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MORTALITY OF SWAMP BUFFALO CALVES DURING THE LACTATION PERIOD

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Abstract: This study was conducted to determine mortality of swamp calves under semi-intensive rearing conditions at Shtiha government Station in Al-Ghab District, Syria. A total of 501 buffalo calves born throughout period 2008-2018 were analyzed for suckling calf mortality rate study. SAS software (2012) was used to compare four mathematical models [basic and full linear fixed models, logistic, and logarithmic (Log linear) models] to estimate calf mortality during nursing period from birth to 90 days of age. Overall mean mortality rate ranged from 14.5±3.3 to 19.2±2.7%. Sex effect of a calf was significant in the basic model. Calving period and calf weight at birth were significant according to the basic and full models. Interaction between the calving periods and birth weight was highly significant by full linear fixed and Log linear models, confirming that birth weights during the calving period were heterogeneous. The maximum likelihood analysis shows that calf sex, calving period, and birth weight were significant in the logistic model. The parity and birth weight were significant in the linear Log linear model. Effect of birth weight was significant in all models, which confirms its importance in calf mortality. In conclusion, management of buffalo calves and improvement of birth weight to be greater than 30 kg is required to reduce mortality rates during the lactation period of buffalo calves.

Keywords: Bubalus bubalis, Buffalo calves, Mortality rate

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1. Introduction

In Syria, a total number of buffaloes is about 7500 heads (FAO, 2019) which supply more than 620 and 6195 tons of meat and dairy products, respectively (MOAAR, 2011). Environmental factors greatly affect buffalo production. Among these factors, the death of calves before weaning is a major problem for breeders. Each calf surviving from a dam is an advantage in increasing productivity of farm because it saves more weight at weaning and more milk. It is accepted that calf deaths occur during nursing period. Many studies have reported that environmental factors affect calf survival before weaning. Unfavorable calving seasons lead to the mortality of calves, so health management and proper nutrition are key factors in calves' survival. Calf mortality control is vital, not only to improve calf welfare but to increase productivity. Higher calf mortality rates can be associated with higher numbers of calves in herd, breeder performance, severe weather, and nursing period covering first two months of life. Diseases appear in calves when breeders fail to transfer immunoglobulins to them, resulting in increased neonatal mortality (Katsuji, 2019). Maximum mortality has been found in young calves due to diseases as infections so special care is needed for such pathological problems (Dinesh et al., 2015). To control the mortality of calves, the effects of environmental factors should not be ignored to improve the care of calves.

This study was, therefore, conducted to estimate the mortality rate of calves, the effect of calf sex, year of calving, parity, and weight of calves at birth during the nursing period under the conditions of semi-intensive Syrian rearing.

2. Material and Methods

2.1. Animal Materials

This study was conducted at Shatiha station for buffaloes; which is located about 73 km northwest of the Al-Ghab area in Syria. Buffaloes are raised freely in the pastures located on the sides of water channels. Where they go out to pasture in the morning and return in the evening and spend the daytime napping in waters of canals from eleven in the morning until four in the afternoon to escape from the high summer heat. Napping in canal waters in autumn and spring, while almost non-existent in winter. At night, buffaloes take haven in open pens. The calving takes place in the winter and the newborns are weaned in the spring to go out with their dams to the pastures. Newborn calves were left with their dams for a week to feed colostrum. After that, the calves received milk from their mother twice a day, morning and evening, for a month. Calves gradually get used to green grass, concentrated feed, and hay during the first month of age. Then the amount of milk that the newborn takes from his dam gradually absorbs what remains after milking and continues until the dam rejects it.

2.2. Statistical Analysis

A total of 501 records of buffalo calves were analyzed during the period 2008-2018. Each record included the identity of a calf, the sex, parity, year of birth, birth weight, and the fate of the calf whether it was weaned or dead during the lactation period. The data were analyzed using SAS (2012) software according to four mathematical models to study the non-genetic factors that influence the mortality rate of buffalo calves:

$$Y_{ijklm} = \mu + X_i + PR_j + PE_k + BW_l + E_{ijklm} \quad (1)$$

$$Y_{ijklm} = \mu + X_i + PR_j + PE_k + BW_l + XPR_{ij} + XPE_{ik} + XBW_{il} + PRPE_{jk} + PRBW_{jl} + PEBW_{kl} + E_{ijklm} \quad (2)$$

Where, Y_{ijklm}=calf mortality during the suckling period of the $ijklm^{th}$ records, μ=grand mean. X_i=effect of i^{th} sex of calf, PR_j =effect of j^{th} parity, PE_k=effect of k^{th} period of calving, and BW_l=effect of l^{th} birth weight of calf [first=BW<=30, second=BW>=31/kg). E_{ijklm}=random error term associated with the Y_{ijklm}observations with zero mean and variance $I\ \sigma^2 e$.

