0.009	0.032	0.028	0.015	0.553	7.049	12.695	13.400	5.968	0.985
G15	29.35	0.006	0.010	0.007	0.032	0.013	4.671	7.605	7.871	13.661	3.763	1.101
G16	29.32	0.043	0.022	0.025	0.023	0.010	12.785	12.785	14.522	15.104	1.195	1.954
G17	33.38	0.116	0.071	0.081	0.065	0.023	20.960	22.509	25.623	26.014	2.446	3.402
G18	30.84	0.094	0.107	0.081	0.075	0.014	18.808	25.895	26.690	27.922	4.099	3.800
G19	33.96	0.034	0.030	0.021	0.033	0.006	11.310	13.944	14.079	16.722	2.210	2.071
G20	30.10	0.000	0.002	0.030	0.023	0.015	0.738	3.015	11.571	11.820	5.746	0.423
G21	31.18	0.108	0.054	0.039	0.048	0.031	20.161	20.184	20.489	22.484	1.113	3.085
G22	28.61	0.062	0.038	0.043	0.041	0.011	15.243	16.420	18.683	19.705	2.043	2.480
G23	30.37	0.007	0.018	0.018	0.014	0.005	5.163	10.046	11.384	11.384	2.956	1.439
G24	31.14	0.000	0.008	0.041	0.042	0.022	0.246	6.468	14.015	15.795	7.172	0.903
G25	33.52	0.013	0.031	0.051	0.039	0.016	6.946	13.251	17.605	17.669	5.057	1.900

Based on type 1 parameters, genotypes G7, G10, G14, G20, and G24 (cv. Agria) were found to be the most stable ones due to their lowest values. According to type 2 parameters, genotypes G6, G7, G14, G15, G20, and G24 (cv. Agria) were selected as the most stable genotypes. Genotypes G2, G6, G7, G14, G15, G20, and G23 (cv. Luta) were the stable ones based on the type 3 parameters. Genotypes G2, G6, G7, G20, and G23 (cv. Luta) accounted for the lowest values based on the type 4 parameters. The standard deviation was computed for all parameters. Genotypes G2, G7, G6, and G23 (cv. 'Luta') showed the lowest standard deviation of the EV parameter. Cultivar Caesar and genotypes G4, G6, and G16 obtained the lowest value of the standard deviation of D and Za parameters. The standard deviation value of the parameter SIPC for genotypes G2, G4, G6, and G13 (cv. Caesar) and Da for genotypes G4, G6, G13 (cv. Caesar), and G21 were the lowest. Genotypes G4, G6, G7, and G13 (cv. Caesar) acquired the lowest value of standard deviation in terms of the FA criterion.

Since genotypes G6, G7, G14, G15, G20, and G24 (cv. Agria) had the lowest values of ASV parameter, they were considered as the stable genotypes. On the other hand, genotypes G5, G12, G17, and G18 had the highest values of this parameter. Thus, they were selected as the unstable ones (Table 7 and 8). Based on the MASV parameter, genotypes G6, and G7 were selected as the stable genotypes. However, genotypes G5, G10, G12, G17, and G18 were found to be unstable.

GEI was found to be significant in the present study and was six times greater than genotype main effect. This reflects its complexity and high effect on tuber yield in various environments. The large magnitude of GEI causes more dissimilarity in the genetic systems that control the physiological processes that are conferring yield stability in different environments (Karimizadeh et al. 2016, 2019).

Additionally, the significance of GEI is indicative of the genotypes' evaluation in several environments to identify the general and specific adaptation. Thus, considering the significance of GEI, the AMMI method and its parameters were utilized to select the stable genotype. GEI stability is in biological or agricultural forms (Tollenaar & Lee 2002). Breeders tend to use agricultural and dynamic concept of stability instead of its static concept. According to this concept, there is a predictable response in relation to the environmental factors, and the yield of genotypes is likely to be improved through enhancing the environmental conditions. The genotype yield matches the estimated or predicted levels in all environments (Tollenaar & Lee 2002). Sabbaghniya et al. (2008, 2013) expressed that the AMMI method and its parameters were useful for investigating static stability. Zali et al. (2012) investigated the AMMI parameters and classified them into two groups. While the first group included EV, MASV, DZ, SIPC, and FA, the second group consisted of ZA, ASV, and Da. They stated that both of the groups were closely associated with the mean yield. Karimizadeh et al. (2016) stated that ASV features agricultural concepts of stability.

According to the parameters of type I ($SIPC_1$, FA_1, Za_1, Dz_1, EV_1, and Da_1), genotypes G7, G10, G14, G20, and G24 were stable although their yields were below the grand mean (Table 9). Hence, they cannot be considered as the ideal breeding lines. These breeding lines possess the Type I stability, which is equivalent to the biological stability (Lin et al. 1986). The tuber yields of genotypes G14 and G24 were nearly equal to the grand mean. Thus, these breeding lines can be used in breeding programs where the qualitative characteristics are of interest.

Table 9- The two-way table of the environment and genotype means

Genotypes	Ardabil (2016) E1	Hamedan (2016) E2	Karaj (2016) E3	Isfahan (2016) E4	Razavi Khorasan (2016) E5	Ardabil (2017) E6	Hamedan (2017) E7	Karaj (2017) E8	Isfahan (2017) E9	Razavi Khorasan (2017) E10	Genotype mean
G1	54.273	49.443	20.373	34.853	18.957	42.180	30.587	30.217	42.700	32.067	35.565
G2	43.323	31.847	19.957	23.593	33.557	25.893	24.567	26.770	29.217	34.310	29.303
G3	36.193	48.187	31.613	23.593	27.297	42.513	30.347	29.130	28.520	32.533	32.993
G4	46.540	36.440	22.503	29.490	20.800	33.457	30.910	27.853	24.147	24.933	29.707
G5	44.067	80.960	24.167	27.883	43.257	31.633	28.653	39.750	53.830	37.863	41.206
G6	39.930	44.390	27.117	28.957	27.180	37.430	30.790	31.130	35.047	25.277	32.725
G7	42.043	45.053	17.693	18.767	30.910	29.430	23.877	29.350	34.120	28.897	30.014
G8	52.570	41.210	20.497	39.143	33.027	28.487	34.543	37.463	27.957	35.617	35.051
G9	33.290	58.830	21.290	19.570	36.180	25.230	23.413	32.823	32.563	41.200	32.439
G10	40.507	46.863	19.187	19.300	22.373	35.060	18.452	28.670	44.650	28.833	30.390
G11	52.443	33.923	19.317	26.813	36.543	33.983	32.653	24.180	31.440	22.350	31.365
G12	29.223	38.780	29.383	25.203	51.907	18.770	33.457	25.797	27.660	33.533	31.371
Caesar	42.250	34.670	25.297	18.763	17.667	30.917	21.300	23.463	29.050	32.166	27.554
G14	47.343	33.040	19.000	22.520	34.537	27.023	30.233	32.870	41.903	27.333	31.580
G15	34.470	40.987	17.567	22.520	23.783	24.873	23.653	30.130	46.573	28.917	29.347
G16	30.090	41.560	19.740	21.447	34.073	21.340	22.350	20.070	41.837	40.660	29.317
G17	39.247	41.340	16.830	25.470	55.800	23.877	23.990	32.533	45.747	28.967	33.380
G18	27.510	31.890	20.000	27.347	46.803	21.550	36.680	22.960	49.123	24.500	30.836
G19	50.857	55.210	22.950	32.707	25.407	30.170	33.100	29.997	30.540	28.700	33.964
G20	38.413	42.930	10.973	20.373	29.667	31.743	34.793	28.260	39.717	24.100	30.097
G21	25.517	44.480	23.997	22.520	36.130	18.936	25.567	33.460	51.040	30.100	31.175
Satina	36.947	27.895	22.667	24.667	16.820	31.607	30.790	26.500	27.920	40.267	28.608
Luta	35.080	48.540	21.333	23.325	26.913	22.323	21.653	32.153	37.860	34.500	30.368
Agria	36.043	38.233	33.133	26.770	26.667	21.337	31.123	21.930	39.750	36.383	31.137
Savalan	43.690	39.517	45.530	30.543	29.723	23.903	33.440	28.743	35.010	25.100	33.520
Env. mean	40.074	43.049	22.885	25.445	31.439	28.547	28.437	29.048	37.117	31.164	31.720

According to the bi-plot, genotypes G6, G7, G10, G13 (cv. Caesar), G14, G16, G20, G21, and G24 (cv. Agria) had general adaptation in the entire studied regions. Among the all stable genotypes in the study, genotype G6 produced higher yield than average yield of all genotypes tested. In addition, G14 and G24 (cv. Agria) had tuber yield near to mean tuber yield of all genotypes. The availability of cultivars that are highly adaptive to a vast range of regions is one of the important goals of the breeding programs (Mohebodini et al. 2006). Hassanpanah et al. (2018) investigated 11 genotypes in five regions and selected genotypes 397031-16, 397045-13, and 397009-8 for their yield stability and dry matter as well as the other qualitative and quantitative traits. In another study using multivariate methods and qualitative characteristics, Hassanpanah et al. (2016) selected clones 1 and 2 as the stable clones with high tuber yield and for uses as chips, French fries, and roasting.

4. Conclusions

Yield-stable G6 performed high tuber yield with 32.73 t/ha, and the other two yield-stable genotypes G14 and G24 (cv. Agria) produced moderate tuber yield with 31.58 t/ha and 31.14 t/ha, respectively. In conclusion, potato breeding lines G6 and G14 could be considered as candidate for registration in Iran. In addition, Agria might be proposed as a suitable variety for regions such as Ardebil, Hamadan, Razavi Khorasan, Isfahan, and Karaj.

References

Annicchiarico P (1997). Joint regression vs AMMI analysis of genotype × environment interactions for cereals in Italy. *Euphytica* 94: 53-62

Byarugaba A A, Benon M, Tibanyedera D & Barekye A (2018). Genotype by environment interaction (GxE) as a measure of yield stability of Dutch potato varieties in Uganda. *African Journal of Agricultural Research* 13(17): 890-896 https://doi.org/10.5897/ajar2018.13061

Crossa J, Gauch H G & Zobel R W (1990). Additive main effects and multiplicative interaction analysis of two international maize cultivar trials. Crop Science 30: 493-500 https://doi.org/10.2135/cropsci1990.0011183x003000030003x

Crossa J, Fox P N, Pfeiffer W H, Rajaram S & Gauch H G (1991). AMMI adjustment for statistical analysis of an international wheat yield trial. Theoretical and Applied Genetics 81: 27-37 https://doi.org/10.1007/bf00226108

Devaux A, Kromann P & Oriza O (2014). Potatoes for sustainable global food security. *Potato Research* 57: 185-199 https://doi.org/10.1007/s11540-014-9265-1

Fufa M (2013). AMMI analysis of tuber yield of potato genotypes grown in bale, southeastern Ethiopia. Advances in Crop Science and Technology 2: 1-3 https://doi.org/10.4172/2329-8863.1000120

Gauch H G (1988). Model selection and validation for yield trials with interaction. *Biometrics* 44: 705-715 https://doi.org/10.2307/2531585.

Gauch H G & Zobel R W (1988). Predictive and postdictive success of statistical analyses of yield trials. Theoretical and Applied Genetics 76: 1-10 https://doi.org/10.1007/bf00288824

Hassanabadi H, Mosapor-Gorgi A, Hasanpanah D, Ahmadvand R, Parvizi KH, Kazemi M, Hajianfar R & Abdi H R (2013). Khavaran, a new potato cultivar with high yielding and quality. *Research Achievements for Field and Horticulture Crops* 2(1): 67-79

Hassanpanah D, Hassanabadi H, Hosseinzadeh A, Soheili B & Mohammadi R (2016). Factor Analysis, AMMI Stability Value (ASV) Parameter and GGE Bi-Plot Graphical Method of Quantitative and Qualitative Traits in Potato Genotypes. *Journal of Crop Ecophysiology* 10(3): 731-748

Hassanpanah D, Hassanabadi H, Parvizi K, Kazemi M, Jalali A H, Mobser S, Kahbazi M, Hasani M, Mohammadi R & Khashmi M R (2018). Evaluation of quantitative and qualitative traits and stability of commercial cultivars and promising clonese of potato. *Seed and Plant Improvment Journal* 3: 455-474

Kadhem F A & Baktash F Y (2016). Analysis of adaptability and yield stability of promising lines of bread wheat *(Triticum aestavum* L.). Iraqi *Journal of Agricultural Science* 47: 35-43

Karimizadeh R, Mohammadi M, Sabaghnia N & Shefazadeh M K (2012). Using Huehn's Nonparametric Stability Statistics to Investigate Genotype × Environment Interaction. *Notulae Botanicae Horti Agrobotanici* 40: 195-200 https://doi.org/10.15835/nbha4017593

Karimizadeh R, Asghari A, CHinipardaz R, Sofalian O & Ghaffari A (2016). Determining yield stability and model selection by ammi method in rain-fed durum wheat genotypes. *T*urkish *Journal of Field Crops* 21(2): 174-183 https://doi.org/10.17557/tjfc.17390

Karimizadeh R, Asghari A, CHinipardaz R, Sofalian O, Ghaffari A, Shabazi K, Hosseinpour T, Ghojog H & Armion M (2019). Use of principal coordinate analysis for measuring GE interactions in rain-fed durum wheat genotypes. *Journal of* Agricultural Science 25: 38-46 https://doi.org/10.15832/ankutbd.538993

Khomari A, Mostafavi KH & Mohammadi A (2016). Stability study of yield in sunflower (*Helianthus annuus* L.) cultivars using AMMI method. *Journal of Crop Breeding* 9(23): 117-124

Lin C S, Binns M R & Lefkoveitch L P (1986). Stability analysis: Where do we stand? *Crop* Science 26: 894-900

Mohebodini M, Dehghani H & Sabaghpour S H (2006). Stability of performance in lentil (*Lens culinaris* Medik.) genotypes in Iran. *Euphytica* 149: 343-352 https://doi.org/10.1007/s10681-006-9086-7

Omrani S, Naji A M & Esmaeil Zadeh Moghadam M (2018). Evaluation of yield stability of bread wheat (Triticum aestivum L.) genotypes using additive main effects and multiplicative Interaction (AMMI). *Journal of Crop Breeding* 10: 73-80 https://doi.org/10.29252/jcb.10.25.73

Pourdad S S & Jamshid Moghaddam M (2013). Study on genotype×environment interaction through GGE Biplot for seed yield in spring rapeseed (*Brassica Napus* L.) in rain-fed condition. *Journal of Crop Breeding* 5(12): 1-14

Purchase J L (1997). Parametric analysis to describe G × E interaction and yield stability in winter wheat. PhD Thesis, Department of Agronomy, Faculty of Agriculture, University of the Orange Free State (Published), Bloemfontein, South Africa

Raju B M K (2002). A study on AMMI model and its biplots. *Journal of the Indian Society of Agricultural* Statistics 55: 297-322

Sabaghnia N, Sabaghpour S H & Dehghani H (2008). The use of an AMMI model and its parameters to analyse yield stability in multi-environment trials. *Indian Agricultural Science* 146: 571-581 https://doi.org/10.1017/s0021859608007831

Sabaghnia, N, Mohammadi M & Karimizadeh R (2013). Parameters of AMMI model for yield stability analysis in durum wheat. *Agriculturae conspectus Scientificus* 78: 119-124

Safavi S M & Bahraminejad S (2017). The evaluation of genotype × environment interactions for grain yield of oat genotypes using AMMI model. Journal *of Crop Breeding* 9: 125-132 https://doi.org/10.29252/jcb.9.22.125

Shafii B & Price W J (1998). Analysis of genotype-by-environment interaction using the additive main effecta and multiplicative interaction model and stability estimates. *Journal of Agricultural Biological and Environmental Statistics* 3: 335-345 https://doi.org/10.2307/1400587

Sneller C H, Kilgore Norquest L & Dombek D (1997). Repeatability of yield stability statistics in soybean. *Crop Science* 37: 383-390 https://doi.org/10.2135/cropsci1997.0011183x003700020013x

Tarakanovas P & Ruzgas V (2006). Additive main effect and multiplication analysis of grain yield of wheat varieties in lithunia. *Agricultural Research* 4: 91-94

Tollenaar M & Lee E A (2002). Yield potential, yield stability and stress tolerance in maize. *Field Crop Research* 75: 161-169 https://doi.org/10.1016/s0378-4290(02)00024-2

Worku A, Mulugeta G, Berhun B, Abebe T, Giorgis G W, Chindie A & Kebede G (2018). Performance and yield stability analysis of potato genotypes in ethiopia. *Advances in Crop Science and Technology* 6(1): 1-8. https://doi.org/10.4172/2329-8863.1000336

Zali H, Farshadfar E, Sabaghpour S H & Karimizadeh R (2012). Evaluation of genotype × environment interaction in chickpea using measures of stability from AMMI model. *Annals of Biological Research* 3(7): 3126-3136

Zhang Z, Lu C & Xiang Z H (1998). Stability analysis for varieties by AMMI model. *Acta Agronomica Sinica* 24: 304-309

Zobel R W (1994). Stress resistance and root systems. In: 1-4 Aug. 1993. Of the Workshop on Adaptation of Plants to Soil Stresses, INTSORMIL Publ. 94-2. Inst., Univ Nebraska, Lincoln pp. 80-99

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Effects of Scalding Parameters and Ripening on the Chemical, Textural and Microstructural Properties of Urfa Cheese

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ABSTRACT

The objectives of this study were to determine the effect of cheese scalding temperature, scalding time, and ripening time on the chemical, textural and microstructural properties of ovine milk Urfa cheese. Dry salted fresh cheeses were scalded in whey at 70 °C, 80 °C, and 90 °C for 5 and 10 minutes and ripened for 90 days, respectively. Scalding temperature significantly affected fat in dry matter and salt in dry matter ($P<0.05$), total solids and nitrogen, hardness, cohesiveness, gumminess, and chewiness ($P<0.001$). Scalding time significantly affected total nitrogen and gumminess ($P<0.05$), total solids, and hardness ($P<0.001$). Ripening time significantly affected chemical, textural and color properties of ovine milk Urfa cheese ($P<0.001$), except fat in dry matter ($P<0.05$). The color properties of Urfa cheese were not significantly affected by the scalding temperature and time ($P>0.05$). Besides, scalding treatments have improved the microstructure of ovine milk Urfa cheeses. Urfa cheese exhibited a more compact, coarser, and uniform structure with increasing scalding temperature and time. As a result, scalding treatments and ripening on the chemical, textural and microstructural characteristics of Urfa cheeses were substantially effective.

Keywords: Urfa cheese; Scalding; Ripening; Microstructure; Texture

1. Introduction

Textural features are an expression of the rheological properties of the physical structure of food. Cheese quality and identity are largely dependent on textural properties. Cheese texture is very important on consumer acceptability and the end-use of the cheese (Hort & Le Grys 2001; Alinovi et al. 2018). Furthermore, there is a relationship between the microstructure and rheological properties of the cheese, and both are influenced by the chemical cheese composition (El Bakry & Sheehan 2014). On the other hand, numerous reactions take place during the ripening of cheese. It is known that the texture of the cheese is extensively affected by the chemical composition of cheese, cheese production, and biochemical changes during the ripening (Tomaszewska et al. 2019).

Urfa cheese is a traditional semi-hard brined Turkish cheese variety, which is manufactured mainly in the southeast Anatolia region of Turkey from raw bovine milk or mixtures of ovine and caprine milk. However, the industrial Urfa cheese has been made from cow's milk, because of the very short lactation period of ewe's and goat's milk in Turkey. Urfa cheese is consumed fresh and/or mature. The unripened Urfa cheese is only used for the production of traditional cheese dessert products, such as "cheese helva", "cheese bread", and "kadayif" (Atasoy et al. 2013). Urfa cheese is produced without milk pasteurization and starter bacteria, and cheese microflora consists of indigenous microorganisms obtained from raw milk or transmitted from the environment. The presence of natural microorganisms provides the cheese with unique characteristics and cheeses made from raw milk are preferred by consumers (Atasoy et al. 2008; Kırmacı 2016). The microbial safety of Urfa cheese is supplied by dry salting technology by the manual spreading of salt onto the cheese surface. Alternatively, scalding of fresh cheese blocks in boiling whey is an alternative practical way of reducing microbial counts in Urfa cheese. Although there is no standard scalding temperature and time in the traditional Urfa cheese production, general scalding temperature and time are applied as 65-90 °C and about 5-10 minutes, respectively. These methods have different effects on natural microflora and also chemical, textural, and microstructural properties during storage. Following scalding and/or dry salting, Urfa cheese is ripened in brine.

Although numerous studies on the microstructural and textural characteristics of many cheese varieties were conducted, studies on the microstructural and textural characteristics of Urfa cheese are limited (Özer et al. 2003). As far as we know, no study has been conducted on the color characteristics of Urfa cheese up to now. Moreover, any study investigated the effect of

scalding time on the properties of Urfa cheese was not encountered. The objective of this study was to investigate the effects of scalding temperature, scalding time, and ripening time on the color, chemical, textural and microstructural properties of Urfa cheese made from raw ewe milk.

2. Material and Methods

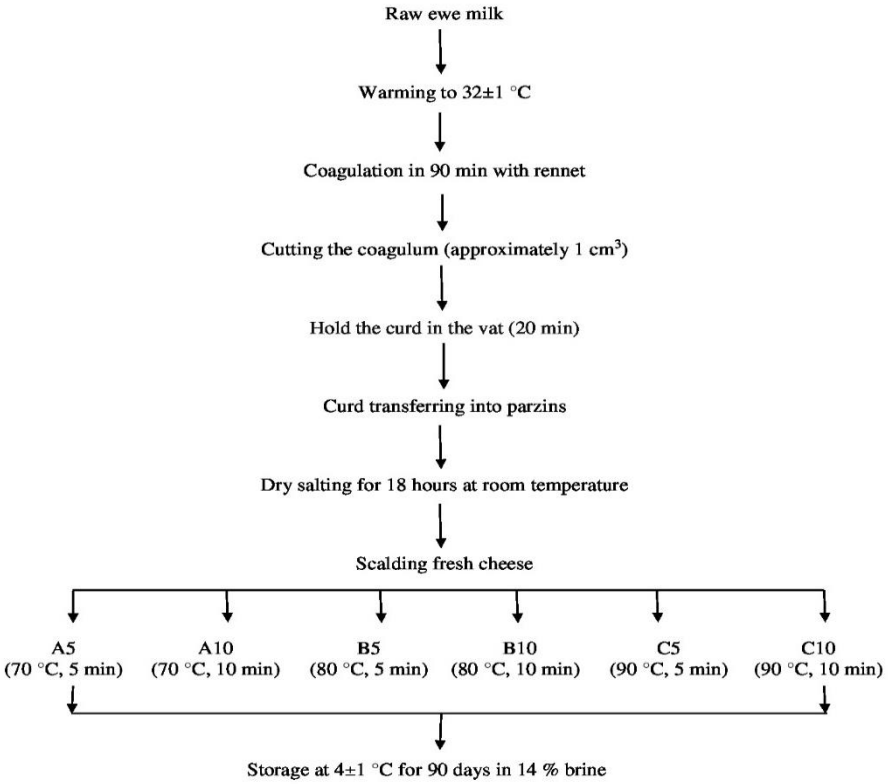
2.1. Materials

Ewe milk used in cheese production was obtained from Şanlıurfa province. The commercial rennet (Surer, Konya, Turkey) was used to coagulate the milk.

2.2. Cheesemaking and sampling

Fresh dry salted and scalded cheeses were designated as A5 (scalded at 70 °C for 5 min), A10 (scalded at 70 °C for 10 min), B5 (scalded at 80 °C for 5 min), B10 (scalded at 80 °C for 10 min), C5 (scalded at 90 °C for 5 min) and C10 (scalded at 90 °C for 10 min). Cheese production is carried out according to Atasoy et al. (2013) by the traditional method. The flow diagram of experimental Urfa cheeses is demonstrated in Figure 1. The Urfa cheese production was replicated 2 times on different days. From each batch, 1, 30, 60, and 90-day old cheese were sampled and analyzed. Each batch consisted of four cheese blocks (each block contained approximately 250 g of cheese).

Figure 1- Flow chart of traditional Urfa cheese production



2.3. Cheese analyses

The titratable acidity, total solids, and salt analyses were carried out according to Turkish Standards (1989). The IDF (1993) method for total nitrogen was used. pH was measured by pH meter (Hanna, HI 2215). The fat analysis was performed with the Gerber method according to Turkish Standards (TS 1978).

Texture profile analyses were performed using Texture Analyzer Model LF Plus (Lloyd Instruments Ltd., Hampshire, UK). Color values were determined using the Hunter Lab instrument (color Quest XE, UK). Lightness, redness, yellowness values of samples were measured according to the CIE system and the ΔE value of samples was calculated.

Microstructures of samples were determined as described by Hayaloglu et al. (2014). Images were monitored with a scanning electron microscope (LEO, EVO 40 Model, Carl Zeiss SMT, Oberkochen, Germany) at 20 kV.

2.4. Statistical analyses

Results were performed by analysis of variance using the Minitab version 16 packet statistic programs. The differences between the samples were determined by Tukey's test.

3. Results and Discussion

3.1. Chemical composition

The effects of scalding parameters and ripening time on the chemical compositions of Urfa cheese are presented in Table 1 and the chemical compositions of the Urfa cheese samples during ripening are demonstrated in Table 2. Total solid contents of the samples were significantly ($P<0.001$) affected by scalding temperature, scalding time, and ripening time. Also, the effects of scalding temperature ($P<0.001$), scalding time ($P<0.05$) on total nitrogen contents were found to be significant. Also, the total solid and total nitrogen contents of Urfa cheese were significantly increased with increasing scalding temperature and time. This is likely to be due to whey expulsion during scalding treatments. Kahyaoglu & Kaya (2003) stated that heat treatment applied to curds reduced the moisture content of Gaziantep cheese. Similar results have been reported by Tunick et al. (1993) for Mozzarella cheeses. Moreover, total solids and total nitrogen contents of Urfa cheese were significantly decreased during ripening. Changes in total solids can be attributed to the breakdown of peptide bonds and new ionic groups. These decreases may be due to the transition of the soluble nitrogen fractions into the brine during cheese storage. Further losses are referred to as hydrolysis of caseins by proteolytic enzymes to lower molecular weight compounds (Grappin & Beuvier 1997) and scattering of water-soluble nitrogen into the brine (Prasad & Alvarez 1999). Previous studies (Atasoy & Türkoğlu 2008) showed that total nitrogen and solid contents of Urfa cheese declined during storage.

While the effect of ripening time on pH and titratable acidity of Urfa cheeses were found to be significant ($P<0.001$), the effects of scalding parameters were insignificant ($P>0.05$). Also, titratable acidity and pH values of Urfa cheese decreased and increased ($P<0.05$), respectively, until the 30th day of ripening. However, no any change was found during the rest of the storage period. These can be explained by the continued fermentation during the maturation, completed by the end of the first month. Additionally, the decrease in titratable acidity may be due to the assimilation of lactic acid and the production of ammonia (Kırmacı et al. 2014). These results are in agreement with those of Atasoy & Türkoğlu (2008) for Urfa cheese.

Fat in dry matter contents of Urfa cheese was significantly affected by scalding temperature, and ripening time ($P<0.05$). In particular, the C5 sample was found to have lower fat content than others. A group of researchers (Ozer et al. 2004) have reported that scalded Urfa cheeses contained higher fat than unscalded ones. Furthermore, fat contents (as dry matter) of B10, C5, and C10 samples decreased ($P<0.05$) during the first 30 days of ripening and remained almost constant after this day. Sahingil et al. (2014) reported that fat contents in dry matter of white cheeses depended on the ripening time.

Table 1- The variance analysis results on the effect of scalding temperature, scalding time, and ripening on the chemical composition, *L*, *a*, *b*, *ΔE* values, and textural properties of Urfa cheese samples (F-values of independent variables and interactions)

	Sources of variance						
	STE	*STI*	*R*	*STE x STI*	*STE x R*	*STI x R*	*STE x STI x R*
Chemical composition							
Total solids	64.87***	54.88***	393.10***	0.06	1.17	0.11	0.57
pH	0.65	0.00	4.61***	0.07	0.19	0.11	0.22
Titratable acidity	0.66	2.22	85.59***	0.92	0.22	0.58	1.38
Total nitrogen	98.92***	7.82*	1283.12***	1.50	2.06	2.38	1.10
Fat in dry matter	5.28*	0.13	3.38*	1.01	0.94	0.59	0.36
Salt in dry matter	3.96*	0.56	785.56***	0.21	4.35**	3.53*	1.29
Texture							
Hardness	84.21***	19.29***	1219.70***	1.18	4.06***	0.43	0.31
Cohesiveness	54.58***	0.38	127.23***	1.31	8.17***	0.13	1.14
Springiness	0.71	0.55	135.67***	1.37	1.42	0.32	0.72
Gumminess	112.93***	5.43*	574.40***	1.15	25.12***	0.38	1.10
Chewiness	78.55***	4.10	548.53***	1.32	24.00***	0.45	1.09
Fracturability	0.06	0.34	29.02***	0.09	0.98	0.08	1.08
Adhesiveness	4.65*	1.34	9.78***	2.75	2.84*	3.87*	0.52
Color							
L	0.16	0.16	15.52***	0.53	0.26	0.12	0.42
a	2.75	1.99	31.09***	1.53	0.23	0.79	0.61
b	1.89	1.51	69.24***	1.26	1.55	0.27	1.26
ΔE	0.47	0.35	6.02***	1.27	1.02	0.56	0.65

*, $P<0.05$ significance level; **, $P<0.01$ significance level; ***, $P<0.001$ significance level; STE, Scalding temperature; STI, Scalding time; R, Ripening time

Salt in the dry matter contents was significantly affected by scalding temperature (P<0.05), ripening time (P<0.001), the interaction of the scalding temperature and ripening time (P<0.01), and scalding time and ripening time (P<0.05). Salt contents in the dry matter of samples increased (P<0.05) during the ripening. In particular, the salt contents in the dry matter of the cheese samples increased until the 60th day of ripening then almost unchanged (except for the A5 sample). The changes in salt contents of Urfa cheeses were similar in previous studies (Atasoy et al. 2008). When cheese is placed in brine, salt molecules transfer from the brine into the cheese as a result of the osmotic pressure difference between the cheese and the brine. Until the osmotic balance between cheese and brine is reached, salt molecules diffusion continues. Thus, salt diffusion was faster into the cheese from brine at the beginning of the ripening.

Table 2- The chemical composition of Urfa cheese samples during the ripening

Cheese samples		Ripening Time (days)			
		1	30	60	90
Total solids (g 100 g^{-1})	A5	58.73±0.03^{Ad}	56.80±0.64^{ABb}	54.92±0.26^{Bc}	52.92±0.44^{Ca}
	A10	59.61±0.15^{Ac}	58.03±0.28^{Aab}	55.22±0.06^{Bbc}	53.56±0.76^{Ba}
	B5	60.71±0.09^{Ab}	58.09±0.29^{ABab}	55.86±0.60^{BCabc}	53.88±0.77^{Ca}
	B10	61.49±0.19^{Aab}	58.63±0.28^{Bab}	56.74±0.01^{Cabc}	54.29±0.02^{Da}
	C5	61.57±0.26^{Aab}	58.68±0.31^{Bab}	56.43±0.03^{Cab}	54.61±0.31^{Da}
	C10	61.85±0.05^{Aa}	59.41±0.01^{Ba}	57.42±0.29^{Ca}	55.47±0.08^{Da}
pH	A5	5.25±0.00^{Ba}	5.42±0.03^{Aa}	5.43±0.22^{Aa}	5.47±0.01^{Aa}
	A10	5.26±0.10^{Ba}	5.32±0.07^{Aba}	5.46±0.12^{Aa}	5.49±0.09^{Aa}
	B5	5.21±0.11^{Ba}	5.44±0.01^{Aa}	5.42±0.14^{Aa}	5.52±0.04^{Aa}
	B10	5.23±0.07^{Ba}	5.34±0.15^{ABa}	5.40±0.01^{Aa}	5.58±0.16^{Aa}
	C5	5.15±0.12^{Ba}	5.31±0.09^{ABa}	5.42±0.16^{Aa}	5.40±0.18^{Aa}
	C10	5.24±0.00^{Aa}	5.42±0.20^{Aa}	5.35±0.18^{Aa}	5.38±0.18^{Aa}
Titratable acidity (g 100 g^{-1} la)	A5	0.20±0.01^{Aa}	0.10±0.01^{Ba}	0.09±0.01^{Ba}	0.09±0.01^{Ba}
	A10	0.21±0.02^{Aa}	0.09±0.00^{Ba}	0.11±0.01^{ABa}	0.08±0.01^{Ba}
	B5	0.20±0.01^{Aa}	0.07±0.01^{Ba}	0.11±0.02^{ABa}	0.08±0.01^{ABa}
	B10	0.21±0.02^{Aa}	0.08±0.01^{Ba}	0.08±0.01^{Ba}	0.06±0.01^{Ba}
	C5	0.21±0.01^{Aa}	0.12±0.02^{ABa}	0.08±0.01^{Ba}	0.07±0.01^{Ba}
	C10	0.19±0.01^{Aa}	0.05±0.01^{Ba}	0.09±0.02^{Ba}	0.06±0.01^{Ba}
Total nitrogen (g 100 g^{-1})	A5	22.81±0.19^{Acd}	19.51±0.48^{Bb}	17.24±0.24^{Cb}	15.75±0.02^{Cc}
	A10	22.61±0.15^{Acd}	19.98±0.01^{Ba}	17.98±0.01^{Cab}	16.31±0.01^{Dbc}
	B5	23.20±0.07^{Abc}	20.21±0.03^{Bab}	18.18±0.31^{Cab}	16.88±0.17^{Dbc}
	B10	23.42±0.02^{Abc}	20.12±0.34^{Bab}	18.28±0.00^{Cab}	16.95±0.02^{Db}
	C5	24.29±0.18^{Aa}	20.69±0.06^{Bab}	18.36±0.39^{Cab}	17.76±0.14^{Ca}
	C10	23.97±0.12^{Aba}	20.96±0.12^{Ba}	18.96±0.04^{Ca}	18.01±0.24^{Da}
Fat in dry matter (g 100 g^{-1})	A5	45.44±0.40^{Aa}	45.51±0.56^{Aa}	46.09±0.12^{Aa}	45.81±0.33^{Aa}
	A10	45.18±0.42^{Aa}	45.02±0.21^{Aa}	45.67±0.33^{Aa}	45.97±0.28^{Aa}
	B5	45.86±0.02^{Aa}	45.13±0.39^{Aa}	45.48±0.55^{Aa}	45.39±0.56^{Aa}
	B10	45.48±0.19^{Aa}	44.76±0.21^{Ba}	45.27±0.56^{Aa}	45.71±0.14^{Aa}
	C5	44.99±0.08^{Ab}	44.46±0.40^{Bb}	45.07±0.57^{Aa}	44.91±0.37^{Ab}
	C10	45.87±0.26^{Aa}	44.50±0.32^{Bb}	45.01±0.06^{Aa}	45.06±0.60^{Aa}
Salt in dry matter (g 100 g^{-1})	A5	14.63±0.06^{Da}	18.75±0.20^{Ca}	21.38±0.57^{Ba}	23.48±0.16^{Aa}
	A10	15.66±0.54^{Ca}	19.29±0.15^{Ba}	20.99±0.59^{ABa}	22.55±0.11^{Aab}
	B5	14.94±0.24^{Ca}	19.48±0.37^{Ba}	21.49±0.19^{Aa}	22.39±0.06^{Ab}
	B10	14.94±0.28^{Ca}	20.30±0.44^{Ba}	21.71±0.25^{ABa}	22.15±0.21^{Ab}
	C5	14.44±0.27^{Ca}	19.33±0.31^{Ba}	21.50±0.16^{Aa}	21.85±0.27^{Ab}
	C10	14.51±0.07^{Ca}	19.63±0.03^{Ba}	21.27±0.23^{Aa}	21.76±0.01^{Ab}

$^{A-D}$ Means in each row with different uppercase letters were significantly affected by storage periods (P<0.05). $^{a-d}$ Means with different lowercase letters were significantly different among cheese samples within the column of the similar ripening period (P<0.05). A5 (scalded at 70 °C for 5 min), A10 (scalded at 70 °C for 10 min), B5 (scalded at 80 °C for 5 min), B10 (scalded at 80 °C for 10 min), C5 (scalded at 90 °C for 5 min) and C10 (scalded at 90 °C for 10 min).

3.2. *Textural properties of Urfa cheeses*

The effects of scalding parameters and ripening time on the textural properties of Urfa cheese are shown in Table 1. Also, textural properties of Urfa cheese samples during the ripening period are given in Table 3. The hardness values of Urfa cheese were significantly influenced (P<0.001) by scalding temperature, scalding time, ripening time, and interaction of scalding temperature and ripening time. Also, the hardness of Urfa cheese was significantly increased with increasing scalding temperature and time. This is likely to be due to an increase in the protein concentration per unit area in the cheese matrix during scalding treatments. Tunick et al. (1993) reported that the hardness of Mozzarella cheese was influenced by curd scalding temperature. Similar results were determined in Gaziantep cheese (Kahyaoglu & Kaya 2003). Besides, the hardness of Urfa cheese samples significantly decreased (P<0.05) during storage, and mature Urfa cheese was found less hard than fresh cheese. Reduction in hardness during storage may be due to proteolysis of the casein network. There is a positive correlation between the hardness of cheese and the quantity of intact α_{s1}-casein (Lawrence et al. 1987). The products of proteolysis are generally water-soluble and cannot contribute

to the protein network (Lawrence et al. 1987). The decreases in cheese during the ripening period were also observed by some researchers (Sahan et al. 2008). Reduction in the hardness of cheeses was not surprising considering the decreased total nitrogen and total solid contents (Table 2) during the ripening.

Table 3- The change of textural properties of Urfa cheese samples during the ripening

Cheese samples		*Ripening Time (days)*			
		1	*30*	*60*	*90*
Hardness	A5	28.66 ± 0.54^{Acd}	20.02 ± 0.96^{Bcd}	10.25 ± 0.73^{Cb}	6.22 ± 0.39^{Db}
	A10	31.63 ± 1.31^{Abc}	22.44 ± 0.37^{Bbcd}	12.77 ± 0.04^{Cab}	6.81 ± 0.50^{Dab}
	B5	32.71 ± 1.26^{Aabcd}	24.13 ± 0.68^{Babc}	14.39 ± 0.75^{Cabc}	6.98 ± 0.84^{Dab}
	B10	33.48 ± 1.10^{Aabc}	25.63 ± 0.85^{Bab}	15.70 ± 1.13^{Ca}	8.09 ± 0.55^{Dab}
	C5	36.97 ± 1.03^{Aab}	26.16 ± 0.82^{Bab}	16.01 ± 0.78^{Ca}	8.91 ± 0.49^{Dab}
	C10	38.48 ± 1.00^{Aa}	27.08 ± 0.16^{Ba}	16.81 ± 0.61^{Ca}	9.62 ± 0.57^{Da}
Cohesiveness	A5	0.16 ± 0.01^{Ab}	0.14 ± 0.01^{Aa}	0.13 ± 0.01^{ABa}	0.08 ± 0.00^{Bb}
	A10	0.16 ± 0.01^{Ab}	0.14 ± 0.01^{ABa}	0.14 ± 0.01^{ABa}	0.11 ± 0.01^{Bab}
	B5	0.26 ± 0.01^{Aa}	0.18 ± 0.01^{Ba}	0.17 ± 0.01^{BCa}	0.11 ± 0.01^{Cab}
	B10	0.24 ± 0.01^{Aa}	0.19 ± 0.01^{ABa}	0.16 ± 0.01^{BCa}	0.11 ± 0.01^{Cab}
	C5	0.26 ± 0.01^{Aa}	0.19 ± 0.01^{Ba}	0.16 ± 0.01^{Ba}	0.12 ± 0.01^{Ba}
	C10	0.28 ± 0.01^{Aa}	0.21 ± 0.01^{ABa}	0.16 ± 0.01^{BCa}	0.11 ± 0.01^{Cab}
Springiness	A5	0.48 ± 0.01^{Aa}	0.29 ± 0.01^{Ba}	0.32 ± 0.01^{BCa}	0.35 ± 0.01^{Ca}
	A10	0.45 ± 0.01^{Aa}	0.30 ± 0.01^{Ba}	0.31 ± 0.01^{Ba}	0.34 ± 0.01^{Ba}
	B5	0.44 ± 0.01^{Aa}	0.31 ± 0.01^{Ba}	0.31 ± 0.01^{Ba}	0.33 ± 0.01^{Ba}
	B10	0.46 ± 0.02^{Aa}	0.31 ± 0.01^{Ba}	0.31 ± 0.01^{Ba}	0.36 ± 0.02^{Aa}
	C5	0.46 ± 0.01^{Aa}	0.34 ± 0.01^{Ba}	0.29 ± 0.02^{Ba}	0.33 ± 0.01^{Ba}
	C10	0.48 ± 0.01^{Aa}	0.33 ± 0.01^{Ba}	0.32 ± 0.01^{Ba}	0.35 ± 0.02^{Ba}
Gumminess	A5	4.79 ± 0.42^{Ac}	2.89 ± 0.05^{Bb}	1.33 ± 0.07^{Cb}	0.56 ± 0.03^{Cb}
	A10	5.25 ± 0.53^{Ac}	3.19 ± 0.19^{Bb}	1.88 ± 0.09^{BCab}	0.76 ± 0.03^{Cab}
	B5	8.71 ± 0.05^{Aba}	4.37 ± 0.25^{Bab}	2.51 ± 0.00^{Ca}	0.79 ± 0.13^{Dab}
	B10	8.16 ± 0.56^{Ab}	4.88 ± 0.59^{Bab}	2.57 ± 0.34^{BCa}	0.89 ± 0.11^{Cab}
	C5	9.83 ± 0.23^{Aab}	5.11 ± 0.59^{Bab}	2.58 ± 0.04^{Ca}	1.15 ± 0.01^{Ca}
	C10	11.06 ± 0.56^{Aa}	5.66 ± 0.43^{Ba}	2.82 ± 0.21^{Ca}	1.09 ± 0.06^{Ca}
Chewiness	A5	2.33 ± 0.15^{Ac}	0.85 ± 0.01^{Bb}	0.42 ± 0.01^{BCb}	0.19 ± 0.02^{Ca}
	A10	2.41 ± 0.25^{Ac}	0.97 ± 0.08^{Bb}	0.58 ± 0.01^{Bab}	0.26 ± 0.02^{Ba}
	B5	3.87 ± 0.07^{Aabc}	1.38 ± 0.02^{Bab}	0.79 ± 0.04^{Cab}	0.27 ± 0.05^{Da}
	B10	3.76 ± 0.47^{Abc}	1.50 ± 0.08^{Bab}	0.80 ± 0.14^{Bab}	0.32 ± 0.05^{Ba}
	C5	4.55 ± 0.03^{Aab}	1.76 ± 0.23^{Ba}	0.75 ± 0.07^{Cab}	0.38 ± 0.01^{Ca}
	C10	5.33 ± 0.37^{Aa}	1.90 ± 0.19^{Ba}	0.91 ± 0.01^{BCa}	0.38 ± 0.01^{Ca}
Fracturability	A5	0.91 ± 0.08^{Aa}	0.82 ± 0.01^{Aa}	0.71 ± 0.03^{Aa}	0.62 ± 0.04^{Aa}
	A10	0.85 ± 0.08^{ABa}	0.89 ± 0.01^{Aa}	0.57 ± 0.01^{ACa}	0.61 ± 0.02^{BCa}
	B5	0.82 ± 0.16^{Aa}	0.96 ± 0.01^{Aa}	0.59 ± 0.05^{Aa}	0.64 ± 0.01^{Aa}
	B10	0.89 ± 0.14^{Aa}	0.94 ± 0.07^{Aa}	0.58 ± 0.04^{Aa}	0.61 ± 0.01^{Aa}
	C5	0.90 ± 0.02^{Aa}	0.89 ± 0.09^{Aa}	0.61 ± 0.01^{Aa}	0.62 ± 0.01^{Aa}
	C10	0.84 ± 0.08^{Aa}	0.75 ± 0.01^{Aa}	0.67 ± 0.06^{Aa}	0.68 ± 0.02^{Aa}
Adhesiveness	A5	0.09 ± 0.01^{Aa}	0.04 ± 0.01^{ABa}	0.01 ± 0.01^{ABa}	0.03 ± 0.02^{Ba}
	A10	0.08 ± 0.01^{Aab}	0.09 ± 0.01^{Aa}	0.06 ± 0.01^{Aa}	0.04 ± 0.03^{Aa}
	B5	0.10 ± 0.01^{ABa}	0.07 ± 0.01^{BAa}	0.04 ± 0.01^{ABa}	0.02 ± 0.01^{Ba}
	B10	0.06 ± 0.01^{ABabc}	0.10 ± 0.01^{Aa}	0.03 ± 0.02^{ABa}	0.01 ± 0.01^{Ba}
	C5	0.03 ± 0.01^{Abc}	0.03 ± 0.01^{Aa}	0.02 ± 0.01^{Aa}	0.02 ± 0.01^{Aa}
	C10	0.02 ± 0.01^{Ac}	0.07 ± 0.02^{Aa}	0.02 ± 0.01^{Aa}	0.05 ± 0.02^{Aa}
Stiffness	A5	87.61 ± 1.42^{Ab}	29.47 ± 0.88^{Ba}	28.96 ± 0.14^{Ba}	26.03 ± 2.62^{Ba}
	A10	96.81 ± 4.15^{Aab}	30.44 ± 1.30^{Ba}	30.48 ± 0.25^{Ba}	26.34 ± 2.52^{Ba}
	B5	93.03 ± 5.46^{Aab}	30.14 ± 0.89^{Ba}	28.43 ± 1.27^{Ba}	27.78 ± 1.93^{Ba}
	B10	108.10 ± 8.25^{Aab}	34.68 ± 2.01^{Ba}	32.01 ± 2.40^{Ba}	26.49 ± 2.39^{Ba}
	C5	119.07 ± 9.70^{Aab}	33.01 ± 2.57^{Ba}	35.45 ± 1.03^{Ba}	25.71 ± 0.89^{Ba}
	C10	121.11 ± 5.21^{Aa}	33.62 ± 0.83^{Ba}	34.68 ± 1.50^{Ba}	26.80 ± 0.84^{Ba}

[A-D] Means in each row with different uppercase letters were significantly affected by storage periods ($P<0.05$). [a-d] Means with different lowercase letters were significantly different among cheese samples within the column of the similar ripening period ($P<0.05$). A5 (scalded at 70 °C for 5 min), A10 (scalded at 70 °C for 10 min), B5 (scalded at 80 °C for 5 min), B10 (scalded at 80 °C for 10 min), C5 (scalded at 90 °C for 5 min) and C10 (scalded at 90 °C for 10 min).

The effects of scalding temperature, ripening time, and interaction of scalding temperature and ripening time on cohesiveness values of cheeses were found to be effective ($P<0.001$). Cohesiveness values of fresh cheeses generally increased with increasing scalding temperature. It has been expressed that scalded Urfa cheeses had a higher cohesiveness value than unscalded ones (Özer et al. 2003). Moreover, cohesiveness values slightly decreased ($P<0.05$) with the ripening period. These changes of cohesiveness during the ripening are in agreement with the results of some researchers (Akalın & Karaman 2010; Eroglu et al. 2016). This may be due to proteolysis. Because proteolysis breaks the structural integrity of the protein network, leading to decreased cohesiveness (Awad et al. 2005).

Even though springiness was affected ($P<0.001$) by the ripening time, it was not affected by the scalding parameters. However, some researchers (Hayaloglu et al. 2014) reported that the scalding process is required to provide elasticity in cheese. Özer et al. (2003) observed that scalding treatment increased the springiness value of Urfa cheeses. Also, springiness values of samples decreased ($P<0.05$) until the 30 days of ripening, then remained constant (except for A5 and B10 samples). The decreases in springiness during cheese storage may be due to degradation of the protein, especially the hydrolysis of para κ-caseinate molecules (Awad et al. 2005), and reduction of free water. Hort & Le Grys (2001) reported that the springiness of Cheddar cheese decreased during the ripening, but also somewhat remained constant in the middle period of ripening.

The effects of ripening time, scalding temperature, the interaction of scalding temperature and ripening time ($P<0.001$), and scalding time ($P<0.05$) on the gumminess values of Urfa cheese were significantly found. This textural parameter of Urfa cheese was significantly increased with increasing scalding temperature. The variation in the aforementioned textural characteristic during the scalding was due largely to the variations in the scores for hardness. Similar results were also obtained from Kahyaoglu et al. (2005) for Gaziantep cheese which is scalded and ripened in brine like Urfa cheese. Moreover, gumminess values of Urfa cheese statistically declined ($P<0.05$) during cheese ripening. Increasing the level of NaCl during cheese maturation may be decreasing in the gumminess parameter due to increased water retention in the cheese. Eroglu et al. (2016) reported that the gumminess value of Kashar cheese was influenced by ripening time. Gumminess is the product of hardness and cohesiveness (Famenin et al. 2019). For this reason, the variables affecting cohesiveness and hardness also affect values of the gumminess during the ripening.

Chewiness values of Urfa cheese were influenced by the ripening time, scalding temperature, interaction of scalding temperature, and ripening time ($P<0.001$). As the scalding temperature increased, chewiness values of Urfa cheeses were generally increased. Furthermore, a decrease in chewiness values of cheeses was observed during the first 60 days of ripening ($P<0.05$). The chewiness is one of the secondary textural parameters of the cheeses and it is calculated by the hardness, cohesiveness, and springiness of the product (Famenin et al. 2019). Thus, changes in chewiness values may be associated with the variations of the hardness, cohesiveness, and springiness values during the ripening.

Fracturability was only influenced by ripening time ($P<0.001$). Nuñez et al. (1991) reported that fracturability value of ewe's milk Manchego cheese was affected by ripening time. Only fracturability value of A10 sample showed a fluctuation during the ripening ($P<0.05$). The higher fracturability value means the lower fracturability of the product (Aminifar et al. 2010). The fracturability value of the A10 sample decreased from the 30th day of maturation, which means that the A10 sample was more fragile during the maturation. This may be related to pore formation during maturation (Aminifar & Emam-Djomeh 2014). These findings are in good agreement with previous studies for different brined cheese (Aminifar & Emam-Djomeh 2014).

Ripening time ($P<0.001$), scalding temperature, interactions of scalding temperature and ripening time, and scalding time and ripening time ($P<0.05$) on the adhesiveness of samples were found effective. Adhesiveness value of only A5 cheese significantly reduced ($P<0.05$) during the ripening. Sahingil et al. (2014) pointed out that the adhesiveness values of white cheeses slightly decreased during maturation, but these declines were not significant. Also, the increase in scalding temperature caused a decrease in the adhesiveness values of 1-day old cheeses. It is known that the adhesiveness value is closely related to the fat and total solid content of the cheese (Motevalizadeh et al. 2018). This change in adhesiveness was following the changes in the total solid (Table 2) and fat (data not shown) during the ripening period.

Stiffness values, in other words, firmness, were influenced by the ripening time, scalding temperature, interaction of scalding temperature and ripening time ($P<0.001$), and scalding time ($P<0.05$). Although stiffness values of samples decreased ($P<0.05$) until the 30th day of ripening, they did not change during the rest of the maturation period. The decreases in springiness during the first month of storage may be due to increased moisture content and pH and breakdown of proteins (Table 2). It is known that the firmness of fresh cheeses decreases after the ripening due to biochemical and physical changes (Aminifar et al. 2010). Furthermore, there is a close relationship between the firmness of cheese and intact α_{s1}-casein. This reduction of stiffness was not surprising due to proteolysis (Lawrence et al. 1987). Also, stiffness values of Urfa cheeses increased with the scalding treatment. This is likely to be due to whey removal and increasing total solid content of Urfa cheese. Additionally, the curd shrinks in size and becomes firmer during the scalding process. Especially, the stiffness value of fresh C10 samples was found higher than fresh A5 sample.