$XPR_{ij} + XPE_{ik} + XBW_{il}$=the interaction of the calf sex with parity, a period of year and birth weight, respectively. $PRPE_{jk} + PRBW_{jl}$=the interaction of the parity with a period of year and birth weight, respectively. $PEBW_{kl}$=the interaction of the period of years with birth weight.

Duncan's (1955) multiple-range test was used to detect differences between the means of effects. Statistical significance was declared at (P<0.05).

$$RES_{ijklm} = A + X_i + PR_j + PE_k + BW_l + LR_{ijklm} \quad (3)$$

$$RES_{ijklm} = X_i \times PR_j \times PE_k \times BW_l \times RES_{ijklm} + LR_{ijklm} \quad (4)$$

Where, RES=Response (Mortality, Survival) of the $ijklm^{th}$ records that were 16 samples for Logistic and 29 Responses for Log-linear models. A=Intercept, LR=Likelihood Ratio, Other symbols are explained in the previous models.

3. Results and Discussion

The overall calf mortality rate ranged from 14.5±3.3 to 19.2±2.7% according to models (Table 1). These values are relatively high due to poor management and unfavorable environmental conditions during the lactation period as 15-25% before weaning calf mortality is seen as an indicator of poor calf management (Moran, 2011). The overall mortality rates for buffalo calves were found at 9.4 and 11.05 in Pakistan and Egypt, respectively, and these values display accepted management of calves during the suckling period (Zaman et al., 2006; El-Regalaty and Aboul-Ela, 2014). On the other hand, there were total mortality rates for buffalo calves of 17.98%, 18.78%, and 15.89% in Peshawar, Punjab, and Nagpur (Zaib Ullah et al., 2007; Bilal et al., 2019; Kharkar et al., 2019), respectively; These values indicate poor management during the suckling period. Some studies reported high mortality values of 31%, 38.8%, and 42.11% before weaning (Oswin, 1999; Thiruvenkadan and Devendran, 2014; Amit et al., 2017), respectively, and these values may point to poor management and unfavorable environmental conditions for the care of buffalo calves. In Pakistan, the mortality rate of buffalo calves was 60% and 79.51% due to worms, lack of preventive measures, and male neglect due to low expected return (Ahmad et al. 2009; Bilal et al. 2009), respectively. Therefore, the calf needs to improve various aspects of calf breeding such as nutrition, housing, and weaning (Bilal et al., 2019). Archana et al. (2020) noted that parasites killed 34.21% of buffalo calves, of which 58.33% were males, 23.07% were females, and 75% of dead calves had diarrhea.

In the basic model (equation 1), the male mortality rate was found to be 8.1% higher than females. Calves' mortality in the second period (2014-2018) was 11.1% higher than the first period (2008-2013).

Table 1. Least Square Means (LSM) and Standard Errors (SE) of the calf mortality rates from birth to weaning at 90 days of age, in buffaloes

Source of Variance		Obs.	LSM±SE	
			Main Fixed Model	Full Fixed Model
Overall mean (μ)		501	0.192±0.027	0.145±0.033
Sex	Male	249	0.232±0.024^a	0.222±0.028
	Female	252	0.151±0.023^b	0.181±0.026
Parity	1st	127	0.177±0.029	0.189±0.030
	+2nd	374	0.207±0.021	0.213±0.024
Calving period	2008-2013	228	0.086±0.022^b	0.060±0.024^b
	2014-2018	273	0.297±0.025^a	0.343±0.030^a
Birth weight of calf, kg (BW)	BW<=30	117	0.270±0.031^a	0.291±0.031^a
	BW>=31	384	0.113±0.020^b	0.113±0.022^b

^{a,b} Mean values with different superscripts in the same effects indicate a significant difference (P<0.05).