3.3. Microstructures of Urfa cheeses

Scanning electron micrographs of samples are presented in Figure 2. The microstructures of cheeses were evaluated before the ripening process. It was observed that scalding treatment was affected microstructures of Urfa cheeses. Scalding treatment resulted in a coarser and more uniform microstructure in Urfa cheeses. It has been stated that the scalding process gives a more compact structure in Malatya cheese (Hayaloglu et al. 2010). Özer et al. (2003) also reported that the scalding provided a more homogeneous microstructure in Urfa cheeses. Especially, it has been found that the cheeses have a more compact structure with increasing scalding time. Scalded cheeses for 10 minutes were characterized by a compact protein network, with small pores. The relationship between cheese microstructure and rheology is known (Buffa et al. 2001). These changes in the microstructure can be associated with textural changes. Thus, it has been determined that some of the textural properties of the Urfa cheeses are

changed by scalding (Table 1). Akalın & Karaman (2010) pointed out that the compactness of the protein network demonstrates the structural development of the cheese responsible for the increased hardness value.

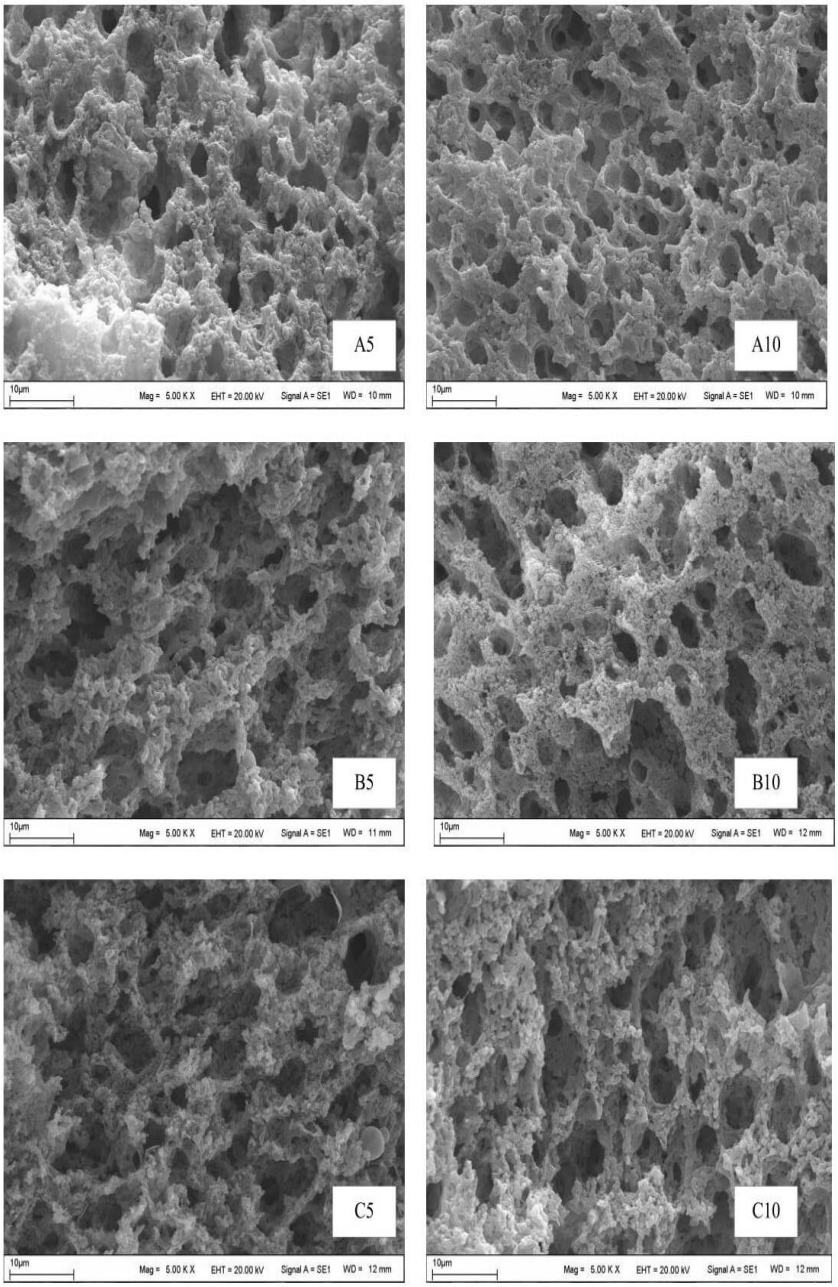


Figure 2- Scanning electron micrographs of Urfa cheese samples. Magnification is 5,000x

3.4. Color values of Urfa cheeses

The effects of scalding parameters and ripening time on *L*, *a*, *b*, and ΔE values of samples are depicted in Table 1. The changes in *L*, *a*, *b* and ΔE values of the Urfa cheeses during the maturation are shown in Table 4. The color properties of Urfa cheeses were only influenced by ripening time ($P<0.001$). It was reported that the effect of ripening time on color values of cheeses was found significant by some researchers (Buffa et al. 2001). The *L*, *b*, *a*, and ΔE values of cheeses did not show a definite trend throughout the ripening. The change in *L* value of the only B10 sample was found to significant during ripening ($P<0.05$). On the other hand, *a* values of cheeses (except for A5 and B10) were observed fluctuating ($P<0.05$) during the maturation. 90-day old cheese generally exhibited higher *a* value than fresh cheeses. *b* values were changed ($P<0.05$) from 1st day of the ripening period and they showed a higher yellowness value than 90-day old cheese. Finally, ΔE values of B10 and C5 samples were found to decline ($P<0.05$) at the end of maturation. Color changes may be attributed to microstructural changes of Urfa cheeses. The scattering of light is connected to its heterogeneousness at the microstructural and molecular levels. In a firm material such as cheese, light diffuses the superficial layers and penetrates largely at the interfaces of the fat globule and the edges of whey pockets (Madadlou et al. 2006).

Table 4- The changes in *L, a, b,* and *ΔE* values of Urfa cheese samples during the ripening

Cheese samples		*Ripening Time (days)*			
		1	*30*	*60*	*90*
L	A5	82.52±0.01^{Aa}	80.32±1.67^{Aa}	82.67±0.51^{Aa}	83.10±1.99^{Aa}
	A10	85.27±1.75^{Aa}	82.10±0.01^{Aa}	82.49±0.40^{Aa}	82.55±0.12^{Aa}
	B5	84.79±2.47^{Aa}	81.40±1.12^{Aa}	82.63±0.84^{Aa}	80.68±1.05^{Aa}
	B10	86.09±1.09^{Aa}	81.43±0.53^{Ba}	82.90±0.58^{ABa}	82.53±0.03^{ABa}
	C5	85.87±1.80^{Aa}	82.10±2.08^{Aa}	82.47±0.46^{Aa}	81.87±0.30^{Aa}
	C10	85.37±0.24^{Aa}	81.66±1.44^{Aa}	82.32±0.44^{Aa}	81.94±0.38^{Aa}
a	A5	-1.41±0.07^{Aa}	-1.50±0.05^{Aa}	-1.50±0.04^{Ab}	-1.33±0.13^{Aa}
	A10	-1.38±0.06^{Aa}	-1.53±0.01^{Ba}	-1.46±0.03^{Ba}	-1.11±0.02^{Ba}
	B5	-1.36±0.07^{ABa}	-1.50±0.03^{Ba}	-1.44±0.03^{ABa}	-1.22±0.02^{Aa}
	B10	-1.33±0.12^{Aa}	-1.45±0.03^{Aa}	-1.37±0.02^{Aa}	-1.16±0.05^{Aa}
	C5	-1.30±0.01^{ABa}	-1.48±0.04^{Ba}	-1.38±0.07^{ABa}	-1.17±0.03^{ABa}
	C10	-1.36±0.04^{ABa}	-1.50±0.01^{Ba}	-1.36±0.02^{Ba}	-1.19±0.02^{Aa}
b	A5	16.17±0.36^{Aa}	13.12±0.91^{ABa}	12.77±0.37^{Ba}	13.82±0.21^{ABa}
	A10	15.75±0.09^{Aa}	13.41±0.34^{Ba}	13.32±0.12^{Ba}	13.09±0.07^{Bab}
	B5	16.23±0.31^{Aa}	13.95±0.42^{Ba}	13.56±0.06^{Ba}	12.71±0.11^{Bb}
	B10	15.53±0.90^{Aa}	13.64±0.08^{ABa}	12.51±0.05^{ABa}	12.91±0.35^{Bab}
	C5	15.60±0.51^{Aa}	12.62±0.53^{Ba}	12.56±0.34^{Ba}	13.11±0.16^{Bab}
	C10	16.05±0.36^{Aa}	12.35±0.85^{Ba}	13.39±0.04^{ABa}	12.81±0.09^{Bab}
ΔE	A5	88.05±0.06^{Aa}	81.40±1.80^{Aa}	83.66±0.45^{Aa}	84.25±2.00^{Aa}
	A10	86.71±1.75^{Aa}	69.71±13.42^{Aa}	83.57±0.37^{Aa}	83.72±0.13^{Aa}
	B5	86.35±2.36^{Aa}	82.61±1.04^{Aa}	83.75±081^{Aa}	81.81±0.94^{Aa}
	B10	87.49±0.91^{Aa}	82.47±0.42^{Ba}	83.93±0.65^{Ba}	83.54±0.09^{Ba}
	C5	87.29±1.67^{Aa}	80.66±0.08^{Ba}	83.33±0.41^{ABa}	82.17±1.06^{ABa}
	C10	86.91±0.14^{Aa}	82.61±1.55^{Aa}	83.42±0.45^{Aa}	82.95±0.39^{Aa}

$^{A-D}$ Means in each row with different uppercase letters were significantly affected by storage periods (P<0.05). $^{a-d}$ Means with different lowercase letters were significantly different among cheese samples within the column of the similar ripening period (P<0.05). A5 (scalded at 70 °C for 5 min), A10 (scalded at 70 °C for 10 min), B5 (scalded at 80 °C for 5 min), B10 (scalded at 80 °C for 10 min), C5 (scalded at 90 °C for 5 min) and C10 (scalded at 90 °C for 10 min).

4. Conclusions

The results of this research showed that ripening time was effective on chemical, textural and color parameters of Urfa cheese. Scalding parameters on textural properties (except springiness and fracturability) of Urfa cheese were found to be important at the beginning of maturation. However, scalding parameters on hardness, cohesiveness, and gumminess were significant at the end of storage. Urfa cheese gained a more compact and uniform structure with the scalding parameters. Increasing scalding temperature and time positively improved the textural and microstructural properties of Urfa cheese. However, the effects of scalding temperature and time on the proteolysis, lipolysis, and volatiles compounds of Urfa cheese were not yet known. For this reason, further studies should be focused on the determination of the effect of scalding temperature and time on biochemical properties of lipolysis and proteolysis in Urfa cheese.

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References

Akalın A S & Karaman A D (2010). Influence of packaging conditions on the textural and sensory characteristics, microstructure and color of industrially produced Turkish white cheese during ripening. *Journal of Texture Studies* 41(4): 549-562 https://doi.org/10.1111/j.1745-4603.2010.00241.x

Alinovi M, Cordioli M, Francolino S, Locci F, Ghiglietti R, Monti L, Tidona F, Mucchetti G & Giraffa G (2018). Effect of fermentation-produced camel chymosin on quality of Crescenza cheese. *International Dairy Journal* 84: 72-78 https://doi.org/10.1016/j.idairyj.2018.04.001

Aminifar M, Hamedi M, Emam-Djomeh Z & Mehdinia A (2010). Microstructural, compositional and textural properties during ripening of Lighvan cheese, a traditional raw sheep cheese. *Journal of Texture Studies* 41(4): 579-593 https://doi.org/10.1111/j.1745-4603.2010.00244.x

Aminifar M & Emam-Djomeh Z (2014). Changes of Texture, Microstructure and free fatty acid contents of Lighvan cheese during accelerated ripening with lipase. *Journal of Agricultural Science and Technology* 16: 113-123

Atasoy A F & Türkoğlu H (2008). Changes of composition and free fatty acid contents of Urfa cheeses (a white-brined Turkish cheese) during ripening: Effects of heat treatments and starter cultures. *Food Chemistry* 110(3): 598-604 https://doi.org/10.1016/j.foodchem.2008.02.046

Atasoy A F, Yetişmeyen A, Türkoğlu H & Özer B (2008). Effects of heat treatment and starter culture on the properties of traditional Urfa cheeses (a white-brined Turkish cheese) produced from bovine milk. *Food Control* 19(3): 278-285 https://doi.org/10.1016/j.foodcont.2007.04.004

Atasoy A F, Hayaloglu A A, Kırmacı H, Levent O & Türkoğlu H (2013). Effects of partial substitution of caprine for ovine milk on the volatile compounds of fresh and mature Urfa cheeses. *Small Ruminant Research* 115(1-3): 113-123 https://doi.org/10.1016/j.smallrumres.2013.09.002

Awad S, Hassan A N & Muthukumarappan K (2005). Application of exopolysaccharide-producing cultures in reduced-fat cheddar cheese: Texture and melting properties. *Journal of Dairy Science* 88(12): 4204-4213 https://doi.org/10.3168/jds.s0022-0302(05)73106-4

Buffa M N, Trujillo A J, Pavia M & Guamis B (2001). Changes in textural, microstructural, and colour characteristics during ripening of cheeses made from raw, pasteurized or high-pressure-treated goats' milk. *International Dairy Journal* 11(11): 927-934 https://doi.org/10.1016/s0958-6946(01)00141-8

El Bakry M & Sheehan J (2014). Analysing cheese microstructure: A review of recent developments. *Journal of Food Engineering* 125: 84-96 https://doi.org/10.1016/j.jfoodeng.2013.10.030

Eroglu A, Toker O S & Dogan M (2016). Changes in the texture, physicochemical properties and volatile compound profiles of fresh Kashar cheese (<90 days) during ripening. *International Journal of Dairy Technology* 69(2): 243-253 https://doi.org/10.1111/1471-0307.12250

Famenin B K, Hosseini H, Zayeri F, Ghanati K, Lorenzo J M, Barba F J & Mousavi Khaneghah A (2019). Effect of mechanically deboning of chicken on the rheological and sensory properties of chicken sausages. *Journal of Food Processing and Preservation* 43(5): e13938 https://doi.org/10.1111/jfpp.13938

Grappin R & Beuvier E (1997). Possible Implications of Milk Pasteurization on the Manufacture and Sensory Quality of Ripened Cheese. *International Dairy Journal* 7(12): 751-761 https://doi.org/10.1016/s0958-6946(98)00006-5

Hayaloglu A A, Deegan K C & Mcsweeney P L (2010). Effect of milk pasteurization and curd scalding temperature on proteolysis in Malatya, a Halloumi-type cheese. *Dairy Science & Technology* 90(1): 99-109 https://doi.org/10.1051/dst/2009052

Hayaloglu A A, Karatekin B & Gurkan H (2014). Thermal stability of chymosin or microbial coagulant in the manufacture of Malatya, a Halloumi type cheese: Proteolysis, microstructure and functional properties. *International Dairy Journal* 38(2): 136-144 https://doi.org/10.1016/j.idairyj.2014.04.001

Hort J & Le Grys G (2001). Developments in the textural and rheological properties of UK Cheddar cheese during ripening. *International Dairy Journal* 11(4-7): 475-481 https://doi.org/10.1016/s0958-6946(01)00074-7

IDF (1993). Milk Determination of Nitrogen Content. IDF Standard 20B, International Dairy Federation, Brussels

Kahyaoglu T & Kaya S (2003). Effects of heat treatment and fat reduction on the rheological and functional properties of Gaziantep cheese. *International Dairy Journal* 13(11): 867-75 https://doi.org/10.1016/s0958-6946(03)00113-4

Kahyaoglu T, Kaya S & Kaya A (2005). Effects of fat reduction and curd dipping temperature on viscoelasticity, texture and appearance of Gaziantep cheese. *Food Science and Technology International* 11(3): 191-198 https://doi.org/10.1177/1082013205055002

Kırmacı H A, Hayaloğlu A A, Özer H B & Türkoğlu H (2014). Evolution of proteolysis in Urfa cheese made from ewe's milk by wild type starter culture systems. *Small Ruminant Research* 119(1-3): 120-129 https://doi.org/10.1016/j.smallrumres.2014.01.006

Kırmacı H A (2016). Effect of Wild Strains Used as Starter Cultures on Free Fatty Acid Profile of Urfa Cheese. *Polish Journal of Food and Nutrition Sciences* 66(4): 303-310 https://doi.org/10.1515/pjfns-2015-0034

Lawrence R C, Creamer L K & Gilles J (1987). Texture development during cheese ripening. *Journal of Dairy Science* 70(8): 1748-1760 https://doi.org/10.3168/jds.s0022-0302(87)80207-2

Madadlou A, Khosroshahi A, Mousavi S M & Djome Z E (2006). Microstructure and rheological properties of Iranian white cheese coagulated at various temperatures. *Journal of Dairy Science* 89(7): 2359-2364 https://doi.org/10.3168/jds.s0022-0302(06)72308-6

Motevalizadeh E, Mortazavi S A, Milani E & Hooshmand-Dalir M A R (2018). Optimization of physicochemical and textural properties of pizza cheese fortified with soybean oil and carrot extract. *Food Science & Nutrition* 6(2): 356-372 https://doi.org/10.1002/fsn3.563

Nuñez M, Guillén A M, Rodríguez-Marín M A, Marcilla A M, Gaya P & Medina M (1991). Accelerated ripening of ewes' milk Manchego cheese: The effect of neutral proteinases. *Journal of Dairy Science* 74(12): 4108-4118 https://doi.org/10.3168/jds.s0022-0302(91)78605-0

Ozer H B, Uraz G, Beyzi-Yılmaz E & Atasoy A F (2004). The effects of brine concentration and scalding on survival of some pathogens in Urfa cheese: a traditional white-brined Turkish cheese. *International Journal of Food Science & Technology* 39(7): 727-735 https://doi.org/10.1111/j.1365-2621.2004.00837.x

Özer B H, Robinson R K & Grandison A S (2003). Textural and microstructural properties of Urfa cheese (a white-brined Turkish cheese). *International Journal of Dairy Technology* 56(3): 171-176 https://doi.org/10.1046/j.1471-0307.2003.00100.x

Prasad N & Alvarez V B (1999). Effect of salt and chymosin on the physico-chemical properties of Feta cheese during ripening. *Journal of Dairy Science* 82(6): 1061-1067 https://doi.org/10.3168/jds.s0022-0302(99)75327-0

Sahan N, Yasar K, Hayaloglu A A, Karaca O B & Kaya A (2008). Influence of fat replacers on chemical composition, proteolysis, texture profiles, meltability and sensory properties of low-fat Kashar cheese. *Journal of Dairy Research* 75: 1-7 https://doi.org/10.1017/s0022029907002786

Sahingil D, Hayaloglu A A, Simsek O & Ozer B (2014). Changes in volatile composition, proteolysis and textural and sensory properties of white-brined cheese: effects of ripening temperature and adjunct culture. *Dairy Science & Technology* 94(6): 603-623 https://doi.org/10.1007/s13594-014-0185-2

Tomaszewska J, Cais-Socolinska D, Bierzunska P, Kaczynski L K, Walkowiak W & Baranowska H M (2019). Behaviour of water in different types of goats' cheese. *International Dairy Journal* 95: 18-24 https://doi.org/10.1016/j.idairyj.2019.02.015

Tunick M H, Malin E L, Smith P W, Shieh J J, Sullivan B C, Mackey K L & Holsinger V H (1993). Proteolysis and rheology of low fat and full fat Mozzarella cheeses prepared from homogenized milk. *Journal of Dairy Science* 76(12): 3621-3628 https://doi.org/10.3168/jds.s0022-0302(93)77703-6

Turkish Standards (1978). Determination of Fat in Cheese. TS 3046, Turkish Standards Institute, Ankara, Turkey

Turkish Standards (1989). White Cheese Standard. TS 591, Turkish Standards Institute, Ankara, Turkey

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The occurrence of *Paraneognathus wangae* (Fan & Li) (Acari: Caligonellidae) and *Raphignathus gracilis* Rack (Acari: Raphignathidae) of stored products in Turkey

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ABSTRACT
The occurrence of *Paraneognathus wangae* (Fan & Li) (Acari: Caligonellidae) was reported for the first time in Turkey. This predatory species was found associated with stored wheat and collected from south-eastern part of Anatolia. Measurements of female and male are provided along with their taxonomic characteristics and illustrations. This is also the first report on the occurrence of the genus *Paraneognathus* (Fan 2000) in Turkey. *Raphignathus gracilis* Rack (Acari: Raphignathidae) was also collected during this study as predatory species and rendered here.

Keywords: Mites, Caligonellidae, *Paraneognathus*, Mardin, Stored product, Turkey

1. Introduction

The Raphignathoidea Grandjean, 1944 (Acari: Prostigmata) includes 11 families: Barbutiidae, Caligonellidae, Camerobiidae, Cryptognathidae, Dasythyreidae, Eupalopsellidae, Homocaligidae, Mecognathidae, Raphignathidae, Stigmaeidae and Xenocaligonellididae, with 62 genera and 900 species (Fan & Zhang 2005, Zhang et al. 2011). In Turkey, this superfamily is represented by 8 families, 25 genera and 192 species (Akyol 2017; Bingül et al. 2017; Doğan et al. 2019).

Caligonellids are small predators, that feed on small invertebrates. They mostly live on cracks of the tree, in litter and also associated with mosses, stored products, bird nests and soil (Summers & Schlinger1955; Meyer & Ueckermann 1989; Fan 2000). The position of the peritremal plate and shape of stylophore are important for the identification of the genus *Paraneognathous* (Ueckermann & Khanjani 2003). *Paraneognathus wangae* (Fan & Li) (Acari: Caligonellidae) described in firstly in *Sinognathus* Fan & Li (1995) and collected from stored rice and the species later replaced in *Paraneognathus* (Fan 2000).

The genus *Paraneognathus*, can be identified as follows; idiosoma without eyes, with 11 pairs of dorsal setae, stylophore long and elongate, genital and anal opening are close to each other, peritreme confined to the edge of the stylophore.

In this paper, the genus *Paraneognathus* and the species *P. wangae* is reported for the first time from Turkey. *P. wangae* was found associated with stored products in Diyarbakır, Mardin and Muş provinces, which are located in the south-eastern part of Turkey. Besides, *R. gracilis* Rack was also collected in Calligonellidae as predatory species and included here.

2. Material and Methods

The mite samples were collected from stored products at Diyarbakır, Mardin and Muş provinces which are situated in the south-eastern part of Turkey (Figure 1). The samples were taken randomly from different wheat storages with monthly intervals during 2013-2014.

Figure 1- Mite samples collection locations

Wheat samples were taken with a split probe from the bulk, depending on the size of the stack in 4 kg. All the samples were collected by the second author.

The mites were obtained by Berlese funnel and preserved in ethanol 70% for further studies. Mite samples were kept in lactic acid for clarification and mounted in Hoyer's medium with microscopic slides for identification. using a Leica compound microscope.

Measurements were obtained with a Leica ICC50 HD soft imaging system. Gnathosoma was measured from the base to tip of the chelicerae. Palpi; from palp trochanter to tip of tarsi; the length of the idiosoma, from the base of gnathosoma to posterior end. The width of the body, was measured at the level of (*c2*). The length of setae were considered from the base to their tips. The legs were measured from the base of the femur to the tip claw. Both setae and solenidia evaluated in the setal counts. All measurements are in micrometres (µm).

The mite samples were deposited at Ankara University and, Diyarbakır Plant Protection Research Institute (Ministry of Agriculture and Forestry).

3. Results and Discussion

Raphignathoidea
Caligonellidae Grandjean 1944
Paraneognathus (Fan 2000)
Sinognathus (Fan & Li 1995:326).
Paraneognathus wangae (Fan & Li 1995)

Diagnosis: Idiosoma does not include dorsal shield or eyes. Stylophore conical and elongated, peritremes (W) shapes and lying the edge to stylophore. Genital pore has 3 (*g1-g3*) pairs setae and with 4 pairs of aggenital setae.

Paraneognathus wangae (Fan & Li 1995)

Female (Figures 2-9)

Oval shaped, body length 520, width 349, Palpus 223; gnathosoma (excluding palp) 153, width of gnathosoma 132.

Dorsum (Figures 2-9)

Dorsum soft and without shield and there are no eyes, integument striated simply. Prodorsum with four pairs setae; *vi*, *ve*, *sci* and *sce*. Dorsal setae barbed. Idiosomal setae measurements; *vi* 43; *ve* 44; *sci* 66; *sce* 58; *c1* 44; *c2* 70; *d1* 34; *e1* 38; *f1* 43; *h1* 49; *h2* 66; *ps1* 24; length of between setae*: vi–vi* 37; *vi–ve* 40; *vi–sci* 61; *vi-sce* 43*; sci–sci* 90; *sci–sce* 74; *sce–sce* 222; sce-c2 72; *c1–c1* 91; *c1–c2* 120; *c1–d1* 46; *c2–d1* 49; *c1-sce* 105*; d1–d1* 106; *d1–e1* 95; *e1–e1* 104; *e1–f1* 67; *f1–f1* 70; *f1–h1* 80; *h1–h1* 27; *h1–h2* 39; *h2–h2* 95*; h2-ps1* 41*; ps1-ps1* 28.

Gnathosoma (Figures 7, 9)

Chelicerae elongated with a very well developed stylophore plate which is conical at the base and surrounded by elongated peritremes (ω) shape including 9-10 septum of each side, at tip 3-4 septum sharply bent down. Subcapitular (*m*, *n*) and adoral setae (*or1*, *or2*); the length of setae: *m* 50, *n* 47; *or1* 15, *or2 22*.

Palpus: (from the femur to tip of tarsus) 223; palp tarsus has with a very well developed claw. Palpal chaetotaxy: femur and genua with 2 simple setae; tibia with 2 setae + 1 well developed claw + 1 accessory claw; tarsus with 1 tridentate eupathidium + 1 solenidion + 5 setae (Figures. 9).

Venter (Figure 4)

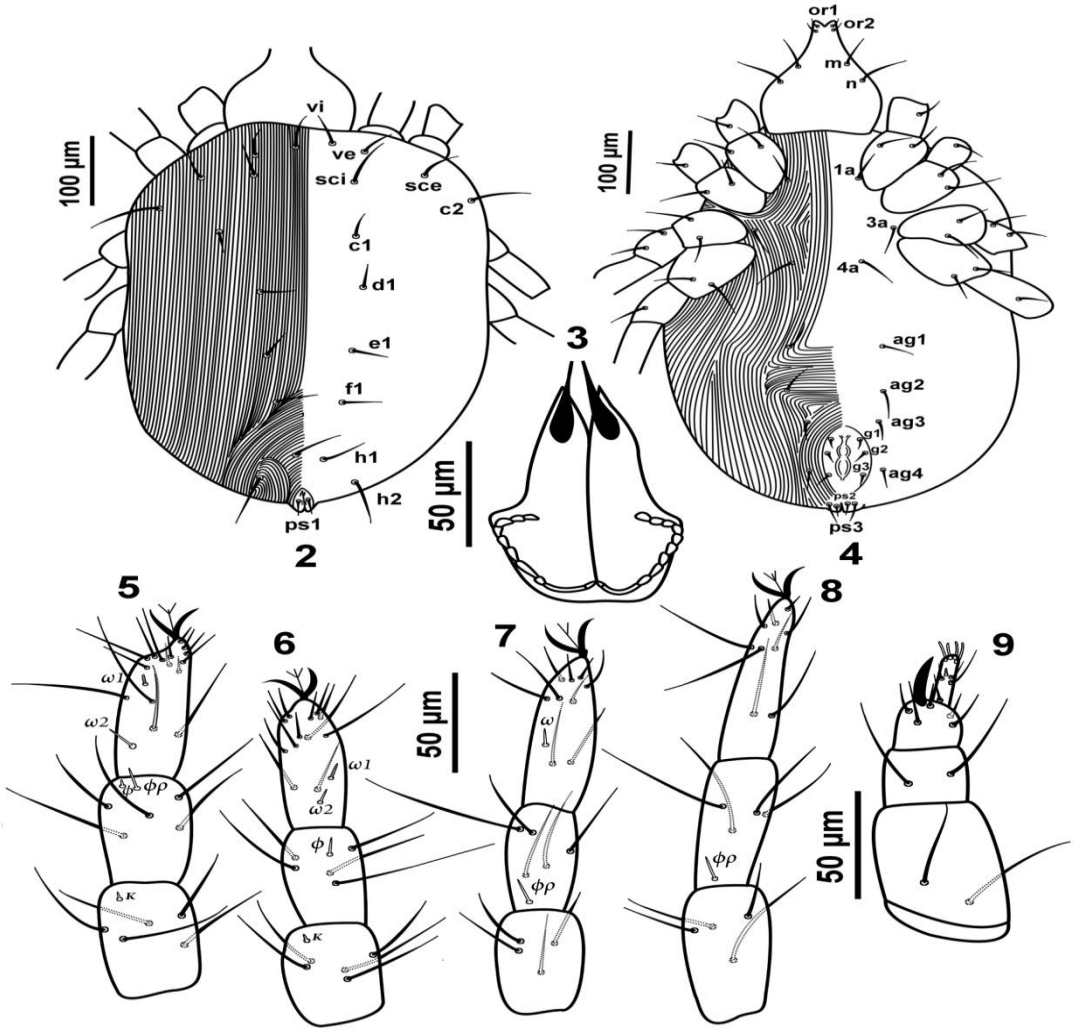
Coxisternal area includes *1a*, *3a* and *4a*; the length of setae: *1a* 53, *3a* 47, *4a* 46; with 4 pairs of agenital setae (*ag1-4*), aggenital shields covered by striae; genital shields with 3 pairs setae (*g1-3*) and pseudanal setae (*ps1-3*); length of setae: *ag1* 37, *ag2* 25, *ag3* 27, ag*4* 23; *g1* 17, g*2* 19; g*3* 17, *ps1* 24, *ps2* 25 and *ps3* 19.

Legs (Figures 5-8)

Lengths; leg I 334; leg II 302; Leg III 284; Leg IV 404.

Legs chaetotaxy (setae and solenidia in brackets) (I–V): coxae 2–2–2–2; trochanters 1–1–2–1; femora 6–6–3-2; genua 5(+1κ)–5(+1κ)–4–4; tibiae 5(+2ω)–5(+1ω)–5(+1ω)–4(+1ω); tarsi:15(+2ω) –12(+2ω)–9 (+1ω)–9.

TaI: ωI 11; ωII 18; Ta II ωI 9; ωII 14; TaIII ω 14.



Figures 2- 9. *Paraneognathus wangae* (Female), 2. Dorsum, 3. Chelicera and Peritreme, 4. Venter, 5. Leg I, 6. Leg II, 7. Leg III, 8. Leg IV, 9. Palp.

Male (Figures. 10-17)

Body length 433, width 220, Palpus 185; gnathosomal length (excluding palp) 137, width of gnathosoma 131.

Dorsum (Figure 10)

Setae measurements; *vi* 37; *ve* 38; *sci* 55; *sce* 46; *c1* 35; *c2* 53; *d1* 29; *e1* 29; *f1* 44; *h1* 22; *h2* 68; *ps1* 24; measurements of distance dorsal setae*: vi–vi* 39; *ve–ve* 108; *vi–ve* 42; *ve–sci* 37; *sci–sci* 91; *sci–sce* 47; *sce–sce 1*70; sce-*c2* 63; *c1–c1* 64; *c1–c2* 97; *c2–c2* 222; *c1–d1* 76; *d1–d1* 73; *d1-e1* 57*; e1–e1* 92; *e1–f1* 25; *f1–f1* 92; *f1–h1* 31; *f1–h2* 79*; h1–h2* 89; *hı-hı* 78*; h1–h2* 75; *h2–h2* 89.

Gnathosoma (Figure 11)
Chelicerae with a conical stylophore and surrounded by elongated peritremes (ω) shapes including 9-10 septum of each side. Subcapitular setae (*m*, *n*) and adoral setae (*or1*, *or2*); subcapitular setae: *m* 33, *n* 43.

Palpus (from the base of femur to tip of tarsus) 185**;** Palp tarsus have with a very well developed claw. Palpal tarsus: 1 tridentate eupathidium + 1 solenidion + 5 simple setae; tibia: 2 setae + 1 developed claw + 1 accessory claw; genua: 2 and femur include 2 tiny setae (Figure 15).

Venter (Figure 12)
Coxisternal area includes (*1a*, *3a* and *4a*); the length of these setae: 34, 29 and 30.

Aggenital setae 4 pairs (*ag1-4*), anogenital shields with 6 pair l setae (*g1-6*) and pseudanal setae (*ps1-3*); lengths: *ag1* 37, *ag2*40, *ag3* 60, ag4 31; *ps1-3* 26; *ps3* 26

Legs (Figures.13-17)

Lengths; leg I 334, leg II 302; Leg III 332; Leg IV 404;

Male leg segments setal formula (setae and solenidia in parantheses) (I─V):

Femur one has sensory setae of tarsi I and II which are subequal in length.

The setal formula of leg segments as (I─V): coxae 2─2─2─2; trochanters 1─1─2─1; femora 6─6─3-2; Femora III with a flange-like apopyhse; genua 5(+1κ)─5(+1κ)─4─4; tibiae 5(+2ω)─5(+1ω)─5(+1ω)─4(+1ω); tarsi:15(+2ω) ─ 12(+2ω) ─ 9 (+1ω) ─ 9. Ge: I.(κ) 7; II (κ) 7; Ti: II (φρ) 11; III (φρ) 11; IV (φρ) 13; Ta I: (ωI) 8; (ωII) 12; Ta II (ωI) 7; (ωII) 13; TaIII (ω) 13.

Material Examined: 7♀♀ and 6♂♂ from whole wheat Mardin (37120950N:40362365E), 06-06-2014, and 1 ♀ from stored wheat, Muş (Kayapınar) (38425333N:41373057E), 13-06-2014.

Distribution: China, Iran, Brazil and Turkey (with this study) (Ardeshir et al. 2014; Fan & Li 1995; Silva et al. 2015).

Remarks. The Turkish specimens resemble the original description of *P. wangae* in all aspects. *P. wangae* is very close to *Paraneognathus summersi*, which has tarsi I with 16 setae and genu I with 7 setae while *P. wangae* has tarsi I 17 and genu I with 6 setae respectively. This species is also very close to *Paraneognathus oblongus*, which has a very strong spine-like sensory setae of tarsus I which is longer than tarsus II while it has subequal length in *P. wangae*. For coxal chaetotaxy, it was mentioned that 2─2─2─2 in Iranian specimen (Fan & Li 1995; Ardeshir et al. 2014). Our specimens have the same coxal chaetotaxy as Iranian specimen have.

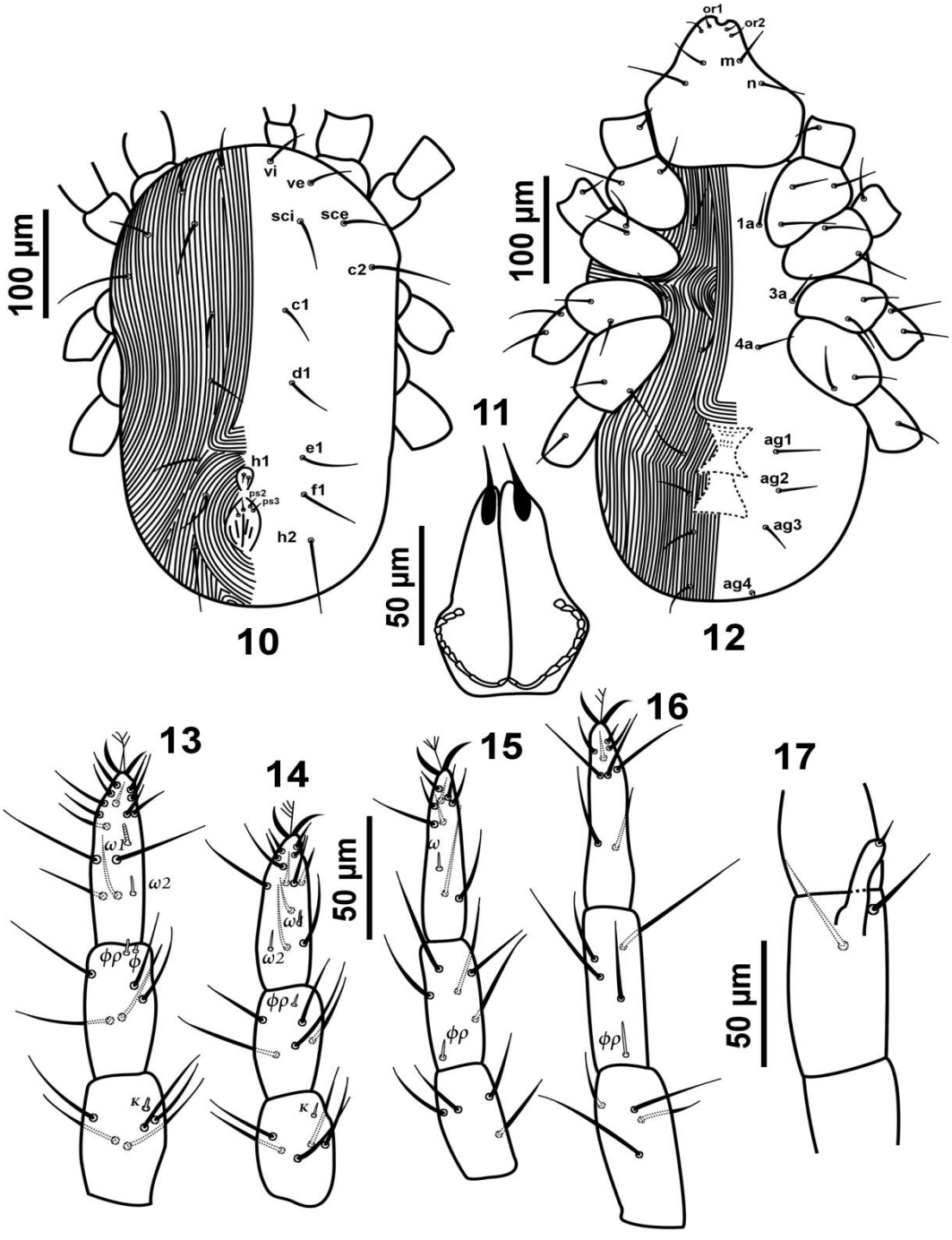
Raphignathidae
Raphignathus Dugés
Raphignathus gracilis (Rack 1962)
Acheles gracilis Rack 1962: 281.
Raphignathus gracilis (Rack), (Koç & Ayyıldız 1996: 210; Doğan 2003: 145. Doğan 2007)

Material Examined. 2 ♀♀, 1♂; 11-06-2014, from litter of mill (animal feed) Muş (38425333N:41373057E); 4♀♀; 06-06-2014, Diyarbakır (Mill) wheat (37565066N:40140041E); 2♀, 13-06-2014 from stored wheat, Diyarbakır (Kayapınar) (37544774N:40051301E), Turkey

Distribution: Algeria, China, Egypt; former U.S.S.R., Germany, Israel, Japan, New Zealand; Turkey; U.S.A., (Meyer & Ueckermann 1989; Li et al. 1992; Hu et al 1995; Fan & Yin 2000; Zaher & Gomaa 1979; Wainstein & Kuznetsov 1978; Rack

1962; Gerson 1968; Ehara 1980; Fan & Zhang 2005; Charlet & McMurtry 1977; Koç & Ayyildiz 1996; Doğan 2007; Yeşilayer & Çobanoğlu 2013).

Remarks; This species was collected previously in Artvin, Denizli, Erzurum, Erzincan and Istanbul. Its habitats are; litter and soil under *Alnus* sp. (Betulaceae), *Castanea* sp. (Fagaceae), *Euonymus fortunei* (Turcz.) (Apocynaceae); *Pistacia* sp. (Anacardiaceae), *Quercus* sp. (Quercaceae), *Rhododendron* sp. (Ericaceae), *Tamarix* sp. (Tamaricaceae), *Ulmus* sp. (Ulmaceae) and *Vitis* sp. (Vitaceae); grassy soil from olive grove; moss on soil and stone (Doğan 2007; Yeşilayer & Çobanoğlu 2013).



Figures 10-17. *Paraneognathus wangae* (Male), 10. Dorsum, 11. Chelicera and Peritreme, 12. Venter, 13. Leg I, 14. Leg II, 15. Leg III, 16. Leg IV, 17. Femur III

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References

Akyol M (2017). Fauna of Coastal Aegean Rafignatoid Mites (Acari: Prostigmata: Raphignathoidea). *Plant Protection Bulletin* 57(2): 205-229 https://doi.org/10.16955/bitkorb.305402

Ardeshir F, Bagherş M & Saeej S P (2014). Redescription of male and female of *Paraneognathus wangae* (Fan & Li) (Acari: Caligonellidae) with a key to all known species of the genus *Paraneognathus. Persian Journal Acarology* 3(2): 145-151 https://doi.org/10.22073/pja.v3i2.17132

Bingül M, Doğan S & Dilkaraoğlu S (2017). Contributions to the knowledge of the mite genus *Stigmaeus* Koch (Acari: Stigmaeidae) of Turkey. *European Journal of Taxonomy* 307: 1-16 https://doi.org/10.5852/ejt.2017.307

Charlet I D & McMurtry J A (1977). Systematics and bionomics of predaceous and phytophagous mites associated with pine foliage in California. *Hilgardia* 45: 173-210 DOI:10.3733/hilg.v45n07p173

Doğan S (2003) Descriptions of three new species and two new records of *Raphignathus* Dugés (Acari: Raphignathidae) from Turkey. *Archives des Sciences* 56: 143-153

Doğan S (2007). Checklist of raphignathoid mites (Acari: Raphignathoidea) of Turkey. *Zootaxa* 1454: 1-26 https://doi.org/10.11646/zootaxa.1454.1.1

Doğan S, Doğan S & Erman O (2019). Mites of *Raphignathus* Dugés (Acari: Raphignathidae) from Harşit Valley (Turkey), with taxonomic notes on other members of the genus. *Plant Protection Bulletin* 59 (1): 25-36 https://doi.org/10.16955/bitkorb.419431

Ehara S (1980). Illustrations of the mites and ticks of Japan. *Zenkoku Noson Kyoiku Kyokai* pp. 562

Fan Q H (2000). A phylogenetic analysis of the family Caligonellidae (Acari: Prostigmata) with description of two new species. *Acta Entomologica Snica* 43: 421-428

Fan Q H & Li L S (1995). A new genus and a new species of Caligonellidae associated with food from China. *Acta Zootaxonomica Sinica* 20(30): 323-327

Fan Q H & Yin X M (2000). The genus *Raphignathus* (Acari: Raphignathidae) from China. *Systematic and Applied Acarology* 5: 83-98

Fan Q H & Zhang Z Q (2005). Raphignathoidea (Acari: Prostigmata). *Fauna of New Zealand* 52: 1-400

Gerson U (1968). Some raphignathoid mites from Israel. *Journal of Natural History* 2: 429-437 https://doi.org/10.1080/00222936800770411

Grandjean F (1944). Observations sur les acariens de la famille des Stigmaeidae. *Archives des Sciences Physiques et Naturelles* 26: 103-131

Hu C Y, Jing Z Q & Liang L R (1995). Two new species and one new record of the genus *Raphignathus* Duges (Acari: Raphignathidae). *Journal of Suzhou Railway Teachers College* 12(3): 21-26

Koç K & Ayyıldız N (1996). Türkiye faunası için yeni iki *Raphignathus* Dugès (Acari, Prostigmata, Raphignathidae) türü. *Turkish Journal of Zoology* 20: 209-214

Meyer M K P & Ueckermann E A (1989). African Raphignathoidea. *Entomology Memoir Department of Agriculture and Water Supply*, Republic of South Africa 74: 1-58 https://doi.org/10.7931/J2/FNZ.52

Rack G (1962). Milben aus Taubennestern mit Beschreibung einer neren Art, *Acheles gracilis* (Acarina, Raphignathidae). *Zoologischer Anzeiger* 168(7-10): 275-292

Ueckermann E A & Khanjani M (2003). Iranian Caligonellidae (Acari: Prostigmata), with description of two new species and re description of *Molothrognathus fulgidus* Summers and Schlinger, with a key to genera and species. *Acarologia* 43: 291-298

Silva D E, da Silva I C O, Horn T B & Ferla N J (2015). First record of *Paraneognathus wangae* (Acari: Caligonellidae) in Brazil. *Symposio Brasileiro de Acarologia, Acarologia integrative* ISBN 978 85 66836-08-08

Summers F M & Schlinger E I (1955). Mites of family Caligonellidae (Acarina). *Hilgardia* 23: 539-561 DOI:10.3733/hilg.v23n12p539

Wainstein B A & Kuznetsov N N (1978). Family Raphignathidae. In: Gilyarov, M. S. (ed.) Identification Key of Soil Inhabiting Mites. Trombidiformes. Nauka,Moscow pp. 149-150

Yeşilayer A & Çobanoğlu S (2013). Determination of raphignathoid mites (Acari: Prostigmata: Raphignathoidea) ornamental plants of Istanbul (Turkey). *Turkish Journal of Entomology* 37: 93-103

Zaher M A & Gomaa E A (1979). Three new species of the genus Raphignathus in Egypt (Prostigmata: Raphignathidae). *Acarologia* 21(2): 187-203

Zhang Z-Q, Fan Q-H, Pesic V, Smit H, Bochkov AV, Khaustov AA, Baker A, Wohltmann A, Wen T-H, Amrine JW, Beron P, Lin J, Gabrys G & Husband R (2011). Order Trombidiformes Reuter,1909. In: Zhang Z.-Q. (ed.) Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. *Zootaxa* 3148: 129-138 https://doi.org/10.11646/zootaxa.3148.1.24

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Genetic Diversity and Phylogenetic Relationships of Turkish Local Popcorn (*Zea mays* everta) Populations analyzed by Simple Sequence Repeats (SSRs) Markers

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ABSTRACT
Maize (*Zea mays* everta) is preferred as a good dietary in Turkey and it is important to know its genetic diversity to improve the yield. Genetic markers are very important in determining genetic diversity in popcorn populations. The aim of this study was to evaluate the genetic diversity of landraces popcorn populations by simple sequence repeats (SSR) markers. A hundred seventy five accessions of popcorn from thirty five populations grown in Turkey were analyzed using twenty SSR markers.

As a result of molecular analysis, 65 of 66 alleles obtained were showed polymorphisms and the polymorphism rate was 98.5%. The average number of alleles for each SSR loci was 3.3, and this the number of alleles varied from 1 to 5. The average the polymorphism information content (PIC) value was calculated to be 0.57 for SSR locus ranging from 0.00 to 0.89. The number and percentage of polymorphic loci of the genotypes were determined to vary between 29/47% and 43.94/71.21 % and the mean values were calculated as 39.114 and 59.265 % respectively. The value of genetic change in the phylogenetic tree obtained from landraces popcorn populations was determined as 0.05, and the genetic difference among genotypes varied from 14.7 to 97.1%. Among the markers used in the study, it was observed that code 'phi064' was the most effective marker for determining genetic diversity in popcorn and the highest allele frequency also on this marker was obtained.

Keywords: Genetic diversity, Landrace, Molecular characterization, Popcorn, SSR

1. Introduction

Maize is the major cereal growing all over the world and it is a considerable cereal ranked as the third in world cereal production after wheat and rice (Adjanohoun et al. 2011). One of the commercially produced plants in the corn variety is popcorn. Popcorn (*Zea mays* everta) can be easily distinguished by plant and seed characteristics among other maize varieties.

Popcorn is preferred in terms of its rich nutritional content, vitamins and minerals. Also, corn is a good dietary product with stomach acidic absorption properties, low calorie and whole grain. The multi-purpose use of the corn has led to intensive research on the plant. Through the breeding studies in popcorn, different breeding methods are applied to ensure suitability for the purpose of efficiency such as adaptation, aquaculture and quality criteria. In order to obtain the necessary variation in breeding activities, registered varieties, local varieties and wild relatives should be screened and the appropriate genes should be transferred to the cultivars by improved techniques. The success in plant breeding is first and foremost provided by an efficient, accurate and rapid selection (Frankel 1972).

Maize spread from Central America to the other regions of the world. In course of time, it adapted to the extreme climatic conditions and thus, now it is characterized by a high degree of genetic variability. Morphological characterization is highly affected by the environment conditions therefore it indicates variability (Aci et al. 2013). On the other hand, molecular characterization is not affected by the environmental conditions and it provides valuable genetic information (Gauthier et al. 2002). In order to reveal the crucial genetic information, molecular markers such as microsatellites (SSR) are used (Yao et al. 2008; Liu et al. 2009; Eschholz et al. 2010) and have been very helpful for determining the diversity.

The aim of this study was to analyze the genetic diversity among different popcorn populations via SSRs markers on the samples collected from the different regions in Turkey.

2. Material and Methods

2.1. Plant material

In this study, one hundred and seventy five accessions of popcorn were used from total 35 population that grown in various cities of Turkey including 34 landraces populations and one standard variety (Table 1). This collection was provided by Department of Plant Gene Resources in Ege Agricultural Research and Antalya West Mediterranean Agricultural Research Institute (Nermin Cin 98 as Standard variety).

Table 1- Genotype No, Registration No, Province, Region, Altitude and Material Color Information of the material used in the research

Genotype No	*Registration No*	*Province*	*Region*	*Altitude*	*Material Color*	*Genotype No*	*Registration No*	*Province*	*Region*	*Altitude*	*Material Color*
1	TR79913*	Canakkale	Biga	40	Yellow	19	TR78053*	Kutahya	Simav	950	Yellow
2	TR79947*	Balikesir	Gonen	120	Red	20	TR78181*	Usak	Sivas	970	Yellow
3	TR79947*	Balikesir	Gonen	120	Yellow	21	TR76375*	Diyarbakir	Cungus	939	Yellow
4	TR79947*	Balikesir	Gonen	120	Pied	22	TR73761*	Eskisehir	Gunyuzu	916	Yellow
5	TR79987*	Balikesir	Bigadic	437	Dark Red	23	TR73698*	Eskisehir	Beylikova	789	Yellow
6	TR79987*	Balikesir	Bigadic	437	Orange	24	TR74311*	Kayseri	Hacilar	1479	Yellow
7	TR73836*	Eskisehir	Gunyuzu	991	Yellow	25	TR85132*	Tokat	Erbaa	---	Yellow
8	TR73836*	Eskisehir	Gunyuzu	991	Orange	26	TR37977*	Tokat	Merkez	560	Light Yellow
9	TR79988*	Balikesir	Bigadic	437	White	27	Ordu - Dogulu	Ordu	Dogulu	---	Red
10	TR79988*	Balikesir	Bigadic	437	Yellow	28	Konya Pop	Konya	---	---	Red
11	TR73746*	Eskisehir	Gunyuzu	916	Orange	29	Nermin Cin**	---	---	---	Yellow
12	TR73746*	Eskisehir	Gunyuzu	916	Light Orange	30	Tokat Erbaa	Tokat	Erbaa	---	Yellow
13	TR39601*	Artvin	Ardanuc	1300	Red	31	Samsun Bafra	Samsun	Bafra	---	Orange
14	TR79932*	Canakkale	Can	103	White	32	Ordu-Akpinar	Ordu	Akpinar	---	Light Yellow
15	TR78115*	Afyon	İncehisar	1140	Yellow	33	Ordu-Kovanli	Ordu	Kovanli	---	Yellow
16	TR76741*	Tekirdag	Sarkoy	120	Dark Red	34	TR54215*	Mugla	Fethiye	1130	Yellow
17	TR38027*	Amasya	Sukuova	400	White/ Yellow	35	TR54215*	Mugla	Fethiye	1130	White
18	TR74236*	Kastamonu	Taskopru	896	Orange						

*, Genotype codes obtained from the Plant Gene Resources Department of Ege Agricultural Research Institute; **, Standard variety. Others populations was collected from various parts of Turkey

2.2. DNA extraction

DNA isolation and SSR analysis were carried out in Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Field Crops Laboratory and University - Industry - Public Cooperation Development, Application and Research Center (USKIM).

Maize seeds were grown in the greenhouse. Five plants (at 4-5 leave stage) were taken randomly from each population and stored at -80 ˚C. Single-plant samples were ground to powder in liquid nitrogen using a mortar and pestle. A total genomic DNA was extracted following a modified procedure by Doyle & Doyle (1987).

2.3. SSR analysis

Twenty SSR primer pairs of maize were selected from two of each chromosome, however it was used in previous studies and reported to be effective (Table 2).

Table 2- Name of the SSR markers, Chromosome locations, DNA Sequences and Base Numbers used in the study

Primer Name	*Chromosome*		*DNA Sequence (5'→3')*	*Primer Name*	*Chromosome*		*DNA Sequence (5'→3')*
Umc1186	6.02	F	TCAAGAACATAATAGGAGGCCCAC	Phi015	8.00	F	GCAACGTACCGTACCTTTCCGA
		R	AGCCAGCTTGATCTTTAGCATTTG			R	ACGCTGCATTCAATTACCGGGAAG
Umc1622	2.00	F	CGCTACAAATCCTACTGGTGCTTT	Phi021	4.00	F	TTCCATTCTCGTGTTCTTGGAGTGGTCCA
		R	CCTCGGATTTTCCAAAACATTTCT			R	CTTGATCACCTTTCCTGCTGTCGCCA
Umc1550	4.03	F	CGGGGTAATTGGGTACATAACCTC	Phi022	9.00	F	TGCGCACCAGCGACTGACC
		R	GTGCCTCCAACGCCTAGTTTTT			R	GCGGGCGACGCTTCCAAAC
Phi095	1.03	F	CCGATCGGCTTTATCACTGTTTAGC	Phi027	9.03	F	CACAGCACGTTGCGGATTTCTCT
		R	ATGCACCATTCTAGCACTATAGCAACACT			R	GCGTACGTACGACGAAGACAC
Umc2101	3.00	F	CCCGGCTAGAGCTATAAAGCAAGT	Phi034	7.00	F	TAGCGACAGGATGGCCTCTTCT
		R	CTAGCTAGTTTGGTGCGTGGTGAT			R	GGGGAGCACGCCTTCGTTCT
Umc1255	4.11	F	GGACTACATCACGCCGGAGAT	Phi064	1.00	F	CCGAATTGAAATAGCTGCGAGAACCT
		R	TTTGGGAGAACAATCGGTTCTGTA			R	ACAATGAACGGTGGTTATCAACACGC
Phi017	9.02	F	CGTTGGCGACCAGGGTGCGTTGGAT	Phi084	10.0	F	AGAAGGAATCCGATCCATCCAAGC
		R	TGCAACAGCCATTCGATCATCAAAC			R	CACCCGTACTTGAGGAAAACCC
Phi057	7.01	F	CTCATCAGTGCCGTCGTCCAT	Phi127	2.00	F	ATATGCATTGCCTGGAACTGGAAGGA
		R	CAGTCGCAAGAAACCGTTGCC			R	AATTCAAACACGCCTCCCGAGTGT
Umc1164	4.01	F	AAATAAACGCTCCAAAGAAAGCAA	Umc2050	3.00	F	CTCCTGCTGTGATTCTAGGACGA
		R	GCACGTGTGTGTGTGTTGTTTTTA			R	CTGGATCTCGGCATGGTCTT
Umc1173	4.09	F	ATCCGCCAAAAAGGGGAAAA	Umc2292	5.00	F	AGCAGAAGAGGACAAACCAGATTC
		R	TAGAAGTAGCACACGCGCCG			R	ACTTCCGGCATGTCTTGTGTTT

The total volume of PCR mixture was 20 µL containing 2 µL ddH_2O, 3.5 µL 10X PCR buffer (Mg^{+2} added), 1.2 µL dNTP (5 mM), 4 µL F primer (20 µM), 4 µL R primer (20 µM), 5 µL Genomic DNA (100 ng), 0.3 µL DNA Taq polymerase (5 U μL^{-1}, Fermantes).

The PCR reaction was performed in a thermal cycler (Eppendorf Mastercycler Gradient) using an initial 94 ˚C denaturing step for 5 min followed by 34 cycles of [denaturation at 94 ˚C for 1 min, annealing for 1 min at the primer's annealing temperature, extension at 72 ˚C for 1 min] and a final extension at 72 ˚C for 5 min.

2.4. Data analysis

The presence (1) or absence (0) of PCR amplicons were coded. Then the data base was registered in an MS Excel spreadsheet in order to generate the analysis matrix. Genetic diversity parameters such as Polymorphism Information Content (PIC) as previously described by Laborda et al. (2005); polymorphism rate (P), number of alleles (Na), expected heterozygosity (He) and Shannon's phenetic index (H) were estimated according to the method used by Nei 1972. Cluster analysis by Un-weighted Pair Group Method using Arithmetic Averages (UPGMA) were estimated according to the method used by Rohlf (1992) and genetic variation patterns among the maize genotypes were identified using PopGen32 (Population Genetic Analysis System, Version 32V) and MEGA (Molecular Evolutionary Genetics Analysis) 6 databases software, respectively.

3. Results and Discussion

3.1. SSR polymorphism

The SSR markers selected to analyze the genetic diversity of the maize accessions displayed different characteristic profiles. Thus, different numbers of polymorphic bands, percentage of polymorphism, Polymorphism Information Content (PIC), and expected heterozygosis have been generated using the SSR markers.

SSR markers used to molecular characterization, allele size, allele number and PIC values included 175 popcorn accessions occurring from 35 landraces popcorn population are reported in Table 3.

Table 3- Allele size, number and PIC value information of SSR markers used in molecular characterization of landraces popcorn populations

Primer	*Allel Size*	*Number of Alleles*	*PIC*	*Primer*	*Allel Size*	*Number of Alleles*	*PIC*
phi015	80-120	4	0.88	phi127	110-129	3	0.40
phi017	100-110	4	0.76	phi064	73-110	5	0.89
phi021	90-120	4	0.87	phi057	160-170	3	0.57
phi034	110-150	4	0.70	umc1550	140-280	3	0.43
umc2292	130-170	5	0.89	phi095	140-180	3	0.56
umc2101	150-180	3	0.50	phi022	150-180	4	0.62
umc2050	120-160	4	0.82	phi027	150-180	4	0.87
umc1622	40-90	2	0.10	umc1164	140-160	2	0.09
umc1186	220-240	2	0.12	umc1173	150-170	3	0.64
				umc1255	130-280	3	0.65

Nineteen of 20 SSR markers were noted to be polymorphic while one marker (phi084) was found to be monomorphic. Total numbers of alleles were detected as 66 and 65 of them were polymorphic. The polymorphism rate was calculated to be as high as 98.5%.

Vivodík et al. (2017) characterizing 40 maize populations with 10 SSR markers determined that they had 65 alleles in total, and that the number of these alleles changed between 4-8 (mean 6.5 alleles) and the PIC value varied from 0.734 to 0.848 (mean PIC 0.810). Riberio et al. (2017) analyzed 48 single hybrid maize varieties commercially used in Brazil with 20 SSR markers and determined the average allele number as 9.8 and the average PIC value as 0.84. Atanda & Olaove (2017) analyzed 24 inbred maize lines with 20 SSR markers and identified total of 101 alleles and found that the average allele number was 5.5 and the average PIC was 0.46.

Allele numbers observed for each locus ranged from 1 (phi084) to 5 (phi064 and umc2292) with an average of 3.3 alleles per locus. The PIC value ranged from 0.00 (phi084) to 0.89 (umc2292 and phi064) with average value of 0.57. Number and percentage of polymorphic loci of populations were observed to range from 29 and 43.94% (population 21) to 47 and 71.21% (population 13) with an average of 39.11 and 59.27%, respectively (Table 4).

Table 4 - Number and percentage of polymorphic loci of maize populations by Nei 1973 method

Population No	*Polymorphic Locus Number*	*Polymorphic Locus Percentage (% P)*	*Population No*	*Polymorphic Locus Number*	*Polymorphic Locus Percentage (% P)*	*Population No*	*Polymorphic Locus Number*	*Polymorphic Locus Percentage (% P)*
1	40	60.61	13	47	71.21	25	39	59.09
2	39	59.09	14	41	62.12	26	35	53.03
3	38	57.58	15	38	57.58	27	41	62.12
4	38	57.58	16	43	65.15	28	41	62.12
5	39	59.09	17	38	57.58	29	41	62.12
6	39	59.09	18	42	63.64	30	40	60.61
7	38	57.58	19	44	66.67	31	36	54.55
8	41	62.12	20	33	50.00	32	39	59.09
9	37	56.06	21	29	43.94	33	39	59.09
10	41	62.12	22	40	60.61	34	43	65.15
11	37	56.06	23	36	54.55	35	44	66.67
12	40	60.61	24	33	50.00	Average	39.114	59.265

Molin et al. (2013) analyzed 48 local popcorn populations in Rio Grande do Sul and Parana in Brazil with 47 SSR markers and identified the polymorphic index as 78.3%. Sharma et al. (2010) analyzed 48 local maize varieties in India with 42 SSR primers and as a result recorded 60% polymorphism rate.

3.2. Genetic Differentiation

The parameters n_a, n_e, h, I and gen frequency revealed the genetic structure of the accessions (Table 5). Among polymorphic loci, the mean observed number of alleles ($n_{a)}$ varied from 1 (phi084**)** to 2 (all other alleles) and mean n_a was 1.99; the mean effective number of alleles (n_e) varied between 1.00 (phi084) and 1.99 (phi015-3) with an average of 1.65 for all accessions. The total gen diversity (h) made according to Nei (1973) method was ranging from 0.0 to 0.5 with the average value of 0.36. The Shannon's information index (I) varied between 0.00 and 0.69 with an average of 0.53 for all accessions. Finally, the gene frequency (*f*) values; f_0 value ranged 0.00 to 0.98 with an average of 0.58 and the f_1 value ranged 0.0171 to 1.0000 with an average of 0.42.

Polymorphism limit of alleles is accepted as 95%, thus alleles are considered to be monomorphic if the frequency is 95% or more, however it is polymorphic when the allele frequency is below 95%.

Accordingly, the allele frequencies in phi017-4 (f_0=0.9714), phi034-1 (f_0=0.9829), umc2292-1 (f_0=0.9714), umc2292-2 (f_0=0.9714) and umc2050-4 (f_0=0.9829) were 95%, therefore, they were regarded as monomorphic. Polymorphic alleles are evaluated in their own, the allele frequency for 0 allele ranged from f_0=0.0000 (phi084) to f_0=0.9314 (phi064-5) and the allele frequency for 1 allele ranged from f_1=0.0686 (phi064-5) to f_1=1.0000 (phi084).

The investigated parameters, total genetic diversity (H_t), genetic diversity within the population (H_s), inter-population genetic differentiation (G_{st}) and gene flow (N_m) revealed the genetic structure of the accessions (Table 6).

Table 5- The mean observed number of alleles, mean effective number of alleles, genetic diversity according to Nei (1973) and Shannon's information index values of genetic variation of all loci

Loci	*Number of Accession*	n_a*	n_e*	*h**	*I**	*Gene Frequency*		*Loci*	*Number of Accession*	n_a*	n_e*	*h**	*I**	*Gene Frequency*	
						Allel 0	*Allel 1*							*Allel 0*	*Allel 1*
phi015-1	175	20.00	12.127	0.175	0.319	0.903	0.097	phi127-3	175	20.00	19.144	0.478	0.671	0.394	0.606
phi015-2	175	20.00	19.994	0.499	0.693	0.509	0.491	phi064-1	175	20.00	13.243	0.245	0.410	0.857	0.143
phi015-3	175	20.00	19.999	0.500	0.693	0.497	0.503	phi064-2	175	20.00	19.767	0.494	0.687	0.446	0.554
phi015-4	175	20.00	13.968	0.284	0.458	0.829	0.171	phi064-3	175	20.00	18.760	0.467	0.660	0.629	0.371
phi017-1	175	20.00	14.115	0.291	0.467	0.823	0.177	phi064-4	175	20.00	19.716	0.493	0.686	0.440	0.560
phi017-2	175	20.00	19.660	0.491	0.685	0.434	0.566	phi064-5	175	20.00	11.464	0.128	0.250	0.931	0.069
phi017-3	175	20.00	19.054	0.475	0.668	0.389	0.611	phi057-1	175	20.00	18.654	0.464	0.657	0.366	0.634
phi017-4	175	20.00	10.588	0.055	0.130	0.971	0.029	phi057-2	175	20.00	19.890	0.497	0.690	0.463	0.537
phi021-1	175	20.00	19.600	0.489	0.683	0.571	0.429	phi057-3	175	20.00	16.897	0.408	0.598	0.714	0.286
phi021-2	175	20.00	19.890	0.497	0.690	0.463	0.537	umc1550-1	175	20.00	18.861	0.470	0.663	0.623	0.377
phi021-3	175	20.00	18.434	0.457	0.650	0.646	0.354	umc1550-2	175	20.00	15.151	0.340	0.523	0.217	0.783
phi021-4	175	20.00	14.410	0.306	0.484	0.811	0.189	umc1550-3	175	20.00	17.959	0.443	0.635	0.669	0.331
phi034-1	175	20.00	10.349	0.033	0.087	0.983	0.017	phi095-1	175	20.00	15.299	0.346	0.531	0.777	0.223
phi034-2	175	20.00	19.054	0.475	0.668	0.611	0.389	phi095-2	175	20.00	16.037	0.376	0.564	0.251	0.749
phi034-3	175	20.00	16.614	0.398	0.588	0.274	0.726	phi095-3	175	20.00	15.743	0.365	0.551	0.760	0.240
phi034-4	175	20.00	11.595	0.137	0.265	0.926	0.074	phi022-1	175	20.00	15.743	0.365	0.551	0.240	0.760
umc2292-1	175	20.00	10.588	0.055	0.130	0.971	0.029	phi022-2	175	20.00	12.818	0.220	0.378	0.874	0.126
umc2292-2	175	20.00	10.588	0.055	0.130	0.971	0.029	phi022-3	175	20.00	13.100	0.237	0.400	0.863	0.137
umc2292-3	175	20.00	19.890	0.497	0.690	0.463	0.537	phi022-4	175	20.00	19.854	0.496	0.690	0.543	0.457
umc2292-4	175	20.00	13.100	0.236	0.400	0.863	0.137	phi027-1	175	20.00	18.202	0.451	0.643	0.657	0.343
umc2292-5	175	20.00	19.465	0.486	0.679	0.417	0.583	phi027-2	175	20.00	19.854	0.496	0.690	0.457	0.543
umc2101-1	175	20.00	19.231	0.480	0.673	0.400	0.600	phi027-3	175	20.00	19.231	0.480	0.673	0.600	0.400
umc2101-2	175	20.00	18.202	0.450	0.643	0.343	0.657	phi027-4	175	20.00	15.596	0.359	0.544	0.766	0.234
umc2101-3	175	20.00	11.595	0.137	0.265	0.926	0.074	umc1164-1	175	20.00	13.100	0.237	0.400	0.137	0.863
umc2050-1	175	20.00	19.968	0.499	0.692	0.520	0.480	umc1164-2	175	20.00	19.392	0.484	0.677	0.589	0.411
umc2050-2	175	20.00	19.716	0.492	0.686	0.440	0.560	umc1173-1	175	20.00	19.767	0.494	0.687	0.446	0.554
umc2050-3	175	20.00	19.392	0.484	0.677	0.589	0.411	umc1173-2	175	20.00	19.535	0.488	0.681	0.577	0.423
umc2050-4	175	20.00	10.349	0.033	0.087	0.983	0.017	umc1173-3	175	20.00	19.968	0.499	0.692	0.520	0.480
umc1622-1	175	20.00	13.968	0.284	0.458	0.829	0.171	umc1255-1	175	20.00	18.202	0.451	0.643	0.657	0.343
umc1622-2	175	20.00	11.464	0.127	0.250	0.069	0.931	umc1255-2	175	20.00	19.231	0.480	0.673	0.400	0.600
umc1186-1	175	20.00	11.595	0.137	0.265	0.074	0.926	umc1255-3	175	20.00	19.767	0.494	0.687	0.554	0.446
umc1186-2	175	20.00	13.676	0.268	0.440	0.840	0.160	Average	175	19.848	16.435	0.361	0.530	0.589	0.420
phi127-1	175	20.00	17.833	0.439	0.631	0.326	0.674	Standard Error	175	0.340	0.1543	0.193			
phi127-2	175	20.00	14.706	0.320	0.500	0.200	0.800								

* na = The mean observed number of alleles; * ne = The mean effective number of alleles (Kimura & Crow 1964); * h = Genetic diversity according to Nei (1973); * I = Shannon's information index (Lewontin 1972).

While H_t varied between 0.0000 (phi084) and 0.5000 (phi015-3) with an average of 0.3606; H_s ranged from 0.0000 (phi084) to 0.4206 (umc1255) and the average was 0.2391. G_{st} values were identified to vary between 0.1290 (phi095-1) and 0.6706 (phi017-4) with an average of 0.3369. N_m values of genotypes ranged from 0.2456 to 3.3772 with a whole average value of 0.9840; the highest values on phi095-1 allele and the lowest on phi017-4 allele were observed. In general, it was observed that the phi095 coded marker was the most polymorphic marker in determining the diversity of genotypes for gene flow than other markers. On the other hand, phi084 coded marker revealed that it was not an effective marker for the determination of gene flow under this study.

In our study the genetic variation determined was lower than other that Vivodik et al. (2017) found for the 40 maize genotypes. Similarly, Zhang et al. (2016) examined 290 inbred maize lines by 201 SSR markers, and the diversity they determined was 0.70. The study accomplished by Tahir & Maeruf (2016) on 9 corn genotypes with 18 SSR markers were reported that genetic diversity was between 0.20 and 0.82.

Table 6- Total genetic diversity, intra-population genetic diversity, inter-population genetic differentiation and gene flow data in determined SSR loci by Nei 1978 method

Loci	H_t	H_s	G_{st}	N_m*	*Loci*	H_t	H_s	G_{st}	N_m*	*Loci*	H_t	H_s	G_{st}	N_m*
phi015-1	0.175	0.109	0.374	0.835	umc2101-2	0.450	0.338	0.249	1.505	umc1550-2	0.3400	0.2057	0.3949	0.7661
phi015-2	0.499	0.260	0.478	0.544	umc2101-3	0.137	0.077	0.434	0.649	umc1550-3	0.4432	0.2971	0.3295	10.174
phi015-3	0.500	0.306	0.387	0.790	umc2050-1	0.499	0.306	0.386	0.793	phi095-1	0.3464	0.3017	0.1290	33.772
phi015-4	0.284	0.128	0.549	0.410	umc2050-2	0.492	0.315	0.359	0.889	phi095-2	0.3764	0.3246	0.1378	31.297
phi017-1	0.291	0.187	0.357	0.900	umc2050-3	0.484	0.347	0.282	1.269	phi095-3	0.3648	0.3154	0.1353	31.944
phi017-2	0.491	0.324	0.339	0.973	umc2050-4	0.033	0.022	0.321	1.054	phi022-1	0.3648	0.1463	0.5990	0.3347
phi017-3	0.475	0.224	0.528	0.445	umc1622-1	0.284	0.096	0.662	0.255	phi022-2	0.2198	0.1143	0.4801	0.5415
phi017-4	0.055	0.018	0.670	0.245	umc1622-2	0.127	0.054	0.570	0.376	phi022-3	0.2367	0.1646	0.3046	11.413
phi021-1	0.489	0.352	0.281	1.277	umc1186-1	0.137	0.054	0.601	0.331	phi022-4	0.4963	0.3337	0.3276	10.261
phi021-2	0.497	0.342	0.310	1.110	umc1186-2	0.268	0.123	0.540	0.424	phi027-1	0.4506	0.2331	0.4826	0.5360
phi021-3	0.457	0.374	0.180	2.267	phi127-1	0.439	0.274	0.375	0.831	phi027-2	0.4963	0.2834	0.4289	0.6656
phi021-4	0.306	0.246	0.193	2.086	phi127-2	0.320	0.214	0.328	1.021	phi027-3	0.4800	0.3474	0.2762	13.103
phi034-1	0.033	0.013	0.593	0.343	phi127-3	0.477	0.269	0.435	0.648	phi027-4	0.3588	0.2469	0.3120	11.027
phi034-2	0.475	0.306	0.355	0.906	phi064-1	0.244	0.137	0.440	0.636	umc1164-1	0.2367	0.1829	0.2274	16.990
phi034-3	0.398	0.242	0.391	0.777	phi064-2	0.494	0.310	0.370	0.848	umc1164-2	0.4843	0.3474	0.2826	12.691
phi034-4	0.137	0.096	0.302	1.155	phi064-3	0.466	0.297	0.363	0.875	umc1173-1	0.4941	0.3977	0.1951	20.630
umc2292-1	0.055	0.036	0.341	0.965	phi064-4	0.492	0.283	0.424	0.676	umc1173-2	0.4881	0.3200	0.3444	0.9518
umc2292-2	0.055	0.027	0.505	0.488	phi064-5	0.127	0.086	0.320	1.062	umc1173-3	0.4992	0.3200	0.3590	0.8929
umc2292-3	0.497	0.306	0.384	0.802	phi057-1	0.463	0.352	0.241	1.572	umc1255-1	0.4506	0.3520	0.2188	17.848
umc2292-4	0.236	0.146	0.381	0.809	phi057-2	0.497	0.402	0.191	2.118	umc1255-2	0.4800	0.3886	0.1905	21.250
umc2292-5	0.486	0.292	0.398	0.755	phi057-3	0.408	0.338	0.171	2.420	umc1255-3	0.4941	0.4206	0.1488	28.597
umc2101-1	0.480	0.338	0.295	1.193	umc1550-1	0.469	0.352	0.250	1.493	Average	0.3606	0.2391	0.3369	0.9840
										Std. Error	0.0238	0.0137		

H_t, Total genetic diversity; H_s, Genetic diversity within the population; G_{st}, Inter-population genetic differentiation; * N_m = Gene flow. E.g.; N_m = 0.5 (1 - G_{st}) / G_{st}; (McDermott & McDonald 1993)

3.3. Genetic relationship and cluster analyses

The amount of genetic change was determined as 0.05 and genetic differences among genotypes ranged between 14.7 and 97.1%. According to our findings, while the lowest genetic distance was observed between 8.3 and 9.3 (Eskisehir-Balıkesir); 18.5 and 22.5 (Kastamonu-Eskisehir); 33.3 and 34.4 (Kovanli/Ordu-Mugla) coded genotypes with the average of 14.7%, the highest genetic distance was determined between 15.3 and 26.4 (Afyon-Tokat) genotypes with 97.1%.

Generally, when the genetic distance values are examined, variations among genotypes were observed to be very high and even among individuals of the same population, genetic differences were large.

A hundred seventy five genotypes were classified in two large clusters (Figure 1). While the first group had genotypes of population 15 (Incehisar/Afyon), the second group had all other genotypes. Then, second group divided into two sub-groups. The first branch of the second group had genotypes 35.2 and 1.2; the second branch of the second group had other genotypes with two sub-groups and then each group was divided into other sub-groups.

When the local populations were compared with the standard variety, it was relationship with all genotypes except for 15 number genotype. Because parental of standard varieties were obtained from populations collected from Turkey. This state is evidence that the local variety is selected from the country's populations and also the accuracy of the research conducted by us. It can be explained by cause moving to different region of the country with open pollination of popcorn genotypes as reason for being sub-groups under 2 groups and interconnected groups. Generally with telling, the distribution of local populations into groups was predominantly by provinces close to each other. In also, some populations obtained from different regions of Turkey were seen to be within the same group. Comertpay (2008), in analysis of the local corn according to the UPGMA method determined that the distribution of genotypes in groups does not show specific distribution; Warburton et al. (2005) reported that the elite corn lines were not divided into groups according to environmental factors and morphological characteristics. Our study

was supported by these two studies, while populations except for 15 number population were being in the groups and subgroup, population of 15 number placed in a separate group.

The reasons of the high genetic diversity of genotypes are; the genotypes used are open fertilizer material, be grown in different places, be high adaptability and it can be concluded that hundreds of seeds can be taken from one plant.

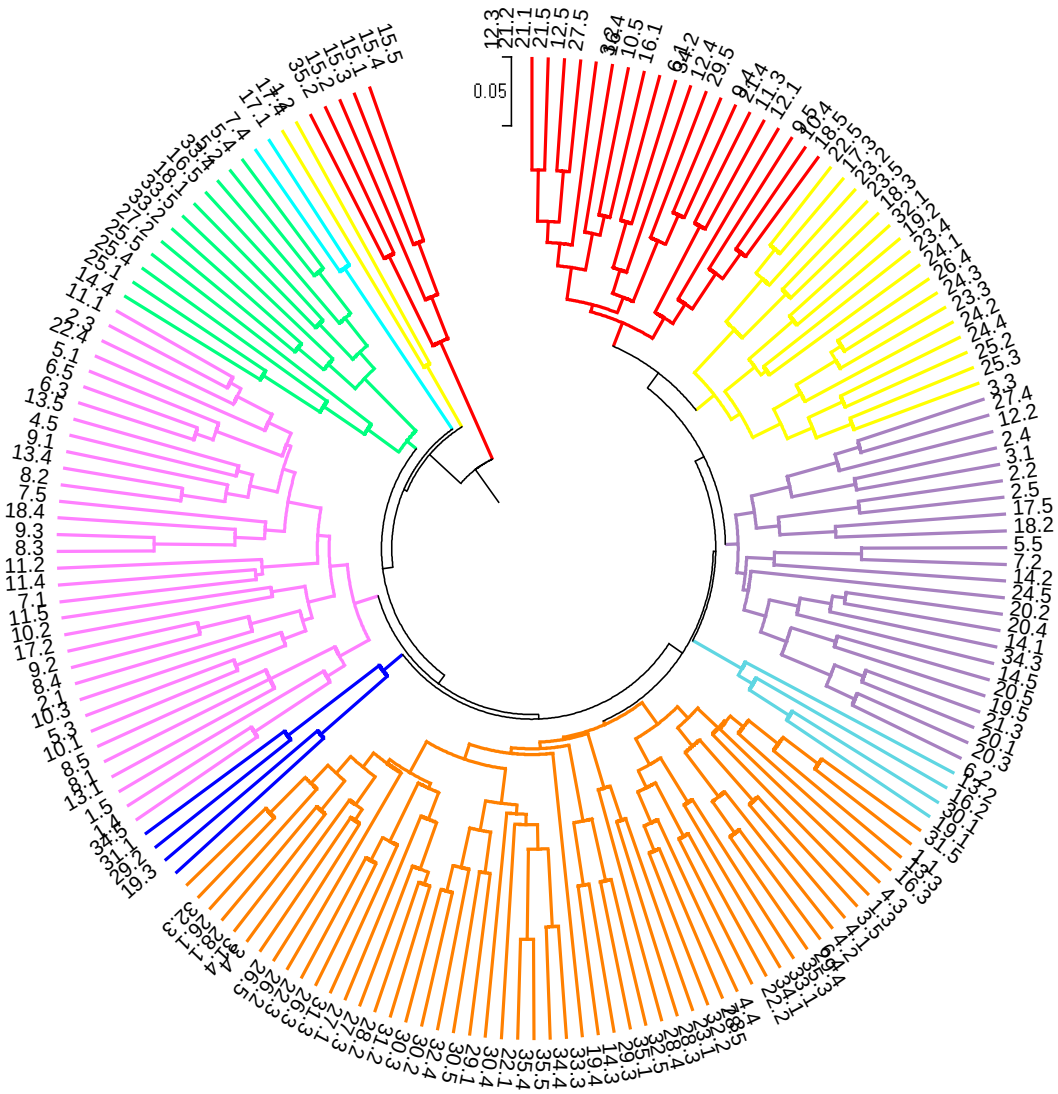

Figure 1- Cluster analysis of 175 accessions of popcorn based on UPGMA difference index through SSR markers polymorphism

4. Conclusions

Local popcorn populations are known to be richer in their genetic diversity than their hybrid counterparts. The use of genotypes in different groups in the breeding studies and having high evolution is beneficial in obtaining healthier and more effective results. At each stage of the study, examining genetic differences or richness within and between populations were observed high differences in each parameter. It was concluded that these differences would be an important resource of providing with a wide crop variety in terms of breeding and researchers.

This study was be first time for reveal of genome map of the genetic diversity on popcorn populations in Turkey. In addition, this study will give direction local hybrid seed production.

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References

Aci M M, Revilla P, Morsli A, Djemel A, Belalia N, Kadri Y, Khelifi-Saloui M, Ordás B & Khelifi L (2013). Genetic diversity in Algerian maize (*Zea mays* L.) landraces using SSR markers. Maydica 58: 304-310

Adjanohoun A, Allagbe M, Noumavo P A, Gotoechan-Hodonou H, Sikirou R, Dossa K K, GleleKakaï R, Kotchoni S O & Baba-Moussa L (2011). Effects of plant growth promoting rhizobacteria on field grown maize. Journal of Animal & Plant Sciences 11(3): 1457-1465

Atanda A S & Olaoye G (2017). Multiplex-Ready PCR assay of SSR marker diversity among quality protein maize inbred parental lines. South African Journal of Plant and Soil 34(2): 149-154 https://doi.org/10.1080/02571862.2016.1211325

Comertpay G (2008). Characterization of open polinated Turkish maize populations using morphological traits and SSRs molecular markers. Cukurova University of Cukurova Institute of Natural and Applied Science Field Crops Department, PhD Thesis, Adana (Published)

Doyle J J & Doyle J L (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin v.19 pp.11-15

Eschholz T W, Stamp P, Peter R, Leipner J & Hund A (2010). Genetic structure and history of swiss maize (*Zea mays* L. ssp. mays) landraces. Genetic Resources and Crop Evolution 57(1): 71-84 https://doi.org/10.1007/s10722-009-9452-0

Frankel O H (1972). The significance, utilization and conservation of crop genetic resources. FAO, Rome

Gauthier P, Gouesnard B, Dallard J, Redaelli R, Rebourg C, Charcosset A, Boyat A (2002). RFLP diversity and relationships among traditional european maize populations. Theoretical and Applied Genetics 105(1): 91-99 https://doi.org/10.1007/s00122-002-0903-7

Kimura M & Crow J F (1964). The Number of alleles that can be maintained in a finite population. Genetics 49(4): 725

Laborda P R, Oliveira K M, Garcia A A F, Paterniani M E A G Z, De Souza A P (2005). Tropical maize germplasm: What can we say about its genetic diversity in the light of molecular markers? Theoretical and Applied Genetics 111(7): 1288-1299 https://doi.org/10.1007/s00122-005-0055-7

Lewontin R C (1972). The Apportionment of human diversity. Evolutionary Biology 6: 381-398

Liu W S, Dong M, Song Z P, Wei W (2009). Genetic diversity pattern of *Stipapurpurea* populations in the hinterland of Qinghai-Tibet Plateau. Annals of Applied Biology 154: 57-65 https://doi.org/10.1111/j.1744-7348.2008.00274.x

McDermott J M & McDonald B A (1993). Gene flow in plant pathosystems. Annual Review of Phytopathology 31(1): 353-373

Molin D, Coelho C J, Máximo D S, Ferreira F S, Gardingo J R, Matiello R R (2013). Genetic diversity in the germplasm of tropical maize landraces determined using molecular markers. Genetics and Molecular Research 12(1): 99-114

Nei M (1972). Genetic distance between populations. The American Naturalist 106(949): 283

Nei M (1973). Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences 70(12): 3321

Nei M (1978). The theory of genetic distance and evolution of human races. Japanese Journal of Human Genetics 23(4): 341

Ribeiro C A G, Pinto M D O, Maciel T E F, Pastina M M, Barros E G D, Guimarães C T (2017). Universal tail sequence-SSR applied to molecular characterization of tropical maize hybrids. Scientia Agricola 74(2): 163-168 https://doi.org/10.1590/1678-992x-2016-0079

Rohlf F J (1992). Program numerical taxonomy and multivariate analysis system. Version 1.70 New York

Sharma L, Prasanna B M, Ramesh B (2010). Analysis of phenotypic and microsatellite-based diversity of maize landraces in India, especially from the North East Himalayan region. Genetica 138(6): 619-631 https://doi.org/10.1007/s10709-010-9436-1

Tahir N A & Maeruf M S (2016). Assessment of salinity tolerance and SSR profile differentiation in nine maize genotypes (*Zea mays* L). Maydica 61 M18: 1-8

Vivodík M, Gálová Z, Balážová Ž, Petrovičová L (2017). Genetic variation of European maize genotypes (*Zea mays* L.) detected using SSR markers. Potravinarstvo Slovak Journal of Food Sciences 11(1): 126-131

Warburton M L, Ribaut J M, Franco J, Crossa J, Dubreuil P, Betrán FJ (2005). Genetic characterization of 218 elite CIMMYT inbred maize lines using RFLP markers. Euphytica 142:97-106 https://doi.org/10.1007/s10681-005-0817-y

Yao Q L, Yang K C, Pan G T, Rong T Z (2008). Genetic diversity of maize (*Zea mays* L.) landraces from southwest China based on SSR data. J. Genet. Genomics 34: 851-860 https://doi.org/10.1016/S1673-8527(07)60096-4

Zhang J, Guo J, Liu Y, Zhang D, Zhao Y, Zhu L, Huang Y, Zhang Z, Chen J (2016). Genome-wide association study identifies genetic factors for grain filling rate and grain drying rate in maize. Euphytica 212(2): 201-21 https://doi.org/10.1007/s10681-016-1756-5

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The Effects of Unsaturated Fatty Acid Supplementation to Ration on Superovulation Performance and Embryo Quality of Donor Cows

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ABSTRACT
This study was conducted to determine the effects of omega-3 (α-linolenic acid) fatty acid supplementation to donor cow rations on superovulation performance, embryo number and quality, and blood-progesterone levels. The study was carried out with two groups, each consisting of 10 black-and-white breed dairy cows. Control group was fed with the basic ration prepared, and the experimental group was fed with the ration added with omega-3 polyunsaturated fatty acid (PUFA) source at a level of 3.82% of the basic ration dry matter (DM) for 60 days training period. Thus, each of experimental group's cow consumed 900 g-day feed additives consisting 149.4 g omega-3 daily. The superovulation protocol was started at the 40th day of feeding period. According to the findings of the study, the difference between the groups in terms of the response to superovulation was found to be insignificant (P>0.05). Similarly, there was no significant difference in blood progesterone levels between the groups (P>0.05). However, the difference between the control group and the experimental group in terms of transferable and non-transferable embryo rates was found to be significantly important (P<0.05). The total number of transferred embryos was determined as 37 in the experimental group and 79 in the control group. The total number of non-transferable embryos was recorded as 78 in the trial group and 43 in the control group. At the end of the study, it was concluded that supplementation to donor cow rations with omega-3 fatty acid sources might have a negative effects on transferrable embryo number and quality.

Keywords: Donor cow; Embryo number; Flaxseed; Omega-3; Quality; Superovulation performance

1. Introduction

Embryo transfer applications are one of the main techniques for making progress in animal breeding. Success in embryo transfer depends on the response of the donor animal to the superovulation and the number and quality of embryos obtained (Bülbül & Dursun 2005). There are many factors affecting the success of superovulation and also the quality of oocytes and embryos (Hasler 2004). These factors include; related to the animal (age, race, genetics, state of the follicles, lactation, etc.), drugs administered, feeding and other environmental factors (temperature, maintenance etc.) (Bülbül & Dursun 2005; Kaymaz 2015).

Nutritional factors may be the most important because they can directly affect reproductive performance and have the ability to change the effects of other factors (Smith & Akinbamijo 2000). Indeed, numerous studies have reported that nutrition of donor cows has an effect on the number and quality of embryos in response to superovulation **(**Boland & Lonergan 2003; Takahashi et al. 2013).

There is a highly complex relationship between nutrition and reproduction. The energy-protein, and mineral-vitamin balance should be well established in dairy cattle (Ergün & Erdoğan 2001).

The energy content of the ration is the most important nutritional factor affecting milk yield, living weight and associated body condition score (BCS) and reproductive function (Boland & Lonergan 2003). One way to improve the energy balance and thus fertility in the early period of lactation is to increase the energy density of the rations by the addition of fat (Staples et al. 1998).

In recent years, it has been accepted that the positive effects of the addition of fat to the ration on fertility are not due to the improvement in the energy status of the cows, but due to the use of high amounts of specific fatty acids (Mattos et al. 2000; Santos et al. 2008; Leroy et al. 2013). In this case, the most emphasized issue has been long-chain unsaturated fatty acids which are omega-3, omega-6 and conjugated linoleic acid (CLA) (Şirin & Kuran 2004; Elis et al. 2016).

Essential fatty acids have important roles in reproductive functions. Because fatty acids are involved in the synthesis of the hormones such as progesterone and prostaglandin ($PGF_{2\alpha}$ and PGE2). Arahidonic acid is a precursor of prostaglandin hormones ($PGF_{2\alpha}$ and PGE2). Linoleic acid (omega-6) is also the precursor of arachidonic acid. Cholesterol is the main precursor to progesterone. Linolenic acid (omega-3) also plays a role in the synthesis of progesterone, as it is a precursor to cholesterol (Mattos et al. 2000; Urlep & Rozman 2013; Tessaro et al. 2015). Indeed, it has been reported that blood progesterone levels increase in cows fed with rations rich in linolenic acid (Mattos et al. 2000). Increased progesterone concentrations during superovulation improve embryo quality and the number of transplantable embryos. The decrease in blood progesterone levels leads to the ovulation of low-quality oocyte, premature onset of meiosis cleavage, and an increase in LH release (Hiçcan & Yıldız 2016). It has also been reported in a study that high progesterone concentration during superovulation application improves embryo quality collected on day 7 after superovulation (Rivera et al. 2011).

There are many studies reporting the effect of feeding on reproduction in cows. Most of these studies show that there is a relationship between feeding and reproduction. The effects of specific nutrients on key issues such as superovulation response in donor cows, oocyte and early embryo development and quality, and conception after embryo transfer are still unclear (Salehi et al. 2016).

The present study aimed to investigate the possible effects of omega-3 fatty acid addition source to the ration of donor cows on follicle development, superovulation performance and quality and number of embryos obtained from the application. Thus, it is aimed to contribute to the literature on the relationship between fertility and fat source added to the ration in donor cows.

2. Material and Methods

This study was carried out in the Department of Animal Breeding of the Eastern Mediterranean Agricultural Research Institute. In this study, a total of 20 Holstein cows having similar live weight (500-550 kg), similar body condition score (2.7-3.0), and free of uterine infections and contagious diseases were used as animal material. The cows used in the trial were at 8-20 weeks of lactation period. The animals were composed with similar lactation age (2-3) and similar milk yield (average 28 kg/day). The cows were in 42-54 months of ages.

Mustafa Kemal University, Animal Experiments Local Ethics Committee's decision dated 15.07.2015 and numbered 2015, 6-11 was approved for conducting the study.

2.1. Supply of feed materials and additives

The concentrate feeds used in Total Mixed Ration (TMR) were prepared in Feeds Unit of East Mediterranean Agricultural Research Institute. Silage and wheat straw were sourced from the farmland owned by the Institute. Vetch dry grass was purchased from the market.

A commercial product called Flaxpro® (Volac International Limited, UK) was used as an additive in the trial Group. The additive containing flaxseed was used as an omega-3 source (C18:3 n-3 linolenic acid) from polyunsaturated fatty acids. The product, which is not commercially available in Turkey, was imported with the support of the firm. Flaxpro® is an energy intensive product (23 MJ-kg KM) consisting of a combination of rumen-protected oil and omega-3 fatty acid-rich flaxseed. In addition, the product has a content of 50% crude oil (CO) and C18:3 n-3 linolenic acid at a rate of 166 g-kg (Table 1).

Table 1- Composition of the additive used in study (%)

DM (Dry Matter)	*CO (Crude Oil)*	*CP (Crude Protein)*	*CF (Crude Cellulose)*	*Ash*	*Ca*	*ME (MJ-kg DM)*
96	50	15	7.5	8	5	23

2.2. Feeding of animals

The study was conducted with control and experimental groups. Experimental group fed with TMR added with supplement as omega-3 fatty acid source. A total of 20 cows were divided into two groups each has 10 black-and-white breed dairy cows. In the study, the animals were fed individually with TMR, which included coarse and concentrated mixed feeds (Table 2). The composition of the TMR used in the experiment was analysed according to the method reported in AOAC (AOAC 1998).

The animal trial period of the study took a total of 60 days, including 10 days pre-feeding period + 30 days feeding period + 20 days superovulation protocol and uterine washing period. The experimental feeding was continued during superovulation applications.

Table 2- Composition and nutrient content of TMR fed to animals in study

Ingredients	*Control group %, DM*	*Trial group %, DM*
Corn silage	26.50	27.04
Wheat straw	12.00	14.36
Vetch dry grass	13.73	14.09
Barley	10.20	10.47
Maize	23.54	13.25
The additive	-	3.82
Corn gluten	1.86	1.91
Soybean meals	11.59	11.89
Wheat bran	-	3.07
Limestone	0.5	0.04
Salt	0.04	0.04
Vit-min. mix*	0.04	0.04
Nutrient content, %		
DM, %	51.5	51.10
CP, % DM	15.1	15.1
CO, % DM	3.27	6.24
Omega-3; % DM	-	0.66
Omega-3 g / day-head	-	149.4
NDF, % DM	38.1	38.3
ADF, % DM	23.3	23.4
NEl, Mcal kg^{-1} DM	35.21	35.21
DM kg / day:	23.2	22.6
Roughage / concentrated	52.1	55.4

*Vitamin-mineral mix; Vitamin A: 12.000.000 IU, Vitamin D_3: 240.000 IU, Vitamin E: 5.000 mg, Vitamin B1: 500 mg, Vitamin B6: 1.000 mg, Niacin: 40.000 mg, Folic Acid: 100 mg, D-Biotin: 200 mg, Antioxidant: 3.500 mg; Cu: 6.000 mg; Zn: 15.000 mg; Fe: 10.000 mg; MCP P: 65.000 mg; I: 100 mg; Co: 40 mg; mg: 20.000 mg; Mn: 15.000 mg; Se: 200 mg; $CaCO_3$: 598.150 mg; Organic Se: 50 mg; Organic Fe: 4.000 mg; Organic Cu: 2.000 mg; Organic Zn: 8.000 mg; Organic Mn: 6.000 mg; Active yeast: $4x10^{10}$; Beta carotene: 4.000 mg; Vanilla aroma: 7.000 mg-5 kg mix.

The control and experimental group rations used in the study were prepared as isocaloric and isonitrogenic (Table 2). When preparing the rations, the groups' average live weight, milk yield, milk fat content, body condition score values were taken into consideration and nutrient requirement and dry matter consumption were calculated through the formulas defined by NRC (NRC 2001). By using these calculated values, TMR was formed by balancing the roughage - concentrate feed ratio to at least 40-60 and maximum 60-40. Daily feed consumption of animals was determined during the exercise period. The average 40 kg-day TMR per animal- was given. The prepared rations were divided into two equal amounts by weighing, and were given in two meals, at 06.30 in the morning and 18.30 in the evening.

2.3. Superovulation protocol

The uterus and ovariums of the donor cows to be used in the study were examined for pathological or cystic structures with ultrasound (Ultrasonic Scanner, HS-101V, Honda, Japan) before starting the feeding program.

The controlled drug released instrument (PRID Delta, Ceva, Turkey) which is the most preferred method (1.55 g progesterone) underwent intravaginal in order to synchronize follicular development prior to superovulation programs. On the 7^{th} day of intravaginal implant administration, FSH (Folltropin-V, Bionech Animal Health Europe Ltd., Ireland) hormone injection was started (Bó et al. 2002). FSH hormone injections were administered intramuscularly (i.m.) in decreasing amounts of 4-4, 3-3, 2-1.5, 1.5-1 cc, twice in 12 hours intervals for 4 days (Kaymaz 2015). At the day of 3 and -4 of the superovulation protocol, 2 cc $PGF_{2\alpha}$ (50 mg cloprostenole, Lutelen, Topkim, Turkey) was injected twice with 24 hours intervals to regress the existing corpus luteum and to ensure ovulation. The controlled drug release material (PRID) was removed at the evening of 3rd day of FSH hormone application. At the 12th, 24th and 48th hours following the last FSH administration, donor cows with estrus were inseminated 3 times (Bó et al. 2002). On the seventh day after the last artificial insemination following the superovulation protocol, the animals were subjected to uterine washing (flushing). On the day of washing of uterus, ovaries were examined with ultrasound (Ultrasonics Convex Scanner, HS-2000, Honda, Japan), and the number and size of corpus luteum on both ovaries were recorded. The superovulation response of the groups was evaluated by the presence of corpus luteum on both ovaries (Albuquerque et al. 2012). At the end of the study superovulation and fertilization rates of groups were calculated as shown below (Childs et al. 2008; Tur 2014).

Superovulation rate: 100 x (the number of corpus luteum with two or more per cow / total number of cows)
Fertilization rate: 100 x (total number of embryos / total number of cell)

2.4. Search and evaluation of embryos

The washing solution brought to embryo transfer laboratory after uterine washing process was examined under a stereo

microscope (Leica, s8apo, Japan) in 90 mL search Petries (Agtech Square Search Dish, VWR, USA). They were classified according to the quality criteria (IETS 2010) and developmental stages (Kanagawa et al. 1995) determined by the International Embryo Transfer Society (IETS).

2.5. Hormone analysis

Blood samples were taken from the 20 donor cows used in the study for the purpose of progesterone hormone analysis. Blood samples were taken 2 times during the study. The first one was taken on the 3rd day of superovulation before $PGF_{2\alpha}$ injection and the second one was taken on the flushing day. Blood progesterone levels were measured by Chemmilunesons method using the DXI800 hormone analyzer (Beckman Coulter, California, USA) and Beckman Coulter progesterone kit at the Balcali Hospital Central Laboratory of Cukurova University.

2.6. Statistical analysis

In the present study, Student t test, Mann Whitney U test and z test were used to compare the data obtained from the groups. Comparison of embryo numbers obtained in Group 1 and Group 2 was performed using Chi-Square test. SPSS 11.5 package program was used to evaluate the data (SPSS 1999).

3. Results and Discussion

Embryos classified as 1st, 2nd and 3rd quality were assessed as transferable embryos, while degenerate embryos and unfertilized oocytes (UFO) and degenerate oocytes were accepted as non-transferable embryos. The number of embryos obtained in the experimental groups are shown in Table 3.

Table 3- The number of transferable and non - transferable embryo, UFO, degenerate oocyte, total embryo and total cell numbers of experimental groups

Groups	*Transferable embryo*	*Non-transferable embryo*	*UFO*	*Degenerate oocyt*	*Total embryo*	*Total cell numbers**
Experiment	37[b]	78[a]	4	56[a]	55	115
Control	79[a]	43[b]	9	12[b]	101	122

*, Total cell numbers; indicates the number of oocytes and embryos obtained.

A total of 23 first quality, 12 second quality, 2 third quality and 18 degenerate embryos were obtained in the experimental group. In the control group, 60 first quality, 18 second quality, 1 third quality and 22 degenerate embryos were obtained. The number of unfertilized oocytes (UFO) was recorded as 4 in the experimental group and 9 in the control group. The number of degenerated oocytes was determined as 56 in the experimental group and 12 in the control group.

The results obtained from the two of research groups were compared with the Chi-Square test in terms of degenerate embryo numbers and degenerate oocyte numbers, There was no statistically significant difference between the groups in terms of degenerate embryo rates ($P>0.05$). However, a statistically significant difference was found between the groups in terms of degenerate oocyte rates ($P<0.05$). The number of degenerated oocytes in the experimental group were found to be higher than the control group.

Similarly, the numbers of transferable embryos and non-transferable embryos between the groups were compared with the Chi-Square test. There was a statistically significant difference between the control group and the experimental group in terms of transferable and non-transferable embryo rates ($P<0.05$). The number of transferable embryos was detected higher in the control group than in the experimental group. However, the number of non-transferable embryos was detected lower in the control group than in the experimental group.

The comparison of the mean blood progesterone levels of experimental and control groups are shown in Table 4. There was no statistically significant difference between the progesterone levels of the groups at the flushing day and during superovulation ($P>0.05$).

Table 4- The mean blood-progesterone levels (ng mL^{-1}) of the experimental groups

Groups	*X±Sx (during superovulation)*	*X±Sx (flushing day)*
Experiment	1.06 ± 0.39	6.79 ± 1.12
Control	2.19 ± 0.48	10.04 ± 2.45

The ovaries of the donor cows in both groups were examined by ultrasound on flushing day and the comparison of the average number and size of the average corpus luteum detected are given in Table 5.

Table 5- The mean corpus luteum numbers and size (mm) of experimental groups

Groups	*X±Sx (CL numbers)*	*X±Sx (CL size)*
Experiment	14.10 ± 2.11	15.79 ± 0.63
Control	14.67 ± 3.03	14.78 ± 0.45

The corpus luteum numbers of the experimental and control groups were compared using Mann Whitney U test and corpus luteum sizes by Student t test. There was no statistically significant difference between groups in terms of corpus luteum number and size of both groups (P>0.05).

While all cows included in the experimental group responded to superovulation, only 9 cows responded to superovulation in the control group. The response rates of superovulation between the groups were compared with z test (hypothesis test of difference of rates). The difference between the groups in terms of response rates to superovulation was found to be insignificant (P>0.05). Superovulation responses of the groups are shown in Table 6.

Table 6- Superovulation Response and Fertilization Rates (%) of Groups

Parameters	*Trial group*	*Control group*
Total Cows	10	10
Number of Cows Flushing	10	9
Response to Superovulation (%)	100	90
Fertilization Rate (%)	47.8	82.7

The fertilization rates of the groups were determined by the ratio of the total number of embryos obtained to the total number of cells. According to this data; the fertilization rate was 47.8% in the experimental group and 82.7% in the control group. Fertilization rates were compared with z test (hypothesis test of difference of rates). A statistically significant difference was observed when the fertilization rates were compared between the experimental and control groups, (P<0.05). The fertilization rates of the groups are shown in Table 6.

In the present study, the effect of the omega-3- rich supplementation to donor cows rations on the number and quality of oocytes and embryos was examined. The total number of cells in terms of oocytes and embryos obtained as a result of the study were determined similar to each other in both groups (experimental group: 115; control group: 122). In donor cows fed with omega-3 source, the number of embryos of transferable quality was recorded lower than the control group. It was interpreted that this difference in transferable embryo numbers between the groups may have been due to the high number of degenerate oocytes and low rates of fertilization in the experimental group. Actually, when the numbers of degenerate oocytes and fertilization rates were compared between the groups, the difference was found to be statistically significant (P<0.05). While the number of degenerate oocytes obtained in the experimental group was 56, it was recorded as 12 in the control group. However, the fertilization rates were determined as 47.8% in the experimental group and 82.7% in the control group.