The mortality rate of calves born with weight equal to or less than 30 kg was higher by 15.7% than that of 31 kg or more during the nursing period based. Whereas the mortality rate in the second period (2014-2018) was 28.3% higher than the first period (2008-2013) and the mortality rate of calves born with weight equal to, less than 30 kg was higher by 17.8% than that of 31 kg or more in the full model (equation 2). The mortality of calves may be due to the higher sensitivity of unfavorable environmental conditions during the nursing period (Table 1).

Table (2) shows that the effect of sex was significant (P<0.01) in the basic model (equation 1). The calving period and calf weight at birth were significant (P<0.01) according to the basic (equation 1) and full models (equation 2). The interaction between a calving period and birth weight was highly significant (P<0.01), confirming that the birth weights during the calving period were heterogeneous. Calving period and birth weight effects demonstrated the importance of environmental factors and management practices on mortality of buffalo calves from birth to weaning at 90 days of age under semi-intense buffalo rearing conditions.

Table 2. Analysis of variance for the mortality rate of calves from birth to weaning at 90 days of age, in buffaloes

Source of Variance	DF	Fixed Model			
		Main		Full	
		MS	Pr.>F	MS	Pr.>F
Gender	1	0.822	0.0070	0.130	0.2760
Parity	1	0.076	0.4104	0.040	0.5434
Period of calving	1	5.436	0.0001	6.072	0.0001
Birth weight	1	1.977	0.0001	2.291	0.0001
Gender×Parity	1	Non-available		0.011	0.7435
Gender×Period of calving	1			0.023	0.6474
Gender×Birth weight	1			0.222	0.1555
Parity×Period of calving	1			0.003	0.8517
Parity×Birth weight	1			0.001	0.9083
Calving period×Birth weight	1			1.339	0.0005
Residual	490	0.112		0.109	

An analysis of the probability of maximum mortality variation showed that the sex of the calf, the period of calving, and the birth weight were significant (P<0.01) through the logistical model (equation 3). The calving period and birth weight were significant (P<0.01) in the Log linear model (equation 4, Table 3). The Log linear model (equation 4) showed that there is real differentiation in mortality response (survival and mortality). The differences between birth weights, calving periods, and sex of calves within the mortality response were heterogeneous. Differences in birth weights in each period of calving, parity, and calf sex were heterogeneous. There is considerable variation in the sex of calves during calving periods (Table 3). Differences in response to mortality, and calving periods, sex calves within parity were homogeneous (Table 3).

In reviews of the literature, Hammad et al. (2013) demonstrated that sex is the most important source of difference in mortality for buffalo calves, with Amit et al. (2017) finding that the male sex is significantly higher in mortality as in the current study. While Zaman et al. (2006) reported that the effect of sex was not significant on the mortality rate of Nili Ravi calves. Amit et al. (2017) found that male calves had a higher mortality rate than females at 60.47% for males compared to 23.53% for females due to male calves being neglected. While Kharkar et al. (2019) reported that female mortality was higher than male mortality, and Khatun et al. (2009) reported that female calves showed 42.85% compared to 28% for males, where females were more likely to be infected.

There is no significant effect of parity (Nitin et al., 2016; Kharkar et al., 2019), respectively as in the current study while El-Regalaty and Aboul-Ela (2014) determined the significant effect of parity on the mortality rate of buffalo calves. Mortality rates of calves born from dams in parities 1 to 6 were similar and ranged between 14% and 16.7%, while mortality rates of calves increased significantly in parities 7 and beyond, reaching the highest value of 27.5%. This may be due to dams getting older, which makes them less caring for the newborn during suckling (Table 1). Zaman et al. (2006) reported that the mortality rate for Nili-Ravi calves was 11.3% at first parity.

Mortality of calves had been significantly affected over the years (El-Regalaty and Aboul-Ela, 2014; Kharkar et al., 2019) in line with the current study, while in other studies this did not affect the mortality of buffalo calves (Hammad et al., 2013; Nitin et al., 2016). Table 1 shows that calf mortality rates were represented by two periods of years. The first period (2008-2014) and the second period (2015-2018) spanned and the differences between them were real. These real differences between the two periods reflect the importance of calf cares to reduce mortality during suckling time.