The results of the present research show no resemblance to the reports of Childs et al. (2008), Petit et al. (2008) and Albuquerque et al. (2012)'s in terms of fertilization rates. Childs et al. (2008), Petit et al. (2008) and Albuquerque et al. (2012) have reported that the addition of n-3 polyunsaturated fatty acids to rations did not affect the number of unfertilized oocytes (UFO) (P>0.05). In the present study, low fertilization rates depending on the high number of unfertilized oocytes is due to the high number of degenerate oocytes in the total number of cells obtained.

While the results of the present study were compatible with Petit et al. (2008) it is different from the results of Childs et al. (2008), Muller et al. (2009), Albuquerque et al. (2012) and Salehi et al. (2016). Albuquerque et al. (2012) found that the average number of the degenerate embryos was statistically higher (P<0.10) in the flaxseed fed group than in the control group in Nellore cattle. Childs et al. (2008), investigated the effects of n-3 polyunsaturated fatty acid supplementation on embryo yield and quality in heifer rations. They found that the number of degenerated embryos obtained in heifers fed with n-3 PUFA was lower than the control group at the end of their study.

The reasons of different results in terms of degenerate embryo numbers, reported in the previous studies might be due to physiological characteristics of donor cows used in the studies (being a heifer or cow, lactation period, etc.) and breeds of the cows might also affect the results.

It could be said that based on to the data obtained from this study, the addition of omega-3 rich unsaturated fatty acid to donor cow rations do not have a positive effect on the number of oocytes and number of transferable quality embryos. The effect of unsaturated fatty acids on embryo quality was investigated in some *in vitro* studies. Fouladi-Nashta et al. (2007), reported that linolenic acid had a positive effect on oocyte and embryo development. However, it has been found that feeding with linolenic acid-rich ration has no positive effect on oocyte and embryo development in donor cows in some of the above-mentioned *in vivo* studies. In this case, it is thought that ovulation may be delayed in superovulated donor cows fed with omega-3 rich ration.

As it is known, a delay in ovulation in cows could lead to low fertility. In this study, it was estimated that the decrease in oocyte quality and fertilization rates may be caused by delay in ovulation. The reason for the decrease in fertility rate due to the delay of ovulation can be explained by aging of the oocyte within the follicle and slow embryo development and early embryonic death and consequently decrease in fertilization. Indeed, it has been reported by Bidarimath & Glover (2015) that ovulation was delayed in cows fed by omega-3-rich rations and this finding supports our results.

Bidarimath & Glover (2015) reported that long-chain unsaturated fatty acids added to the diets may directly or indirectly affect LH waves and estradiol secretion before ovulation. Under normal circumstances, when the estradiol concentration reaches a threshold or peak, the anterior lobe of the pituitary is stimulated to secrete GnRH. In the present study, depending on this information, it is thought that ovulation delays may be due to delay in LH fluctuation and this delay may be caused by insufficiency in estradiol release. Some other studies have also reported that the addition of unsaturated fatty acid sources to dairy cow rations leads to a decrease in plasma estrodiol levels (Fouladi-Nashta et al. 2007).

In this study, the number and dimensions of corpus luteum detected on flushing day were compared in order to evaluate the superovulation performance of both groups. The total number of corpus luteum on both ovaries was accepted as 2 or more in order to develop a response to superovulation in donor cows (Tur 2014). Accordingly, all of the donor cows from the experimental group used in the present study responded to superovulation. However, when the responses of the cows in control and experimental groups to the superovulation applications were compared statistically, the difference between the two groups was found to be insignificant ($P>0.05$). In addition, no statistically significant difference was found between the experimental and control groups in terms of the number and size of corpus luteum ($P>0.05$). In this study, it was found that the effect of feeding with rich omega-3 unsaturated fatty acid sources on the number and size of corpus luteum and superovulation response were insignificant. This result of the present study were supported by Capovilla et al. (2006), Childs et al. (2008), Muller et al. (2009), Ghasemzadeh-Nava et al. (2011), Albuquerque et al. (2012), Salehi et al. (2016), Gandra et al. (2017).

In literature about the research topic, it is reported that progesterone synthesis increase in linolenic acid-rich ration-fed cows in general (Şirin & Kuran 2004; Leroy et al. 2013). However, Ambrose et al. (2006) reported that plasma progesterone concentrations between groups remained unaffected by feeding with a ration enriched with α-linolenic acid. This finding is consistent with the data obtained from the current research.

Similarly, Moriel et al. (2014) did not find any significant difference in serum progesterone levels of the groups in which dietary fatty acid was added to the rations and the control group to evaluate the effects of PUFA due to calcium salts on serum concentrations of progesterone and insulin in their study.

Ghasemzadeh-Nava et al. (2011) investigated the effects of polyunsaturated fatty acid addition to rations on plasma metabolites, ovarian function, and prostaglandin release in lactating cows. They found that the plasma estradiol, progesterone and $PGF_{2\alpha}$ levels were similar between the groups.

The results of the present study in terms of serum progesterone levels were found to be similar with the reports of Ambrose et al. (2006), Ghasemzadeh-Nava et al. (2011) and Moriel et al. (2014). In this case it could be interpreted that n-3 PUFA source used in this study may be converted to stearic acid, i.e. saturated state via biohydrogenation by rumen microorganisms for this different results (Staples et al. 1998).

4. Conclusions

As a result of this study, the effect of omega-3 source addition to donor cow rations on oocyte and embryo number and quality was evaluated and it was determined that feeding rich in unsaturated fatty acid source led to a decrease in quality of oocytes and the number of transferable embryos in donor cows. As a reason of this results it could be interpreted that linolenic acid may affect granulosa cells of follicles and causes a drop in plasma estrodiol levels which leads a delay in ovulation as a result of inadequate LH fluctuations in donor cows applied superovulation.

It is concluded that oocyte quality and embryo development may be adversely affected due to ovulation delay in donor cows fed by ration rich in omega-3 fatty acid. In addition, the possibility of the omega-3 PUFA source which might be converted to saturated stearic acid via biohydrogenation by rumen microorganisms should be considered.

As can be understood from the results of the current research and the other studies about the topic, the effects of unsaturated fatty acid source addition on superovulation performance, embryo number and quality of donor cows ration are not clear. These contradictory results suggest that more in *vivo* advanced studies are needed on the subject.

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References

Albuquerque K P, do Prado I N, do Prado R M, Cavallieri F L B, Rigolon L P & Barbosa O R (2012). Superovulatory response, production and quality of embryos of cows fed on linseed or canola seed supplemented diets. *Acta Scientiarum Animal Sciences* 34(3): 321-327 http://doi.org/10.4025/actascianimsci.v34i3.13372

Ambrose D J, Kastelic J P, Corbett R, Pitney P A, Petit H V, Small J A & Zalkovic P (2006). Lower pregnancy losses in lactating dairy cows fed a diet enriched in linoleic acid. *Journal of Dairy Science* 89: 3066-3074 http://doi.org/10.3168 / jds.S0022-0302 (06) 72581-4

AOAC (1998). Official methods of analysis. 16th ed. 4th revision, Association of Official Analytical Chemists, Washington DC, USA

Bidarimath M & Glover K (2015). Effect of omega-3 fatty acıds on the ovarıes of lactatıng dairy cows. Retrieved in December, 20, 2016 from https://www.researchgate.net-publication-284673150 http://doi.org/10.13140/RG.2.1.3988.1044

Boland M P & Lonergan P (2003). Effects of nutrition on fertility in dairy cows. *Advances in Dairy Technology* 15: 19-33

Bülbül B & Dursun Ş (2005). Factors affecting superovulation response in cows. *Livestock Research Journal* 15(1): 16-25 In Turkısh

Bó G A, Baruselli P S, Moreno D, Cutaia L, Caccia M, Tríbulo R, Tríbulo H & Mapletoft R J (2002). The control of follicular wave development for selfappointed embryo transfer programs in cattle. *Theriogenology* 57(1): 53-72 http://doi.org/10.1016/j.theriogenology.2013.09.020

Capovilla L C T, Rigolan L P, Cavalieri F L B, Macedo F A F, Albuquerquue K P, Lourenço F J & Santello G (2006). Superovulation and embryos viability of santa ınes ewes fed with essential fatty acids. *Academical Journal* 4(1): 65-73

Childs S, Carter F, Lynch C O, Sreenan J M, Lonergan P, Hennessy A A & Kenny D A (2008). Embryo yield and quality following dietary supplementation of beef heifers with n-3 polyunsaturated fatty acids (PUFA). *Theriogenology* 70(6): 992-1003 http://doi.org/10.1016/j.theriogenology.2008.06.008

Elis S, Freret S, Desmarchais A, Maillard V, Cognie J, Briant E, Touze J L & Dupont M (2016). Efect of a long chain n-3 pufa-enriched diet on production and reproduction variables in holstein dairy cows. *Animal Reproduction Science* 164: 121-132 http://dx.doi.org/10.1016/j.anireprosci.2015.11.020

Ergün Y & Erdoğan Z (2001). The effect of feeding on fertility in dairy cattle - I: Energy, protein and fertility relationship. *Bulletin Veterinary Journal* October: 9-12 In Turkısh

Fouladi-Nashta A A, Gutierrez C G, Gong J G, Garnsworthy P & Webb R (2007). Impact of dietary fatty acids on oocyte quality and development in lactating dairy cows. *Biology of Reproduction* 77(1): 9-17 http://doi.org/10.1095/biolreprod.106.058578

Gandra J R, Verdurico L C, Mingoti R D, Takiya C S, Gardinal R, Vendramini T H A, Barletta R V, Visintin J A & Rennóve F P (2017). Whole flaxseed, raw soybeans and calcium salts of fatty acids supplementation for transition cows: Follicle development and embryo quality. *Italian Journal of Animal Scıence* 16(4): 538-545 http://doi.org/10.3168 / jds.2015-9974

Ghasemzadeh-Nava H, Fatahnia F, Nikkhah A & Zamiri M J (2011). Effects of dietary polyunsaturated fatty acids on ovarian function and prostaglandin secretion in lactating dairy cows. *International Journal of Veterinary Research* 5(2): 129-135

Hasler J F (2004). Factors influencing the success of embryo transfer in cattle. Retrieved in November, 25, 2014 from

Hiçcan Ö & Yıldız G (2016). Effects of energy balance on breeding in dairy cows. Retrieved in December, 28, 2016 from News / livestock / dairy-cows-energy-balance-effects on http://www.tarimdanhaber.com/ In Turkısh

IETS (2010). Bovine in vivo embryo slide set tutorial. Retrieved in December, 21, 2016 from http://www.iets.org.

Kanagawa H, Shimohira I & Saitoh N (1995). Manual of bovine embryo transfer. National Livestock Breeding Center MAFF, JICA-Japan

Kaymaz M (2015). Obstetrics and gynecology in farm animals. Kaymaz M, *Assisted reproductive techniques*, 2nd Edition, Medipres Printing Publishing Ltd. Şti., Malatya s. 695-811 In Turkısh

Leroy J L, Sturmey R G, Van Hoeck V, De Bie J, McKeegan P J & Bols P E J (2013). Dietary lipid supplementation on cow reproductive performance and oocyte and embryo viability: A real benefit? *Animal Reprodiction Science* 10(3): 258-267 http://doi.org/10.1111/rda.12308

Mattos R, Staples C R & Thatcher W W (2000). Effects of dietary fatty acids on reproduction in ruminants. *Reviews of Reproduction* 5(1): 38-45 https://doi.org/10.1530 / ror.0.0050038

Moriel P, Cappellozza B I, Ferraretto L F, Aboin A C, Vieira F V R, Rodrigues R O, Cooke R F & Vasconcelos J L M (2014). Effects of supplementation of calcium salts of polyunsaturated fatty acids on serum concentrations of progesterone and insulin of pregnant dairy cows. *Revista Brasileira de Zootecnia* 43(1): 20-26 https://doi.org/10.1590/S1516-35982014000100004

Muller M, Prado I N, Júnior A R L, Silva R R, Capovilla L C T, Rigolon L P, Cavalieri F L B & Marques J A (2009). Ω-3 nd Ω-9 on performance, superovulatory response and embryo production in nellore heifers. *Archivos de Zootecnia* 58(222): 241-252

NRC (2001). Nutrient Requirement of Dairy Cattle. 7th revised ed., National Academy Press, Washington DC

Petit H V, Cavalieri F B, Santos G T, Morgan J & Sharpe P (2008). Quality of embryos produced from dairy cows fed whole flaxseed and the success of embryo transfer. *Journal of Dairy Science* 91(5): 1786-1790 http://doi.org/10.3168/jds.2007-0782

Rivera F A, Mendonça L G, Lopes G J R, Santos J E, Perez R V, Amstalden M, Calderon A C & Chebel R C (2011). Reduced progesterone concentration during growth of the first follicular wave affects embryo quality but has no effect on embryo survival post transfer in lactating dairy cows. *Reproduction* 141(3): 333-342 http://doi.org/10.1530/REP-10-0375

Salehi R, Colazo M G, Tsoi S, Behrouzi A, Tsang B K, Dyck M K, Oba M & Ambrose D J (2016). Morphologic and transcriptomic assessment of bovine embryos exposed to dietary long-chain fatty acids. *Reproduction* 152: 715-726 http://doi.org/10.1530 / REP-16-0093

Santos J E P, Cerri R L & Sartori R (2008). Nutritional management of the donor cow. *Theriogenology* 69(1): 88-97 http://doi.org/10.1016/j.theriogenology.2007.09.010

Smith O B & Akinbamijo O O (2000). Micronutritients and reproduction in farm animals. *Animal Reproduction Science* 60-61: 549-560 http://doi.org/10.1016 / s0378-4320 (00) 00114-7

Staples C R, Burke J M & Thatcher W W (1998). Influence of suplemental fats on reproductive tissue and performance of lactating cows. *Journal of Dairy Science* 81(3): 856-871 http://doi.org/10.3168 / jds.S0022-0302 (98) 75644-9

SPSS for Windows (1999). Base System User's Guide, Release11.5, PSS Inc. Chicago, USA

Şirin E & Kuran M (2004). The effect of dietary fatty acids on reproductive functions in ruminants. 4th *National Animal Science Congress*, 1-3 September, İsparta s. 54-61 In Turkısh

Takahashi M, Sawada K, Kawate N, Inaba T & Tamada H (2013). Improvement of superovulatory response and pregnancy rate after transfer of embryos recovered from japanese black cows fed rumen bypass polyunsaturated fatty acids. *Journal of Veterinary Medical Science* 75(11): 1485-1490 http://doi.org/10.1292/jvms.12-0235

Tessaro F H, Ayala T S & Martins J O (2015). Lipid mediators are critical in resolving ınflammation: A review of the emerging roles of eicosanoids in diabetes mellitus. *Biomed Research International* 2015: 8 http://doi.org/10.1155 / 2015/568408

Tur İ (2014). Koyun rasyonundaki ham protein oranının kan üre nitrojen, ovaryum fonksiyonu, fertilizasyon, uterus fizyoloji ve embriyo kalitesi üzerine etkisi. Doktora tezi, Basılmamış, Selçuk Üniversitesi Sağlık Bilimleri Enstitüsü, Konya

Urlep Z & Rozman D (2013). The ınterplay between circadian system, cholesterol synthesis, and steroidogenesis affects various aspects of female reproduction. *Front Endocrinol (Lausanne)* 4: 111 http://doi.org/10.3389 / fendo.2013.00111

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Heritability and Genetic Parameters of Some Antioxidant Enzyme Activities in Barley (*Hordeum vulgare* L.) Cultivars under Salinity Stress

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ABSTRACT

In order to study the heritability and genetic parameters of antioxidant activity in barely (*Hordeum vulgare* L.) under salinity stress, a seven-parent half diallel (F_1 crosses + parents) was conducted in the non-stress and salt stress (8 and 12 dS m^{-1}) conditions in the greenhouse, during 2016-17, Ardabil, Iran. In this experiment, antioxidant enzymes ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) were measured. The results showed that the salinity had increased the expression of all of the three enzymes and the activity of enzymes were differed under different salinity levels. The average degree of dominance was higher than unity for all cases, suggesting the control of traits by over-dominance. Under saline condition heritability in narrow sense (h^2n) was found low to medium (0.11-0.41) but their broad-sense heritability (h^2b) was estimated relatively high (0.74-0.90). The results suggested the lack of heterosis in control of these traits except for APX activity in 8 dS m^{-1} salinity. Results showed that in APX activity recessive alleles were favorable, in CAT activity, under non-stress condition, dominant alleles, and under 12 dS m^{-1} salinity, recessive alleles were desirable; although, such relations were not clearly revealed in SOD activity. Due to the importance of dominance, it was indicated that the evaluation of genotypes must be done at progressive breeding program. Based on general combining ability effects, it was concluded that under salinity, Rihane and Nosrat had favorable alleles for APX activity. In CAT activity, Nosrat had favorable alleles. In case of SOD, Afzal and Valfajr had favourable alleles. In spite of the importance of physiological traits as selection criteria in breeding of salinity tolerance, presence of large dominance effects should not be neglected and selection for these traits should be delayed until after some inbreeding.

Keywords: Heritability; Ascorbate peroxidase; Catalase; Superoxide dismutase, Salinity

1. Introduction

Soil salinity is considered as a substantial factor persuading crop production and agricultural sustainability in the arid and semi-arid regions, reducing soil value and its productivity (Schleiff 2008; Ashraf 2010) and is considered as a serious abiotic stress in agriculture (Mahajan & Tuteja 2005). It is reported that over 6.5% of the total worldwide land area and 19.5% of irrigated land area salt-affected (FAO 2018). The salinity limits the economic exploitation of land for crop production and reduces the growth and fertility of plants (Frary et al. 2010). Despite the low diversity in plants salinity tolerance, some levels of tolerance and genetic diversity have been presented in some crops such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Flower & Yeo 1995; Flower 2004). Barley is the most salt tolerant cereal crop with a tolerance value of 8 dS m^{-1} (Katerji et al. 2006). The salt tolerance of plant genotype expresses its ability to grow and produce appropriate yield in a saline environment (Munns et al. 2002). Similar to other agronomic traits, breeding to tolerate salt stress, requires economic justification, genetic diversity, fast and reliable methods for selection, as well as genetic control of traits (Genec et al. 2010). The effects of salinity stress on plant physiology are well documented. High salinity can cause oxidative stress, leading to lipid peroxidation, protein oxidation and production of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Tanou et al. 2009). Plants have extensive defense systems that destroys or neutralize these ROS. These defense systems includes enzymatic and non-enzymatic mechanisms (Loggini et al. 1999). The enzymes of this defense system include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). The non-enzymatic system contains ascorbic acid (ASA), glutathione, alfatucophorol (Vitamin E) and carotenoids (Blokhin et al. 2003). The superoxide dismutase enzyme converts free oxygen radical (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen (O_2), which is the first reaction of ROS detoxification. In the next step, hydrogen peroxide can be detoxified by the catalase and ascorbate peroxidase (DaCosta & Huang 2007). APX has a major role in detoxification of hydrogen peroxide. Therefore, the damage caused by oxidative stress is minimized (Arora et al. 2002; Kocsy et al. 2005). There are many

reports about increasing the APX activity under the salinity stress (Roychoudhury et al. 2010). The role of the SOD enzyme is very important for eliminating O_2^- because the phospholipid membrane is impermeable to superoxide radicals (Alscher et al. 2002). Catalase is one of the enzymes with the maximum output in removing H_2O_2 (Garratt et al. 2002). Most results have shown that antioxidant enzymes activity in barely acts to reduce the oxidative stress damage caused by salinity (Kim et al. 2005) and antioxidant activity in the salt tolerant barely varieties was higher than the sensitive varieties (Xiaoli et al. 2009). The activities of antioxidant enzymes were increased in both roots and shoots under salinity in barley, But the increase was more significant and consistent in the roots. Among the antioxidant enzymes, activity of CAT was increased the most drastically (Kim et al. 2005). Determining the heritability and genetic variance components of traits, is one of the most important parts of each breeding program and helps to breeders in choosing suitable selection methods for identification of superior genotypes. Combining ability analysis also helps to identify superior parents with favorable alleles in construction of breeding populations through hybridization. This experiment was conducted to determine the heritability of some antioxidant enzymes activities in barley under salinity stress.

2. Material and Methods

In this research, 7 salt tolerant and sensitive barley cultivars were selected based on published data in research area, then were evaluated in a pot experiment under non-stress and 12 dS m^{-1} salinity (data not shown), and stress tolerance index (STI) of the cultivars was determined based on grain yield (Table1). In a half diallel, parents were crossed to make hybrids. The F_1 hybrids and their parental lines were grown under three salinity stress (non-stress, 8 and 12 dS m^{-1}) in completely randomized design with three replications at greenhouse of Islamic Azad University, Ardabil, Iran during 2016-17. Five seeds were sown in 25×30 cm plastic pots filled with sterilized sand, garden soil and compost in a 1:1:1 ratio. Salinity stress was exposed by irrigating the pots with the above mentioned saline water in the four-leaf stage. To control the salinity level, electric conductivity of the drained water from pots was measured. Applying salinity gradually completed within two weeks. After reaching to the desired EC, subsequent irrigations done with tap water and saucers were used to return extra water to the pot to prevent salt washing. Pots in non-stress condition were irrigated by the fresh water. After flowering and exposure of plants to salinity, samples were taken from flag leaves and were immediately transferred to the -80 °C freezer. Then, the samples were powdered in liquid nitrogen and enzymes was extracted by Sairam et al. (1998) method. Fresh leaf (0.5 g) were ground in a mortar with pestle in 5mL of 50 mM phosphate buffer (pH 7.8) at 4 °C. The homogenate was centrifuged at 13,000×g for 15 min. The activity of SOD was measured according to the method of Giannopolities & Ries (1977). The 3-mL reaction mixture contained 13 mM methionine, 75 µM nitro blue tetrazolium chloride, 2 µM riboflavin, 50 mM phosphate buffer (pH 7.8), and 0-50 µL enzyme extract. The reaction mixture was incubated for 10 min below two 15-watt fluorescent bulbs. Then the reaction solution was wrapped through the black cloth to measure absorption. Absorbance were measured at a wavelength of 560 nm with a Spectrophotometric. One unit of SOD activity were considered as the amount of enzyme required for 50% preventing of photochemical revival of Nitro Blue tetrazolium Chloride and was calculated according to the equation: Unit SOD=(V/v)-1 where V and v represent the rate of the absorption in absence and in presence of enzyme, respectively. Catalase was assayed by measuring the initial rate of disappearance of hydrogen peroxide by the method of Chance & Maehly (1955). For measurement of CAT activity assay solution (3 mL) contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 and 100 µL enzyme extract. The reaction was initiated by adding the enzyme extract. Decrease in absorbance of the reaction solution at 240 nm was recorded. Absorbance converted to H_2O_2 concentration following this formula: (H_2O_2(*Mmol*)=0.024×Absorbtion+0.011). One unit of CAT activity was defined as the amount of enzyme required to oxidize 1 mM of H_2O_2 per minute. APX activity were assayed using modified method of Nakano & Asada (1981). The 3-mL reaction solution of APX contained 50 mM phosphate buffer (pH 7.0), 0.5 mM Acid ascorbic, 0.1 mM H_2O_2, and 100 µL enzyme extracts. APX activity was measured by its absorbance at 290 nm. One unit of APX activity was defined as the oxidation of 1 mM of ascorbate per minute. Absorbance converted to acid ascorbic concentration following this formula: (Ascorbat (*Mmol*) =1.255×Absorbtion+0.097). Its absorbance was measured at 560 nm with a spectrophotometer (SHIMADZU, Model UV-120-02, Japan). Enzyme activity expressed in per unit (mg) protein basis.

Table 1- Source / Pedigree of studied barley cultivars

Parent	*cultivar*	*STI**	*Tolerance*	*Pedigree,Origin*
1	Afzal	0.529	tolerant	Chahafzal
2	Nosrat	0.544	tolerant	Karoon/Kavir, Iran
3	Valfajr	0.373	Semi-tolerant	CI-108985, Egypt
4	Kavir	0.416	Semi-tolerant	Arivat, USA
5	Rihane	0.247	sensitive	Atlas 46 /Arivat //Athenais ICB76-2L-1AP-0AP, ICARDA
6	Sahra	0.242	sensitive	L.B. LRAN/ Una8271// Giorias "s" Com, CIMMYT
7	Yusef	0.272	sensitive	Ligne527/chn-01//Gustoe/4/Rhn-08/3/DeirAlla 106//DI71/strain 205

*, determined based on previous work (unpublished data)

2.2. Statistical analysis

Diallel was analyzed by Hayman (1954) graphical method and Griffing (1956) fixed model. The goodness of fit of the additive-dominant model was evaluated by linear regression of Wr on Vr (H_0: b = 1 vs. H_1: b ≠1) (Hayman 1954). The genetic parameters:

D, H_1, H_2 and F were estimated by method of Singh & Singh (1984). Average degree of dominance, broad-sense heritability and narrow-sense heritability were estimated using method proposed by Mather & Jinks (1971). The diallel analysis was done based on the method of Hayman (1954) and calculated by DIAL98 software (Ukai 1989). Genetic components were estimated by electronic spread sheets in the Excel 2010 program.

3. Results and Discussion

The result analysis of variance revealed significant differences among 28 genotypes (7 parents +21 F_1's) for all the studied traits, suggesting the presence of adequate genetic variability to proceed to diallel analysis (Table 2). Significant Salinity×Genotype interaction mean squares indicates the different expression of traits under different salinity levels (Table 2). Salinity had significantly increased the expression of all of the three enzymes activity (Table 2). The results of the goodness of fit of the additive-dominant model are presented in Table 3. The slope of linear regression of W_r on V_r was significantly greater than 0 and had not significant difference with 1 in all cases except for SOD activity under non-stress condition (Table 3), indicating the adequacy of additive-dominance model for traits (Mather & Jinks 1971). Rohman et al. (2006) reported that epistasis effects significantly play a role in controlling wheat morphological traits, and additive-dominance model aren't sufficient for traits fitting. The significance of "a" component in table 4 (except SOD in non-stress) was in accordance with the significance of additive effects (D component) in table 5 except for CAT activity. Singh et al. (2006) showed that the additive and non-additive effects in both F_1 and F_2 generations are important in barley. The dominance genetic effects (b source of variation) was significant for all traits, indicating the presence of dominance effects in the control of traits (Table 4). Non-corrected and corrected dominance variances (H_1 and H_2 components) were also significant confirming the effects of dominance in controlling of the traits (Table 5). The "b_1" component, was non-significant in all studied traits (except APX in 8 ds m^{-1}). The "b_1" item measures the mean deviations of the F_1's from the mid-parental values and becomes significant when the dominance effects at various loci are predominantly in dominance effect. That is, there is a directional dominance effect and measures the average heterosis (Singh & Singh 1984). The "b_2" component was significant in all of the traits, except for APX activity in 8 ds m^{-1} salinity. The significance of the "b_2" item indicated that the mean dominance deviations of the F_1's from their mid parental values differed significantly over the F_1 arrays; this implies the presence of asymmetry in the distribution of alleles among the parents (Hayman 1954). This also means that some parents had a significantly better performance than others (Ramalho et al. 1993). Similar results were reported by Singh & Singh (1992) and Sharma (1998). Since "b_2" is significant for most cases, the "a" item will not measure additive variance unambiguously, but it will be contaminated with non-additive variance also (Singh & Singh 1984). The "b_3" component which is equivalent to specific combining ability variance was significant in all of cases except for APX activity in 8 ds m^{-1} salinity. Significant "b_3" exhibited residual dominance effect (b_3) resulted from additive× additive, additive× dominance and dominance×dominance interaction effects (Chaudhry et al. 1977). The proportion of positive and negative genes was estimated by calculating ($H_2/4H_1$) in table 5. This ratio was found to be less than 0.25 in all of the traits, indicating unequal proportions of positive and negative alleles in loci with asymmetrical distribution of genes in the parents. This is also substantiated by "H_1" being greater than "H_2"in these traits. Similar results were reported in other studies (Bouzerzour & Djakoune 1998; Roy 2000). The estimate of the genetic component "F" was significant in APX activity which is an indication of asymmetry in the distribution of dominant and recessive alleles in the parents. Positive and significant values for this indicator indicate a higher prevalence of dominance alleles among parents (Table 5). The ratio of the total number of dominant and recessive alleles in the parents (KD/KR) was higher than one in all of traits, indicated that parents carry more dominant alleles. Positive values for F substantiated by (KD/KR) being greater than one (Table 5). This finding is in agreement with earlier reports (Ciulca et al. 2000; Bhatnagar et al. 2001; Dharam & Sanjay 2009). According to the results, the average degree of dominance was higher than unity for all traits, indicating the presence of over-dominance in controlling the traits under study (Table 5). The regression line intersected below the point of origin suggesting over dominance for controlling the trait (Figure 1). Similar conclusion was reported by Rebetzke et al. (2003) and Shahbazi et al. (2013) in the genetic control of physiological traits in wheat and barley (Chowdhry et al. 2002; Singh et al. 2006). The narrow-sense heritability of traits was low to moderate (0.11 to 0.41), however, their broad-sense heritability was high (Table 5). Tuberosa (2012) estimated the heritability of most the traits of crops under drought conditions low (0.3-0.4) or intermediate (0.4-0.7). The high broad-sense heritability can be attributed to the low environmental effects in the appearance of these traits. The low narrow-sense heritability of these traits indicated that non-additive effects were primarily responsible for the genetic variation in these hybrids and also the traits were highly influenced by the growing environment. Similar results reported in *brassica napus* (Khan & Khan 2005). For almost all the traits, the parental array points were scattered all along the regression line in the Wr/Vr graphs. The parents with most dominant genes are nearest to the origin and with most recessive genes, farthest from the origin and with equal frequencies of dominant and recessive genes, fall in the middle. Parents along the regression line show genetic interactions or epistasis, more distance more interaction. Parents above regression line, show duplicate epistasis and below regression line, show complementary epistasis (Figure 1). According to parents distance from the origin of the regression line W_r (parent offspring co-variance) on V_r (parental variance), it can be concluded that for APX in non-stress and 8 ds m^{-1}, cultivar Sahra has more dominant alleles, cultivars Kavir and Rihane have more recessive alleles, in 12 ds m^{-1}, Yoosef had more dominant alleles and Nosrat had more recessive alleles. In CAT activity under non-stress condition, Valfajr had more dominant and Kavir had recessive alleles respectively. In 8 and 12 ds m^{-1}, Rihane and Yoosef had more dominant alleles. Moreover, in SOD under 8 ds m^{-1}, Kavir had more dominant alleles and Sahra had more recessive alleles; in 12 ds m^{-1}, cultivar Sahra had more recessive alleles (Figure 1). The correlation coefficients between the parental means and order of dominance "rYr (Wr + Vr)" which indicates the relation between the favorability of alleles and dominance, were significantly positive in APX activity indicating that recessive alleles are favorable. For CAT activity, under

non-stress condition dominant alleles were favorable but under stress (12 ds m^{-1}) recessive alleles were favorable (table 5). Result of combining ability analysis showed that the general combining ability variance is significant (except APX in 8 ds m^{-1}, CAT in non-stress and SOD in 12 ds m^{-1}). The specific combining of traits was significant in all cases except SOD in 12 ds m^{-1} salinity, showing the higher importance of dominance variance than additive variance (Table 6). Joshi et al. (2004) reported that the heterotic effect in the wheat autogamous plant has a negligible effect in breeding the traits based on the specific combining ability. Based on GCA effects (Table 7) it was concluded that in APX activity: Afzal, under non-stress; Rihane, under 8 ds m^{-1} and Nosrat in 12 ds m^{-1} had favorable alleles. In CAT activity; Afzal, under non-stress; Nosrat, under 8 and 12 ds m^{-1} had favorable alleles. In case of SOD: Afzal, under 8 ds m^{-1} and Valfajr, under 12 ds m^{-1} have favorable alleles because of high GCA values. Parents with good general combining ability can be used in hybridization program for varietal improvement.

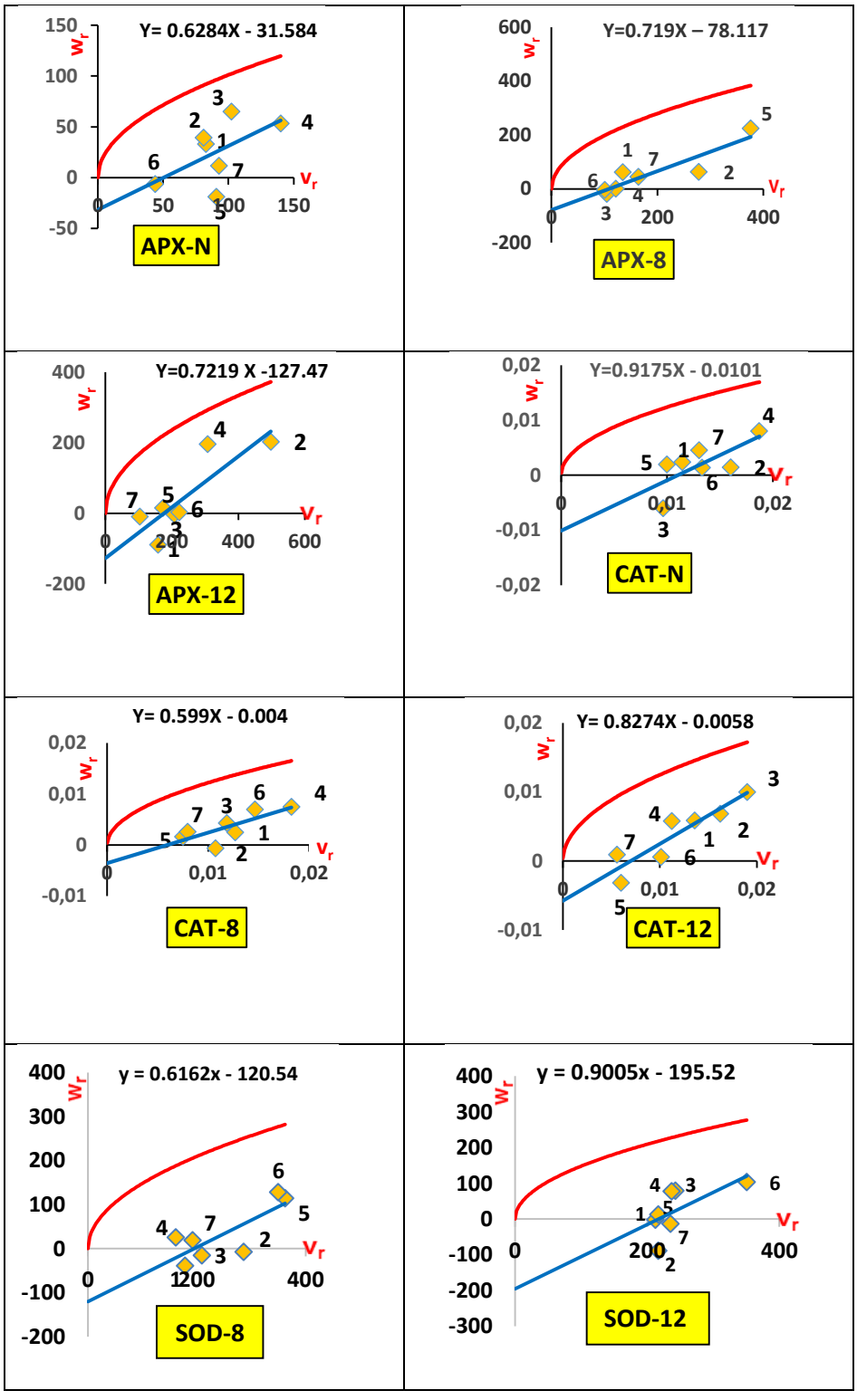

Figure 1- Regression line W_r/V_r

Table 2- Analysis of variance of studied traits under different salinity stress levels

Source of variation	*Degrees of freedom*	*Mean squares*		
		Ascorbate peroxidase	*Catalase*	*Superoxide dismutase*
Genotype	27	486.94**	0.036**	418.72**
Salinity	2	1006.11**	0.080**	696.32*
Salinity× Genotype	54	301.32**	0.019**	333.10**
Error	168	88.21	0.007	150.65
CV%		13.81	1.81	26.81

** and *, significant at P<0.01 and P<0.05

Table 3- Analysis of additive -dominant model by regression of w_r on v_r for traits

Null Hypothesis	*Ascorbate peroxidase*			*Catalase*			*Superoxide dismutase*		
	0	*8*	*12*	*0*	*8*	*12*	*0*	*8*	*12*
b = 0	0.63* ±0.24	0.72** ±0.14	0.722* ±0.21	0.92* ±0.33	0.6* ±0.22	0.83** ±0.16	0.18 *±0.05	0.62* ±0.21	0.90* ±0.37
b = 1	0.63^{NS} ±0.24	0.72^{NS} ±0.14	0.722^{NS} ±0.21	0.92^{NS} ±0.33	0.6^{NS} ±0.22	0.83^{NS} ±0.16	0.18** ±0.05	0.62^{NS} ±0.21	0.90^{NS} ±0.37

NS, Non-significant; ** and *, significant at P<0.01 and P<0.05; b- regression coefficient; 0, 8 and 12 ds m^{-1}- salinity stresses

Table 4- Gene interactions' ANOVA results

Source of variation		*Degrees of freedom*	*Mean Squares*							
			Ascorbate peroxidase			*Catalase*			*Superoxide dismutase*	
			0	*8*	*12*	*0*	*8*	*12*	*8*	*12*
rep		2	8.4^{NS}	55.7^{NS}	3.2^{NS}	0.008^{NS}	0.005^{NS}	0.004^{NS}	0.07^{NS}	0.98^{NS}
a		6	236.1**	468.3**	712.3**	0.016*	0.036**	0.031**	0.16**	0.86**
b		21	235.6**	275.5*	485**	0.025**	0.023**	0.026**	1.4**	0.72**
	b_1	1	25^{NS}	964.3**	137^{NS}	0.01^{NS}	0.008^{NS}	0.063^{NS}	0.24^{NS}	1.94^{NS}
	b_2	6	258.5**	277.1^{NS}	388.8**	0.033**	0.018**	0.022**	0.29**	0.33**
	b_3	14	240.8**	225.7^{NS}	551.1**	0.023**	0.025**	0.025**	1.9**	0.81**
Error		54	34.1	148	89.8	0.007	0.006	0.007	0.11	0.96

NS, Non-significant; ** and *, significant at P<0.01 and P<0.05; a: additive effect; b: non- additive effect; b_1: direct of dominance; b_2- gene frequency balance; b_3- particular dominance; 0, 8 and 12 ds m^{-1}- salinity stresses

Table 5- Estimates of genetic components and related statistics in half- diallel design

Parameters	*Ascorbate peroxidase*			*Catalase*			*Superoxide dismutase*	
	0	*8*	*12*	*0*	*8*	*12*	*8*	*12*
D	91**±4.7	342**±6	249**±7.5	0.01^{NS}±0.1	0.01^{NS}±0.04	0.01^{NS}±0.04	97**±6.4	352**±6.5
H_1	313**±11.3	691**±14.4	921**±18	0.046^{NS}±0.2	0.04^{NS}±0.1	0.04^{NS}±0.1	701**±15.4	1025**±15.5
H_2	224**±10	492**±12.7	758**±15.8	0.037^{NS}±0.1	0.03^{NS}±0.04	0.03^{NS}±0.1	540**±13.6	654**±13.7
F	87**±11.3	499**±14.4	334**±18	0.018^{NS}±0.2	0.01^{NS}±0.1	0.01^{NS}±0.1	105**±15.3	639**±15.5
$\overline{D}$	1.86	1.42	1.93	1.94	1.75	1.67	2.68	1.7
$H_2/4H_1$	0.18	0.18	0.21	0.2	0.21	0.18	0.192	0.159
KD/KR	1.7	3.1	2.1	2.4	1.9	1.8	1.51	3.27
h_n	0.41	0.11	0.15	0.12	0.27	0.37	0.27	0.16
h_b	0.9	0.74	0.88	0.78	0.85	0.85	0.74	0.77
$rY_r(w_r+v_r)$	0.89**	0.79*	0.66^{NS}	-0.73*	-0.59^{NS}	0.73*	0.40^{NS}	0.48^{NS}

NS- Non-significant; ** and * - Significant at P<0.01 and P<0.05; D- additive genotypic variance; H_1- in-corrected dominance variance; H_2- corrected dominance variance; F- average covariance between additive and dominance effects; $\overline{D}$ - average degree of dominance; $H_2/4H_1$- relative distribution of positive and negative alleles between parents; KD/KR- relative distribution of dominant and recessive alleles among parents; h_n- narrow-sense heritability; h_b- broad-sense heritability; $rY_r(w_r+v_r)$- correlation between Y_r and (w_r+v_r); 0, 8 and 12 ds m^{-1}- Salinity stresses

Table 6 - Result of analysis of variance

Source of variation	*Degrees of freedom*	*Ascorbate peroxidase*			*Catalase*			*Superoxide dismutase*	
		0	*8*	*12*	*0*	*8*	*12*	*8*	*12*
GCA	6	493**	269^{NS}	526**	0.015^{NS}	0.05**	0.05**	1.21**	1.58^{NS}
SCA	21	162**	333*	538**	0.023**	0.02**	0.02**	1.05**	0.52^{NS}
Error	54	34.1	148	89.8	0.009	0.01	0.01	0.11	0.96

NS - Non-significant; ** and * - Significant at P<0.01 and P<0.05; 0, 8 and 12 ds m^{-1}- Salinity stresses GCA- General Combining Ability; SCA- Specific Combining Ability

Table 7- General combining ability estimates of parents

Parents	*General combining ability*							
	Ascorbate peroxidase			*Catalase*			*Superoxide dismutase*	
	0	*8*	*12*	*0*	*8*	*12*	*8*	*12*
Afzal	4.58	-0.52	0.74	0.03	0.02	0.03	0.28	0.12
Nosrat	-0.22	0.86	8.08	0.02	0.08	0.06	0.18	-0.2
Valfajr	3.8	3.2	2.19	0.01	0.003	0.05	0.14	0.5
Kavir	3.58	0.04	0.22	-0.04	-0.02	-0.01	-0.04	-0.12
Rihane	-4.96	3.89	-5.42	0.01	-0.01	-0.06	-0.13	-0.15
Sahra	-6.02	-5.39	-2.61	-0.02	-0.03	-0.04	-0.33	-0.11
Yoosef	-0.77	-2.08	-3.19	-0.01	-0.05	-0.03	-0.11	-0.04

0, 8 and 12 ds m^{-1}- Salinity stresses

4. Conclusions

The results suggested that most of the traits adequately can be described by additive-dominance model. Results showed that additive effects and dominant effects were significant in most of traits. In general, all of traits had high broad-sense heritability, indicating the accuracy and precision of the data measured. The degree of dominance was greater than one for all traits, indicated that these traits under the influence of over dominance gene action evidence of the decline in narrow sense heritability. Importance of dominant gene effects was suggested by Dashti et al. (2010) in wheat under salinity stress. We found that dominance was more important, hence, more generations need to be generated and evaluated. In spite of the importance of physiological traits as selection criteria in breeding programs, presence of large dominance effects should not be neglected and selection for these traits should be delayed until after some inbreeding. However, the potential exists for these dominance effects to be exploited in development of F_1 hybrids in cross pollination crops.

References

Alscher R G, Erturk N & Heath L (2002). Role of superoxide dismutases (SOD_s) in controlling oxidative stress in plant. *Journal of Experimental Botany* 153: 1331-1341 http://doi.org/ 10.1093/jxb/53.372.1331

Arora A, Sairam R K & Srivastava G C (2002). Oxidative stress and antioxidant system in plants. *Annual Review of Current Science* 82: 1227-1238

Ashraf M (2010). Registration of 'S-24' spring wheat with improved salt tolerance. *Journal of Plant Registration* 4: 34-37 http://doi.org/10.3198/jpr2008.05.0252crc

Bhatnagar V K, Sharma S N & Sastry E V D (2001). Genetics of quantitative characters in six rowed barley over environments. *Indian Journal of Genetics* 61: 358-359

Blokhin O, Virolainen E & Fagerstedt K (2003). Antioxidant oxidative damage and oxygen deprivation stress. *Annual Review of Botany* 91: 179-194 http://doi.org/10.1093/aob/mcf118

Bouzerzour H & Djakoune A (1998). Inheritance of grain yield components in barley. *Rachis* 16: 9-16

Chance B & Maehly A C (1955). Assay of catalases and peroxidases. *Methods Enzymol* 2: 764-775 http://doi.org/10.1016/S0076-6879(55)02300-8

Chaudhry F M, Alam S M, Rashid A & Latif A (1977). Mechanism of differential susceptibility of two rice varieties to zinc deficiency. *Plant and soil* 46: 637-642

Chowdhry M A, Ambreen A & Khaliq I (2002). Genetic control of some polygenic traits in vulgare species. *Asian Journal of Plant Science* 1: 235-237

Ciulca S, Nedelea G & Mandosa E (2000). Combining ability study of plant height in two- rowed barley. Lucrai- Stiintifice- Agricultura,- Universitateade- Stiinte- Agricole- si- Medicina Veterinara- aBanatului- Timisoara 32: 853-858 Frey, K.J. 1975

DaCosta M & Huang B (2007). Changes in antioxidant enzyme activities and lipid peroxidation for bent grass species in response to drought stress. *Journal of the American Society for Horticultural Science* 132: 319-326 http://doi.org/10.21273/JASHS.132.3.319

Dashti H, Naghavi M R & Tajabadipour A (2010). Genetic Analysis of Salinity Tolerance in a Bread Wheat Cross. *Journal of Agricultural Science and Technology* 12: 347-356

Dharam P & Sanjay K (2009). Genetic analysis of forage yield and other traits in barley (*Hordeum vulgare* L.). *Barley Genetics Newsletter* 39: 13-19

FAO (2018). FAO Soil Portal, Extents of Salt Affected Soils. Retrieved in June, 5, 2018 from http://www.fao.org/soils-portal/soil-management/management-ofsome-problem-soils/salt-affected-soils/more-information-on-saltaffected-soils/en/

Flower T J (2004). Improving crop salt tolerance. *Journal of Experimental Botany* 55: 307-319

Flower T J & Yeo A R (1995). Breeding for salinity resistance in crop plants: where next? *Australian Journal of Plant Physiology* 22: 875-884 http://doi.org/10.1071/PP9950875

Frary A, Gol D, Keles D, Okmen B, Pinar H, Sigva H, Yemenicioglu A & Doganlar S (2010). Salt tolerance in *Solanum pennellii*: Antioxidant response and related QTL. *BMC Plant Biology* 10: 1-58 http://doi.org/10.1186/1471-2229-10-58

Garratt L C, Janagoudar B S, Lowe K C, Anthony P, Power J B & Davey M R (2002). Salinity tolerance and antioxidant status in cotton cultures. *Free Radical Biology and Medicine* 33: 502-511

Genec Y, Oldach K, Verbyla A, Lott G, Hassan M, Tester M, Wallwork H & McDonald G K (2010). Sodium exclusion QTL associated with improved seedling growth in bread wheat under salinity stress. *Theoretical and Applied Genetics* 121: 877-894 http://doi.org/10.1007/s00122-010-1357-y

Giannopolities C N & Ries S K (1977). Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiology* 59: 309-314 http://doi.org/10.1104/pp.59.2.309

Griffing B (1956). A generalized treatment of the use of diallel crosses in quantitative inheritance. *Heredity* 10: 31-50 http://doi.org/10.1038/hdy.1956.2

Hayman B I (1954). The theory and analysis of diallel crosses. *Genetics* 39: 789-809

Joshi S K, Sharma S N, Singhania D L & Sain R S (2004). Combining ability in the F_1 and F_2 generations of the diallel cross in hexaploid wheat (*Triticum aestivum* L. em. Thell). *Hereditas* 141: 115-121 http://doi.org/10.1111/j.1601-5223.2004.01730.x

Katerji N, Van Hoorn J W, Hamdy A, Mastrorilli M & Fares C (2006). Classification and salt tolerance analysis of barley varieties. *Agricultural Water Management* 85(1-2): 184-192

Khan F A & Khan R S A (2005). Inheritance pattern of quantitative characters in *Brassica napus*. *International Journal of Agriculture and Biology* 7(3): 420-423

Kim S Y, Lim J H, Park M R, Kim Y J & Park T T (2005). Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress. *Journal of Biochemistry and Molecular Biology* 38(2): 218-224

Kocsy G, Laurie R, Szalai G, Szilagyi V, Simon-Ssarkadi L, Galiba G & Deronde J A (2005). Genetic manipulation of prolin levels affects antioxidant in soybean subjected to simultaneous drought and heat stresses. *Physiologia Plantarum* 124: 227-235 http://doi.org/10.1111/j.1399-3054.2005.00504.x

Loggini B, Scartazza A, Brugnoli E & Navaru-Izzo F (1999). Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiology* 119: 1091-1099

Mahajan S & Tuteja N (2005). Cold, salinity and drought-stressed: an overview. *Archives of Biochemistry and Biophysics* 444:139-158 http://doi.org/10.1016/j.abb.2005.10.018

Mather K & Jinks J L (1971). Biometrical Genetics. Second edition. Chapman Hall, London 382 pp

Munns R, Husain S, Rivelli A R, James R A, Condon A G, Lindsay M P, Lagudah E S, Schahtman D P & Hare R A (2002). Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. *Plant Soil* 247: 93-105 http://doi.org/10.1023/A:1021119414799

Nakano Y & Asada K (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiology* 22: 867-880 http://doi.org/10.1093/oxfordjournals.pcp.a076232

Ramalho M A P, Santos J B & Zimmermann M J O (1993). Genética quantitativa em plantas autógamas: aplicações ao melhoramento do feijoeiro. UFG, Goiânia, 271 pp

Rebetzke G J, Condon A G, Richards R A & Farquhar G D (2003). Gene action for leaf conductance in three wheat crosses. *Australian Journal of Agricultural Research* 54: 381-87

Rohman M M, Sultana R, Podder R, Tanjimul Islam A T M, Kamrul Islam M & Islam M S (2006). Nature of gene action in barley (*Hordeum vulgare* L.). *Asian Journal of Plant Sciences* 5: 170-173

Roy D (2000). Plant breeding analysis and exploitation of variation. *Alfa Science International Ltd* 701 pp

Roychoudhury A, Basu S & Sengupta D N (2010). Amelioration of salinity stress by exogenously applied spermidine or spermine in three varieties of indica rice differing in their level of salt tolerance. *Journal of Plant Physiology* 168: 317-328 http://doi.org/10.1016/j.jplph.2010.07.009

Sairam R K, Deshmukh P S & Saxena D C (1998). Role of antioxidant systems in wheat genotypes tolerance to water stress. *Biologia Plantarum* 41(3): 387-394 http://doi.org/10.1023/A:1001898310321

Schleiff U (2008). Analysis of water supply of plants under saline soil conditions and conclusions for research on crop salt tolerance. *Journal of Agronomy and Crop Science* 194: 1-8 http://doi.org/10.1111/j.1439-037X.2007.00290.x

Shahbazi H, AAli E, Imani A A & Zaeefizadeh M (2013). Inheritance of cell membrane stability under heat and osmotic stresses in bread wheat. *SABRAO Journal of Breeding and Genetics* 45(2): 187-194

Sharma R (1998). Statistical and Biometrical techniques in plant breeding. Publishers H. S. Poplar for New Age International Limited, New Delhi, pp. 178-197

Singh S K, Singh H C & Singh H L (2006). Inheritance of quality traits in barley (*Hordeum vulgare* L.). *International Journal of Plant Sciences* 1: 304-305

Singh R P & Singh S (1992). Estimation of genetic parameters through generation means analysis in bread wheat. *Indian Journal of Genetics and Plant Breeding* 52: 369-375

Singh M & Singh R K (1984). A comparison of different methods of half-diallel analysis. *Theoretical and Applied Genetics* 67: 323-326 http://doi.org/10.1007/BF00272868

Tanou G, Molassiotis A & Diamantidis G (2009). Induction of reactive oxygen species and necrotic death-like destruction in strawberry leaves by salinity. *Environmental and Experimental Botany* 65: 270-281 http://doi.org/10.1016/j.envexpbot.2008.09.005Tuberosa R (2012). Phenotyping for drought tolerance of crops in the genomics era. *Frontiers in Physiology* 19: 1-26 http://doi.org/10.3389/fphys.2012.00347

Ukai Y (1989). A microcomputer program DIALL for diallel analysis of quantitative characters. *Japanese Journal of Breeding* 39: 107-109 http://doi.org/10.1270/jsbbs1951.39.107

Xiaoli J, Youzong H, Fanrong Z, Meixue Z & Guoping Z (2009). Genotypic difference in response of peroxidase and superoxide dismutase isozymes and activities to salt stress in barley. *Acta Physiologiae Plantarum* 31: 1103-1109 http://doi.org/10.1007/s11738-009-0328-x

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Agronomic Performance of the Alternative Cereal Species in the Highest Plain of Turkey

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ABSTRACT

Due to their important nutrition and health potential, the interest in einkorn (*Triticum monococcum* L.) and emmer (*Triticum dicoccum* Schrank) wheat, as well as naked barley (*Hordeum vulgare* L. var. *nudum*) species is increasing. The study examined the agricultural properties related to three einkorn wheats, three emmer wheats and two naked barleys, one bread wheat and one hulled barley in spring sowing under irrigated and rain-fed conditions. Depending on irrigated and rain-fed agriculture conditions, the vegetative period of genotypes varied between 59.3-71.7 and 58.2-71.0 days, grain filling period varied between 29.8-38.0 and 26.7-33.8 days, plant height varied between 79.6-105.2 and 79.1-99.0 cm, the number of spike per square-meter varied between 533.3-682.5 and 457.5-573.3, the number of grains per spike varied between 16.1-22.6 and 13.6-20.0, the 1000-kernel weight varied between 31.2-54.6 g and 28.0-47.6 g, the grain yield varied between 2410-4099 kg ha^{-1} and 1716-2660 kg ha^{-1}, and the crude protein content varied between 10.1-13.5% and 10.4-14.8%, respectively. The highest grain yield was obtained from Tokak 157/37 barley cultivar, while the highest crude protein contents were obtained from einkorn genotypes. The number of spike per square-meter, the number of grains per spike, the 1000 kernel weight and the grain yield decreased by 14.6%, 9.4%, 8.7%, and 26.2% respectively, while the crude protein content increased by 8.2% under rain-fed agriculture conditions. It was determined that Özen and Yalın barley varieties could not be an alternative to Tokak 157/37 barley cultivar due to low grain yield and protein content. Einkorn cv. Çatalyazı and emmer wheat cv. Çağlayan in irrigated conditions, and all the einkorn and emmer genotypes in rain-fed conditions were superior to Kırik wheat genotype in terms of grain yield. The genotypes of the einkorn had a significantly higher grain protein content compared to the Kırik and emmer genotypes. It is possible to note that Çatalyazı and Çağlayan genotypes are promising cereals in Erzurum region.