Table 3. Maximum Likelihood analysis of variance for the mortality rate of calves from birth to weaning at 90 days of age, in buffaloes

Source of Variance	DF	Logistic Model		Log linear Model	
		Chi-Square	Pr>ChiSq	Chi-Square	Pr>ChiSq
Gender	1	6.53	0.0106	1.91	0.1674
Parity	1	0.66	0.4151	29.43	<.0001
Period of calving	1	37.26	<.0001	0.69	0.4057
Birth weight	1	16.25	<.0001	11.77	0.0006
RES	1			90.71	<.0001
Birth weight×RES	1			16.89	<.0001
Period of calving×RES	1			29.00	<.0001
Parity×RES	1			0.57	0.4505
Gender×RES	1			8.86	0.0029
Calving Period×Birth weight	1	Non-available		8.06	0.0045
Parity×Birth weight	1			39.44	<.0001
Gender×Birth weight	1			4.86	0.0275
Parity×Period of calving	1			0.71	0.3995
Gender×Period of calving	1			6.10	0.0135
Gender×Parity	1			1.43	0.2317
Likelihood Ratio	13	14.50	0.2065	13.34	0.4219

The mortality rate was significantly affected by the weight of the calf (El-Regalaty and Aboul-Ela, 2014) as in this study, while the effect of birth weight was not significant on the mortality of Neil Rafi calves (Zaman et al., 2006). Table 1 shows that calves weighing more than 30 kg had a 17% lower mortality rate compared to calves of equal or less birth weight and could withstand unfavorable conditions during the lactation period. Therefore, work must be done to improve birth weight. Elsayed et al. (2020) showed in a study on the same herd that the estimate of genetic change was very low and non-significant; confirming that effective selection for birth weight was not present during the study period.

4. Conclusion

The results of present study indicated that all models gave an odds ratio for the effect of birth weight on calf mortality, taking into account other explanatory variables. Whereas the interaction between birth weight and birth periods was heterogeneous in both the full and Log linear models. Therefore, good care and management are essential for reducing calf mortality. Moreover, selection for calf weight at birth to be more than 30 kg should be applied to avoid the risk of mortality.

Author Contributions

A single author made all tasks and reviewed and approved the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethical permission was taken from the Syrian General Commission for Scientific Agricultural Research (GCSAR) (2018-1).

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METRIC CHARACTERISTICS OF THE ZEBU (*Bos Indicus*) GUDALI VARIETY BANYO IN THE HIGH GUINEA SAVANNAH AREA OF CAMEROON

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Abstract: This work aims to assess the genetic diversity of local cattle breeds in Cameroon. For this purpose, biometric data were collected in six (6) localities of Mayo-Banyo, at random, on a sample of 321 adult Gudali variety Banyo zebus (234 cows and 87 bulls) aged 6 to 16 years old, with the body condition score varying from 2 to 4. Body measurements (cm) are presented as follows: Height at the withers (129.34 ± 0.50), height at the sacrum (134.56 ± 0.37), chest depth (67.37 ± 0.33), head length (47.62 ± 0.42), forehead length (20.33 ± 0.20), horn length (34.45 ± 1.56), ear length (22.66 ± 0.26), body length (187.74 ± 2.45), trunk length (130.5 ± 1.27), scapulo-ischial length (137.88 ± 1.33), pelvic length (42.87 ± 0.31), tail length (100.52 ± 0.88), pelvis width (38.31 ± 0.32), face width (17.61 ± 0.27), muzzle circumference (44.60 ± 0.44), chest circumference (167.81 ± 1.46), barrel circumference (18.60 ± 0.16), hock circumference (38.72 ± 0.35), hump circumference (73.37 ± 1.92) and live weight (350.24 ± 8.70). The main biometric indices are: The massivity index (2.69 ± 0.06 kg / cm), proportionality (94.42 ± 1.06), cephalic (37.13 ± 0.55), body profile (0.69 ± 0.01), surface (1.79 ± 0.02m2), format (1.44 ± 0.01), scapulo-ischial (1.06 ± 0.01), typist-thoracic (0.11 ± 0.00), thoracic development (0.77 ± 0.00), framing (0.14 ± 0.00). Bulls and other cattle reared in controlled systems presented a large format. The discriminant factor analysis made it possible to detect three morphometric types identifiable with two phenotypes.