Keywords: Einkorn, Emmer, Naked barley, Yield, Protein

1. Introduction

The first culture forms and local populations of wheat and barley are important as a genetic source for the improvement of these species (Zaharieva & Monneveux 2014) and for breeding quality (Lachman et al. 2012 a, b; Longin & Würschum 2016) and resistance to stress factors (Jaradat 2011; Aslan et al. 2016 a, b) in modern varieties. Einkorn (*Triticum monococcum* L.) and emmer (*Triticum dicoccum* Schrank) hulled wheat species, the first plants that were cultured by humankind, are still cultivated in some countries such as Turkey, the Balkans, Italy, France, Spain, Germany, Switzerland and Austria today (Konvalina et al. 2013). Einkorn populations in Kastamonu, Karabük, Samsun and Bilecik provinces of Turkey have been cultivated, while emmer populations in Kars, Ardahan and Kayseri have generally been produced in high altitude and barren-unproductive areas. They are mainly utilized for cracked wheat, bread, pasta, noodles, tarhana soup and animal feed.

Archaeological findings indicate that hulled wheat species, which have different names such as siyez, kabılca, kavılca, gacer, have grown in Turkey for over 10,000 years. Sowing areas of einkorn and emmer wheat species that cannot compete with modern varieties in yield and gains are gradually decreasing. Statistical data demonstrated that hulled wheat producing area in Turkey was 137,000 ha in 1953, while it was 38,000 ha in 1983 and 3076 ha in 2017. Morgounov et al. (2016) reported that the decrease of genetic diversity of the local wheat population in Turkey ranged from 50% to 70% compared to 1920 and the main reason why local populations are still grown in limited areas is high grain quality and resistance to abiotic stress factors. Einkorn and emmer wheat, also known as ancient wheat species, have been the subject of numerous studies in recent years. These wheats are defined as functional foods with significant potential for human nutrition and health because of their high protein, fiber, mineral, antioxidant and carotenoid values (Borghi et al. 1996; Brandolini et al. 2008; Chatzav et al. 2010; Lachman et al. 2012 a, b). Moreover, these are recommended as alternative crops for low input agriculture and organic farming conditions due to their resistance to some fungal diseases and pests (Konvalina et al. 2010; Zaharieva & Monneveux 2014) and their adaptation to poor nutrient soils (Troccoli & Codianni 2005; Konvalina et al. 2012). Aslan et al. (2016 a) reported that einkorn wheat populations

were more tolerant of cold stress than bread wheat cultivars during germination period while Feng et al. (2018) pointed out that emmer wheat was a potential gene source in salt stress breeding.

The non-adherent husk status of naked barley is a trait that occurs as a result of mutation after cultivation of the barley and is controlled by a recessive single gene locus (nud) (Taketa et al. 2004). Naked barley is the most common culture in the world in China's Tibetan Plateau and Australia, Mexico, Canada, Japan, Syria and the UK are listed as other producer countries (Zeng 2015). The development of naked barley (*Hordeum vulgare* L. var. *nudum*) varieties increases the use of barley in human nutrition with its addition different proportions to bread, pasta and noodles and in the form of soup, porridge, cookies and beverages (Altan et al. 2006; Köten et al. 2013). The interest in naked barley that is a rich source of essential vitamins, minerals and beta-glucan soluble fiber has increased as it is highly recommended as a suitable nutritional option for people with diabetes and cholesterol problems (Kinner et al. 2011; Yan et al. 2016; Sterna et al. 2017).

Although there is an increasing interest in einkorn and emmer wheat as well as naked barley, which have significant potential in terms of nutrition and health, the data on the adaptation of these species to different environmental conditions is limited. Einkorn and emmer populations, which have significant potential for low input agriculture and organic farming are likely to be easily accepted by farmers in the Erzurum region due to their resistance to some diseases and pests and their ability to adapt to poor soils. Three einkorn populations, three emmer populations and two naked barley cultivars, Kırik bread wheat and Tokak 157/37 barley cultivars were tested under Erzurum irrigated and rain-fed agricultural conditions, and some agricultural characteristics and their potential to be alternative products were investigated in this study.

2. Material and Methods

This study was carried out during 2017 and 2018 at Ataturk University Plant Production Application and Research Centre in Erzurum (latitude 39º 55' N, longitude 41º 61' E, 1853 m above sea level), Turkey. The experimental material consisted of 10 cereal genotypes, belonging to different species and including local landraces and the modern varieties, were used in this study (Table 1). In the region, the most common varieties of Kırik and Tokak 157/37 were used as checks. The seeds of landraces were sampled in-place from farmers' current crop year harvest stocks. During the vegetation period, the total rainfall was 138.4 and 285.6 mm, the average temperature was 14.9 and 14.6 °C in 2017 and 2018, respectively (Table 2). The experimental soil was a clay-loam with pH of 7.65-7.81, organic matter content 1.51-1.52%, and total N content 0.088-0.091. Available P and K contents of the soils were 79.2-83.3 and 1237-1267 kg ha^{-1}, respectively. The experiments were sown on 21th April, 2017 and 20th April 2018 in spring. The experimental design was a randomized complete block in a split-plot configuration with three replications. Main plots were irrigated and rain-fed treatments and subplots were the genotypes. In irrigated treatment, plots were irrigated at booting stage and milk stage of plants. In rain-fed treatment, plants were grown under natural conditions. Plots were sown with a planter. The seeding rate in the experiments for all the genotypes was 500 viable seeds per m^{-2}. Subplots consisted of six plant rows spaced 20 cm apart, with a row length of 6.0 m. N as ammonium sulphate was applied to the plots of 60 kg ha^{-1} and P as TSP to the plots of 50 kg ha^{-1}. Half of N and all P were applied at sowing; the second half of N was applied at the beginning of stem elongation. Weeds were controlled by hand.

Table 1- Names and some traits of the alternative cereal species included in the field experiments

	Genotypes	*Origin*	*Some traits*
Einkorn landraces	Çatalyazı	Kastamonu İhsangazi - Çatalyazı Village	Light brown spike, awny, amber grain
	Enbiya	Kastamonu İhsangazi - Enbiya Village	Yellow spike, awny, amber grain
	Musasofular	Bolu Seben - Musasofular Village	White spike, awny, amber grain
Emmer landraces	Yıldırımtepe	Ardahan Çıldır - Yıldırımtepe Village	Brown spike, awny, amber grain
	Çağlayan	Kars - Çağlayan Village	Light brown spike, awny, amber grain
	Şahmelik	Kayseri Develi - Şahmelik Village	White spike, awny, amber grain
Naked barley	Özen	Field Crops Central Research Institute - Ankara	Spring, 2-rows, medium early, medium height, awny, white-amber grain
	Yalın	Field Crops Central Research Institute - Ankara	Alternative, 2-rows, medium late, medium height, awny, white-amber grain
Bread wheat	Kırik	East Anatolian Agricultural Research Institute - Erzurum	Alternative, awnless, white-hard grain
Hulled barley	Tokak 157/37	Field Crops Central Research Institute - Ankara	Alternative, 2-rows, medium early, medium height, awny, white grain

Table 2- Some climate data of study years and long-term mean (LTM: 1990-2016) in Erzurum province

Months	*Total rainfall (mm)*			*Average temperature (°C)*			*Minimum temperature (°C)*		*Maximum temperature (°C)*	
	2017	*2018*	*LTM*	*2017*	*2018*	*LTM*	*2017*	*2018*	*2017*	*2018*
April	44.8	11.0	57.5	5.6	7.4	5.5	-8.4	-18.1	12.7	19.8
May	59.0	140.0	66.3	10.6	11.3	10.5	-1.1	2.3	17.9	21.8
June	12.6	76.8	43.5	15.7	14.6	14.9	1.2	3.7	24.3	30.8
July	6.8	24.8	23.4	20.8	20.1	19.2	6.0	4.2	30.6	24.4
August	15.2	33.0	15.7	21.6	19.8	19.5	4.4	6.3	31.1	32.3
Total/Average	138.4	285.6	206.4	14.9	14.6	13.9				

The length of the vegetative period (VP) was taken as the number of days from sowing to anthesis. The length of the grain-filling period (GFP) was taken as the number of days from anthesis to physiological maturity. Anthesis was defined as the period when 50% of the spikes had anthers extruding, and physiological maturity was defined as the period when 50% of the glumes of the spikes had turned yellow. Plant height was measured from soil surface to top of spikes. Spikes per m^2 were determined from a 1-m row sample. Ten spikes were randomly harvested from within plots for the determination of kernels per spike. At maturity, the plots were trimmed to 4.5 m, and the four inner rows were harvested with a plot combine, and the weight of cleaned grain from each plot was recorded. Kernel weight was determined based on 4x100 kernel samples. Grain protein content was determined by near-infrared spectroscopy (model NIRS DS2500, Foss, Denmark) calibrated based on official AACC method 39-10 (AACC 2010). Grain samples of the einkorn and emmer landraces were dehulled before protein was determined.

The years and treatments were considered to be random, while genotypes were considered to be fixed. The analysis of variance was performed with the SAS GLM (SAS Inst., Cary, NC) software package. When significant genotype effects were detected, Duncan's Multiple Range Test was used to determine the differences among the genotypes. Data were combined over the years and presented as a 2-year mean values.

3. Results and Discussion

The soil moisture content at sowing time was sufficient, and crops usually germinated normally in both years. In the irrigated plots, lodging occurred at the early grain-filling stage and it was visually scored as a percent of the plot. Lodging in the einkorn landraces plots was 20-30%, emmer landraces plots were 40-60%, and the other cultivars plots was 10-20%. In 2017, there was no damage related to pests or diseases. In 2018, rust diseases were observed on all the genotypes and no disease control was done.

The results of the analysis of variance showed that most of the agronomic characteristics (except 1000-kernel weight) were significantly influenced depending on year. Favorable climatic conditions during the growth cycle in 2018 increased vegetative period, grain filling period, plant height, number of spike per square-meter, grain number per spike, grain yield, and harvest index, but decreased crude protein content (Tables 3, 4 and 5). The genotype and treatment factors had a significant effect on all the traits studied. Except for grain number per spike, year x genotype interactions were highly significant in terms of the investigated traits. Year x treatment interactions were significant in terms of the vegetative period, plant height, number of spike per square-meter and 1000-kernel weight. Genotype × treatment interaction effect was significant in terms of the vegetative period, grain filling period, plant height, number of spike per square-meter, 1000-kernel weight, grain yield and harvest index.

3.1. Vegetative period, grain filling period and plant height

Vegetative period and grain-filling period are critical periods in terms of accumulation of adequate storage capacity for grain filling and kernel weight. Vegetative periods of genotypes ranged between 59.3-71.7 days in irrigated and 58.2-71.0 days in rain-fed conditions (Table 3). In both growing conditions, Tokak 157/37 cultivar had the shortest while Enbiya and Yıldırımtepe genotypes had the longest vegetative period. Karagöz & Zencirci (2005) pointed out that the vegetative period of einkorn, emmer and bread wheat were 17.0 days, 11.1 days and 14.0 days, respectively, and there were significant variations among them. Moreover, they reported that the average vegetative period was the shortest bread wheat and longest einkorn type. Ear of emmer wheat emerged eight days before einkorn wheat under Italian conditions (Troccoli & Codianni 2005). Ottekin et al. (1996) reported that naked barley genotypes matured earlier in comparison with hulled barley genotypes. It was determined in this study that einkorn and emmer wheat genotypes had a similar vegetative period with Kırik bread wheat genotype while naked barley had a similar vegetative period with Tokak 157/37 cultivar. Irrigation extended the vegetative period one more day as an average of genotypes, when compared to rain-fed condition.

Grain filling period of genotypes ranged between 29.8-38.0 days in irrigated conditions while it was 26.7-33.8 days in rain-fed (Table 3). The einkorn genotypes had the same grain filling period as the Kırik cultivar whereas naked barley cultivars had a longer grain filling period than Tokak 157/37. The average grain filling period was 34.2 days in irrigation conditions and decreased to 31.0 days in dry farming conditions. It is obvious that moisture insufficiency limits the grain filling period (Frederick & Camberato 1995; Öztürk 1999 a). The longest grain filling period in both irrigated and dry farming conditions was observed

in Yalın and Özen varieties while the shortest grain filling period was observed in Yıldırımtepe and Şahmelik genotypes. The response of genotypes to growing conditions was different in terms of the grain-filling period. Rain-fed conditions reduced the grain filling period to 4.2 days in Özen variety and 1.8 days in Çağlayan population.

Table 3- Vegetative period, grain filling period and plant height of the alternative cereal genotypes grown under irrigated and rain-fed conditions[1]

Genotypes	Vegetative period (days)			Grain filling period (days)			Plant height (cm)		
	Irrigated	Rain-fed	Mean	Irrigated	Rain-fed	Mean	Irrigated	Rain-fed	Mean
Çatalyazı*Ein*	71.2	70.7	70.9^{a}	34.7	31.0	32.8^{b}	97.8	93.5	95.7^{bc}
Enbiya*Ein*	71.5	71.0	71.3^{a}	34.5	31.5	33.0^{b}	105.2	99.0	102.1^{a}
Musasofular*Ein*	71.2	70.3	70.8^{a}	34.5	32.2	33.3^{b}	98.9	95.2	97.1^{b}
Yıldırımtepe*Em*	71.7	71.0	71.3^{a}	29.8	26.7	28.3^{d}	89.3	93.1	91.2^{d}
Çağlayan*Em*	65.7	64.3	65.0^{c}	34.0	32.2	33.1^{b}	94.1	90.8	92.5^{cd}
Şahmelik*Em*	71.2	67.5	69.3^{b}	30.8	26.7	28.8^{d}	93.7	92.0	92.8^{cd}
Yalın*Nb*	64.3	64.2	64.3^{d}	37.5	33.7	35.6^{a}	79.6	79.1	79.3^{e}
Özen *Nb*	64.7	64.8	64.8^{cd}	38.0	33.8	35.9^{a}	96.8	86.2	91.5^{d}
Kırık*Bw*	71.2	70.3	70.8^{a}	34.7	32.2	33.4^{b}	99.8	96.5	98.1^{b}
Tokak 157/37*Hb*	59.3	58.2	58.8^{e}	33.7	30.5	32.1^{c}	83.3	80.3	81.8^{e}
Mean	68.2^{a}	67.2^{b}	67.7	34.2^{a}	31.0^{b}	32.6	93.8^{a}	90.6^{b}	92.2
2017	67.0	65.5	66.3^{b}	33.4	30.2	31.8^{b}	84.1	76.0	80.1^{b}
2018	69.4	69.0	69.2^{a}	35.1	31.8	33.5^{a}	103.6	105.1	104.4^{a}
F values									
Year (Y)			5104.2**			280.0**			1906.9**
Genotype (G)			770.9**			196.1**			47.1**
Treatment (T)			541.5**			1042.3**			34.2**
Y x G			10.5**			3.6**			25.6**
Y x T			81.5**			0.3			74.9**
G x T			11.6**			5.0**			3.3**
Y x G x T			9.4**			2.9**			2.2*
CV (%)			0.8			1.9			3.9

[1]The means marked with the same letter are not significantly different; F values marked with * and ** are significant at 0.05 and 0.01 levels, respectively.
(*Ein: einkorn, Em: emmer, Nb: naked barley, Bw: bread wheat, Hb: hulled barley)*

Plant heights of genotypes were measured between 79.6 and 105.2 cm in irrigated conditions and 79.1and 99.0 cm in rain-fed conditions. (Table 3). In both growing conditions, the longest plant height was measured in Enbiya genotype and the shortest height in Yalın cultivar. Genotypes may differ in terms of plant height depending on vegetative period length, the number of internodes and internode length. It is possible to note that Troccoli & Codianni (2005) measured a longer plant height with 116 cm in einkorn and 127 cm in emmer, when compared to the results of this study. Karagöz & Zencirci (2005) determined that the plant height of einkorn and emmer wheat genotypes changed between 79.1-104.4 and 63.8-102.1 cm, respectively. In addition, the plant height of hulled and naked barley genotypes was determined to change between 50-84 cm and 38-82 cm, respectively by Ottekin et al. (1996). The value of hulled barley was respectively measured as 46.9-73.7 cm and 40.9-56.1 cm by Tobiasz-Salach et al. (2012) and Öztürk et al. (2001). The findings obtained in our study in terms of plant height was generally in agreement with the findings of these researchers. Plant height as the average of genotypes was 93.8 cm in irrigated conditions. However, it decreased significantly in rain-fed conditions and was determined as 90.6 cm. Plant height, which is a drought-sensitive character (Gomez-Macpherson & Richards 1995), is mainly affected by environmental conditions between booting and heading. During this period, the lack of moisture reduces plant height by shortening the nodes. Although the lack of moisture in late development periods had a less negative impact on plant height, plant height decreased significantly in rain-fed conditions.

3.2. Spike number per m^2, grain number per spike and 1000-kernel weight

The spike number per m^2 of genotypes ranged between 533.3-682.5 in irrigated and 457.5-573.3 in dry farming conditions. The highest number of spikes per square meter was recorded in Musasofular and Çatalyazı and the lowest was in Özen and Yalın genotypes (Table 4). The number of spike per square-meter of genotypes may diverge depending on the degree of tillering and the ability to maintain fertile tiller until harvest. The spike number of einkorn and emmer genotypes in this study was higher than the values (416-442) obtained by Troccoli & Codiannni (2005) under Italian conditions. The spike number of naked genotypes was higher than the values (196-389) obtained in Iran conditions by Balouchi et al. (2005). However, it was similar to the values (413-513) obtained in Poland conditions by Tobiasz-Salach et al. (2012). Number of spike per square-meter, which was 593.3 in irrigated conditions, was 506.8 due to the decrease in the fertility tiller rate in rain-fed conditions. The number of potential spikes in the unit area is mainly determined by the development processes and environmental conditions up to the beginning of the booting. However, the ability to maintain the number of fertile tillers until harvest is an important attribute that contributes to grain yield in rain-fed conditions (Öztürk 1999 b).

Table 4- Spike number per m^2, grain number per spike and 1000-kernel weight of the alternative cereal genotypes grown under irrigated and rain-fed conditions[1]

Genotypes	Spike number per m^2			Grain number per spike			1000-kernel weight (g)		
	Irrigated	Rain-fed	Mean	Irrigated	Rain-fed	Mean	Irrigated	Rain-fed	Mean
Çatalyazı*Ein*	618.3	573.3	595.8^{ab}	16.4	14.3	15.4^{d}	32.0	28.0	30.0^{g}
Enbiya*Ein*	638.3	552.5	595.4^{ab}	16.1	13.6	14.8^{d}	34.5	30.0	32.2^{f}
Musasofular*Ein*	682.5	546.7	614.6^{a}	16.6	14.2	15.4^{d}	34.4	31.2	32.8^{ef}
Yıldırımtepe*Em*	605.0	540.8	572.9^{bc}	18.9	17.0	18.0^{c}	36.2	33.3	34.7^{d}
Çağlayan*Em*	579.2	514.2	546.7^{cd}	19.7	18.7	19.2^{bc}	35.2	32.9	34.0^{de}
Şahmelik*Em*	580.8	468.3	524.6^{d}	21.0	19.3	20.1^{ab}	38.5	36.1	37.3^{c}
Yalın*Nb*	533.3	483.3	508.3^{d}	21.0	18.5	19.8^{abc}	41.6	39.3	40.5^{b}
Özen *Nb*	575.8	457.5	516.7^{d}	20.0	18.9	19.5^{abc}	42.0	39.9	40.9^{b}
Kırık*Bw*	566.7	466.7	516.7^{d}	22.6	20.0	21.3^{a}	31.2	29.1	30.2^{g}
Tokak 157/37*Hb*	552.5	464.2	508.3^{d}	20.1	19.7	19.9^{ab}	54.6	47.6	51.1^{a}
Mean	593.3^{a}	506.8^{b}	550.0	19.2^{a}	17.4^{b}	18.3	38.0^{a}	34.7^{b}	36.4
2017	538.3	478.2	508.3^{b}	17.6	16.7	17.1^{b}	38.7	34.0	36.3
2018	648.2	535.3	591.8^{a}	20.9	18.2	19.5^{a}	37.4	35.5	36.4
F values									
Year (Y)			428.1**			45.2**			0.1
Genotype (G)			18.6**			25.5**			295.6**
Treatment (T)			459.5**			26.5**			86.8**
Y x G			9.8**			0.9			58.5**
Y x T			42.6**			6.4			16.0*
G x T			2.6*			0.7			4.2**
Y x G x T			2.2*			0.2			3.6**
CV (%)			6.0			8.7			3.6

[1]The means marked with the same letter are not significantly different; F values marked with * and ** are significant at 0.05 and 0.01 levels, respectively.
(*Ein: einkorn, Em: emmer, Nb: naked barley, Bw: bread wheat, Hb: hulled barley)*

Grain number of genotypes varied from 16.1 to 22.6 in irrigated condition while it varied from 13.6 to 20.0 in rain-fed condition. In this study, it was found that Kırık, Şahmelik and Yalın genotypes in irrigated condition and Kırık, Tokak 157/37 and Şahmelik genotypes in rain-fed condition had the highest grain number (Table 4). Moreover, the lowest grain number per spike was observed in einkorn genotypes under both growth conditions. Since the number of the fertile spikelet in the spike and the number of fertile flowers in the spikelet differ from genotype to genotype, significant differences may occur between genotypes in terms of grain number in the spike. The number of grains per spike for einkorn and emmer wheat was determined to change between 13.6-36.6 and 15.4-39.6, respectively in Ankara condition by Karagöz & Zencirci (2005) and again ranged from 13.8 to 17.8, respectively in Czech Republic condition by Konvalina et al. (2010). In the studies on naked barley genotypes, the number of grains per spike for genotypes was reported to be between 21 and 54 under Iranian condition (Balouchi et al. 2005) and 14.7-23.6 under Polish conditions (Tobiasz-Salach et al. 2012). Drought can limit the number of grains in the spike by reducing both the number of the spikelet in the spike and flowers in the spikelet and causing the death of fertilized flowers. The number of grains per spike was 19.2 in irrigated conditions, whereas it was recorded as 17.4 in rain-fed agriculture. Although the number of potential grains in spike is mainly determined by pre-spike development processes and environmental conditions, the deficiency of moisture after anthesis may reduce the number of fertile flowers in spikes away from the center of the spike. Both Öztürk (1999a) and Bogale & Tesfaye (2011) reported that the late drought significantly reduced the grain number of a spike compared to irrigated conditions.

According to the results obtained in this study, the 1000-kernel weight of genotypes ranged between 31.2 and 54.6 g in irrigated and 28.0 and 47.6 g in rain-fed conditions. Tokak 157/37 cultivar had the highest 1000-kernel weight in both growing conditions. On the other hand, the lowest 1000-kernel weight was determined in Kırık genotype in irrigated condition and Çatalyazı genotypes in rain-fed agricultural condition (Table 4). The grain weight is also influenced by the dynamic balance between the yield components, but is mainly determined by post-flowering development processes and environmental conditions (Wiegand et al. 1981). Grain weight is a common function of the grain filling period and grain filling rate, and genotypic variation in these characters gives rise to significant differences in terms of 1000 grain weight. 1000-kernel weights for einkorn and emmer wheat genotypes were determined between 28.5 and 38.8 g and 29.5 and 34.7 g, respectively, by Karagöz & Zencirci (2005) and these findings were similar to ours. Unlike our findings, Konvalina et al. (2010) reported that the kernel weight was between 23.8 and 28.1 g and 36.9 and 46.1 g for these genotypes, respectively. This value in the studies conducted on genotypes of naked barley was determined to be between 29.0 and 43.3 g, 24 and 47 g and 37.3 and 49.4 g by Ottekin et al. (1996), Balouchi et al. (2005) and Tobiasz-Salach et al. (2012), respectively, and significant genotypic differences were noted. The shortage of moisture decreases 1000 grain weight by reducing grain filling period and increasing leaf senescence (Öztürk 1999 b; Hafsi et al. 2000). The 1000-kernel weight was 38.0 g in irrigated condition with respect to the average of crop years and genotypes, but decreased in all genotypes under rain-fed conditions and was an average of 34.7 g.

3.3. Grain yield, harvest index and crude protein content

The more favorable climatic conditions in 2018 increased number of spike per square-meter and the number of grains per spike, and the grain yield was significantly higher than the one in 2017. Grain yields of genotypes ranged between 2410-4099 kg ha^{-1} in irrigated conditions and 1716-2660 kg ha^{-1} in rain-fed conditions (Table 5). Tokak 157/37 cultivar had the highest grain yield in irrigated and rain-fed conditions, followed by Özen and Yalın naked barley varieties. The lowest grain yields were obtained from Yıldırımtepe and Şahmelik genotypes in irrigated conditions and Kırik and Enbiya genotypes in rain-fed conditions. Grain yield of Kırik genotype under rain-fed condition was similar to the values (1635-1734 kg ha^{-1}) obtained by Öztürk et al. (2006) under Erzurum condition while its grain yield in irrigated condition was higher than the value (2470 kg ha^{-1}) obtained by Salantur et al. (2006). Grain yield of Tokak 157/37 cultivar is higher than the values obtained by Öztürk et al. (2001) and Çağlar et al. (2009) (2576 and 2250 kg ha^{-1}, respectively) in Erzurum irrigated agricultural conditions. In this study, grain yields obtained from einkorn (1895-3025 kg ha^{-1}) and emmer (2228-3095 kg ha^{-1}) genotypes were lower than the yields determined in einkorn (1895-3025 kg ha^{-1}) and emmer (2228-3095 kg ha^{-1}) genotypes by Kaplan et al. (2014) under Kayseri condition. Troccoli & Codianni (2005) obtained 1690 and 3850 kg ha^{-1} grain yields from einkorn and emmer populations in Italy condition, respectively. The grain yields of emmer genotypes were determined to range between 2579-3293 kg ha^{-1} in the Czech Republic (Konvalina et al. 2012) and 2480-2500 kg ha^{-1} in the US condition (Kucek et al. 2017). The grain yield in naked barley was reported between 1617 and 3420 kg ha^{-1} in Ankara condition (Ottekin et al. 1996) and 3390 and 4510 kg ha^{-1} in England condition (Dickin et al. 2012). Grain yield of 3007 kg ha^{-1} in irrigated condition decreased by 26.2% as a result of the negative effect of moisture insufficiency on yield components (spike number per m^2, grain number per spike and grain weight reduction rates 14.6%, 9.4% and 8.7%, respectively), and it was identified as 2218 kg ha^{-1} under rain-fed conditions. Öztürk (1999a) determined that grain yield of winter bread wheat decreased by 30.5% in rain-fed agricultural conditions compared to irrigated agricultural conditions. The grain yields of all genotypes in this study reduced in rain-fed conditions compared to irrigated agricultural conditions. Grain yield losses in rain-fed conditions may vary according to genotypes owing to differences in the response of genotypes to rain-fed conditions in terms of grain yield. The decrease rates of grain yield in rain-fed agricultural conditions were the lowest in Yıldırımtepe (7.6%) and Şahmelik (8.3%) genotypes while the highest in Kırik (40.4%) and Tokak 157/37 (35.1%) varieties.

Table 5- Grain yield, harvest index and crude protein content of the alternative cereal genotypes grown under irrigated and rain-fed conditions[1]

Genotypes	Grain yield (kg ha^{-1})			Harvest index (%)			Crude protein content (%)		
	Irrigated	Rain-fed	Mean	Irrigated	Rain-fed	Mean	Irrigated	Rain-fed	Mean
Çatalyazı^{Ein}	3025	1989	2507^{bcd}	25.8	20.8	23.3^{b}	13.0	14.7	13.8^{a}
Enbiya^{Ein}	2793	1895	2344^{d}	24.6	21.7	23.2^{b}	13.2	14.8	14.0^{a}
Musasofular^{Ein}	2749	2027	2388^{cd}	24.4	22.6	23.5^{b}	13.5	14.5	14.0^{a}
Yıldırımtepe^{Em}	2410	2228	2319^{d}	24.7	24.5	24.6^{b}	11.7	13.0	12.3^{cd}
Çağlayan^{Em}	3095	2259	2677^{bcd}	25.8	22.4	24.1^{b}	12.8	13.8	13.3^{ab}
Şahmelik^{Em}	2576	2363	2469^{cd}	25.7	24.1	24.9^{b}	12.3	13.0	12.6^{bc}
Yalın^{Nb}	3097	2536	2816^{bc}	30.1	27.3	28.7^{a}	10.1	10.4	10.3^{e}
Özen ^{Nb}	3346	2508	2927^{b}	28.4	27.8	28.1^{a}	10.9	12.7	11.8^{d}
Kırik^{Bw}	2881	1716	2299^{d}	27.8	19.7	23.7^{b}	12.5	13.4	13.0^{bc}
Tokak 157/37^{Hb}	4099	2660	3380^{a}	34.1	22.7	28.4^{a}	11.6	12.0	11.8^{d}
Mean	3007^{a}	2218^{b}	2612	27.1^{a}	23.4^{b}	25.3	12.2^{b}	13.2^{a}	12.7
2017	2613	1727	2170^{b}	23.6	20.3	22.0^{b}	12.5	13.6	13.0^{a}
2018	3401	2709	3055^{a}	30.7	26.4	28.5^{a}	11.8	12.9	12.4^{b}
F values									
Year (Y)			459.5**			55.0**			27.5**
Genotype (G)			10.8**			2.9**			33.3**
Treatment (T)			365.4**			18.2*			72.3**
Y x G			6.9**			2.8**			2.3*
Y x T			5.5			0.3			0.1
G x T			3.5**			1.8			1.6
Y x G x T			1.2			1.2			0.6
CV (%)			14.0			18.0			5.6

[1]The means marked with the same letter are not significantly different; F values marked with * and ** are significant at 0.05 and 0.01 levels, respectively.
(*Ein: einkorn, Em: emmer, Nb: naked barley, Bw: bread wheat, Hb: hulled barley)*

Harvest indexes of genotypes ranged between 24.4 and 34.1% in irrigated and 19.7 and 27.8% in rain-fed conditions. The highest harvest indexes in irrigated agricultural conditions were obtained from Tokak 157/37, Özen and Yalın genotypes, respectively, whereas the lowest ones were obtained from Musasofular and Enbiya genotypes. Yalın and Özen genotypes had the highest harvest index in rain-fed agricultural condition while Kırik and Çatalyazı genotypes had the lowest harvest index. As a result of differences in total dry matter production and assimilate distribution, genotypes may be significantly different in point of harvest index (Karimi & Siddique 1991). The recent breeding strategy has increased the number of grains per unit area and the harvest index with high plant fertility, stiffness of the plant, shortening of plant height, and high spike fertility. The recent breeding strategy in cereals has led to an increase in the resistance to lodging due to shortening of plant height, and also in harvest index and grain number per unit area due to more spike fertility (Guarda et al. 2004). Öztürk (1999 a) found that wheat genotypes

were significantly different in way of harvest index and also calculated them between 29.9-41.3% in irrigated and 30.4-38.5% in rain-fed conditions. The harvest indexes in einkorn genotypes and emmer genotypes, respectively, were determined to range from 29.9% to 36.3% and from 27.6% to 33.3% by Kaplan et al. (2014), while they respectively changed from 30% to 40% and from 30% to 40% according to Konvalina et al. (2010). These values are higher than our findings. Furthermore, while the harvest index was 27.1% in irrigated conditions, it significantly decreased and was 23.4% in rain-fed agricultural conditions. The negative effect of moisture deficiency on grain yield is more than the negative effect on stem yield. Other researchers also reported that a moisture deficiency reduced the harvest index (Öztürk 1999a; Bogale & Tesfaye 2011).

The fact that the post-heading period was cooler and more humid in the second year of the research caused the crude protein ratio to be lower compared to 2017. While the crude protein contents of grain genotypes ranged between 10.1% and 13.5% in irrigated condition; 10.4% and 14.8% in rain-fed condition. The lowest crude protein content was determined in Yalın cultivar in both growing conditions. Musasofular had the highest crude protein ratio in irrigated condition, followed by other Enbiya and Çatalyazı einkorn wheat genotypes. As in irrigated condition, the highest crude protein content in rain-fed condition was obtained from einkorn wheat populations such as Enbiya, Çatalyazı and Musasofular. The grain protein content is the most important indicator of the nutritional value and quality of the product and it is likely to be considered as alternative cereal genotypes in this aspect. In terms of grain protein ratio, the einkorn wheat type was the most superior, emmer genotypes and Kırik cultivar was similar in the middle order and barley varieties were in the last order with the lowest crude protein contents. The crude protein contents obtained from einkorn species were similar to the values (7.30-15.99%, 14.2-16.6% and 11.6-13.9%, respectively) reported by Kaplan et al. (2014), Konvalina et al. (2013) and Geisslitza et al. (2018), while they were lower than the value (15.5-22.8%) reported by Brandolini et al. (2008). Crude protein contents of emmer populations were significantly lower than the values that varied between 16.1%-19.0% according to Konvalina et al. (2012), 14.7%-18.9% according to Konvalina et al. (2013), and 14.2%-15.0% according to Kucek et al. (2017), while they were similar to the values (11.2-12.4%) reported by Geisslitza et al. (2018). In naked barley genotypes, the values which were determined as 13.2-19.5% by Ottekin et al. (1996), 12.55-15.92% by Helm & Francisco (2004) and 12.6-16.1% by Tobiasz-Salach et al. (2012) were higher than ours. However, Balouchi et al. (2005) determined lower protein contents (7.21-11.61%) in comparison with our results. The crude protein content was 12.2% in irrigated condition, whereas it significantly increased and reached up to 13.2% in rain-fed conditions. It is clear that lack of moisture after anthesis increases the amount of nitrogen accumulated in the grain per unit starch by reducing the synthesis and storage of carbohydrates (Panozzo & Eagles 2000, Öztürk & Aydin 2004).

4. Conclusions

This study provides important data about the agricultural potential of einkorn wheat, emmer wheat and naked barley genotypes which were grown in spring under irrigated and rain-fed agriculture conditions. The highest grain yield was obtained from Tokak 157/37 barley cultivar and the highest protein ratio from einkorn genotypes. When the average of genotypes was taken into consideration, it is possible to state that the number of spike per square-meter, grain number per spike, 1000-kernel weight and grain yield decreased by 14.6%, 9.4%, 8.7% and 26.2%, respectively in rain-fed condition compared to irrigated condition while crude protein content increased by 8.2%. It was concluded that naked barley cultivars of Özen and Yalın cannot be an alternative to Tokak 157/37 barley cultivar due to low grain yield and protein ratios. Çatalyazı and Çağlayan genotypes in irrigated condition and all einkorn and emmer genotypes in rain-fed agriculture condition were superior to Kırik cultivar in terms of grain yield. The genotypes of the einkorn had a significantly higher grain protein content compared to the Kırik and emmer genotypes, which had similar protein content. It is possible to state that Çatalyazı and Çağlayan genotypes are more promising than Kırik cultivar to get more economical summer cereal production in Erzurum region. The applicability of winter planting, optimum seeding rate and nitrogen dose may be useful for achieving higher yields in these genotypes. The most important problem regarding these genotypes is the high risk of lodging. Considering their high adaptability to poor soils, the einkorn and emmer genotypes that are appropriately chosen have the potential to be important alternative for farmers in summer planting under low input farming and organic farming conditions.

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References

Altan A, Yağcı S, Maskan M & Göğüş F (2006). Evaluation of barley products. 9th *Turkey Food Congress*, 24-26 May 2006, Bolu s. 495-498 (in Turkish)

American Association of Cereal Chemist (2010). Approved methods of the AACC. St. Paul, MN, USA

Aslan D, Ordu B & Zencirci N (2016 a). Einkorn wheat (*Triticum monococcum* ssp. *monococcum*) tolerates cold stress better than bread wheat (Triticum aestivum L.) during germination. *Journal of Central Research Institute for Field Crops* 25(2): 182-192 https://doi.org/10.1016/j.jcs.2021.103177

Aslan D Zencirci N, Etöz M, Ordu B & Bataw S (2016 b). Bread wheat responds salt stress better than einkorn wheat does during germination. *Turk J Agric For* 40: 783-794 https://doi.org/10.3906/tar-1604-59

Balouchi H R, Sarvestani Z T & Sanavy S A M M (2005). Agronomic factors on selected hulles barley genotypes. *Journal of Agronomy* 4(4): 333-339

Bogale A & Tesfaye K (2011). Relationships between kernel ash content, water use efficiency and yield in durum wheat under water deficit induced at different growth stages. *African J. Basic Appl. Science* 3(3): 80-86

Borghi B, Castagna R, Corbellini M, Heun M & Salamini F (1996). Breadmaking quality of einkorn wheat (*Triticum monococcum* ssp. *monococcum*). *Cereal Chem* 73(29): 208-214 https://doi.org/10.1016/s1161-0301(14)80038-5

Brandolini A, Hidalgo A & Moscaritolo S (2008). Chemical composition and pasting properties of einkorn (*Triticum monococcum* L. ssp. *monococcum*) whole meal flour. *Journal of Cereal Science* 47: 599-609 https://doi.org/10.1016/j.jcs.2007.07.005

Chatzav M, Peleg Z, Ozturk L, Yazici A, Fahima T, Cakmak I & Saranga Y (2010). Genetic diversity for grain nutrients in wild emmer wheat: potential for wheat improvement. *Annals of Botany* 105: 1211-1220 https://doi.org/10.1093/aob/mcq024

Çağlar Ö, Öztürk A & Bulut S (2006). Adaptation of some bread wheat cultivars in Erzurum Plain conditions. *Atatürk University, Journal of Agriculture Faculty* 37(1): 1-7 (in Turkish with abstract in English)

Dickin E, Steele K, Edwards-Jones G & Wright D (2012). Agronomic diversity of naked barley (*Hordeum vulgare* L.): A potential resource for breeding new food barley for Europe. *Euphytica* 184: 85-99 https://doi.org/10.1007/s10681-011-0567-y

Feng K, Cui L, Lv S, Bian J, Wang M, Song W & Nie X (2018). Comprehensive evaluating of wild and cultivated emmer wheat (Triticum turgidum L.) genotypes response to salt stress. *Plant Growth Regul* 84: 261-273 https://doi.org/10.1007/s10725-017-0337-5

Frederick J R & Camberato J J (1995). Water and nitrogen effects on winter in the Southeastern Coastal Plain: II. Physiological respons. *Agronomy Journal* 87: 521-526 https://doi.org/10.2134/agronj1995.00021962008700030021x

Geisslitza S, Wieser H, Scherfa K.A & Koehler P (2018). Gluten protein composition and aggregation properties as predictors for bread volume of common wheat, spelt, durum wheat, emmer and einkorn. *Journal of Cereal Science* 74: 19-27 https://doi.org/10.1016/j.jcs.2018.08.012

Gomez-Macpherson H & Richards R A (1995). Effects of sowing time on yield and agronomic characteristics of wheat in south-eastern Australia. *Aust J. Agric. Research* 46(7): 1381-1399 https://doi.org/10.1071/ar9951381

Guarda G, Padovan S & Delogu G (2004). Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. *European Journal of Agronomy* 21(2): 181-192 https://doi.org/10.1016/j.eja.2003.08.001

Hafsi M, Mechmeche W, Bouamama L, Djekoune A, Zaharieva M & Monneveux P (2000). Flag leaf senescence, as evaluated by numerical image analysis, and its relationship with yield under drought in durum wheat. *Journal of Agronomy and Crop Science* 185(4): 275-280 https://doi.org/10.1046/j.1439-037x.2000.00436.x

Helm C V & Francisco A D (2004). Chemical characterization of brazilian hulless barley varieties, flour fractionation, and protein concentration. *Sci. Agric. (Piracicaba, Braz.)* Nov./Dec 61(6): 593-597 https://doi.org/10.1590/s0103-90162004000600005

Jaradat A A (2011). Ecogeography, genetic diversity, and breeding value of wild emmer wheat (*Triticum dicoccoides*). *AJCS* 5(9): 1072-1086

Kaplan M, Akar T, Kamalak A & Bulut S (2014). Use of diploid and tetraploid hulled wheat genotypes for animal feeding. *Turk J Agric For* 38: 838-846 https://doi.org/10.3906/tar-1401-20

Karagöz A & Zencirci N (2005). Variation in wheat (*Triticum* spp.) landraces from different altitudes of three regions of Turkey. *Genetic Resources and Crop Evolution* 52: 775-785 https://doi.org/10.1007/s10722-004-3556-3

Karimi M M &Siddique K H M (1991). Crop growth and relative growth rates of old and modern wheat cultivars. *Australian Journal of Agricultural Research* 42(1): 13-20 https://doi.org/10.1071/ar9910013

Kinner M, Nitschko S, Sommeregger J, Petrasch A, Linsberger-Martin, G, Grausgruber H, Berghofer E & Siebenhandl-Ehn S (2011). Naked barley optimized recipe for pure barley bread with sufficient beta-glucan according to the EFSA health claims. *Journal of Cereal Science* 53: 225-230 https://doi.org/10.1016/j.jcs.2011.01.001

Konvalina P, Capouchova I, Stehno Z & Moudry J (2010). Agronomic characteristics of the spring forms of the wheat landraces (einkorn, emmer, spelt, intermediate bread wheat) grown in organic farming. *J. Agrobiol* 27(1): 9-17 https://doi.org/10.2478/s10146-009-0002-3

Konvalina P, Capouchova I & Stehno Z (2012). Agronomically important traits of emmer wheat. *Plant Soil Environ* 58(8): 341-346 https://doi.org/10.17221/174/2011-pse

Konvalina P, Capouchová I & Stehno Z (2013). Baking quality of hulled wheat species in organic farming. *Nutrition and Food Engineering* **7**(6): 370-373 https://doi.org/10.5772/29330

Köten M, Ünsal A S & Atlı A (2013). Evaluation of barley as human food. *Turkish Journal of Agriculture-Food Science and Technology* 1(2): 51-55 (in Turkish with abstract in English) https://doi.org/10.24925/turjaf.v1i2.51-55.25

Kucek L K, Dyck E, Russell J, Clark L, Hamelman J, Burns-Leader S, Senders S, Jones J, Benscher D, Davis M, Roth G, Zwinger S, Sorrells M E & Dawson J C (2017). Evaluation of wheat and emmer varieties for artisanal baking, pasta making, and sensory quality. *Journal of Cereal Science* 74: 19-27 https://doi.org/10.1016/j.jcs.2016.12.010

Lachman J, Orsak M, Pivec V & Jiru K (2012 a). Antioxidant activity of grain of einkorn (*Triticum monococcum* L.), emmer (*Triticum dicoccum* Schuebl [Schrank]) and spring wheat (*Triticum aestivum* L.) varieties. *Plant Soil Environ* 58(1): 15-21 https://doi.org/10.17221/300/2011-pse

Lachman J, Musilova J, Kotikova Z, Hejtmankova K, Orsak M & Pribyl J (2012 b). Spring, einkorn and emmer wheat species – potential rich sources of free ferulic acid and other phenolic compounds. *Plant Soil Environ* 58(8): 347-353 https://doi.org/10.17221/289/2012-pse

Longin C F H & Würschum T (2016). Back to the future - Tapping into ancient grains for food diversity. *Trend Plant Science* 21: 731-737 https://doi.org/10.1016/j.tplants.2016.05.005

Morgounov A, Keser M, Kan M, Küçükçongar M, Özdemir F, Gummadov N, Muminjanov H, Zuev E & Qualset C O (2016). Wheat landraces currently grown in Turkey: distribution, diversity, and use. *Crop Sci*ence 56: 3112-3124 https://doi.org/10.2135/cropsci2016.03.0192

Ottekin A, Akar T, Tosun H, Ozan A N & Demir Z (1996). Determination of agronomic and quality characteristics of hulless barley. 4th National Nuclear Agriculture and Livestock Congress, 25-27 September 1996, Bursa pp.103-108 (in Turkish with abstract in English)

Öztürk A (1999a). Drought resistance in bread wheat genotypes. *Turk J. Agric. For*. 23 (supplement issue 5): 1237-1247 (in Turkish with abstract in English)

Öztürk A (1999b). The effect of drought on the growth and yield of winter wheat. *Turk J. Agric. For*. 23(5): 531-540 (in Turkish with abstract in English)

Öztürk A, Çağlar Ö & Tufan (2001). Adaptation of some barley cultivars in Erzurum condition. *Atatürk University, Journal of Agriculture Faculty* 32(2): 109-115 (in Turkish with abstract in English)

Öztürk A & Aydın F (2004). Effect of water stress at various growth stages on some quality characteristics of winter wheat. *Journal of Agronomy and Crop Science* 190(2): 93-99 https://doi.org/10.1046/j.1439-037x.2003.00080.x

Öztürk A, Çağlar Ö & Bulut S (2006). Growth and yield response of facultative wheat to winter sowing, freezing sowing and spring sowing at different seeding rates. *J. Agronomy & Crop Science* 192: 10-16 https://doi.org/10.1111/j.1439-037x.2006.00187.x

Panozzo J F & Eagles H A (2000). Cultivar and environmental effects on quality characters in wheat. II. Protein. *Aust. J. Agric. Research* 51: 629-636 https://doi.org/10.1071/ar99137

Salantur A, Öztürk A, Akten Ş, Şahin F & Dönmez M F (2006). Growth and yield response of spring wheat Triticum aestivum L to inoculation with rhizobacteria. *Plant Soil Environ* 3: 111-118 https://doi.org/10.17221/3354-pse

Sterna V, Zute S, Jansone I & Kantane I (2017). Chemical composition of covered and naked spring barley varieties and their potential for food production. *Pol. J. Food Nutr. Science* 67(2): 151-158 https://doi.org/10.1515/pjfns-2016-0019

Taketa S, Kikuchi S, Awayama T, Yamamoto S, Ichii M & Kawasaki S (2004). Monophyletic origin of naked barley inferred from molecular analyses of a marker closely linked to the naked caryopsis gene (nud). *Theor Appl Genet* 108: 1236-1242 https://doi.org/10.1007/s00122-003-1560-1

Tobiasz-Salach R, Jankowska D, Bobrecka-Jamro D & Buczek J (2012). Grain yield and chemical composition of new, naked lines of barley grown in conditions of subcarpathian region. *Acta Sci. Pol., Agricultura* 11(1): 99-111 https://doi.org/10.2478/v10081-009-0004-2

Troccoli A & Codianni P (2005). Appropriate seeding rate for einkorn, emmer, and spelt grown under rainfed condition in southern Italy. *Europ J Agron* 22: 293-300 https://doi.org/10.1016/j.eja.2004.04.003

Wiegand C L & Cuellar J A (1981). Duration of grain filling and kernel weight of wheat as affected by temperature 1. *Crop Science* 21(1): 95-101 https://doi.org/10.2135/cropsci1981.0011183x001100010027x

Yan W, Yao H, Nie S & Li Y (2016). Mineral analysis of hulless barley grown in different areas and its β-glucan concentrates. *Cogent Food and Agriculture* 2: 1-8 https://doi.org/10.1080/23311932.2016.1186139

Zaharieva M & Monneveux P (2014). Cultivated einkorn wheat (*Triticum monococcum* L. ssp. *monococcum*): the long life of a founder crop of agriculture. *Genet Resour Crop Evol* 61:677-706 https://doi.org/10.1007/s10722-014-0084-7

Zeng X Q (2015). Genetic variability in agronomic traits of a germplasm collection of hulless barley. *Genetics and Molecular Research* 14(4): 18356-18369 https://doi.org/10.4238/2015.december.23.23

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Determining the Effects of Climate Change and Market Prices on Farm's Structure by Using an Agent Based Model

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ABSTRACT
In this study, an agent-based model was used to simulate structure change of farms during 20 years period of climate and market price changes in the rural area of Eslamshahr City in Iran. Decision rules that used in the model are based on the information that collected by direct interviews with farmers. So the model includes rules that define the relationship between agents and their environment. Results clearly showed that farmers' behavior patterns and the cover of agricultural land in the region affected by environmental and market factors changes. Comparison of the results of model implementation for various scenarios has shown that the highest yield and income loss has occurred in scenarios where there was a 10% reduction in access to water. Also, there is a less decrease in the crops land size in groups which includes small and medium farmers.

Keywords: Agent based, Climate, Farm Structure, Land use, Iran

1. Introduction

The social, economic and spatial dynamics of rural regions are often affected by the processes and dynamics of the agricultural farm structure changes. These dynamics are complex processes caused by the interaction between natural and social systems at different scales. The role that a farm takes within this complex process depends not only on the farm's characteristics, the characteristics of the farmer or farm manager but also on local competition, as well as the economic, institutional and environmental conditions. For an adequate understanding of the underlying processes, it is important to capture not only the interactions amongst and between farms and their environment but also the farms' behavior, and their decision processes (Appel & Balmann 2018).

The ongoing internal and external pressure on farmers has resulted in the fluctuation of gross margins, income, and a continuous change in the number of farmers in the region. Understanding these significant trends and their impact on the farm structure requires a deeper knowledge of the mechanisms involved and the impacts of different policy measures (Beckers et al. 2018). The core theme of agriculture structural change science is to understand the dynamics of the farmer's decision-making rules according to these trends (Schindler 2009). As mentioned above, the driving forces can be categorized as endogenous and exogenous processes of a region. Endogenous processes are socio-economic and biophysical conditions of farms in a specific region including farmer's experience, preferences, economic condition, and land fertility. Exogenous processes are those occurring at global, national and regional scales, varying from changes in the market prices to climate change and policy frameworks (Valbuena et al. 2010).

Agriculture is one of the most climate-sensitive sectors and directly affected by changes in climate conditions. Farmers may implement climate change adaptation measures to reduce or avoid adverse developments and take advantage of emerging opportunities. Others may forbear to adapt which results in a lack of timely adaptation. On the other hand, the volatility and price imbalance of agricultural products and inputs as an exogenous factor influences farmer's income and expenditure situation and, consequently, the welfare of their lives. The change in farmers' welfare status will shape the decision-making process and the selection of activity options in the upcoming period. Farmers' adaptation decisions -such as other human behavior- is influenced by the individual characteristics and economic and social conditions of the farmer (Mitter et al. 2019). Agriculture in Iran is also affected by market prices and climatic conditions, which can lead to changes in farmer preferences and behavior, and change in agricultural cover and economic outcomes in the region. Iran is one of the world's water-scarce regions and is extremely vulnerable to the impacts of climate change due to its high dependency on climate-sensitive agriculture (Karimi et al. 2017). A new approach to analyze and simulate farm structural changes according to exogenous and endogenous factors is the use of agent-based simulation models. Behavioural or 'process-based' models such as agent-based models (ABMs) have received

increasing attention because they allow the simulation of emergent social and economic conditions from underlying external factors such as climate changes and market prices fluctuations and internal factors like behavioral processes of farmer's decision making and land use changes (Seo et al. 2018). Hailegiorgis et al. (2018), used an agent-based model to find the impact of climate change on the adaptive capacity of rural communities in Ethiopia and showed that climate effects caused farmers to migrate from the region. Lamperti et al. (2017) introduced an agent-based model to assess and monitor the Coupled Climate and Economic Dynamics. Wossen et al. (2017) provided an ex-ante assessment of the impacts of climate and price variability on household income and food security in Ethiopia and Ghana.

The ABM model in this research includes a socio-ecological system representing the farm region of "Eslamashahr" in Iran that is informed by empirical data from a social survey about the behavior and heterogeneity of farmers. This area covers 4 rural main districts and 49 villages. The dominate cultivation crops are wheat, barley, corn, and alfalfa. According to Iran's third national report to UNFCC (The United Nations Framework Convention on Climate Change, 2017), the projections of mean temperature based on scenarios for Iran show that the mean temperature will increase in the whole country in future decades compared to the baseline period. So, the temperature is estimated to increase up to 1 degree in some parts of the country that the current study was conducted. Also, precipitation changes in the area will be up to -4.4%. Meanwhile, according to the data provided by the statistics of the Ministry of Agriculture and Statistics of Iran in different years, farmers in this region like other parts of the country face annual changes in prices of products and production costs, as in recent years, the average prices of agricultural products has grown 9.66%, and the average annual cost of production has grown by 13.08%. Therefore, the purpose of this study is to understand how the interaction between agents or farmers in agricultural land with climate change in the region and market fluctuations, and to simulate and measure the economic outcomes of this interaction for 20 years. Whilst ABM is increasingly applied to assess farm structural changes in several regions and countries but to our knowledge, no similar agent-based studies have been so far conducted in Iran.

2. Material and Methods

This research adopted an agent-based model as a suitable approach to quantify the agricultural systems, their structural change, and endogenous adjustment to climate changes and price volatilities in "Eslamshahr" as an example of a traditional agricultural landscape during a period of 20 years from 2016 to 2036. The model was constructed using the NetLogo software. The framework of this model and the type of variables for entry into the simulation model Were determined after the review of previous similar studies (Lobanco & Esposti 2010; Bert et al. 2011; Lamperti et al. 2017) and according to the conditions of the farmers in the region. Farmers' economic and social heterogeneity and their differences in reaction thresholds to external factors lead to their different outcomes in a given period. Here, quantitative models based on aggregated data can't meet the research needs but in agent-based framework, the model is implemented for every individual agent and ultimately the overall agricultural region profile can be simulated. Information was collected from interviews with farmers and agricultural administrators. The environment, which influences farmer's decisions, is defined based on economic and climatic parameters. The economic parameters include product prices and costs of production such as fertilizers, pesticides, labor, and land costs (i.e. rental price). The climatic parameters are represented by the effects of temperature and rainfall on yields. Considering the diversity of farm sizes in the county, a stratified sampling method was selected with proportional allocation. The sample size was 195 (out of 585 households) and the variables used in the research model are defined in Table 1:

Table 1- Variables used in agent based model

Variable	*Description*
$GI_{j,t} = P_{j,t} * Y_{j,t}$	Gross income of each crop per hectare (Tomans)
CL_J	Land size of crop J (hectares)
$TCL_t = \sum CL_{j,t}$	Total farm land size (hectares)
$TGI_t = \sum(GI_{j,t} * CL_{j,t})$	Total farm gross income (Tomans)
$TEXP_t = \sum(EXP_{j,t} * CL_{j,t})$	Total farm costs (Tomans)
$TNI_t = TGI_t - TEXP_t$	Total farm net income (Tomans)
$NI_t = TNI_t/TCL_t$	Net income per hectare (Tomans)
$LABOR_t = \sum_j(LABOR_{j,t} * \ CL_{j,t})$	Total farm labor (man
AL_t	Aspiration level or expected income per hectare (Tomans)
$OC = RP_t + IR_t$	Opportunity cost of a period of use of each hectare (Tomans)
RP_t	Average value of rent per hectare of farm land in the region (Tomans)
$IR_t = 0.15 * (RP_t + EXP_t)$	Interest amount (interest rate=0.15), (Tomans)
$WC_t = TNI_t - SINC_t + TRI_t - TRE_t + WC_{t-1}$	Working capital (Tomans)
$NL = [WC/RP]$	Maximum land that farmer can lease in the next period potentially
$SINC_t$	Farmer's Household Livestock Minimum Cost (Tomans)
$TRI_t = \ RP_t \, . RCL_t$	Total revenue from land rent out (Tomans)
$TRE_t = RP_t \, . LCL_t$	Total cost of leasing farm land (Tomans)
RCL_t	The amount of land rented out (hectares)
LCL_t	The amount of land leased (hectares)

The adaptive decisions of the farmers depend on several rules. These rules were derived from interview results. For example, 1: farmers compute their opportunity cost and compare that with farming income; 2: farmers remember their past income and consider changing land use (crop pattern), If their income gap from the expected income level (say aspiration level) increases; 3: if farming income gap from aspiration level in successive consecutive periods increases (in this research, three periods), then they supply their land for rent or will abandon farming in it. 4) Farmers with enough working capital will demand land for rent potentially. The implementation process of the simulation model and adaptive decisions are presented in the decision tree (fig.), which provides a framework for the empirical application of the ABM.

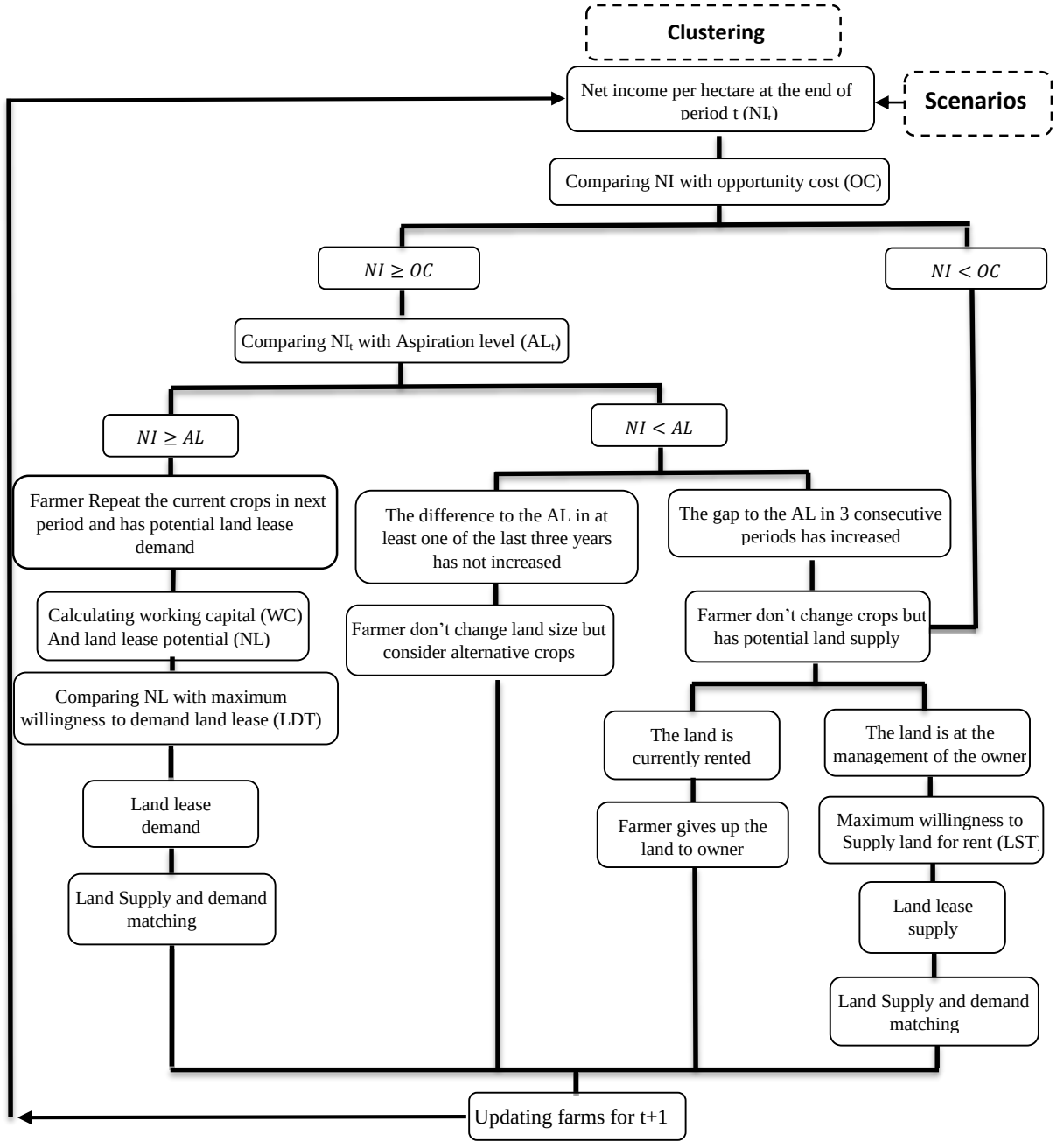

Figure- Decision tree of Agent based model

2.1. Scenario preparation

In this research, it is assumed that the product yield per hectare for each farm, changes due to climate parameters over a given period. So employing the FAO 56 approach, the response of yields of crops in the study region was quantified by the yield-water relations for each farmer (Allen et al. 1998). Thereby actual changes in the yield of various crops can be defined under different climate scenarios over 20 years period (Table 2). All data used for variables in this relation derived from agricultural and Economic vulnerability subdivision of Iran's third national communication to the United Nations Framework Convention on Climate Change.

Table 2- Climate change pre-scenarios

- Percentage of change in annual rainfall: -4.4%					
- Percentage of change in annual temperature: +1%					
	Condition	Annual Change of yields (α)			
		wheat	barley	maize	alfalfa
A1	Fixing access to water as much as the base year	-0.3%	-0.27%	-3.16%	-0.17%
A2	Reducing 10% of access to water	-0.79%	-0.8%	-0.88%	-0.64%

Notes: -In scenario A1 it is assumed that, despite the decrease in rainfall over a period of time, using improved technology, through proper irrigation, the available water is fixed at the base year and main reason of yield decrease is changing climate and crop evapotranspiration, -In scenario A2 it is assumed that the total amount of available water for agriculture will decrease by 10% at the end of the simulation period

In this research, two pre-scenarios are defined about predicted changes in products prices and production cost.

Table 3- Price changes pre-scenarios

	Pre-scenarios	*Annual growth rate of product price*	*Annual growth rate of production costs*
B1	Growth rate of production costs $\leq$ growth rate of product prices	+9.66%	+13.86%
B2	Growth rate of production costs = growth rate of product prices	+13.86%	+13.86%

Notes: - In scenario B1, it is assumed that production costs and prices of the products are the same as the past 15 years (annual change of products prices= +9.66% and annual change of production costs = +13.86%), -In scenario B2, production costs and product prices grow by as much as 13.86 percent.

Based on the above pre-scenarios, the following mixed scenarios are presented:

A1B1: fixed water access as much as the base year + continued trend over the last 15 years in rising product prices and production costs
A1B2: fixed water access as much as the base year + similar changes in product prices and production costs
A2B1: 10% reduction in water access + continued trend over the last 15 years in rising product prices and production costs
A2B2: 10% reduction in water access + similar changes in product prices and production costs

2.2. Economic calculations

This submodel calculates the economic results for a farmer during one full production cycle. After preparing scenarios, by using variables such as total production costs (TEXP), product prices (Pj), yield per hectare (Yj), total gross income (TGI), gross income per hectare (GI), and net income in each hectare (NI) would be calculated at the end of each period t.

Variables that directly would be affected by scenarios include:

$Y_{j,t} = Y_{j,0} * (1+\alpha)^t$: Yield in a hectare of product j in year t
$P_{j,t} = P_{j,0} * (1+\beta)^t$: Market price of product j in year t
$EXP_{j,t} = EXP_{j,0} * (1+\beta)^t$: Production cost in a hectare of product j in year t

A part of the results of the submodel is the financial balance for a farmer and his/her household at the end of a cycle. The balance is expressed as the working capital accumulated by an agent at the end of the cycle (WC). Briefly, the accumulation of working capital is the result of the balance between available capitals from previous cycles, received total income, and incurred total expenses during a production cycle. After calculating the net income per hectare (NI), the farmer calculates the opportunity cost of the agricultural activity (OC) and compares it with the NI. If the net income of each hectare is greater than or equal to the cost of opportunity, then the farmer will compare the net incomes per hectare (NI) with the expected value or Aspiration level (ALt) (Bert et al. 2011). The aspiration level would be calculated by average income per hectares of successful farmers in the region. It is assumed that farmers with the highest technical efficiency are successful farmers. For this purpose, the technical efficiency coefficient of all farms was calculated by the DEA[1] method and the average of NI for farmers in each cluster with an efficiency coefficient above 0.8 determined as aspiration level in that cluster. Now, if the net income is less than the aspiration level, then the process of changing the gap or the difference with this threshold will be the benchmark for the decision. If this gap increases for three consecutive years, the farmer will have a potential supply of land.

$$(NI_t/AL_t) > (NI_{t-1}/AL_{t-1}) > (NI_{t-2}/AL_{t-2}) \quad (2)$$

[1] *Data* envelopment *analysis*

If the net income per hectare is less than the expected value, but the difference does not increase for all three consecutive years, the farmer will only seek to replace the crops according to crop preferences. So he will choose crops for the next year from a discrete set of available options. For this purpose, crop preferences were determined by performing an interview with farmers and utility values obtained for each crop according to their statements. If the land is rented, it will be transferred to the owner and if the land is a property, then the land offered for rent is equivalent to the maximum willingness to rent out (LST), that previously obtained by interviewing each farmer. If NI ≥ AL, the current period cropping pattern will be repeated in the next period and the farmer will have a potential land lease demand. For farmers who have a potential land lease demand, at first the working capital (WC) will be calculated and then the maximum land that farmers can lease (NL) will be determined (Bert et al. 2011). Comparing (NL) with the maximum willingness to lease land (LDT) determines the actual demand for land lease. If NL ≥ LDT then actual demand is equal to LDT and if NL < LDT then it is equal to NL.

3. Results and Discussion

Before running the simulation model, farmers were divided into three classes to obtain different classes proportional to the size and scale of production, using K-Means clustering method. In this method, every data point is allocated to each of the clusters through reducing the in-cluster sum of squares. In other words, the K-means algorithm identifies k number of centroids, and then allocates every data point to the nearest cluster, while keeping the centroids as small as possible. So, Cluster 1 with 105 farmers, cluster 2 with 378 farmers and cluster 3 with 378 farmers were identified.

3.1. Simulation model results

The results show that in A1B1 scenario the total cropping land size in cluster 2 and cluster 1 have the highest decrease at the end of the simulation period. Large-scale farmers are more flexible while reducing economic benefits due to increased production costs (in this scenario, the rate of increase in production costs is higher than the rate of increase in prices of products), but smaller farmers with lower income earnings respond quicker. According to the model decision tree, which compares net income per hectare with the opportunity cost and also net income per hectare with expected income (Aspiration level) over consecutive periods, a larger percentage of small farmers Due to the lack of desirability, reduced their cultivated land or stop cultivating to use the released capital in other businesses. Comparison of yield changes per hectare of the products indicated that the highest yield loss was due to the wheat product, which is reduced by 5.8%, and in all three clusters this reduction value is the same. The yield of barley and alfalfa products in cluster 3, which includes large-scale farmers, has declined less. It seems that farmers have been able to compensate for the decline in yields due to climate change, given access to more machinery and equipment and the use of agronomic methods.

Table 4- Simulation results for A1B1 scenario

	Cluster 1			*Cluster 2*			*Cluster 3*		
	A1B1	*A0B0*	*Change percent*	*A1B1*	*A0B0*	*Change percent*	*A1B1*	*A0B0*	*Change percent*
Total cropping land size (hectares)	304.5	327	-6.8	1179	1275	-7.5	4884	4989	-2.1
Yield in hectare of wheat (kg)	3651	3877	-5.8	4025	4275	-5.8	4915	5220	-5.8
Yield in hectare of barley (kg)	3631	3833	-5.2	3719	3926	-5.2	4823	5071	-4.8
Yield in hectare of maize (kg)	40363	40800	-1.07	42892	44288	-3.1	50355	52000	-3.1
Yield in hectare of alfalfa (kg)	13531	14000	-3.35	13628	14100	-3.3	14134	14579	-3.05
Income/cost index	1.54	2.72	-43.3	1.58	2.98	-45.3	2	3.8	-47.3
Average labor work in each farm (labors*Days)	136	151	-9.9	140	152	-7.8	2058	2076	-0.86

To compare the economic indicators of farmers in three clusters, the ratio of total income to total cost is used. As can be seen, the value of this index in cluster 3 is higher than the other two clusters. The lowest employment reduction rate is for cluster 3 farmers, which was only 0.86% lower than the beginning of the period.

In A1B2 scenario, total cropping land size in the cluster 2 has the highest decrease. The highest yield in hectare loss is due to wheat production, which is reduced by 5.8%, and in all three clusters this reduction is similar. The value of the income/expense ratio in cluster 2 and 3 is higher than cluster 1.

In the A2B1 scenario, it is assumed that the total water consumption of crops will decrease by 10%. As a result, the yield loss is not only due to an increase in evapotranspiration but also a reduction in water availability. On the other hand, in this scenario, it is assumed that the changes in annual product prices and production costs are the same as in the last 15 years, with the price of products rising by 9.66% annually and production costs by 13.068%. Comparison of yield changes per hectare of the products

showed that the highest yield loss was related to alfalfa. The value of the ratio of income to expense at the end of the simulation period in cluster 2 and 3 is higher than cluster 1.

Table 5- Simulation results for A1B2 scenario

Variable	*Cluster 1*			*Cluster 2*			*Cluster 3*		
	A1B1	*A0B0*	*Change percent*	*A1B1*	*A0B0*	*Change percent*	*A1B1*	*A0B0*	*Change percent*
Total cropping land size (hectares)	321	327	-1.8	1221	1275	-4.2	4878	4989	-2.2
Yield in hectare of wheat (kg)	3651	3877	-5.8	4025	4275	-5.8	4915	5220	-5.8
Yield in hectare of barley (kg)	3631	3833	-5.2	3719	3926	-5.2	4823	5071	-4.8
Yield in hectare of maize (kg)	40363	40800	-1.07	42892	44288	-3.1	50355	52000	-3.1
Yield in hectare of alfalfa (kg)	13532	14000	-3.3	13628	14100	-3.3	14134	14579	-3.05
Income/cost index	2.81	2.72	3.3	2.9	2.98	-2.6	3.7	3.8	-2.6
Average labor work in each farm (labors*Days)	144	151	-4.6	145	152	-4.6	2058	2076	-0.86

Table 6- Simulation results for A2B1 scenario

Variable	Cluster 1			Cluster 2			Cluster 3		
	A1B1	A0B0	Change percent	A1B1	A0B0	Change percent	A1B1	A0B0	Change percent
Total cropping land size (hectares)	259	327	-20.7	1223	1275	-4.07	4774	4989	-4.3
Yield in hectare of wheat (kg)	3309	3877	-14.6	3647	4275	-14.6	4454	5220	-14.6
Yield in hectare of barley (kg)	3264	3833	-14.8	3343	3926	-14.8	4336	5071	-14.4
Yield in hectare of maize (kg)	34924	40800	-14.4	37111	44288	-16.2	43569	52000	-16.2
Yield in hectare of alfalfa (kg)	12313	14000	-12.05	12400	14100	-12.05	12861	14579	-11.7
Income/cost index	1.37	2.72	-49.6	1.39	2.98	-53.42	1.77	3.8	-53.42
Average labor work in each farm (labors*Days)	117	151	-22.5	144	152	-5.2	2016	2076	-2.89

In the A2B2 scenario, as in the previous, a 10% reduction in water availability will exacerbate this decline in yields. Also, the annual price changes of products and production costs are the same and grow as much as 13.68% annually. Results showed that total cropping land size of the area in cluster 2 and cluster 1 have the greatest decrease.

Table 7- Simulation results for A2B2 scenario

Variable	*Cluster 1*			*Cluster 2*			*Cluster 3*		
	A1B1	*A0B0*	*Change percent*	*A1B1*	*A0B0*	*Change percent*	*A1B1*	*A0B0*	*Change percent*
Total cropping land size (hectares)	294	327	-10.09	1214	1275	-4.7	4872	4989	-2.3
Yield in hectare of wheat (kg)	2308	3877	-14.6	3647	4275	-14.6	4454	5220	-14.6
Yield in hectare of barley (kg)	3264	3833	-14.8	3343	3926	-14.8	4336	5071	-14.4
Yield in hectare of maize (kg)	34924	40800	-14.4	37111	44288	-16.2	43569	52000	-16.2
Yield in hectare of alfalfa (kg)	12312	14000	-12.05	12400	14100	-12.05	12861	14579	-11.7
Income/cost index	2.51	2.72	-7.72	2.58	2.98	-13.4	3.29	3.8	-13.4
Average labor work in each farm (labors*Days)	132	151	-12.5	143	152	-5.9	2055	2076	-1.01

4. Conclusions

In this research, the results of the implementation of the agent-based simulation model to study farm structural changes in the rural area of “Eslamshahr” for 20 years are presented in detail. The simulation results clearly showed that farmers' behavior patterns and the agricultural land cover affected by environmental and market factors variation. This is consistent with numerous studies related to the underlying agent-based simulation (Lobianco & Esposti 2010; Bert et al. 2011; Acosta et al. 2014; Lamperti et al. 2017; Wossen et al. 2017, Seo et al. 2018). Results also suggested that the preferences and subjective priorities of each farmer in determining the cropping pattern and selection of products have a significant effect on the final agricultural cover in the region, which is similar to the results of Valbuena et al. (2010). Results indicated that at the end of the simulation period, due to climate change, water scarcity and the change of the prices of products and production costs, the number of active agricultural farms in the cluster 1, which includes small-scale farmers, will be further reduced. Also, the employment in the agricultural sector across all scenarios and the total cropping land size in the number of scenarios will decrease. Such a decrease intensifies under the scenario of a 10 percent reduction in water access and the continuation of the past trend in the annual change in prices and production costs (A2B1). In scenario A2B1 and A2B2, where the 10% reduction in access to water occurs during the simulation period, there is the highest yield loss, for example, wheat yields fall by more than 14% in cluster 3. This has resulted

in numerous consequences such as declining production and employment, changing the dominant agricultural profile in the region, and increasing the likelihood of land use change. It is essential to create adequate incentives for farmers by the government to compensate for the adverse effects of possible scenarios and encouraging the development of modern agricultural practices to reduce the functional effects of climate change and the lack of access to water.

Encouraging the development of products that demonstrate greater flexibility and adaptation to climate change can be one of the suggested strategies. The promotion and training of modern agricultural practices and the provision of mechanization needed by farmers in the region will help to improve the performance of agricultural farms. The highest drop in employment was observed in small and medium-size agricultural units. There is also the highest income/cost ratio in large agricultural units. Appropriate policies and support orientation towards small and medium-size farms are necessary to increase competitiveness and sustainability in situations where production performance is reduced in the simulation horizon. In a situation where in the coming years, small scale farms will have a more economic vulnerability to climate change and market fluctuations, encouraging the creation and development of production cooperatives in the region with the participation of small-scale farms can lead to increased productivity and reduced costs.

References

Acosta L, Rounsevell M, Bakker M, Van Doorn A, Montserrat G & Delgado M (2014). An agent-based assessment of land use and ecosystem changes in the traditional agricultural landscape of Portugal. *Journal of Intelligent Information Management* 6(2):55-80 https://doi.org/10.4236/iim.2014.62008

Allen R, Pereira L S, Raes D & Smith M (1998). Crop evapotranspiration, Guidelines for computing crop water requirements. FAO Irrigation and drainage, paper 56, Food and Agriculture Organization of the United Nations

Appel F & Balmann A (2018). Human behavior versus optimizing agents and the resilience of farms-Insights from agent-based participatory experiments with Farm AgriPoliS. *Ecplogical Complexity* 40(100731) https://doi.org/10.1016/j.ecocom.2018.08.005

Beckers V, Beckers J, Vanmaecke M & Van Hecke, E (2018). Modelling farm growth and its impact on Agricultural land use: A country scale application of an agent-based model. *Journal of Land* **7**(3): 109 https://doi.org/10.3390/land7030109

Bert F, Podesta P, Rovere S & Menendez A (2011). An agent-based model to simulate structural and land-use changes in agricultural systems of the Argentine pampas. *Journal of Ecological Modelling* 22(19): 3486-3499 https://doi.org/10.1016/j.ecolmodel.2011.08.007

Hailegiorgis A, Crooks A & Cioffi-Revilla C (2018) An agent-based model of rural households' adaptation to climate change. *Journal of Artificial Societies and Social Simulation* 21(4): 4 https://doi.org/10.18564/jasss.3812

UNFCC (2017). Iran's third national report, National climate change office at the department of environment on behalf of the government of the Islamic Republic of Iran

Karimi V, Karami E & Keshavarz M (2017). Climate change and agriculture: Impacts and adaptive responses in Iran. *Journal of Integrative Agriculture* 17(1): 1-15 https://doi.org/10.1016/s2095-3119(17)61794-5

Lamperti F, Dosi G, Napoletano M, Roventini A & Sapio A (2017). Faraway, so close: Coupled climate and economic dynamics in an agent-based integrated assessment model. *Journal of Ecological Economics* 150: 315-339 https://doi.org/10.2139/ssrn.2944328

Lobianco A & Esposti R (2010). The regional multi-agent simulator (RegMAS): An open-source spatially explicit model to assess the impact of agricultural policies. *Journal of Computers and Electronics in Agriculture* 72(1): 14-26 https://doi.org/10.1016/j.compag.2010.02.006

Mitter H, Larcher M, Schonhart M, Stottinger M & Schmid E (2019). Exploring farmers' climate change perceptions and adaptation intentions: Empirical evidence from Austria. *Journal of Environmental management* 63**(**6): 804-821 https://doi.org/10.1007/s00267-019-01158-7

Schindler J (2009). A multi-agent system for simulating land-use and land-cover change in the Atankwidi catchment of Upper East Ghana. Unpublished PhD thesis, Faculty of mathematics and natural sciences, University of Bonn, Germany http://hdl.handle.net/20.500.11811/4160

Seo B, Crown C, Rounsevell M (2018). Evaluation and calibration of an agent based land use model using remotely sensed land cover and primary productivity data. In: proceedings of *the IEEE International Geoscience and Remote Sensing Symposium*, 22-27 July, Valencia, Spain https://doi.org/10.1109/igarss.2018.8518023

Valbuena D, Verbung P H, Bregt A & Lightenberg A (2010). An agent-based approach to model land-use change at a regional scale. *Journal of Landscape Ecology* 25(2): 185-199 https://doi.org/10.1007/s10980-009-9380-6

Wossen T, Berger T, Mekbib G H & Troost C (2017). Impacts of climate variability and food price volatility on household income and food security of farm households in East and West Africa. *Journal of Agricultural systems* 163: 7-15 https://doi.org/10.1016/j.agsy.2017.02.006

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Application of Different Drying Techniques on Peach Puree

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ABSTRACT
In this study, six various applications were performed to dry peach puree using methods of convective drying (CD), microwave drying (MW1, MW2 and MW3) and combined convective-pulsed microwave drying (CD+MW2 and CD+MW3). Effect of drying on time, color, pH, Brix and micrographs were evaluated. The data of total drying time revealed that the maximum value was belonged to "CD" (220 min). The minimum value was obtained by "MW1" (10 min). By comparison of total color change (ΔE), the highest values were achieved with "CD+MW3", whereas the lowest values were achieved with "MW2". Under all drying applications, the maximum pH and Brix changes were observed with "CD+MW2". From the microstructure, the samples to which the microwave method was applied displayed a collapsed structure as to the sample dried by the convective method.

Keywords: Drying time, pH, Brix, Color, SEM

1. Introduction

Peach (*Prunus persica* L.) parts of the family of *Rosaceae* (Liu et al. 2017) and is now cultivated widely in subtropical regions of the World (Zhang et al. 2017). Total worldwide production was approximately 22.8 million tons in 2014 (FAO 2017). However, high moisture content causes rapid perishability (Zhu & Shen 2014), so peaches should be eaten as fresh throughout the year (Golisz et al. 2013) or be preserved in some form (Kingsly et al. 2007) for instance by using drying methodologies to extend their shelf life (Lyu et al. 2017). Usually, dried peaches are used in bakery, cake, fruit leather and sauces (Doymaz 2014). Peach fruit is also a source of vitamins, minerals and beneficial plant compounds (Doymaz & Bilici 2014) and a helpful ingredient of the diet (Fuentes-Pérez et al. 2014).

The drying process associates simultaneous coupled heat-mass transfer (Cui et al. 2004). Meanwhile, the drying process has a significant influence on the sensory and nutritional characteristics of the end-product (Lyu et al. 2017). Hence to obtain a consistent quality dried product, various dryers should be utilized (Golisz et al. 2013). Drying technologies such as far-infrared and microwave (Wang & Sheng 2006), hot air (Doymaz & Bilici 2014), convection (Zhu & Shen 2014), short and medium infrared under vacuum (Chayjan & Allai 2016), freezing (Pieniazek & Messina 2017), infrared with explosion puffing (Lyu et al. 2017) and osmotic pretreated infrared (Zhang et al. 2017) have been applied to peaches. Due to the dense physical structure and chemical composition factors, sliced fruits dry quite slowly and considerable darkening occurs during drying (Sankat & Castaigne 2004). In view of the drying method problem mentioned above, the puree drying process is a comparably inexpensive and simple process that requires shorter drying periods and lower drying temperatures (Karim & Wai 1999). For the food industry, peach purees are substantial and used as ingredients in many products such as juices, beverages, jellies, jams and baby foods (Massa et al. 2010).

In this research, the main objective was to compare the effect of convective, microwave and combined convective-pulsed microwave drying applications on peach puree, and to provide an alternative puree drying application for industrial purposes considering to drying curves, color, pH, Brix and microstructure.

2. Material and Methods

2.1. Drying experiments

Fresh samples of peaches were bought from the local market in the Bursa province of Turkey. The samples stored in a refrigerator at a temperature of +4±0.5 °C to reduce chemical and physiological changes and prevent moisture loss. In experiments, healthy

and matured fruit samples were used. Initial moisture content were specified by keeping the samples in an oven dryer (M3025P, Electromag, Turkey) at 105 °C for 24h (Celen & Kahveci 2013). The moisture content of fruits was determined 4.73 g water.g dry matter^{-1}.

Drying applications were done in a custom-modified pulsed microwave-convective dryer with each 60 g peach purees. The experimental conditions of drying included the following: convective drying of 60 °C (CD), microwave drying at 200 W (MW1), microwave drying at 200 W in pulse ratio of 2 (MW2) and 1.5 (MW3), and combined convective-pulsed microwave drying. The pulse ratio (PR) for each run was computed as PR = (t_{on} + t_{off})/t_{on}. Here, t_{on} is the "on" time of magnetron power and t_{off} is the "off" time of magnetron power (Gunasekaran & Yang 2007). During the study, the 30s of t_{on} and 30s of t_{off} represented PR=2, and 40s of t_{on} and 20s of t_{off} represented PR=1.5. A digital balance (Radwag, Poland) was used for the determination of sample weights that were saved at 5 min intervals (Kumar & Shrivastava 2017).

2.2. Drying curves

The Equation 1 and 2 were applied to calculate the *MR* (moisture ratio) and *DR* (drying rate) at the time of drying experiments (Thorat et al. 2012):

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad (1)$$

$$DR = \frac{M_{t+dt} - M_t}{dt} \quad (2)$$

Where, M_t, M_o, and M_e refer to the moisture content at a given time, the initial moisture content and the equilibrium moisture content, respectively. M_{t+dt} is the moisture content at $t+dt$ and t is the drying time (min). After analyzing the formula, the values M_e are rather small concerning M_t or M_o. Ultimately as proposed by some of the researchers, the moisture ratio formula was shortened as follows:

$$MR = \frac{M_t}{M_o} \quad (3)$$

2.3. Color analysis

A Hunterlab Color Analyzer (MSEZ-4500L, Reston, USA) was used to determine the color attributes of fresh (as the reference value) and dried peach samples regarding to the lightness L* (white [100] - black [0]) and a* (red [+] - green [–]) and b* (yellow [+] - blue [–]). The *C* (Chroma), α° (Hue angle) and ΔE (total color difference) values were determined according to Eq. (4-6) given below where L_0, a_0, and b_0 represent the reference value (Purkayastha et al. 2013).

$$C = \sqrt{(a^2 + b^2)} \quad (4)$$

$$\alpha = \tan^{-1}\left(\frac{b}{a}\right) \quad (5)$$

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (6)$$

2.4. pH and Brix determination

For peach samples, a pH meter (6173, Jenco, USA) was used to determine the pH values at room temperature. Before the measurements, two-point buffers at pH=7.0 and 4.0 were applied to calibrate. On the other hand, a digital refractometer (MA871, Milwaukee, Romania) was used to measure the level of sugar, Brix (Mechlouch et al. 2012).

2.5. Microstructure evaluation

The influence of various drying applications on the microstructures of peach samples was examined by using SEM (EVO 40, Carl Zeiss, Oberkochen, Germany). Particles were extracted from the dried samples and these was coated with gold-palladium. Microphotographs were taken and considered under a high vacuum (20 kV) (SCD-005, Baltec, Wetzlar, Germany) (Tian et al. 2015).

2.6. Statistical analysis

Randomized plots factorial design of experimental type was performed during the study. Triplicate runs were done in experiments. The JMP software (Version 7.0; SAS Institute Inc., USA) was used for the analysis of variance (ANOVA). To compare the mean values at the 5% significance level ($P<0.05$), the LSD (least significant difference) test was applied.

3. Results and Discussion

3.1. Drying curves

The impacts of different drying applications (CD, MW1, MW2, MW3, CD+MW2 and CD+MW3) on the moisture content versus drying time and drying rate in drying period of peach purees are demonstrated in Figure 1 and 2, respectively. As shown, it took 220, 10, 105, 15, 40 and 20 min to reach the final moisture content at "CD", "MW1", "MW2", "MW3", "CD+MW2" and "CD+MW3", respectively. The results express that increasing microwave power "on" time, the drying times of peach purees decreased. Based on the previous peach drying studies, the required drying time reaching the final moisture content was found for air temperatures of 45 °C (765 min), 55 °C (500 min), 65 °C (310) and 75 °C (225 min) (Doymaz & Bilici 2014). Also, the various infrared power levels were needed 400 min (83 W), 240 min (125), 130 min (167) and 90 min (209 W) (Doymaz 2014). Additionally, Eştürk & Soysal (2010) and Soysal et al. (2009) have reported an observation parallel to our study on the effect of microwave with continuous and intermittent microwave-convective drying of dill and oregano, respectively. Depending on both researches, the continuous treatments led to shorter drying times. In the present study, "CD" resulted in the longest drying time, whereas a significant reduction in drying time was observed when "CD+MW2" and "CD+MW3" combinations were used. These results are in relevance with the research of Soysal et al. (2009) on red pepper drying. The duration for the convective drying applications was found approximately 10.4-19.6 and 2.5-11.8 times longer than that in the continuous microwave-convective method and intermittent microwave-convective method depending on the microwave output power and PR, respectively. Similarly, Eştürk (2012) studied the drying of sage leaves with intermittent and continuous microwave-convective drying methods and the drying time of the convective drying was determined to be 64 to 112 times longer than that of the PR=1.

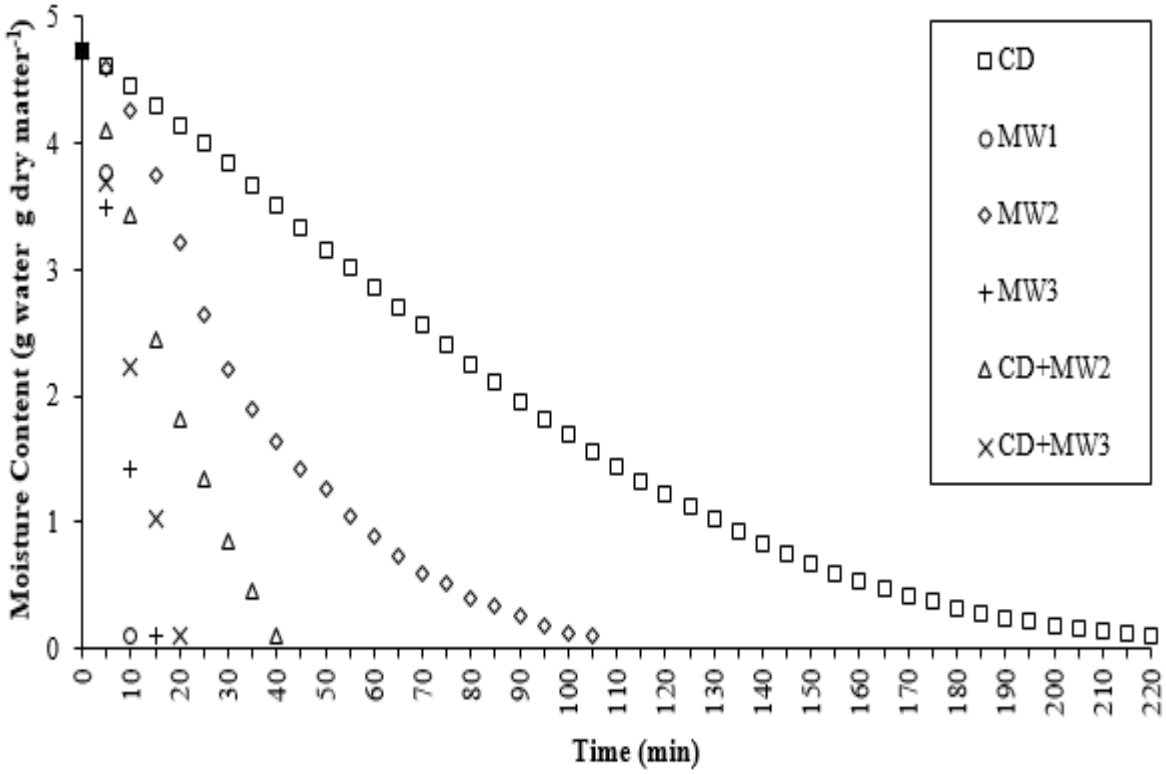

Figure 1- The moisture content vs. time of peach puree at drying applications

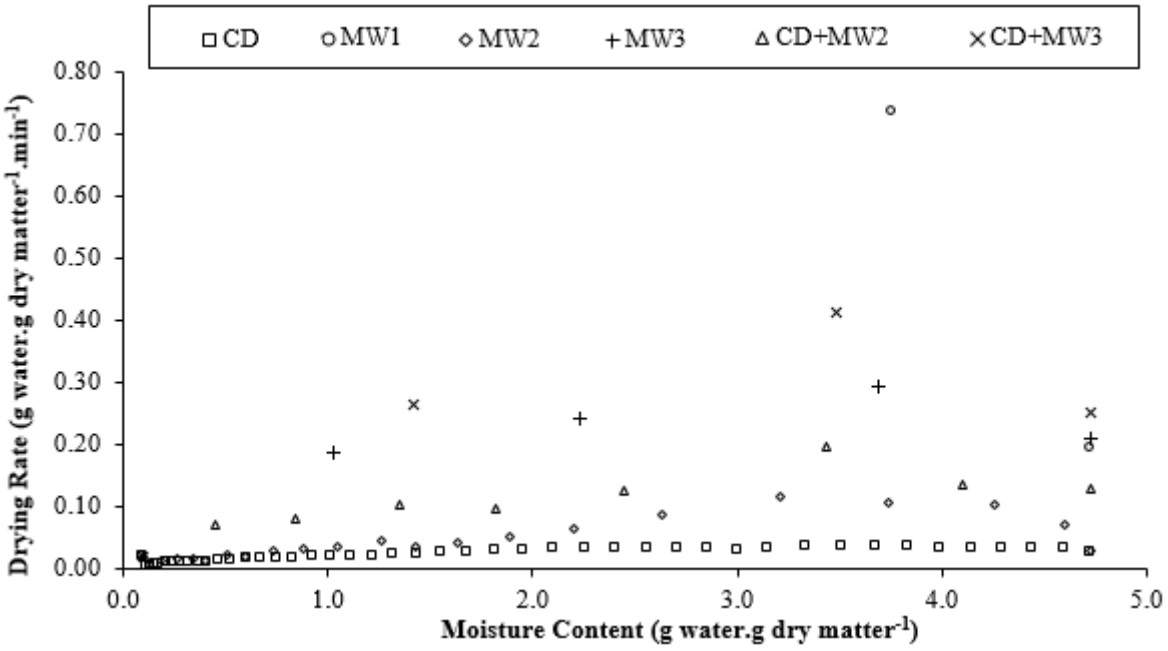

Figure 2- The drying rate vs. moisture content of peach puree at drying applications

3.2. Color analyses

The color changes of the different drying applications are displayed in Figure 3. The parameters of L_0, a_0, and b_0 of peach puree were 69.72, 14.43, and 63.50, respectively. Compared to the fresh sample, drying with "MW2" generated the highest *L* value (58.27) and the lowest ΔE value (13.53). Additionally, any other drying applications in the present study had significantly ($P<0.05$) higher values than the fresh peach puree sample. Of all the drying applications, the *b* value was notably ($P<0.05$) at the highest for the dried sample with "CD+MW2" (60.35). Besides, the lowest values of *C* were found to be similar for peach purees dried either using the "CD" or the "CD+MW3" application ($P<0.05$). There were statistically significant differences between α values of all drying applications ($P<0.05$). Contrary to our study, Pieniazek & Messina (2017) presented different color values (*L*, *a*, and *b)* in both fresh (85.03, 1.02 and 43.11) and freeze-dried (80.33, 0.91 and 40.02) peach samples. Although freeze-drying is recognized with its characteristic to provide high-quality final products (Khampakool et al. 2019), the differences between fresh samples can be explained with the growth conditions, genetic factors and harvesting times (Er & Özcan 2010). Furthermore, Contreras et al. (2008) investigated the microwave method on convective drying for the color parameters of apple and strawberry. The application of higher microwave or air temperature has impacted in lesser color difference in the samples of dried apple. Besides, microwaves had a positive effect on sample lightness (higher *L* values), which could lead to the discoloration at surface level in line with the higher temperature reached at the time of drying for dried strawberry samples. Likewise, Junqueira et al. (2017) studied the microwave, convective, and intermittent microwave–convective methods effect on pumpkin (*Cucurbita moschata* Duch.) slices drying. Lower values of *a* were found after the convective drying process, indicating that losses of red coloration and microwave treatments were suggesting better color quality.

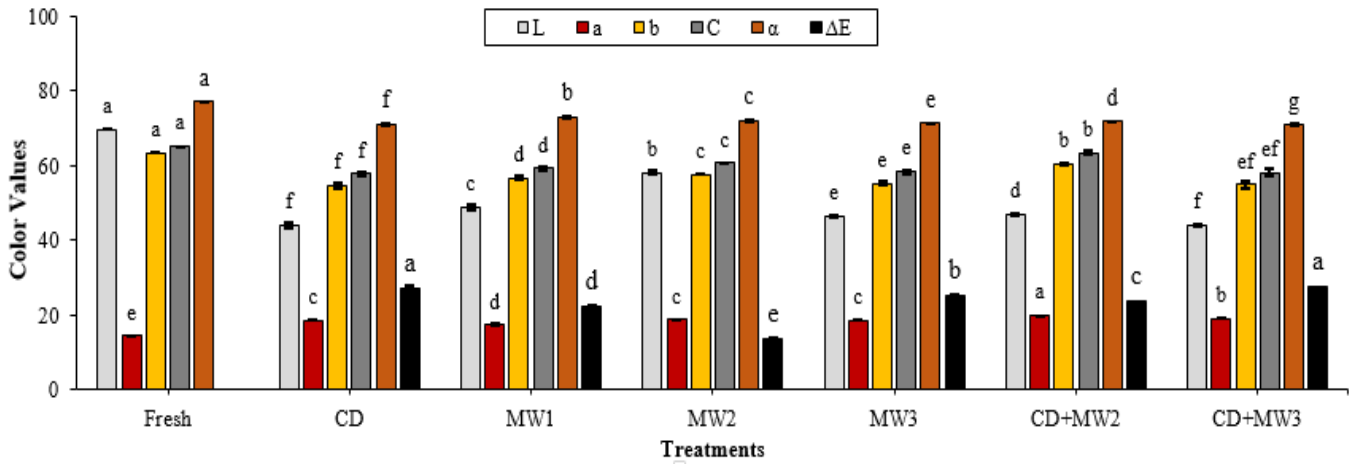

Figure 3- Color changes of peach puree at drying applications

3.3. pH and Brix analyses

Figure 4 shows the pH and Brix values for the dried and fresh samples of peach puree. The results gathered from the pH analysis showed that all drying applications increased the pH content from 4.26 (fresh) to 4.41 (CD+MW2). Although the drying process affected the pH variation, there were no differences in pH values between "CD" and "MW3". Furthermore, the Brix content in the fresh peach puree was 9.00. The changes in Brix values were increased with all drying experiments. Among the six drying experiments, the highest Brix value (78.00) was recorded with "CD+MW2", while the lowest values (40.80) were recorded with "CD". Results of our study were similar by Fuentes-Pérez et al. (2014). The pH and Brix values of six peach cultivars (O'Henry, Ryan Sun, Summer Rich, Ruby Rich, Spring Lady and Royal Glory) were measured between 3.40-4.12 and 7.93-14.08, respectively. Also, Mechlouch et al. (2012) investigated the tomato drying with microwave drying with three output powers density (1, 2 and 3 W g^{-1}) at two temperatures (57 and 67 °C). The pH - Brix values for tomato dried in microwave power of 1, 2 and 3 W g^{-1} at a constant drying air temperature (57 °C) were 4.86 - 1.50, 4.50 - 2.00 and 4.29 - 5.13, respectively. At a constant 67 °C drying air temperature, the pH - Brix values were 4.91 - 1.00, 4.53 - 1.88 and 4.42 - 3.00 at a microwave power of 1, 2 and 3 W g^{-1}, respectively. Zade & Lakade (2017) applied microwave heating and convective hot air drying to produce raisins from grapes with desirable quality aspects. Experiments were performed by changing two process parameters of hot air temperature and specific microwave power density in the range of 35 to 55 °C and 0.15 to 0.35 W g^{-1}, respectively. The optimal combination at 45 °C hot air temperature and 0.25 W g^{-1} specific microwave power density resulted in the 4.03 pH and 77.19 Brix values.

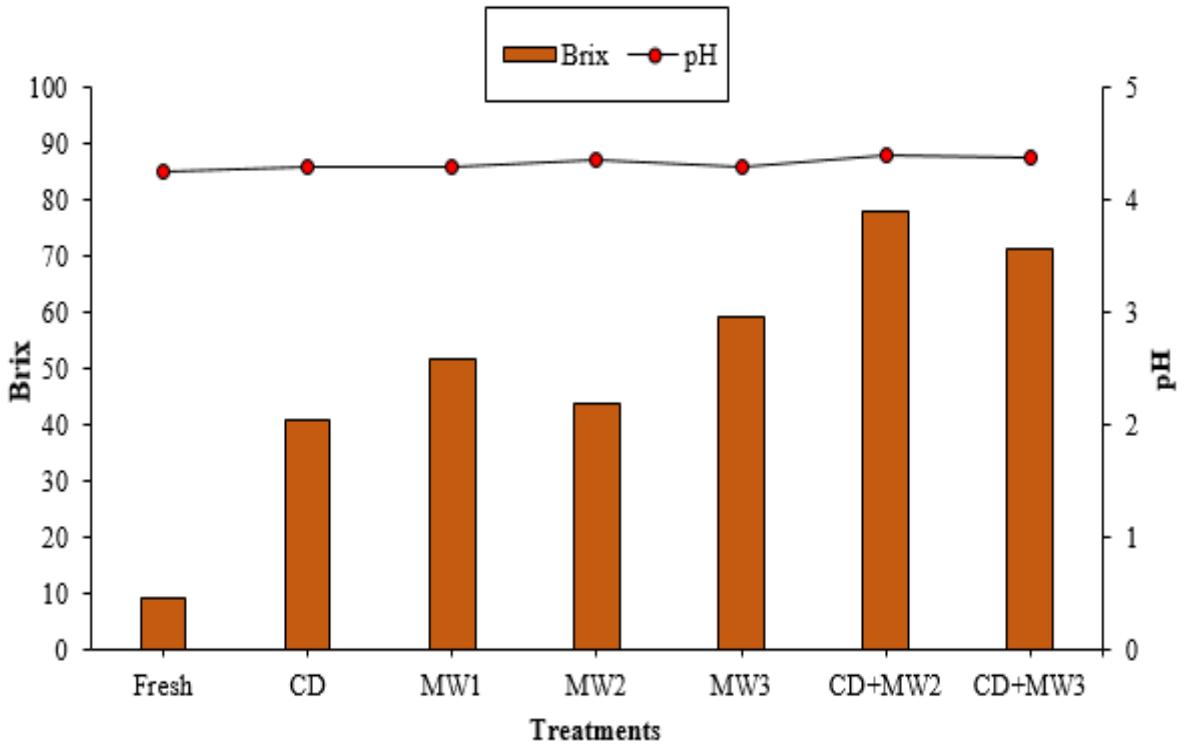

Figure 4- pH and Brix Comparison of peach puree at drying applications

3.4. Microstructure analyses

The impact of different drying applications on the tissue structure of the dried peach puree was determined by using SEM (scanning electron microscopy) (Figure 5a-f). The micrographs were examined through 2000× microscopy. The "CD" dried samples were demonstrated to be distribution that is more regular. Other applications caused the fissure structure. However, the highest distortion was seen in the "MW1" treatment. Lyu et al. (2017) imaged peach samples as well. Although a uniform porous structure was shown in fresh peach samples, the loose structure with the highest sugar penetration existed after infrared drying. Similarly, Witrowa-Rajchert & Rzaca (2009) determined the influence of drying on the internal structure of dried apples. The results present that regarding the structural properties, the apples dried by the convective method are significantly different (small cavities and very high density) from convective-microwave dried apples. In addition, Izli & Isik (2015) researched the microwave, convective and microwave-convective methods for the microstructure characteristics of tomatoes. Although after convective drying at 50 and 75 °C microstructure was detectable, the microwave-convective drying conditions caused structural damages by destroying the external surfaces of the samples.

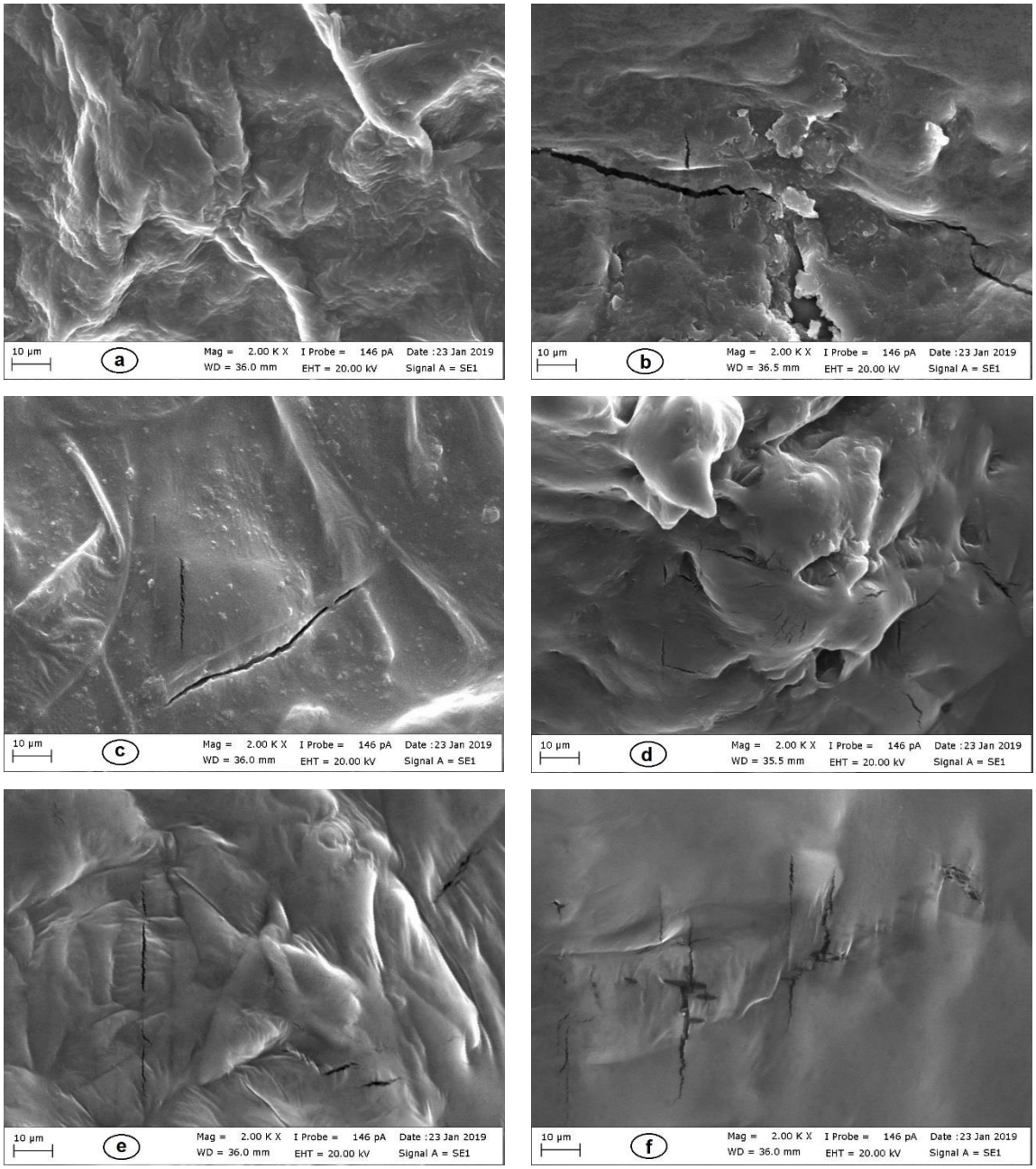

Figure 5- Microstructure of peach puree: CD (a), MW1 (b), MW2 (c), MW3 (d), CD+MW2 (e) and CD+MW3 (f)

4. Conclusions

In the presented study, drying curves, color, pH, Brix and microstructure of peach puree were analyzed by performing convective, microwave, pulsed microwave and pulsed microwave-convective drying. According to the experimental results, the "MW1" drying condition showed the shortest drying time and minimum pH change. Comparing the color values, all applications had a negative effect on the color values except *a* value. Moreover, total color differences (*ΔE)* were found lowest with "MW2". However, the scanning electron micrographs showed that microwave applications disrupted the clear and porous structure. As a conclusion of drying time and drying rate comparisons, it was found that microwave usage had a favorable effect on the drying of peach puree. Furthermore, some quality analysis was also showed that the "CD+MW2" has considerably higher values over the other drying methods in terms of pH, Brix and *a*, *b and C* color values.

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Abbreviations and Symbols	
CD	Convective drying
db	dry basis
M_0	Initial moisture content, g water.g dry matter^{-1}
M_t	The moisture content at a particular time, g water.g dry matter^{-1}
M_e	Equilibrium moisture content, g water.g dry matter^{-1}
MR	Moisture ratio
MW	Microwave drying
PR	Pulse ratio
SEM	Scanning electron microscopy
t_{on}	Magnetron power "on" time
t_{off}	Magnetron power "off" time

References

Celen S & Kahveci K (2013). Microwave drying behaviour of tomato slices. *Czech Journal of Food Science* 31(2): 132-138 https://doi.org/10.17221/30/2012-CJFS

Chayjan A R & Allai B (2016). Comparison of short and medium infrared radiation on drying parameters of peach slices under vacuum conditions. *Food Science and Technology* 13(58): 107-116

Contreras C, Martín-Esparza M E, Chiralt A & Martínez-Navarrete N (2008). Influence of microwave application on convective drying: Effects on drying kinetics, and optical and mechanical properties of apple and strawberry. *Journal of Food Engineering* 88(1): 55-64 https://doi.org/10.1016/j.jfoodeng.2008.01.014

Cui Z W, Xu S Y & Sun D W (2004). Microwave-vacuum drying kinetics of carrot slices. *Journal of Food Engineering* 65(2): 157-164 https://doi.org/10.1016/j.jfoodeng.2004.01.008

Doymaz I (2014). Suitability of thin-layer drying models for infrared drying of peach slices. *Journal of Food Processing and Preservation* 38(6): 2232-2239 https://doi.org/10.1111/jfpp.12277

Doymaz İ & Bilici B (2014). Influence of citric acid pretreatment on drying of peach slices. *International Journal of Food Engineering* 10(4): 829-837 https://doi.org/10.1515/ijfe-2014-0026

Er F & Özcan M M (2010). Chemical compositional properties and mineral contents of some apple cultivars. *South-Western Journal of Horticulture Biology and Environment* 1(2):121-131

Eştürk O (2012). Intermittent and continuous microwave-convective air-drying characteristics of sage (*Salvia officinalis*) leaves. *Food and Bioprocess Technology* 5(5): 1664-1673 https://doi.org/10.1007/s11947-010-0462-x

Eştürk O & Soysal Y (2010). Drying properties and quality parameters of dill dried with intermittent and continuous microwave-convective air treatments. *Journal of Agricultural Sciences* 16(1): 26-36

FAO (2017). *Food and Agricultural Organization Statistica Database*. Retrieved in April, 01, 2019 from http://faostat3.fao.org/download/Q/QC/E

Fuentes-Pérez M C, Nogales-Delgado S, Ayuso M C & Bohoyo-Gil D (2014). Different peach cultivars and their suitability for minimal processing. *Czech Journal of Food Sciences* 32(5): 413-421

Golisz E, Jaros M & Kalicka M (2013). Analysis of convectional drying process of peach. *Technical Sciences* 16(4): 333-343

Gunasekaran S & Yang H W (2007). Effect of experimental parameters on temperature distribution during continuous and pulsed microwave heating. *Journal of Food Engineering* 78(4): 1452-1456 https://doi.org/10.1016/j.jfoodeng.2006.01.017

Junqueira J R D J, Corrêa J L G & Ernesto D B (2017). Microwave, convective, and intermittent microwave-convective drying of pulsed vacuum osmodehydrated pumpkin slices. *Journal of Food Processing and Preservation* 41(6): e13250 https://doi.org/10.1111/jfpp.13250

Izli N & Isik E (2015). Color and microstructure properties of tomatoes dried by microwave, convective, and microwave-convective methods. *International Journal of Food Properties* 18(2): 241-249 https://doi.org/10.1080/10942912.2013.829492

Karim A A & Wai C C (1999). Foam-mat drying of starfruit (*Averrhoa carambol*a L.) puree. Stability and air drying characteristics. *Food Chemistry* 64(3): 337-343 https://doi.org/10.1016/S0308-8146(98)00119-8

Khampakool A, Soisungwan S & Park S H (2019). Potential application of infrared assisted freeze drying (IRAFD) 242 for banana snacks: Drying kinetics, energy consumption, and texture. *LWT - Food Science and Technology* 99: 355-363 https://doi.org/10.1016/j.lwt.2018.09.081

Kingsly R P, Goyal R K, Manikantan M R & Ilyas S M (2007). Effects of pretreatments and drying air temperature on drying behaviour of peach slice. *International Journal of Food Science & Technology* 42(1): 65-69 https://doi.org/10.1111/j.1365-2621.2006.01210.x

Kumar V & Shrivastava S L (2017). Optimization of vacuum-assisted microwave drying parameters of green bell pepper using response surface methodology. *Journal of Food Measurement and Characterization* 11(4): 1761-1772 https://doi.org/10.1007/s11694-017-9557-7

Liu C J, Wang H O, Xue Y L, Zhang Z Y, Niu L Y, Li D J & Liu C Q (2017). Screening quality evaluation factors of freeze-dried peach (*Prunus Persica* L. Batsch) powders from different ripening time cultivars. *Journal of Food Quality* pp. 1-12 https://doi.org/10.1155/2017/7213694

Lyu J, Yi J, Bi J, Chen Q, Zhou L & Liu X (2017). Effect of sucrose concentration of osmotic dehydration pretreatment on drying characteristics and texture of peach chips dried by infrared drying coupled with explosion puffing drying. *Drying Technology* 35(15): 1887-1896 https://doi.org/10.1080/07373937.2017.1286670.

Massa A, González C, Maestro A, Labanda J & Ibarz A (2010). Rheological characterization of peach purees. *Journal of Texture Studies* 41(4): 532-548 https://doi.org/10.1111/j.1745-4603.2010.00240.x

Mechlouch R F, Elfalleh W, Ziadi M, Hannachi H, Chwikhi M, Aoun A B & Cheour F (2012). Effect of different drying methods on the physico-chemical properties of tomato variety 'Rio Grande'. *International Journal of Food Engineering* 8(2): 1-13 https://doi.org/10.1515/1556-3758.2678

Pieniazek F & Messina V (2017). Quality parameters of freeze-dried peach snack. *British Food Journal* 119(12): 2959-2968 https://doi.org/10.1108/BFJ-11-2016-0526

Purkayastha M D, Nath A, Deka B C & Mahanta C L (2013). Thin layer drying of tomato slices. *Journal of Food Science and Technology* 50(4): 642-653 https://doi.org/10.1007/s13197-011-0397-x

Sankat C K & Castaigne F (2004). Foaming and drying behaviour of ripe bananas. *LWT-Food Science and Technology* 37(5): 517-525 https://doi.org/10.1016/S0023-6438(03)00132-4

Soysal Y, Arslan M & Keskin M (2009). Intermittent microwave-convective air drying of oregano. *Food Science and Technology International* 15(4): 397-406 https://doi.org/10.1177/1082013209346588

Soysal Y, Ayhan Z, Eştürk O & Arıkan M F (2009). Intermittent microwave–convective drying of red pepper: Drying kinetics, physical (colour and texture) and sensory quality. *Biosystems Engineering* 103(4): 455-463 https://doi.org/10.1016/j.biosystemseng.2009.05.010

Thorat I. D, Mohapatra D, Sutar R F, Kapdi S S & Jagtap D D (2012). Mathematical modeling and experimental study on thin-layer vacuum drying of ginger (*Zingiber officinale* R.) slices. *Food and Bioprocess Technology* 5(4): 1379-1383 https://doi.org/10.1007/s11947-010-0429-y

Tian Y, Wu S, Zhao Y, Zhang Q, Huang J & Zheng B (2015). Drying characteristics and processing parameters for microwave-vacuum drying of kiwifruit (*Actinidia deliciosa*) slices. *Journal of Food Processing and Preservation* 39(6): 2620-2629 https://doi.org/10.1111/jfpp.12512

Wang J & Sheng K (2006). Far-infrared and microwave drying of peach. *LWT-Food Science and Technology* 39(3): 247-255 https://doi.org/10.1016/j.lwt.2005.02.001

Witrowa-Rajchert D & Rząca M (2009). Effect of drying method on the microstructure and physical properties of dried apples. *Drying Technology* 27(7-8): 903-909 https://doi.org/10.1080/07373930903017376

Zade P P & Lakade S S (2017). Optimization of process parameters for microwave-convective drying of grapes using response surface method. In: *International Conference on Advances in Thermal Systems, Materials and Design Engineering*, 21-22 December, Mumbai pp. 1-6 http://dx.doi.org/10.2139/ssrn.3101687

Zhang P, Zhou L, Bi J, Liu X, Lyu J, Chen Q & Wu X (2017). Drying kinetics and quality attributes of peach cylinders as affected by osmotic pretreatments and infrared radiation drying. *International Journal of Food Engineering* 13(5): 1-10 https://doi.org/10.1515/ijfe-2016-0023

Zhu A & Shen X (2014). The model and mass transfer characteristics of convection drying of peach slices. *International Journal of Heat and Mass Transfer* 72: 345-351 https://doi.org/10.1016/j.ijheatmasstransfer.2014.01.001

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Catching Performance and Catching Efficiency of Siliconized Baits in Handline Fishery

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ABSTRACT
The present study investigated the catching performance and catching efficiency of natural mud shrimp (alive) (*Upogebia pusilla*), siliconized mud shrimp and siliconized pellet in handline fishing. The trials were performed in Kıyıkışlacık Village of Muğla province. The main body of the handline is Ø 0.50 mm, leader and snood are Ø 0.27-0.30 mm, the snood length and distance are 10 cm and 20 cm, respectively. Each handline has 3 hooks and the hook type is 4 no straight. Catches from natural mud shrimp, siliconized shrimp and siliconized pellet bait retained separately, sorted by species and weighed as. 0.01 g sensitivity and measured as the total length. Totally 590 individuals from 30 different species in total were caught including; 28 bony fish (93.33%), and 2 cephalopods (0.66%). While natural mud shrimp captured the 50.7% of fish, 44.7% and 4.6% were caught with siliconized mud shrimp and siliconized pellet, respectively. CPUE values of natural mud shrimp, siliconized shrimp and silicon pellet were calculated as 1.57 *n/h*, 1.38 n/h and 0.14 *n/h*, respectively. In addition, YPUE values were determined as 121.84 g/*h*, 137.73 g/*h* and 7.62 g/*h* for natural mud shrimp, siliconized shrimp and silicon pellet, respectively. Despite the fact that the number of individuals that a live mud shrimp catch in a unit of time is high, it was found out that the weight is more in a unit of time when silicone mud shrimp is used.

It was concluded that using of siliconized mud shrimp has a high capacity of catching performance and catching efficiency and can be used as bait when natural mud shrimp cannot be utilized as alive or unable to be supplied, due to the weather conditions and time restrictions.

Keywords: Handline fishing, Mud shrimp, *Upogebia pusilla*, Siliconized bait, Pellet feed, Aegean sea

1. Introduction

The history of handline fishing dates back to old times (Huse & Fernö 1990). Today, it is commonly used around the world both for commercial and amateur purposes. It has a low cost for high catching efficiency. Due to both easy make and uses it can be used from small lakes to the oceans.

The most important factors that affect on catching performance in handline fishery are hooks and baits. While the size and shape of the hook have distinct characteristics for target species, the efficiency of the bait may change according to the catching area, season and target species etc. Fishes tend to prefer baits that are existed in their habitat which are common for them. Therefore, fishers use these baits as much as possible. However, reasons such as the incapability to provide an ideal bait and the high-cost lead fishers to come up with alternative solutions.

There are some studies conducted on handline fishing in Turkey. Kaykaç et al. (2003) presented catching efficiency of the cross and straight hooks. Akamca & Kiyaga (2009) investigated the prey-predator relationship of sea bass (*Dicentrarchus labrax*) line fishing in İskenderun Bay, Yumurtalık Cove. Aydın (2011) compared the impacts of razor clam (*Solen vagina*) and sardine (*Sardina pilchardus*) on annular seabream (*Diplodus annularis*), picarel (*Spicara flexuosa*), common two-banded seabream (*Diplodus vulgaris*) and bogue (*Boops boops*) catching efficiency. In the sea bass line fishing, catching efficiency was compared to between live fishing baits as annular seabream (*Diplodus annularis*) and grey mullet (*Mugil cephalus*), dead baits picarel (*Spicara sp*.) and cuttlefish (*Sepia elegans*) by Soykan & Kınacıgil (2013). Ateşşahin et al. (2015) determined the relationship between spinner hook sizes (2, 3 and 4) and hook selectivity for rainbow trout (*Oncorhynchus mykiss*), a species important to recreational fisheries in Karakaya Dam Lake in Eastern Turkey.

Despite the fact that mud shrimp (*Upogebia pusilla*) is commonly used in handline fishing in Turkey, there are no studies conducted on its catching performance or efficiency, and there is only one study in the international literature (Erzini et al. 1998).

To take into consideration cost disadvantages and not the availability of natural baits, fishers have started to look for alternative handline bait. In Bodrum region, anglers who practice amateur handline and commercial long line fishing have started to use the bait that is a mixture of pellet bait (ready-made bait to feed fishes in farms) and silicone (adhesive construction material known for its waterproofness). The mixture bait of silicon and pellet is only used in Turkey and there is no study examined the impact of the bait on catching performance.

In this study, catching performance and efficiency of three different bait types; live mud shrimp (natural), silicon pellet bait and siliconized mud shrimp bait, which was developed in this study, were investigated.

2. Material and Methods

Fishing experiments were carried out at monthly sampling periods between May 2016 and April 2017 in Zeytinlikuyu site, which located in Kıyıkışlacık Village of Muğla province, Milas. Catching activities were performed with groups of 3 or 4 people in the morning (beginning of the sunrise and +3 hours) and evening around (sunset -3 and sunset hours). The water depth of the sampling areas varied between 7 and 52 meters and the average depth was 22 meters. In the study bait handline which is named "gadide" in the region was used. The main body of the handline is Ø 0.50 mm, leader and snoods are Ø 0.27-0.30 mm, the snood length and distance are 10 cm and 20 cm, respectively. Each set has 3 hooks and the hook is 4 no straight type. All hook sets used in the study were made as identical (Figure 1). The order of the baits was changed for each use.

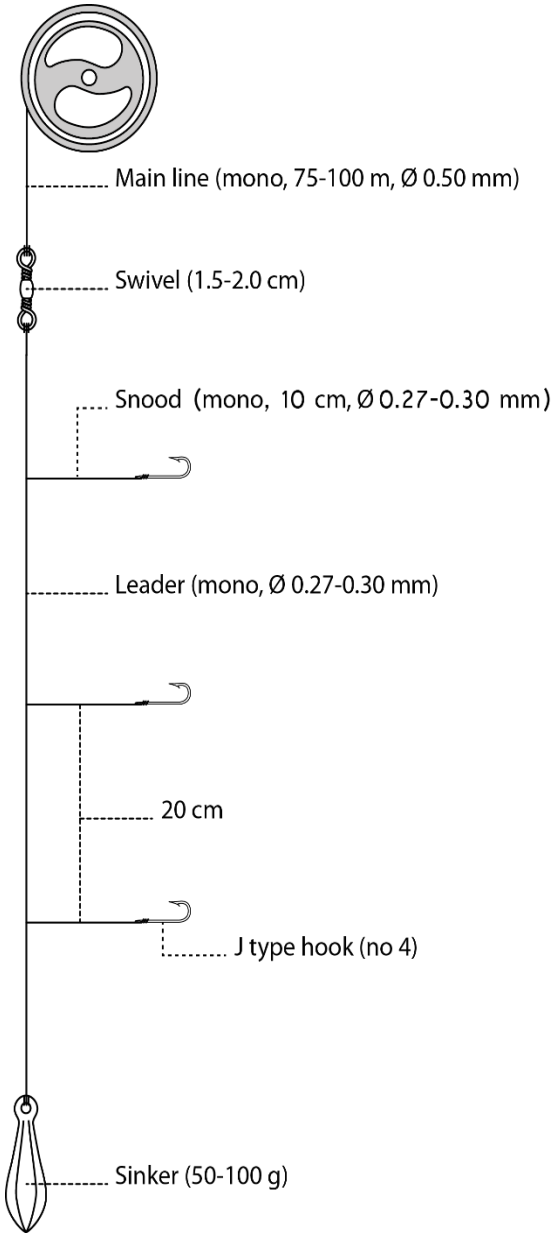

Figure 1- Handline used in experiments

Three different baits were examined in the study;

I- Live (natural) mud shrimp; it was provided via purchasing from the producers. It was kept in cold storage during the fishing period and using as a live.

II- Siliconized mud shrimp; the mud shrimps which were provided by purchasing from producers were put down in a freezer for preventing the pain as much as possible based on the scientific ethical principles. After this process, the bodies of the mud shrimps were pressed with silicone. In this process, syringes filled with transparent aquarium silicone were used. The syringe was injected from the point where the abdomen was close to uropod directly to the mud shrimp (Figure 2) and kept at room temperature.

III- Silicon pellet; It is also sold in the fishing equipment market but not purchased in the study. Silicon pellet was made before fishing. The pellet using as feed was moisturized to soften for 10 hours. Afterwards, the bait embosses with a hammer or pulverized with a mixer, and then mixed with silicone, and re-filled in a silicon tube, and lastly, it was taken out of the silicon tube in the form of strips and allowed to dry. The next day, baits were cut in appropriate sizes for hooks used in the study (Figure 3).

Silicone, which is technically described as “polysiloxane”, is used for wide range of purposes in different sectors such as automotive, electronics, beauty and self-care, food-beverage sector, construction and architecture. Transparent construction silicone aquarium silicone was preferred in the study due to their weak odour.

Figure 2- Siliconized of mud shrimp

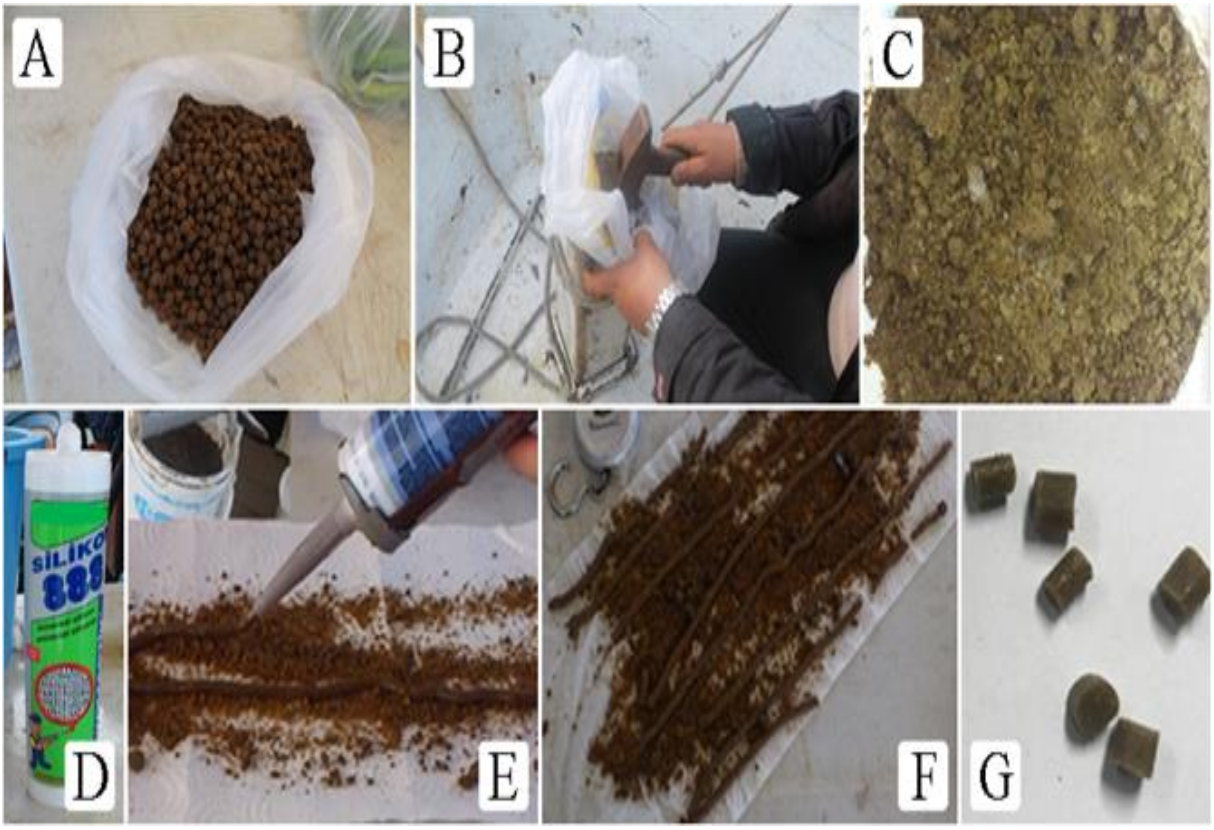

Figure 3- Making stages of the silicon pellet bait (A: Pellet feed, B: Pulverizing, C: Floured pellet, D: Transparent silicone, E: Silicone bait sticks, F: Drying, G: Silicon pellet bait that ready to fishing)

The individuals obtained from experiments were classified as live mud shrimp, silicon mud shrimp and silicon pellet. Each individual was weighed (nearest g) as and their total length (TL) was measured (nearest mm).

Before parametric tests were performed, the data were analyzed for homogeneity of variances and normal distribution using Levene and Kolmogorov-Smirnov one-sample test, respectively. If the homogeneity of variances and normal distribution were not confirmed, the data were not used for the test (Zar 1974).

The question of whether there is a statistical difference between the numbers of the sampled species in the 95% confidence interval or not was calculated with a χ^2 test (chi-square) (Zar 1974);

$$\chi^2 = \sum \frac{(G - B)^2}{B}$$

In the equation; *B* presents the number of expected individuals and *G* presents the number of observed individuals.

The question of whether there is a significant difference between the lengths of sampled individuals in the 95% interval was calculated by a Student's *t*-test (Zar 1974). The given equations were used in the cases that variances of the length groups were equal or non-equal;

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S^2}{n} + \frac{S^2}{n}}} \quad \text{or} \quad t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

In the equations, low index 1 and 2 represent independent groups, $\bar{x}$ represents the arithmetic mean and S^2 represents variance.

Whether there was a significant difference between the bait types used in the tests in the scope of the study and the mean length of the species in the 95% confidence interval or not was determined by using a one-way analysis of variance (ANOVA) (Zar 1974);

Source of the Variance	*SS*	*df*	*MS*	*F*
Between Groups (*B*)	$SS_B = \sum \left[\frac{\left(\sum x_i\right)^2}{n_i} \right] - \frac{\left(\sum x\right)^2}{N}$	$df_B = k - 1$	$MS_B = \frac{SS_B}{df_B}$	$F = \frac{MS_B}{MS_W}$
Within a Group (*W*)	$SS_W = SS_T - SS_B$	$df_W = N - k$	$MS_W = \frac{SS_W}{df_W}$	
Total (*T*)	$SS_T = \sum x_i^2 - \frac{\left(\sum x_i\right)^2}{N}$	$df_T = N - 1$		

In the equations; n_i represents the number of individuals in group i, N represents the number of individuals in all groups, $\bar{x}_i$ represents the arithmetic means of the group i, k represents the total number of groups, SS represents sum the of squares, *df* represents the degree of freedom, MS represents mean square and F represents calculated criteria value.

The Catch per Unit Effort (CPUE) and Yield per Unit Effort (YPUE) were calculated respectively as; a number of individuals that can are catchability with one hook and the amount of the yielded catchability (gram) (Aydın, 2011);

$$\text{CPUE} = \frac{\sum n}{\sum h \times \sum t} \quad \text{and} \quad \text{YPUE} = \frac{\sum W}{\sum h \times \sum t}$$

In the equations, n represents the number of individuals, W represents the total weight of individuals (g), h represents the number of hooks used in a set, and t represents the duration of catching.

Mathematical (meteorological) seasons are used for the northern hemisphere for the seasonal sampling periods. According to this, March, April and May data are spring; June, July and August data are summer, September, October and November data are fall and December, January and February data are used as winter data set (TSMS 2017).

3. Results and Discussion

Results of the catching performance and efficiency of mud shrimp have been revealed for the first time in Turkey. Furthermore, the findings on catching efficiency and catching a performance of mud shrimp have been presented worldwide for the first time with this study. As far as it is known, the silicone pellet mixture is only used in Turkey and the results on its catching performance have been introduced for the first time.

A total of 590 individuals that belongs to 30 species were sampled in the fishing duration of 2427 minutes (40 hours and 27 minutes). These species included; 588 bony fishes (99.66%), and 2 cephalopods (0.34%). According to the results, the most frequently samples were (>5%); annular seabream (*Diplodus annularis*, 23.90%), common pandora (*Pagellus erythrinus*, 18.47%), common two-banded seabream (*Diplodus vulgaris*, 15.93%), gilthead seabream (*Sparus aurata*, 11.53%) and brown comber (*Serranus hepatus*, 9.49%) (Table 1). The first five species that were sampled in the study constituted 79.32% of the total catches.

In the study, 50.7% of 590 individuals (n = 299) were sampled with live Mediterranean mud shrimp, and this bait was respectively followed by silicone Mediterranean mud shrimp (n = 264) with 44.7% and silicone pellet bait (n = 27) with 4.6%.

The study results showed that the species caught with live Mediterranean mud shrimp most frequently were (%>10); annular seabream with 24.41%, common pandora 19.06%, gilthead seabream 14.05%, common two-banded seabream 13.71% and brown comber 11.04% (Table 2). Most frequently were (%>5) species obtained siliconized mud shrimp were; annular seabream 22.35%, common pandora 19.32%, common two-banded seabream 17.42%, gilthead seabream 9.85% and brown cumber 7.95%. Lastly, the species which were caught with silicone pellet bait were (%>10); annular sea bream 33.33%, common two-banded seabream 25.93% and gilthead seabream 11.11%.

Table 1- Experimented species, numbers, percentage distribution with lengths and weights distribution, arithmetic mean and standard error Bony fishes

Family	*Species name*	*Common name*	*n*	%	L_{min}	L_{max}	L_{mean}	L_{se}	W_{min}	W_{max}	W_{mean}	W_{se}
Balistidae	*Balistes capriscus*	Grey triggerfish	5	0.85	17.5	32.5	25.46	2.94	94.0	617.2	351.33	103.55
Caranginae	*Trachurus mediterraneus*	Mediterranean horse mackerel	3	0.51	14.6	24.2	18.37	2.96	26.5	130.0	64.90	32.73
Engraulidae	*Engraulis encrasicolus*	European anchovy	1	0.17	10.5	10.5	10.50		8.1	8.1	8.10	
Gobiidae	*Gobius niger*	Black goby	11	1.86	8.0	12.6	10.52	0.41	6.0	21.6	15.15	1.53
Labridae	*Labrus viridis*	Green wrasse	1	0.17	21.5	21.5	21.50		101.0	101.0	101.00	
Moronidae	*Dicentrarchus labrax*	European seabass	1	0.17	36.0	36.0	36.00		459.0	459.0	459.00	
Mullidae	*Mullus barbatus barbatus*	Red mullet	1	0.17	13.0	13.0	13.00		24.0	24.0	23.98	
Sciaenidae	*Umbrina cirrosa*	Shi drum	1	0.17	25.5	25.5	25.50		193.0	193.0	193.00	
Scombridae	*Scomber japonicus*	Chub mackerel	4	0.68	28.5	33.5	30.53	1.09	175.1	310.8	239.00	27.87
Scorpaenidae	*Scorpaena scrofa*	Red scorpionfish	1	0.17	14.5	14.5	14.50		54.1	54.1	54.10	
Serranidae	*Serranus cabrilla*	Comber	21	3.56	7.4	18.2	12.06	0.75	7.1	85.0	29.97	4.70
	Serranus hepatus	Brown comber	56	9.49	7.2	25.1	9.32	0.31	6.5	255.7	19.29	4.38
	Serranus scriba	Painted comber	6	1.02	12.5	19.4	15.37	1.03	26.5	106.2	58.62	11.83
Siganidae	*Siganus rivulatus*	Marbled spinefoot	1	0.17	13.5	13.5	13.50		75.0	75.0	75.00	
Sparidae	*Boops boops*	Bogue	14	2.37	8.4	18.6	13.71	0.58	5.9	62.1	32.64	3.73
	Dentex maroccanus	Morocco dentex	2	0.34	22.1	26.1	24.10	2.00	144.0	206.0	175.00	31.00
	Diplodus annularis	Annular seabream	141	23.90	8.5	17.8	14.00	0.14	10.0	110.0	50.45	1.61
	Diplodus puntazzo	Sharpsnout seabream	1	0.17	23.8	23.8	23.80		260.0	260.0	260.00	
	Diplodus vulgaris	Common two-banded seabream	94	15.93	8.9	26.5	16.86	0.45	11.9	327.3	96.15	6.69
	Lithognathus mormyrus	Sand steenbras	6	1.02	20.6	24.6	22.55	0.56	113.4	163.9	144.82	7.44
	Oblada melanura	Saddled seabream	2	0.34	18.0	19.0	18.50	0.50	69.0	80.6	74.80	5.80
	Pagellus acarne	Axillary seabream	2	0.34	12.0	12.8	12.40	0.40	21.9	23.2	22.55	0.65
	Pagellus erythrinus	Common pandora	109	18.47	10.0	31.5	20.19	0.49	12.0	392.0	125.45	8.52
	Pagrus caeruleostictus	Bluespotted seabream	4	0.68	7.9	27.5	22.05	4.74	229.0	310.0	272.24	16.74
	Sparus aurata	Gilthead seabream	68	11.53	13.0	23.6	17.52	0.33	5.0	450.0	83.74	7.33
	Spicara maena	Blotched picarel	24	4.07	10.9	16.7	14.47	0.26	14.7	52.5	35.66	1.88
Tetraodontidae	*Lagocephalus spadiceus*	Half-smooth golden pufferfish	6	1.02	23.2	29.4	25.92	1.01	230.0	510.0	352.50	46.15
Trachinidae	*Trachinus draco*	Greater weever	2	0.34	23.4	24.9	24.15	0.75	85.0	123.0	104.00	19.00
		Sum of bony fishes:	588	99.66								
Loliginidae	*Loligo vulgaris*	European squid	1	0.17	23.2	23.2	23.20		310.0	310.0	310.00	
Octopodidae	*Octopus vulgaris*	Common octopus	1	0.17					2680.0	2680.0	2680.0	
		Sum of cephalopods:	2	0.34								
Grand total:			590	100.00								

n, Sample size; %, Ratio in total; *L*, Total length (cm); *ML*, Mantle length (cm); *W*, Total weight (gr). $_{min}$, $_{max}$, $_{mean}$ and $_{se}$: Minimum, maximum, mean and standard error. Species listed in alphabetical order according to family and species name. Scientific and common names are based on FishBase (Froese & Pauly 2019) and SeaLifeBase (Palomares & Pauly 2019).

Table 2- Experimented species with bait types used in the study, numbers and rates with result of statistical tests

Species name	*LM*				*SM*			*SP*			*P*			
	Σn	*n*	*%T*	*%G*	*n*	*%T*	*%G*	*n*	*%T*	*%G*	*T*	*LM-SM*	*LM-SP*	*SM-SP*
Balistes capriscus	5	1	20.0	0.3	3	60.0	1.1	1	20.0	3.7	–	–	–	–
Trachurus mediterraneus	3	0	0.0	0.0	3	100.0	1.1	0	0.0	0.0	+	–	×	–
Engraulis encrasicolus	1	0	0.0	0.0	1	100.0	0.4	0	0.0	0.0	–	–	×	–
Gobius niger	11	3	27.3	1.0	7	63.6	2.7	1	9.1	3.7	–	–	–	+
Labrus viridis	1	0	0.0	0.0	1	100.0	0.4	0	0.0	0.0	–	–	×	–
Dicentrarchus labrax	1	0	0.0	0.0	1	100.0	0.4	0	0.0	0.0	–	–	×	–
Mullus barbatus barbatus	1	0	0.0	0.0	1	100.0	0.4	0	0.0	0.0	–	–	×	–
Umbrina cirrosa	1	1	100.0	0.3	0	0.0	0.0	0	0.0	0.0	–	–	–	×
Scomber japonicus	4	2	50.0	0.7	1	25.0	0.4	1	25.0	3.7	–	–	–	–
Scorpaena scrofa	1	0	0.0	0.0	1	100.0	0.4	0	0.0	0.0	–	–	×	–
Serranus cabrilla	21	10	47.6	3.3	11	52.4	4.2	0	0.0	0.0	+	–	+	+
Serranus hepatus	56	33	58.9	11.0	21	37.5	8.0	2	3.6	7.4	+	–	+	+
Serranus scriba	6	4	66.7	1.3	2	33.3	0.8	0	0.0	0.0	–	–	+	–
Siganus rivulatus	1	1	100.0	0.3	0	0.0	0.0	0	0.0	0.0	–	–	–	×
Boops boops	14	7	50.0	2.3	5	35.7	1.9	2	14.3	7.4	–	–	–	–
Dentex maroccanus	2	2	100.0	0.7	0	0.0	0.0	0	0.0	0.0	–	–	–	×
Diplodus annularis	141	73	51.8	24.4	59	41.8	22.4	9	6.4	33.3	+	–	+	+
Diplodus puntazzo	1	0	0.0	0.0	1	100.0	0.4	0	0.0	0.0	–	–	×	–
Diplodus vulgaris	94	41	43.6	13.7	46	48.9	17.4	7	7.4	25.9	+	–	+	+
Lithognathus mormyrus	6	2	33.3	0.7	4	66.7	1.5	0	0.0	0.0	–	–	–	+
Oblada melanura	2	0	0.0	0.0	2	100.0	0.8	0	0.0	0.0	–	–	×	–
Pagellus acarne	2	1	50.0	0.3	1	50.0	0.4	0	0.0	0.0	–	–	–	–
Pagellus erythrinus	109	57	52.3	19.1	51	46.8	19.3	1	0.9	3.7	+	–	+	+
Pagrus caeruleostictus	4	1	25.0	0.3	3	75.0	1.1	0	0.0	0.0	–	–	–	–
Sparus aurata	68	42	61.8	14.1	26	38.2	9.9	0	0.0	0.0	+	–	+	+
Spicara maena	24	13	54.2	4.4	8	33.3	3.0	3	12.5	11.1	+	–	+	–
Lagocephalus spadiceus	6	4	66.7	1.3	2	33.3	0.8	0	0.0	0.0	–	–	+	–
Trachinus draco	2	0	0.0	0.0	2	100.0	0.8	0	0.0	0.0	–	–	×	–
Loligo vulgaris	1	1	100.0	0.3	0	0.0	0.0	0	0.0	0.0	–	–	–	×
Octopus vulgaris	1	0	0.0	0.0	1	100.0	0.4	0	0.0	0.0	–	–	×	–
Total:	590	299	50.7	100.0	264.0	1741.5	100.0	27.0	99.2	100.0	+	-	+	+

LM, Live Mediterranean mud shrimp; SM, Silicone Mediterranean mud shrimp; SP, Silicone pellet bait; Σ, Total. *n*: Sample size; %T, Ratio in species total; %G, Ratio in group total; *P*, Chi-square (χ^2) test results of 95% confidence limit; +, Statistically difference. –, Statistically no difference; ×, Insufficient data for testing; T, Test results of the all bait types

The study findings showed that among the species caught with different three different baits were statistically different (χ^2, $P<0.05$).

According to the results, no statistically significant difference was found between the sample sizes of other species and bait types (χ^2, $P>0.05$). Moreover, when the total number of caught individuals was taken into the consideration, the number of individuals caught by all three types of bait was statistically different than each other (χ^2, $P<0.05$). In terms of the number of individuals, no difference was found between live Mediterranean mud shrimp and silicone Mediterranean mud shrimp baits in the paired comparison (χ^2, $P>0.05$). On the other hand, a difference was found between live Mediterranean mud shrimp-silicone pellet and also between silicone Mediterranean mud shrimp and silicone pellet (χ^2, $P<0.05$) (Table 2).

In the study, the smallest-size of an individual was 7.2 cm with brown comber (*Serranus hepatus*) and the biggest one was 36 cm TL with European seabass (*Dicentrarchus labrax*). The mean lengths of the samples that caught with different baits were statistics different for grey triggerfish, black goby, chub mackerel, brown comber, comber, bogue, annular seabream, common Pandora and blotched picarel ($P<0.05$), however, it was not statistically different for common two-banded seabream (ANOVA,

P>0.05). In the comparison between live Mediterranean mud shrimp and silicone Mediterranean mud shrimp, no difference was found for the mean lengths of all species (ANOVA, P>0.05). In the comparison made between paired bait groups, the difference was found that silicon Mediterranean mud shrimp-silicone pellet bait and live Mediterranean mud shrimp-silicone pellet bait for common two-banded seabream (ANOVA, P<0.05). Due to the insufficient data, test statistics were not applied for the 20 species (apart from the aforementioned ones) (Table 3).

Table 3- Lengths ranges, mean lengths, standard error and result of statistical test differences of experimented species with bait types used in the study

	Live Med. mud shrimp					*Silicone Med. mud shrimp*					*Silicone pellet bait*					*P*			
Species name	*n*	L_{min}	L_{mak}	L_{ort}	L_{se}	*n*	L_{min}	L_{mak}	L_{ort}	L_{se}	*n*	L_{min}	L_{mak}	L_{ort}	L_{se}	*T*	*LM-SM*	*LM-SP*	*SM-SP*
Balistes capriscus	1	32.5	32.5	32.50		3	17.5	32.0	23.77	4.30	1	23.5	23.5	23.50		–	–	–	–
Trachurus mediterraneus						3	14.6	24.2	18.37	2.96									
Engraulis encrasicolus						1	10.5	10.5	10.50										
Gobius niger	3	9.9	12.6	11.53	0.83	7	8.0	11.8	10.03	0.46	1	10.9	10.9	10.90		–	–	–	–
Labrus viridis						1	21.5	21.5	21.50										
Dicentrarchus labrax						1	36.0	36.0	36.00										
Mullus barbatus barbatus						1	13.0	13.0	13.00										
Umbrina cirrosa	1	25.5	25.5	25.50															
Scomber japonicus	2	30.7	33.5	32.10	1.40	1	29.4	29.4	29.40		1	28.5	28.5	28.50		–	–	–	×
Scorpaena scrofa						1	14.5	14.5	14.50										
Serranus cabrilla	10	7.4	16.0	11.17	0.92	11	8.0	18.2	12.86	1.14							–		
Serranus hepatus	33	7.2	25.1	9.55	0.50	21	7.3	10.3	8.97	0.18	2	8.6	9.5	9.05	0.45	–	–	–	–
Serranus scriba	4	12.5	19.4	15.48	1.43	2	13.3	17.0	15.15	1.85							–		
Siganus rivulatus	1	13.5	13.5	13.50															
Boops boops	7	8.4	18.6	13.66	1.14	5	12.6	15.4	13.72	0.50	2	12.9	14.9	13.90	1.00	–	–	–	–
Dentex maroccanus	2	22.1	26.1	24.10	2.00														
Diplodus annularis	73	9.0	17.0	14.11	0.18	59	8.5	17.4	13.78	0.24	9	11.4	17.8	14.47	0.65	–	–	–	–
Diplodus puntazzo						1	23.8	23.8	23.80										
Diplodus vulgaris	41	9.0	24.1	16.68	0.69	46	9.2	26.5	17.67	0.60	7	8.9	19.0	12.61	1.30	+	–	+	+
Lithognathus mormyrus	2	21.9	23.4	22.65	0.75	4	20.6	24.6	22.50	0.82							–		
Oblada melanura						2	18.0	19.0	18.50	0.50									
Pagellus acarne	1	12.8	12.8	12.80		1	12.0	12.0	12.00								×		
Pagellus erythrinus	57	10.7	30.2	19.14	0.59	51	10.0	31.5	21.35	0.79	1	20.9	20.9	20.90		–	–	–	–
Pagrus caeruleostictus	1	27.2	27.2	27.20		3	7.9	27.5	20.33	6.24							–		
Sparus aurata	42	13.0	23.6	17.17	0.43	26	14.7	23.3	18.07	0.51							–		
Spicara maena	13	13.7	16.7	14.97	0.21	8	12.3	15.4	13.98	0.45	3	10.9	15.2	13.60	1.36	–	–	–	–
Lagocephalus spadiceus	4	23.2	29.4	26.20	1.51	2	24.2	26.5	25.35	1.15							–		
Trachinus draco						2	23.4	24.9	24.15	0.75									
*Loligo vulgaris**	1	23.2	23.2	23.20															
*Octopus vulgaris***						1	2.68	2.68	2.68										
Total:	299					264					27								

n, Sample size. *L*: Total length (cm); *, Mantle length (cm); **, Total weight (kg). $_{min}$, $_{max}$, $_{mean}$ and $_{se}$: Minimum, maximum, mean and standard error; *P*, One way ANOVA test results of 95% confidence limit; +, Statistically difference; –, Statistically no difference; ×, Insufficient data for testing; LM, Live Mediterranean mud shrimp; SM, Silicone Mediterranean mud shrimp; SP, Silicone pellet bait

CPUE value was calculated as 1.03 *n*/*h* and YPUE value was calculated as 89.06 *g*/*h*. According to the seasonal alterations, the most productive season was found as autumn (CPUE; 1.99 *n*/*h*, YPUE: 180.18 *g*/*h*) in spite of this the least productive season

was spring (CPUE; 0.28 *n*/*h*, YPUE: 24.63 *g*/*h*). For the summer and winter seasons, the CPUE values were respectively found as 0.89 and 1.45 *n*/*h*; additionally, the YPUE values were found as 71.77 and 124.92 *g*/*h* (Figure 4).

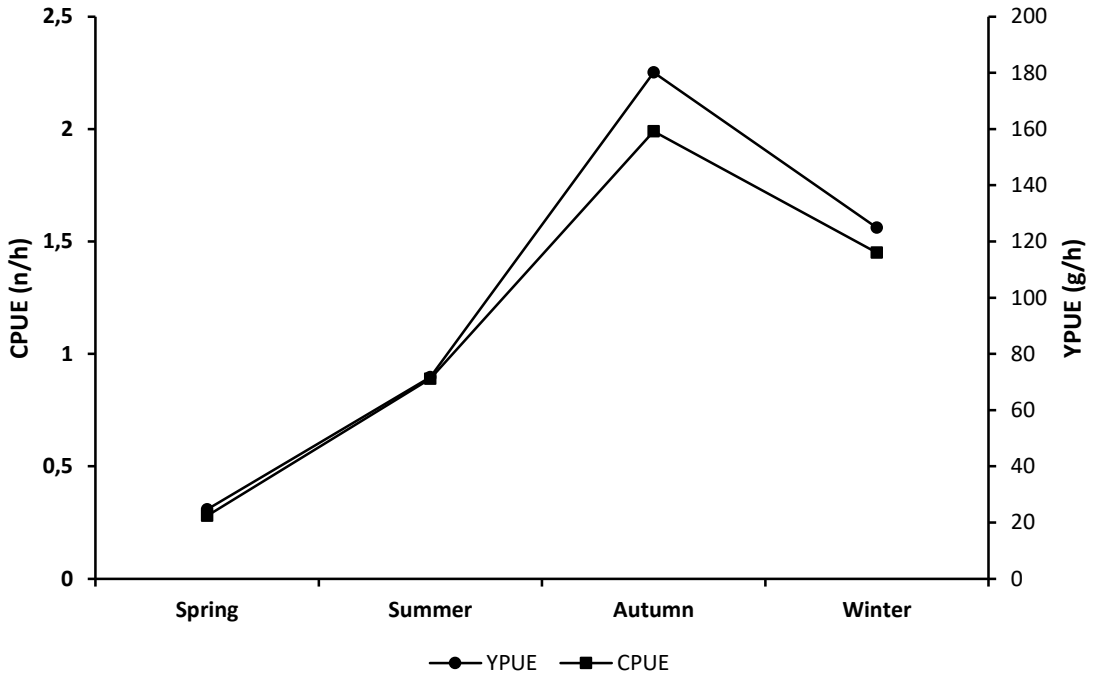

Figure 4- Seasonal changes of CPUE and YPUE values

The CPUE value of handline fishing practised with live Mediterranean mud shrimp was calculated as 1.57, 1.38 for silicone Mediterranean mud shrimp and 0.14 *n*/*h* for silicone pellet bait. On the other hand, the YPUE value of handline fishing was found as 121.84 for live Mediterranean mud shrimp and 137.73 for silicone Mediterranean mud shrimp and 7.62 *g*/*h* for silicone pellet bait (Figure 5).

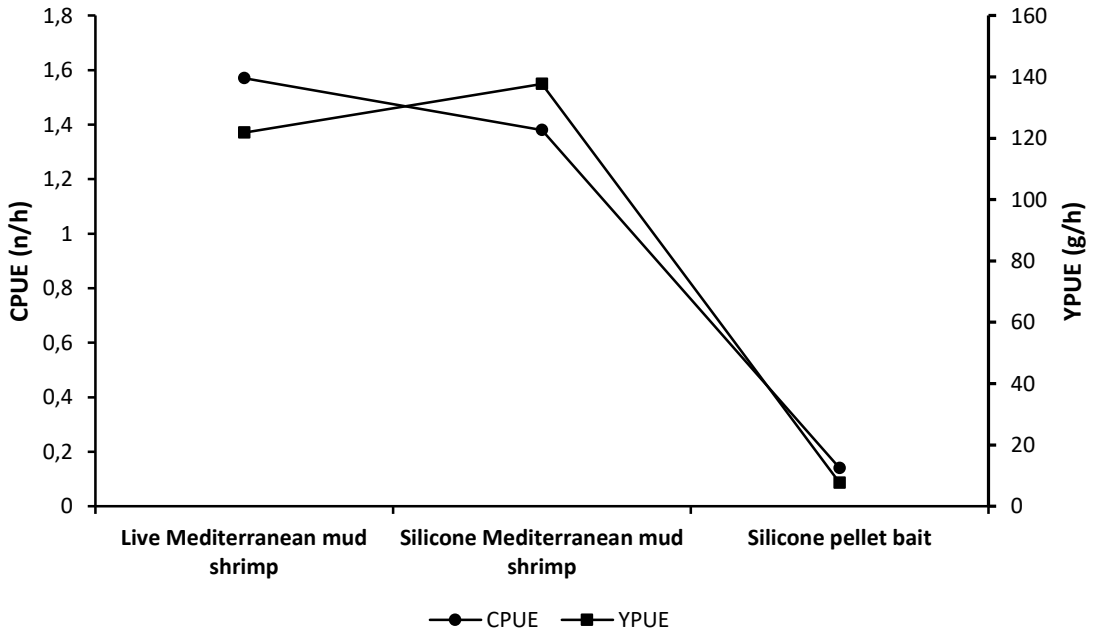

Figure 5- CPUE and YPUE values for bait types

The study findings revealed that the least productive season for all bait types was spring according to the seasonal productivity data of all three types of baits tested in the study in terms of handline fishing. The results showed that autumn was the most productive season for live Mediterranean mud shrimp and silicone Mediterranean mud shrimp. However, for silicone pellet bait, the most productive season was winter.

The numbers of individuals, which were caught with live mud shrimp and silicone mud shrimp, were relatively approximate to each other (50.7 and 44.7%, respectively). This result shows that silicone mud shrimp bait can be considered as an alternative to the live mud shrimp bait. Among the caught species, brown comber, seabream, two banded bream and common pandora have high catching rate for all types of baits. On the other hand, it was observed that mud shrimp which was used as a live bait died in the catching activities and lost the water it contains, and therefore its catching efficiency has tended to decrease. Løkkeborg (1991), minced raw materials as feeding stimulants and nylon bag as reinforcement was tested in fishing trials for cusk (*Brosme brosme*), ling (*Molva molva*), Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). According to study results, compared with natural bait, minced Atlantic herring (*Clupea harengus*) enclosed in a nylon bag gave a higher catch rate for haddock (58%), torsk and ling. The texture of the nylon bag had a negative effect on the catch rate, most pronounced for cod and haddock. Interviews conducted with the fishers in the study area demonstrated that silicone pellet catches more products in certain periods (dark of the moon) and catch certain species more (seabass, mackerel etc.). Therefore, the efficiency of silicone pellet should be investigated in another study.

The seasonal comparison of CPUE and YPUE values showed that fall is the most productive season (CPUE; 1.99 *n*/*h*, YPUE: 180.18 *g*/*h*) followed by winter (CPUE; 1.45 *n*/*h* and 124.92 *g*/*h*,), summer (CPUE; 0.89 *n*/*h*, YPUE: 71.77 *g*/*h*) and spring (CPUE; 0.28 *n*/*h*, YPUE: 24.63 *g*/*h*). The main reason for the given situation was the subnormal temperatures recorded in the winter season of the year 2017. These seasonal differences might stem from temperatures, abiotic factors such as flows, local migration, and biological factors such as abundant nutrition and bait selection. In addition, given factors, the condition of the fish, which may seasonally change, might be cause this differences.

In the context of handline fishing, fish behaviour set is classified within 4 phases as; the presence of bait, searching and findings of the bait, seizing and swallowing the bait (Fernö & Huso 1983; Özdemir & Erdem 2006). The main reason of selecting silicon mud shrimp for testing is to increase mud shrimps' duration of stay on the hook and increase their catching efficiency and catching performance. The most important senses for fishes to detect the bait are smell and visibility. Artificial mud shrimp (dummy mud shrimp) can be found in the market. This type of bait attracts a fish yet the lack of smell decreases its catching performance. Despite the fact that live mud shrimp catch higher number of individuals in handline fishing (13%) in a unit of time (1.57 *n*/*h*) in comparison to silicone mud shrimp (1.38 *n*/*h*); in terms of weight of the product in a unit of time, silicone mud shrimp (137.73 *g*/*h*) showed higher rates (13%) in comparison to live mud shrimp bait (121.84 *g*/*h*). Given the fact that the weight parameter identifies the price and level of economic revenue of the yield, it can be argued that the use of silicone mud shrimp bait is more profitable in handline fishing.

The most important benefit of the silicone mud shrimp in handline fishing was the decrease in the cost by allowing re-use of the bait (more than 5 times re-use than live mud shrimp). Another important contribution of this advantage was enabling to need lower amount of mud shrimps, particularly in the handline fishery. The given situation will offer a more environmentalist approach for catching, as fewer mud shrimps will be caught. In this study, mud shrimp's duration of staying on the hook was not calculated. On the other hand, it was observed that fishes hit the silicone mud shrimp bait to the point where there is only silicone left. The given situation allows the fishing line to stay longer in the water.

One of the most important disadvantages in experiments with live mud shrimp bait is providing of live mud shrimp particularly in the winter season. Mud shrimps bury in the mud in the cold-weather periods (winter) and continue their lives in deeper parts. This situation causes more difficult supplying live mud shrimp and its price increase 3 or 4 times than the normal season. Particularly in these periods, the use of silicone mud shrimp in handline fishing will help to decrease the cost of baits by preventing loses from the catches.

The captured of 590 individuals belonging to 30 species shows that the richness of the study area and the attractiveness of the selected baits. The maximum number of species in the Aegean Sea, in line fishing (including long line), was reached in this study (Kaykaç et al. 2003; Aydın 2011; Soykan & Kınacıgil 2013). On the other hand, the results indicate that more than 70% of the obtained individuals had economic significance and the seabream constituted 20% of the total catched products prove the importance of handline fishing in the region. The cephalopods had a low rate within the catch composition as 6.6%. The main reason for the given situation is the methods that are used only for fish; and different handline and equipment are used for cephalopods (Beğburs et al. 2004; Kaykaç et al. 2012).

In terms of 3 baits of total catches, 9 species have sufficient data set for statistical tests. It was found that there is no significant difference between the lengths groups except two banded bream. Differences were found for two banned breams live mud shrimp-silicon pellet and silicon mud shrimp and silicone pellet. No difference was found for 15 species lengths groups between caught live and siliconized mud shrimp. The communication of the Republic of Turkey Ministry of Agriculture and Forestry No: 4/1, which regulated the commercial catch, imposed weight and lengths restriction species (RTMAF 2016). Among these, the minimum length sized for the species -which are also included in this study- have been announced as 20 cm for seabream, 18 cm for two banded bream and mackerel, 15 cm for common pandora and 21 cm for white seabream. The average total length of the seabreams obtained in experiments found as 17.62 cm (live mud shrimp bait: 17.17, silicon mud shrimp bait: 18.07 cm, which was below the legal length). While both common pandora and mackerel mean lengths were found above the minimum

landing size. Differences were found for two banned breams, which were found different in terms of length groups of live mud shrimp-silicon pellet and silicon mud shrimp and silicone pellet.

There are no studies conducted on this type of bait which was used in the tests both in the Turkish and international literature, therefore direct comparisons could not be performed. There is only one study on the catching performance and catching efficiency of the mud shrimp (Erzini et al. 1998). The catching performance of razor shell (*Ensis siliqua*) and mud shrimp (*Upogebia pusilla*) were tested with round bend, flatted and spade end of 2316 DT hooks (numbers 11.13 and 15). It was found that the bait type did not significantly affect the catch size distribution. Although more fish were caught with the razor shell bait, higher catch rates red sea breams obtained with mud shrimp.

4. Conclusions

Article 32(b) of the communication (communication no: 2016/35) regarding the regulation of commercial fishery, provincial directorates have authorized to regulate catching method, time restriction, annual yield etc. for products to be used as bait in fisheries. According to this regulation, fishing, supplying and commercial sale of mud shrimp (including European razor clam, dye-murex and solitary tube worm) are forbidden within the borders of İzmir province between the dates of 1st February and 30th June. On the other hand, there are no records of statistics kept for this species, and of others, which are used as fish baits (European razor clam, solitary tubeworm etc.). It is stated that the catching pressure has been gradually increasing on this species, and the catching rates have been gradually decreasing. Therefore, population dynamics studies should be investigated.

A transparent silicone was used in the study. An important question is whether the silicon pellet which is commonly used in handline and long line fishing is digested by fish or not. There is no information on the individuals, which swallowed the silicone pellet. An urgent need to carry out studies on the questions of whether silicone pellet is digested (or defecated) or leads to death. In the case that any adverse impacts of silicon pellet will be detected, its commercial use should be forbidden.

The present study includes innovative ideas and methodologies to be used in handline fishing. Despite the fact that the number of individuals catches by mud shrimp in a unit of time in handline fishing is high, it was identified that the weight catches in a unit of time is more with a silicone mud shrimp. Given that the weight parameter is decisive in determining the price of a product and the level of economic input; it can be argued that silicone mud shrimp bait catching in handline fishing is more profitable. The study results lead to the conclusion that the use of silicone mud shrimp bait is useful particularly in the periods that a live mud shrimp cannot be used or provided.

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References

Akamca E & Kiyağa V B (2009). Effects of live bait size on European seabass (*Dicentrarchus labrax*) angling. *XV. National Fisheries Syposium*, 01-04 July, Rize

Ateşşahin T, Duman E & Cilbiz M (2015). Selectivity and catch efficiency of three spinner hook sizes in angling for rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) in Karakaya Dam Lake (Eastern Turkey). *Turkish Journal of Fisheries and Aquatic Sciences* 15(4): 851-859 http://dx.doi.org/10.4194/1303-2712-v15_4_08

Aydın İ (2011). Is natural bait-type a stochastic process for size and condition of fishes in the recreational fishery from the İzmir Bay? *Mediterranean Marine Science* 12(2): 390-400 http://dx.doi.org/10.12681/mms.39

Beğburs C R, Altınağaç U & Hoşsucu H (2004). A study on octopus handlines made from different materials (In Turkish). *Ege Journal of Fisheries and Aquatic Sciences* 21(1-2): 5-8

Erzini K, Gonçalves J M S, Bentes L, Lino G B & Ribeiro J (1998). Species and size selectivity in a 'red' sea bream long line 'métier' in the Algarve (southern Portugal). *Aquatic Living Resources* 11(1): 1-11 http://dx.doi.org/10.1016/S0990-7440(99)80025-4

Fernö A & Huse I (1983). The effect of experience on the behaviour of cod (*Gadus morhua* L.) towards a baited hook. *Fisheries Research* 2(1): 19-28 http://dx.doi.org/10.1016/0165-7836(83)90100-5

Froese R & Pauly D (2019). FishBase. Retrieved in February, 12, 2019 from http://www.fishbase.org

Huse I & Fernö A (1990). Fish behaviour studies as an aid to improved long line hook design. *Fisheries Research* 9(4): 287-279

Kaykaç M H, Arslan E, Göncüoğlu H, Maktay C B, Aydın C, Aydın İ, Bayramiç İ, Özdemir Y, Ünal V & Tokaç A (2012). The Importance of the Color Factor and Squid (*Loligo Vulgaris* Lamarck, 1798) Fishing Methods in Izmir Bay (In Turkish). Ege University Scientific Research Projects Coordination, 2009/SÜF/023, İzmir

Kaykaç M H, Ulaş A, Metin C & Tosunoğlu Z (2003). A study on catch efficiency of straight and kirbed hooks at handline fishing (In Turkish). *Ege Journal of Fisheries and Aquatic Sciences* 20(1-2): 227-231

Løkkeborg S (1991). Fishing experiments with an alternative long line bait using surplus fish products. *Fisheries Research* 12(1): 43-56

Özdemir S & Erdem Y (2006). Fish behaviour at fisheries by passive fishing gears (In Turkish). *Ege Journal of Fisheries and Aquatic Sciences* 23(1/3): 467-471

Palomares M L D & Pauly D (2019). Sea Life Base. Retrieved in April, 23, 2019 from http://www.sealifebase.org

RTMAF (2016). Notification 4/1 Regulating Commercial Fishing. *Republic of Turkey, Minister of Agriculture and Rural Affair, General Directorate of Conservation and Inspection*, Ankara

Soykan O & Kınacıgil H T (2013). Setlining for European Seabass (*Dicentrarchus labrax*, Linnaeus, 1758); technical features and bait preference. *Ege Journal of Fisheries and Aquatic Sciences* 30(2): 45-49 http://dx.doi.org/10.12714/egejfas.2013.30.2.01

TSMS (2017). Meteorological Dictionary-Turkish State Meteorological Service. Retrieved in August, 1, 2019 from https://www.mgm.gov.tr/genel/meteorolojisozlugu.aspx

Zar J H (1974). Biostatistical Analysis. Englewood Cliffs, Prentice-Hall, New Jersey

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Effects of Phytase Enzyme Supplementation to Hazelnut Meal Based Diets on Growth Performance and Nutrient Digestibility of Siberian sturgeon (*Acipenser baerii* Brand, 1869)

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ABSTRACT
This research was carried out to determine that the effects of diets containing 30% hazelnut meal and different proportions of phytase enzymes on the growth performance of Siberian sturgeon (*Acipenser baerii*) (initial mean weight, 960.23 ± 0.55 g). The trial diets consisted of feeds supplemented with 0.25 g kg^{-1} (G2), 0.50 g kg^{-1} (G3), and 1.00 g kg^{-1} (G4) phytase enzyme, and with no enzyme added to the control group (G1), respectively. Experiment groups were performed in 3 replicates and trials were carried out for 90 days.

As a result, adding phytase enzyme to feeds contain 30% hazelnut meal was found to have a positive effect on the weight gain (WG, g), the protein efficiency ratio (PER), feed conversion ratio (FCR), and specific growth rate (SGR, %). Growth performance was found to be more successful in all groups fed feeds supplemented with phytase enzyme compared to the G1 group ($P<0.05$). The G4 was the best group than the others statistically ($P<0.05$). In terms of the digestibility effect of enzyme added groups, the highest total digestion rate (77.14 ± 0.07%) was obtained in the G4 enzyme group and the lowest total digestibility (74.32 ± 0.02%) was estimated in the G1.

Keywords: Phytase; Plant protein; Sturgeon; *Acipenser baerii;* Fish meal

1. Introduction

The feed costs constitute 50-75% of total operational expenses in aquaculture. The high protein-containing raw materials are used in feed production and fish meal is preferred. In recent years, the increase in fish meal prices has escalated feed costs (Akiyama 1995). The fish meal and fish oil import reached up to 123 thousand tons and 40 thousand tons as of 2017 in Turkey. The amount of foreign currency paid for fish meal import is 155 million dollars (Anonymous 2017). The plant-based feed raw materials are shown as a remedy for reducing feed costs (Akiyama 1995). Many studies have been carried out regarding the use of plant-based feed ingredients instead of some or all of the fish meal. In these studies, with the increased protein qualities of legumes and oilseed meal, the negative effects were addressed and the insufficient amino acid structure was tried to be balanced by the addition of various synthetic amino acids and various attractant substances to increase feed intake (Yeşilayer et al. 2013; Morales et al. 2016; Jiang et al. 2018; Arıman Karabulut et al. 2019). During the past two decades, the use of enzymes to reduce the adverse effects of anti-nutritional factors arising from feed additives, to increase feed digestibility, and to reduce environmental impacts is becoming increasingly widespread in animal production (Cao et al. 2007; Zhu et al. 2014; Mireles-Arriaga et al. 2015). The phytic acid content of the plant originated raw materialsis different. For example, it ranges between 0.50 and 1.89% in wheat, 0.40 and 2.06% in legumes, 2.00 and 5.20% in oilseed plant, 1.00 and 2.20% in soybean, and 0.23 and 0.92% in hazelnut (Schlemmer et al. 2009).

In-plant feed ingredients, the amount of phosphorus is sufficient for fish metabolism. However, owing to the presence of phosphorus phytin in soybean meal and by-products, its digestibility is low (Wang et al. 2009). The effectiveness of the fish to benefit from phosphorus can be increased by the soybean meal, which is subjected to pre-treatment with phytase enzyme. It has been reported that the use of phytase enzyme reduces the excretion of phosphorus in aquaculture. Phytase is the enzyme that removes phosphates from the structure of phytic acid by providing hydrolysis (Selle et al. 2000; Zhu et al. 2014; von Danwitz et al. 2016).

The using rate of enzymes at the feed 1-2‰ will have a minor effect on the feed cost. However, positive effects such as improving the digestibility of feeds, increasing feed utilization rate and creating less phosphorus excretion to the environment cover this cost excessively. Commercial enzymes are still used today in poultry feeds. The use of enzymes produced for fish feed is relatively new in the market (Yiğit & Koca 2011). It has been used more and more in fish feeds for the last two decades

to make better use of plant nutritional supplements. The addition of protease, mixed enzyme, and phytase to sea bream feed using soybean meal instead of fish meal (40% soybean meal, 25% fish meal) resulted in significantly better feed evaluation (Ayhan et al. 2008). Farhangi & Carter (2007) reported that in the feed of rainbow trout, which is 16.58 g, the addition of different exogenous enzymes increases the use of plant additives and reaches a better feed conversion ratio (FCR) value than fish meal supplemented feed group. Wang et al. (2009) added different levels of phytase to the feed of *Oncorhynchus mykiss* with soybean meal and determined that the phytase enzyme increased the digestibility of proteins and minerals, decreased the digestion of fats, and also decreased the loss of nutrients by feces. In another study, it has been determined that the addition of multienzyme to the plant additives feed of juvenile *Huso huso* significantly improved weight and increased the SGR and FCR (Ghomi et al. 2012).

The literature on the use of phytase in the feed of sturgeon is limited but the phytase enzyme additive feeds are investigated in *Oncorhynchus mykiss* (Wang et al. 2009; Morales et al. 2016), *Oreochromis niloticus x Oreochromis aureus* (Lin et al. 2007), *Pelteobagrus fulvidraco* (Zhu et al. 2014), *Psetta maxima* (von Danwitz et al. 2016), *Ictalurus punctatus* (Chen et al. 2019) and *Pagrus major* (Biswas et al. 2019).

Sturgeon is a species with a high economic and ecological value, because of the meat and its caviar in terms of aquaculture (Arıman Karabulut & Osmanoglu 2019). In this study, the effect of the use of phytase enzyme with different levels of hazelnut meal that is added to Siberian sturgeon (*Acipenser baerii*) feed was conducted.

2. Material and Methods

2.1. Rearing and facilities

This research was conducted at Recep Tayyip Erdogan University (RTEU) Aquaculture Application and Research Center between December 2018 and March 2019 (90 days) under natural photoperiod conditions. In total, 120 randomly sampled Siberian sturgeon (960.23 ± 0.55 g) were used at 1^{+} years of age. Fishes were placed in 12 tanks with a tank volume of 605 L (water level adjusted to 480 L), with 10 fish in each tank. During the study, the development of fish was monitored by individual length and weight measurements at 15-day intervals. Water temperature, dissolved oxygen, and pH values were routinely measured throughout the experiment. The temperature, pH and oxygen minimum and maximum values were determined between 9.5 and 13.5 °C, 7.1 and 7.4, and 9.8 and 9.9 mg L^{-1}, respectively.

2.2. Experimental diets

The feeds with 45% crude protein (isonitrogenous) and 12.7% crude lipid used in the experiment were produced with fish meal, hazelnut meal, boncalite, corn gluten, fish oil, pellet binder, vitamin-mineral mixes, and different ratios of phytase enzyme (0.25 g kg^{-1}; G2, 0.50 g kg^{-1}; G3 and 1.00 g kg^{-1}; G4). Fish meal, fish oil, hazelnut meal, corn gluten, boncalite, vitamins-mineral mixes were obtained from a commercial feed factory (KAGSAN-Trabzon-Turkey). Similarly, 6-phytase (E.C.3.1.3.26) enzyme (Ronozyme HiPhos (M) produced by the strain *Aspergillus oryzae* DSM 22594) was obtained from DSM Nutritional Products in Istanbul Turkey.

The basic nutritional content of feed ingredients (crude protein (CP), dry matter (DM), crude ash (CA), crude lipid (CL), nitrogen-free extracts (NFE), and crude cellulose) was analyzed in the RTEU Faculty of Fisheries laboratories. The formulation of feeds was calculated using the Microsoft Office Excel program.

The four different feeds, which are control (G1), G2, G3, G4, and contain phytase enzyme 0, 0.25, 0.50, and 1.00 g kg^{-1}, respectively, were prepared (Table 1). First, the feed raw materials were milled and sieved through a 500 µm mesh screen and mixed for 15 minutes until a homogeneous mixture was obtained. Then, phytase enzyme (except G1), fish oil, and 35% distilled water were added and mixed for 15 minutes, and pulped. The dough mixture was passed through a mincing machine and pellets suitable for fish were created. The prepared pellets were dried in the POL-EKO APARATURA brand drying cabinet set at 50 °C for 24 hours (Gimenez et al. 2009). The feeds taken from the oven were kept at normal room temperature and cooled, then stored at -20 °C to prevent enzyme degradation.

Table 1- Ingredients and proximate composition of experimental diets (% dry matter)

Ingredients	*G1*	*G2*	*G3*	*G4*
Fishmeal	30	30	30	30
Hazelnut meal (defatted)	30	30	30	30
Boncalite	12	11.75	11.5	11
Corn gluten	16	16	16	16
Fish oil	8.25	8.25	8.25	8.25
Phytase	0	0.25	0.5	1
Methionine	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5
Vitamin mixture	0.75	0.75	0.75	0.75
Mineral mixture	0.5	0.5	0.5	0.5
Molasses	1	1	1	1
Chromic oxide (Cr_2O_3)	0.5	0.5	0.5	0.5
Proximate composition				
Dry matter (DM) (%)	91.3	90.8	91.1	91.0
Crude protein (CP) (%)	45.10	45.12	45.14	45.19
Crude lipid (CL) (%)	12.69	12.68	12.68	12.67
Crude ash (CA) (%)	8.1	8.2	8.1	8.0
Crude cellulose (CC) (%)	2.10	2.08	1.99	2.00
NFE	23.31	22.01	23.18	23.14
GE (MJ kg^{-1})	19.74	19.72	19.73	19.69

Note: Vitamin mixture**:** Included of per kg; Vitamin A 20.000.000 IU, Vitamin D_3 2.000.000 IU, Vitamin E 200.000 mg, Vitamin $K_3$12.000 mg, Vitamin B_1 20.000 mg, Vitamin B_2 30.000 mg, Vitamin B_6 20.000 mg, Vitamin B_{12} 50 mg, Vitamin C 200.000 mg, Niacin 200.000 mg, Cal.D. Panth. 50.000 mg, Folic acid 6.000 mg, D-Biotin 500 mg, Cholin Chloride 300.000 mg; Mineral mixture: Included of per kg; 60 mg Manganese, 80 mg Zinc, 60 mg Ferro, 5.000 mg Copper, 2.000 mg Iodine, 1.000 mg Cobalt, 200 mg Selenium, 50 mg Magnesium. Nitrogen free extracts (NFE) = matter - (CP + CL + CA) (adapted by Liu et al. 2009) Gross energy (GE) = (CP × 23.9 MJ kg^{-1}) + (CL × 39.8 MJ kg^{-1}) + (NFE × CC × 17.6 MJ kg^{-1}) (adapted by von Danwitz et al. 2016).

2.3. Feeding and feces collection

The feeds were prepared as 2% of the bodyweight after measurements were recorded every 15 days. Feeding was carried out by hand three times a day, 9:00 am, 1:00 pm, and 5:00 pm equal to each meal. After one hour from feeding, the fecal collection was performed by siphoning method (Liu et al. 2009). The collected samples of feces were frozen at -20 °C without waiting and stored in the freezer until analysis was performed (Adapted by Omnes et al. 2017).

2.4. Determination of apparent digestibility coefficient

After three months of the experiment, 0.5% ratio chromium oxide (Cr_2O_3) was added as an indicator to calculate the digestion rates of the nutrients in the trial feeds. To determine the digestion rates in the groups, the fish were adapted to indicator supplemented feeds for 5 days. Following the adaptation process, feces were collected from the tanks with siphoning. Apparent digestibility coefficients for dietary nutrients were calculated as follows:

ADC of nutrient (%) =100 x [1 - (Cr_2O_3 in diet/Cr_2O_3 in faeces) x (nutrient in faeces/nutrient in diet)] (1)

ADC of dry matter (%) = 100 x [1- (Cr_2O_3 in diet/Cr_2O_3 in faeces)] (Jiang et al. 2018). (2)

2.5. Sample collection and chemical analyses

Both at the beginning and the end of the experiment, 3 specimens were taken randomly from each tank and their length and weights were determined. The samples were humanely killed by overdose anesthesia (100 ppm clove oil) based on the procedures of the Recep Tayyip Erdogan University Ethics Committee (Decision No: 2015/17). The carcass weights were determined by

removing the head, fins and internal organs of the fish. (adapted by Jiang et al. 2018). The fish meat composition was defined by the analysis of CP, CL, CA, moisture (M), and DM. The DM ratio of the fish meats was calculated according to "TS 1743 (110 ± 1 °C)", CP according to the "Kjeldahl Method", CL according to the "Soxhlet Method" (Lovell 1981), CA according to "TS 1746" (550 ± 1 °C)" (Lovell 1981; AOAC 2000). The amino acid analysis of the trial feeds was performed by Kazlıçeşme R & D Laboratory and the results were presented in Table 2. The nutritional ratios of experimental feed and feces were determined with respect to AOAC (2000). The chromium oxide ratios in feed and feces samples were determined with respect to Furukawa & Tsukahara (1966).

Table 2- Amino acid content of experimental diets (% of dietary protein)

Essential amino acids	*G1*	*G2*	*G3*	*G4*
Valine	4.47	4.45	4.46	4.45
Leucine	4.60	4.63	4.61	4.61
Isoleucine	3.09	3.10	3.11	3.10
Lysine	7.65	7.64	7.66	7.67
Phenylalanine	5.09	5.10	5.12	5.11
Threonine	3.90	3.89	3.92	3.86
Histidine	3.55	3.55	3.54	3.53
Methionine	2.68	2.71	2.70	2.70
Arginine	5.70	5.73	5.79	5.67
Non-essential amino acids				
Alanin	2.63	2.60	2.64	2.64
Aspartik Acid	4.42	4.46	4.48	4.47
Tyrocine	1.32	1.30	1.33	1.30
Gylcine	2.31	2.29	2.30	2.29
Proline	2.40	2.39	2.41	2.43
Cystine	0.29	0.30	0.31	0.29
Glutamic Acid	6.11	6.10	6.12	6.13
Serine	2.02	1.99	2.04	2.03

2.6. Statistical analysis

Results are presented as determined by the mean ± SE of three replicates. To evaluate the findings of the present study Sigma Plot 11.0 package programs were used. All data were subjected to one-way analysis of variance (ANOVA) and then by TUKEY's multiple range test. it was considered significant with a p-value of <0.05.

3. Results

3.1. Feed utilization and growth performance

At the end of the study, the growth performance data of fish fed with different amounts of enzyme-containing feed for 90 days are presented in Table 3. According to these data, the best specific growth rate (SGR) and weight gain (WG) were observed in the G4 group. The G4 group was followed by G3, G2, and G1, respectively, and the differences between all groups were found to be statistically significant (P<0.05). While the best FCR was found in the G4 group, the worst FCR was obtained in the G1 group. The best protein efficiency ratio (PER) was found in G4 between all groups. While the difference between all groups was statistically significant in terms of PER, there was no difference in terms of condition factor (CF) (P<0.05).

Table 3- Effect of dietary enzyme supplementation on the performance of Siberian sturgeon

Parameters	*G1*	*G2*	*G3*	*G4*
IBW (g)	961.27 ± 4.21	958.73 ± 1.60	959.97 ± 3.43	960.96 ± 9.27
FBW (g)	1119.58 ± 2.61 ^{a}	1273.93 ± 4.33 ^{b}	1370.65 ± 3.21 ^{c}	1433.18 ± 1.85^{d}
WG (g)	158.33 ± 1.51^{a}	315.21 ± 1.28^{b}	410.70 ± 1.74^{c}	472.23 ± 0.68^{d}
WGR (%)	16.45 ± 2.05 ^{a}	32.92 ± 1.89 ^{b}	42.77 ± 1.09 ^{c}	49.35 ± 2.11 ^{d}
SGR (%)	0.17 ± 0.24^{a}	0.33 ± 0.16^{b}	0.41 ± 0.17^{c}	0.46 ± 0.15^{cd}
FCR	1.98 ± 0.10^{a}	1.60 ± 0.03^{b}	1.52 ± 0.05^{c}	1.41 ± 0.03^{d}
FI (% day^{-1})	313.49 ± 2.07	504.34 ± 0.25	624.26 ± 1.38	665.85 ± 1.21
PER	3.52 ± 0.34^{a}	6.98 ± 0.29^{b}	9.11 ± 0.31^{c}	10.43 ± 0.22^{d}
CF (%)	0.35 ± 0.02^{a}	0.38 ± 0.01^{a}	0.42 ± 0.04^{ab}	0.45 ± 0.04^{ab}

Note: Data represent as mean ± *SE* of triplicate tanks (n = 10, r = 3); mean values in the same row with different superscripts are significantly different (P<0.05). IBW: Initial body weight; FBW: Final body weight; DBW: Deat body mass; "t" is the number of culture days. Weight gain (WG, g) = FBW−IBW Weight gain rate (WGR) = 100 × [(FBW−IBW) / IBW], Specific growth rate (SGR, %/day) = 100 × [(ln FBW−ln IBW)/t], Feed conversion ratio (FCR) = [(feed consumed) / (wet weight gain)], Feed intake (FI, %/day) = 100 × (dry feed intake) / [(IBW + FBW + DBW)/2 × t)], Protein efficiency ratio (PER) = wet weight gain / protein intake, Condition factor (CF) = (wet weight / total length cm3) × 100

3.2. Apparent digestibility coefficients

The digestibility values of the nutrients in the groups fed with the experimental feeds are presented in Table 4. The best total digestion rate and protein digestion rate were obtained at the G4 group. As a result of the study, total digestion rate, protein digestion rate, and lipid digestion rate values were found to be statistically significant in the experimental groups to the G1 group ($P<0.05$).

Table 4- Effect of dietary enzyme supplementation on the apparent digestibility coefficient (ADC) of Siberian sturgeon

ADC (%)	*G1*	*G2*	*G3*	*G4*
Dry matter	74.32 ± 0.02^{a}	74.98 ± 0.10^{b}	76.05 ± 0.12^{c}	77.14 ± 0.07^{d}
Protein	81.27 ± 0.27^{a}	82.77 ± 0.11^{b}	84.89 ± 0.09^{c}	85.03 ± 0.12^{d}
Lipid	83.39 ± 0.16^{a}	85.80 ± 0.09^{b}	86.06 ± 0.03^{b}	90.44 ± 0.14^{c}

Note: Data represent as mean ± *SE*. Mean values in the same row with different superscripts are significantly different ($P<0.05$)

3.3. Whole-body index and body composition

The Viscerosomatic index (VSI), Gonadosomatic index (GSI), and Hepatosomatic index (his) values got at the end of the experiment are given in Table 5. In terms of hepatosomatic organs and gonadosomatic organs, the experimental groups were found to have a lower value than the G1 group as well as the digestive performance. The gonad weights of the enzyme-added groups were observed heavier than the G1 group and the difference between the groups was statistically significant ($P<0.05$).

Table 5- Body composition and biometric parameters at the end of the experiment

Parameters	*G1*	*G2*	*G3*	*G4*
HSI (%)	5.29 ± 0.42^{a}	3.80 ± 0.25^{b}	3.72 ± 0.23^{b}	3.28 ± 0.30^{c}
VSI (%)	14.3 ± 2.02^{a}	12.18 ± 1.53^{b}	12.01 ± 1.16^{b}	11.4 ± 0.94^{c}
GSI (%)	0.18 ± 0.13^{a}	0.47 ± 1.10^{a}	0.52 ± 0.84^{b}	0.58 ± 0.03^{b}
Crude matter (CM) (%)	23.04 ± 0.01^{a}	24.88 ± 0.02^{b}	25.02 ± 0.01^{c}	25.37 ± 0.03^{c}
Crude protein (CP) (%)	17.53 ± 0.10^{a}	18.44 ± 0.13^{b}	18.57 ± 0.19^{b}	18.92 ± 0.22^{c}
Crude lipid (CL) (%)	2.69 ± 0.22^{a}	2.58 ± 0.10^{b}	2.56 ± 0.31^{b}	2.57 ± 0.07^{b}
Crude ash (CA) (%)	0.82 ± 0.11^{a}	0.77 ± 0.10^{b}	0.76 ± 0.22^{b}	0.76 ± 0.14^{b}

Note: Data represent as Mean ± *SE* of triplicate tanks (n= 3, r= 3); mean values in the same row with different superscripts are significantly different ($P<0.05$). Hepatosomatic index (HSI) (%) = 100 ×(liver weight (g) / fish weight (g)), Viscerosomatic index (VSI) (%) = 100 ×(viscera weight (g) / whole body weight (g)), Gonadosomatic index (GSI) (%) = 100 ×(gonad weight (g) / whole body weight (g))

4. Discussion

In this research, the potential of phytase enzyme supplemented and hazelnut meal added fish feed in Siberian sturgeon cultivation and its effects on fish growth parameters were investigated.

In some studies, it has been reported that the use of multienzyme and phytase enzyme in omnivorous and herbivorous fed warm-water fish feeds, which can benefit better from plant feed raw materials, achieved successful results (Lin et al. 2007; Chen et al. 2019). Yan et al. (2002) fed channel catfish (*Ictalurus punctatus*), which weighed 12 g, with a phytase-supplementary fish feed. They reported that enzyme-supplemented feed did not affect weight gain, however, feed intake was higher in the control group than in the phytase groups. Lin et al. (2007), the commercial enzyme complex supplemented with soybean and cotton meal containing fish feeds was used and they fed hybrid tilapia (*Oreochromis niloticus*) which weighed 18 g. As a result, they reported that the enzyme-containing groups observed better growth performance than the control group. Chen et al. (2019) investigated the nutrient utilization, growth performance, and phosphorus equivalence in the usage of phytase in plant-based feeds on channel catfish (*I. punctatus*). As a result, they reported that the growth performance of phytase, which is complementary to fish feed, the FCR, the digestibility coefficient of phosphorous increased, and phosphorus content excreted in feces decreased significantly. Ghomi et al. (2012) used multienzyme supplementary feed (250 mg kg^{-1}) at fingerling *Huso huso* with an initial weight of 9.76 ± 0.68 g. As a result of exogenous enzyme addition higher WG (53.03 ± 0.15 g), the best SGR (3.68 ± 0.17%), and the best FCR (3.49 ± 0.14) were found. The same researchers reported that enzyme addition provided significant improvement in comparison with the control group. Ávila et al. (2015) used soybean meal as the main protein source in the juvenile rainbow trout diet and investigated its effects on growth performance, phosphorus excretion, and lysozyme activity by supplementation yeast and phytase enzyme to the diet. As a result of the study, higher values were observed on the SGR, FCR, and Protein conversion ratio (PCR) on the groups fed with yeast and phytase enzyme supplemented diets than those observed in

the commercial diet, but no significant differences were reported. Wang et al. (2009) have fed rainbow trout for 90 days with different levels of phytase enzyme added and soybean meal containing feed in their study. At the end of the study, they reported that the FCR and PER were improved and the SGR did not change to this was not statistically significant. Xu et al. (2020) conducted a study on juvenile hybrid sturgeon (*Acipenser baerii* ♀ × *Acipenser schrenckii* ♂) for 12 weeks. In their study, they created four feed groups (FM 100, FM 100p, FM 250, and FM 250p), which contains 10% and 25% fishmeal and the other protein source (soybean meal, wheat flour, cottonseed protein), with the same proportions phytase supplemented or without. As a result, they reported that there was no significant difference between SGR, WGR, FI, and FCR. Maas et al. (2018) conducted a study on the effect of phytase, xylanase, and their combination on growth performance and nutrient utilization in Nile tilapia (*O. niloticus*). At the end of the study, it was reported that both phytase and xylanase-supported feed groups significantly affected growth. It was stated that growth was similar to control in xylanase-supplemented diets, whereas growth in phytase-supplemented diets improved. Also, the best growth performance was determined with fish fed the diet supplemented with both phytase and xylanase groups. Ayhan et al. (2008) investigated the effects of protease, multienzyme, and phytase additions on growth, feed evaluation and nitrogen-phosphorus excretion in sea bream feeds using soybean meal instead of fishmeal. At the end of the study, there was no significant difference between the groups in terms of WG and the SGR, while the phytase-added group has been reported to have significantly better FCR compared to the control group. As a result of this study, WG, SGR, FCR, PER, and CF were positively affected in all groups in parallel with the increase in enzyme amount in fish fed with Siberian sturgeon feed supplemented with phytase enzyme and hazelnut meal. The best group was identified as G4, which has been fed with feed supplemented with 1.00 g kg^{-1} phytase, and the G4 group was found to be statistically significant compared to all groups ($P<0.05$). In the present study, the inclusion of phytase enzyme in fish feed has improved the rate of utilization of hazelnut meal additive. This study is similar to those studies of Lin et al. (2007), Ghomi et al. (2012), Ávila et al. (2015), Maas et al. (2018), Chen et al. (2019), and Xu et al. (2020) the group FM 100p in terms of WG and SGR and FM 250p in terms of FI and FCR in which the enzyme supplement had a positive effect on the growth parameters. However, the current study is different from the studies of Wang et al. (2009) who have reported that SGR, FCR, and PER are not statistically different. Also, this study is different from the studies of Ayhan et al. (2008) in terms of SGR, Yan et al. (2002) in terms of WG and Xu et al. (2020) the group FM 100p in terms of FI and FCR and FM 250p in terms of WG and FI. It is thought that the difference between the results can be due to the different fish species used, different raw materials of plant origin, the enzyme used in a different amount of use.

The effect of apparent phosphorus digestibility is one of the most important criteria to evaluate the effect of phytase on phosphorus digestibility. Phytase helps to prevent phosphorus contamination by converting the phosphate in the form of phytate into usable phosphorus and enables the use of phosphorus in a more convenient way, reducing inorganic phosphate supplementation in the feed (Selle et al. 2000; Cao et al. 2007; Mireles-Arriaga et al. 2015). Phytate degrading enzymes are recommended to increase the nutritional value of plant material. In recent years, to reduce phosphorus pollution as an environmental waste in intensive animal husbandry, the use of phytase enzyme in animal feed has been brought to the agenda (Asan 2007). In the present study, the effect of phytase enzyme on growth parameters and digestibility has been investigated by participating in different proportions to feed with containing 30% hazelnut meal. The best total digestion rate was observed in the G4 group where the most phytase enzyme was added with 77.14%. Rodehutscord & Pfeffer (1995) added 1000 IU kg^{-1} phytase enzyme to trout feed containing soybean meal and reported that phosphorus digestibility increased from 25% to 57% at 15 °C by adding phytase to the diet. In another study, protease, different enzyme mixtures, and phytase enzymes were added separately to sea bream fish feeds containing fish meal and soybean meal, and their effects on growth parameters and nitrogen-phosphorus pollution were investigated. As a result, it was reported that the best phosphorus digestibility was determined in the phytase supplemented feed group (Ayhan et al. 2008). Wang et al. (2009) conducted a digestibility study with soybean meal containing phytase enzyme supplementation in rainbow trout. They reported that with phytase supplementation, the apparent digestibility coefficient (ADC) of diet protein and minerals was increased, also as a negative effect of phytase on the ADC of lipid. Also, they stated that with phytase supplementation, lipid excretion slightly increased, but nutrient excretion decreased in feces. Xu et al. (2019) reported that the phytase-supplemented FM 100p group showed better ADC than the phytase-free FM 100 group, while the phytase-supplement FM 250p group showed a worse ADC value than the phytase-free FM 250. As the reason for this, it was reported that the plant protein level in the FM 250p diet does not provide sufficient phytate-P and phytate complexing protein, also, the ability of fish to digest and absorb fish meal-rich bone phosphorus be insufficient. Maas et al. (2018) reported that the phytase supplement increased ADC from 90.1% to 91.2% for crude protein, 92.9% to 93.9% for crude lipid, and 49.7% to 56.7% for carbohydrates.

The improvement of the growth performance in fish and well assessment of feed, thus reduction of environmental waste most likely related to the digestibility of nutrients. Similarly, in this study, enzyme use in fish feeds improved growth performance and feed conversion ratio. These observations support the knowledge that phosphorus can be used more conveniently by degrading phytate phosphorus in feeds supplemented by the phytase enzyme. It has been concluded that the use of phytase may contribute to environmental sustainability in connection with less waste release to the receiving environment.

Farhangi & Carter (2007) have added 4 different exogenous enzymes to peeled lupin containing (*Lupinus albus*) feed of the rainbow trout (16.58 g) and they reported no statistically significant change in WG and carcass composition between the groups at the end of a 6-week feeding trial. Imanpoor & Bagheri (2012), in their study on Iranian sturgeon (352.07 g) (*Acipenser persicus*) added 0.5 g kg^{-1} magnesium and 0.5 g kg^{-1} phytase enzyme to the soybean meal supplement and they reported that HSI values were better in enzyme-supplemented groups compared to the control group but there was no difference in body

composition values. Biswas et al. (2019) prepared two different feeds and added phytase enzyme in the second feed trial. They reported that phytase enzyme addition did not make a significant difference on VSI, HSI, crude protein, and crude lipid compared to the control groups and that the groups differed only with the feed group that they prepared with a protein-based diet. In this study with the Siberian sturgeon fed with 30% containing hazelnut meal, a significant difference was detected between the G1 and the G4 group in HSI, VSI, crude protein, and crude lipid ratios depending on the amount of enzyme addition. The findings are similar to the studies of Lin et al. (2007) who used mixed enzyme supplemented in hybrid tilapia (*Oreochromis niloticus* x *O. aureus*). In terms of body composition, this study is similar to studies of Farhangi & Carter (2007), Imanpoor & Bagheri (2012), and Biswas et al. (2019) in some aspects, but is also different in other aspects. It is thought that this difference may be since fish species and weights are different, the sources of plant protein used are different, the amounts of enzymes are different and the studies have been conducted under different environmental conditions.

As a result, the addition of 1 g kg^{-1} of phytase enzyme to the Siberian sturgeon diet with 30% hazelnut meal did not have a negative effect on WG, FCR, PER, CF, nutrient digestion rate, and body composition. The addition of 1 g kg^{-1} phytase enzyme in Siberian sturgeon (mean 960.23 ± 0.55 g) feed with 30% containing hazelnut meal had a positive effect on FCR. Also, the FCR and PER were in harmony with each other. Thus, it is understood that the feed supplemented with phytase enzyme and containing hazelnut meal is better evaluated by the Siberian sturgeon. It is concluded that the addition of phytase will contribute to the interaction of aquaculture and a sustainable environment by decreasing phosphorus excretion by defecation as it will increase the digestibility of phosphorus by degrading phytate in plant feed additives.

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References

Akiyama T (1995). Nutritive value of alternative protein sources. In: *New Feeds for Fish Culture Utilization of Alternative Protein Sources*, Suisangaku Series 102, Koseisha-koseikaku, Tokyo pp. 35-42

Anonymous (2017). Fisheries Statistics. TUİK, Retrieved in December 20, 2019, from www.tuik.gov.tr

Arıman Karabulut H & Osmanoglu M I (2019). The investigation possibilities of using whiting meal instead of pelagic fish meal in Russian sturgeon (*Acipenser gueldenstaedtii* Brandt, 1833) diet. *Fresenius Environmental Bulletin* 28(6): 4935-4948

Arıman Karabulut H, Kurtoğlu İ Z & Kırtan Y E (2019). Effects of the Feeds Containing Hazelnut Meal as Plant Protein Source on Growth Performance and Body Composition of Siberian sturgeon (*Acipenser baerii*) and Economic Profitability Value. *Turkish Journal of Veterinary and Animal Sciences* 43(2): 244-252 https://doi.org/10.3906/vet-1807-7

Association of Analytical Communities (AOAC) (2000). Official Methods of Analysis, 19th ed. Arlington, Virginia, USA

Asan M (2007). Microbial phytases, applications and biotechnology. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* 13(2): 147-155 https://doi.org/10.1501/Tarimbil_0000000438

Ávila D S, Sánchez E A, Hernández L H H, Araiza M A F & López O A (2015). Addition of yeast and/or phytase to diets with soybean meal as main protein source: Effects on growth, P excretion and lysozyme activity in juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum). *Turkish Journal of Fisheries and Aquatic Sciences* 15: 215-222 https://doi.org/10.12714/egejfas.2017.34.1.05

Ayhan V, Diler I, Arabaci M & Sevgili H (2008). Enzyme supplementation to soybean based diet in Gilthead Sea Bream (*Sparus aurata*): Effects on growth parameters and nitrogen and phosphorus excretion. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 14(2): 161-168 https://doi.org/10.9775/kvfd.2008.33-a

Biswas A, Araki H, Sakata T, Nakamori T & Takiia K (2019). Optimum fish meal replacement by soybean protein concentrate from soybean milk and phytase supplementation in diet of red sea bream, *Pagrus major*. *Aquaculture* 506: 51-59 https://doi.org/10.1016/j.aquaculture.2019.03.023

Cao L, Wang W, Yang C, Yang Y, Diana J, Yakupitiyage A, Luo Z & Li D (2007). Application of microbial phytase in fish feed. *Enzyme and Microbial Technology* 40(4): 497-507 https://doi.org/10.1016/j.enzmictec.2007.01.007

Chen A, Liu X, Cui C, Yang C, Wang Y, Bu X & Yang Y (2019). An evaluation of phytase for Channel catfish (*Ictalurus punctatus*) fed all plant‐protein diet: Growth performance, nutrient utilization and P equivalency value. *Aquaculture Nutrition* 25(1): 215-224 https://doi.org/10.1111/anu.12845

Farhangi M & Carter C G (2007). Effect of enzyme supplementation to dehulled lupin-based diets on growth, feed efficiency, nutrient digestibility and carcass composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* 38: 1274-1282 https://doi.org/10.1111/j.1365-2109.2007.01789.x

Furukawa A & Tsukahara H (1966). On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. *Bulletin of the Japanese Society of Scientific Fisheries* 32: 502-506. https://doi.org/10.2331/suisan.32.502

Ghomi M R, Shahriari R, Langroudi H F, Nikoo M & von Elert E (2012). Effects of exogenous dietary enzyme on growth, body composition, and fatty acid profiles of cultured great sturgeon *Huso huso* fingerlings. *Aquaculture international* 20(2): 249-254 https://doi.org/10.1007/s10499-011-9453-9

Gimenez A V F, Diaz A C, Velurtas S M & Fenucci J L (2009). Partial substitution of fishmeal by meat and bone meal, soybean meal, and squid concentrate in feeds for the prawn, *Artemesia longinaris*: Effect on Digestive Proteinases. *The Israeli Journal of Aquaculture - Bamidgeh* 61(1): 48-56 https://doi.org/10.46989/001c.20546

Imanpoor M R & Bagheri T (2012). Effects of replacing fish meal by soybean meal along with supplementing phosphorus and magnesium in diet on growth performance of Persian sturgeon, *Acipenser persicus*. *Fish Physiology and Biochemistry* 38(2): 521-528 https://doi.org/10.1007/s10695-011-9532-x

Jiang H B, Chen L Q & Qin J G (2018). Fishmeal replacement by soybean, rapeseed and cottonseed meals in hybrid sturgeon *Acipenser baerii* ♀ × *Acipenser schrenckii* ♂. *Aquacult Nutrition* 24(4): 1369-1377 https://doi.org/10.1111/anu.12674

Lin S, Mai K & Tan B (2007). Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquaculture research* 38(15): 1645-1653 https://doi.org/10.1111/j.1365-2109.2007.01825.x

Liu H, Wu X, Zhao W, Xue M, Guo L, Zheng Y H & Yu Y (2009). Nutrients apparent digestibility coefficients of selected protein sources for juvenile Siberian sturgeon (*Acipenser baerii* Brandt) compared by two chromic oxide analyses methods. *Aquaculture Nutrition* 15: 650-656 https://doi.org/10.1111/j.1365-2095.2008.00634.x

Lovell T (1981). Laboratory Manual for Fish Feed Analysis and Fish Nutrition Studies. Department af Fisheries and Allied Aquacultures International Center for Aquaculture, Auburn University, US 65 pp

Maas R M, Verdegem M C J, Dersjant-Lib Y & Schrama J W (2018). The effect of phytase, xylanase and their combination on growth performance and nutrient utilization in Nile tilapia. *Aquaculture* 487: 7-14 https://doi.org/10.1016/j.aquaculture.2017.12.040

Mireles-Arriaga A I, Espinosa-Ayala E, Hernández-García P A & Márquez-Molina O (2015). Use of exogenous enzyme in animal feed. *Life Science Journal* 12(2): 23-32

Morales G A, Denstadli V, Collins S A, Mydland L T, Moyano F J & Øverland M (2016). Phytase and sodium diformate supplementation in a plant-based diet improves protein and mineral utilization in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition* 22(6): 1301-1311 https://doi.org/10.1111/anu.12340

Omnes M H, Le Goasduff J, Le Delliou H, Le Bayon N, Quazuguel P & Robin J H (2017). Effects of dietary tannin on growth, feed utilization and digestibility, and carcass composition in juvenile European seabass (*Dicentrarchus labrax* L.). *Aquaculture Reports* 6: 21-27 https://doi.org/10.1016/j.aqrep.2017.01.004

Rodehutscord M & Pfeffer E (1995). Effects of supplemental microbial phytase on phosphorus digestibility and utilization in rainbow trout (*Oncorhynchus mykiss*). *Water Science and technology* 31(10): 143-147 https://doi.org/10.2166/wst.1995.0371

Schlemmer U, Frølich W, Prieto R M & Grases F (2009). Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. *Molecular nutrition and food research* 53: 330-375 https://doi.org/10.1002/mnfr.200900099

Selle P H, Ravindran V, Caldwell R A & Bryden W L (2000). Phytate and phytase: Consequences for protein utilisation. *Nutrition Research Reviews* 13: 255-278 https://doi.org/10.1079/095442200108729098

von Danwitz A, van Bussel C G, Klatt, S F & Schulz C (2016). Dietary phytase supplementation in rapeseed protein based diets influences growth performance, digestibility and nutrient utilisation in turbot (*Psetta maxima* L.). *Aquaculture* 450: 405-411 https://doi.org/10.1016/j.aquaculture.2015.07.026

Wang F, Yang Y, Han Z, Dong H, Yang C & Zou Z (2009). Effects of phytase pretreatment of soybean meal and phytase-sprayed in diets on growth, apparent digestibility coefficient and nutrient excretion of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquaculture International* 17(2):143-157 https://doi.org/10.1007/s10499-008-9187-5

Xu G-L, Xing W, Li T-L, Ma Z-H, Jiang N, Xu S-D, Xu M, Yu H-H & Luo L (2020). The effects of different fishmeal level diets with or without phytase supplementation on growth performance, body composition, digestibility, immunological and biochemical parameters of juvenile hybrid sturgeon (*Acipenser baerii* Brandt ♀ × *A. schrenckii* Brandt ♂). *Aquaculture Nutrition* 26: 261-274 https://doi.org/10.1111/anu.12987

Yan W, Reigh R C & Xu Z (2002). Effects of fungal phytase on utilization of dietary protein and minerals, and dephosphorylation of phytic acid in the alimentary tract of channel catfish *Ictalurus punctatus* fed an all-plant-protein diet. *Journal of the World Aquaculture Society* 33(1): 10-22 https://doi.org/10.1111/j.1749-7345.2002.tb00473.x

Yeşilayer N, Kaymak I E, Gören H M & Karslı Z (2013). Alternative uses of plant protein sources to fish meal in fish feed. *Gaziosmanpaşa Journal of Scientific Research* 4: 12-30

Yiğit N Ö, & Koca S B (2011). The use of enzyme in fish feeds. *Journal of Fisheries Sciences* 5(3): 205-212

Zhu Y, Qiu X, Ding Q, Duan M & Wang C (2014). Combined effects of dietary phytase and organic acid on growth and phosphorus utilization of juvenile yellow catfish *Pelteobagrus fulvidraco*. *Aquaculture* 430: 1-8 https://doi.org/10.1016/j.aquaculture.2014.03.023