Keywords: Gudali, Biometrics, Biometric index, Cameroon

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1. Introduction

In Cameroon, the cattle herd is estimated at just over 7.456.123 head and annually supplies 122,306 tons of meat, which represents an estimated contribution of 54% of all meat products (INS, 2017). This latter is mainly made up of zebus (99%), Taurus (1%) being very poorly represented. The zebus arrived in North Cameroon from Bornu (Nigeria today) almost 200 years ago (Paguem et al., 2020). Today they exist in two breeds: the Gudali zebu (34%) and the M'bororo zebu (66%) (Manjeli and Tchoumboué, 1990). The Gudali zebu exists in three varieties: the Ngaoundere, Tignere and Banyo (Lhoste, 1969).

They are found in Adamaoua where it is mainly reared by breeders of the Peul ethnic group. We estimate between 400.000 and 600.000 persons who derive most of their existence from cattle rearing (Hamadou, 2009). This breed represents for these breeders a very important animal genetic resource because of their immense capacity of adaptation in very varied climates and ecosystems (FAO, 2007) with a carcass yield oscillating between 46 and 52% (Lhoste, 1969), and an average milk yield of 483 liters in 168 days (Tebong, 1985). Despite the interest and importance of this breed, Gudali zebu has only been the subject of very few biometric studies (Lhoste, 1969; Tawah and Rege, 1994; Doba, 2016) compared to other breeds in the country. Metric characterization is most often used as a selection method (Sow et al., 1991). The Strategy Document for the Livestock, Fisheries and Animal Industries sub-sector prepared in 2011 by the Minister of Livestock, Fisheries and Animal Industries (MINEPIA), in line with strategic priority No.1 of the “Plan d’Action Mondial pour la gestion des resources zoogénétiques”, recommends the inventory, characterization of animal genetic resources and monitoring of trends and associated risks. It is in this context that this study was initiated for a better evaluation of these animal genetic resources in Mayo-Banyo Division, Adamaoua region, which contains a little more than ¾ of the cattle population.

2. Materials and Methods

2.1. Study Period and Zone

This study was conducted between May and June 2020 in the Mayo-Banyo Division, Adamaoua region (Figure 1), and more precisely in the Banyo district. The prevailing climate is Sudano-Guinean, characterized by a long rainy season of seven months (from April to October) and a short dry season of five months (from November to March). Rainfall is abundant (1,500 to 1,800 mm) but unevenly distributed (MINEPAT, 2012). The relief is rugged with an altitude varying between 800m and 1800m.

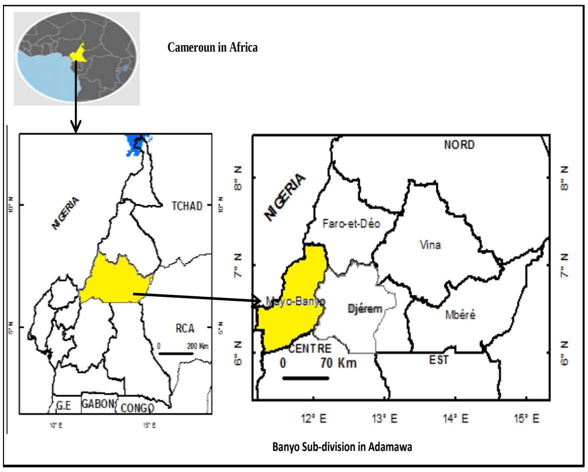

Figure 1. Study zone. Commune de Banyo (2015).

2.2. Sample Size

Data were collected in six (6) localities in Mayo-Banyo Division, in a random fashion, on a sample of 321 adult animals aged between 6 to 16 years with a body condition score ranging from 2 to 4 (Table 1). Pregnant cows, young bulls and sick animals were systematically eliminated from the collection. Age was estimated by dental chronometry and horn ring count, but also by interviewing the herdsman. Collection was done in the morning before the animals went out to pasture.

2.3. Data Collection

A total of 20 measurements were taken from the animal on level ground and in its normal stance (Figure 2), in accordance with the guidelines from AU-IBAR (2015) and FAO (2007). These are: chest perimeter (thoracic perimeter taken just behind the animal's front legs through the passage of the straps), body length (from the neck to the tail attachment), head length (from the bun to muzzle), scapulo-ischial length (measure from point of the shoulder to the ischium), trunk length (distance between the hump and the tail attachment), pelvis length (distance between the point of the hips and the point of the buttocks), tail length (length between the attachment of the tail and its end), length of the horns (longest distance from the root of the horn to its end), length of the forehead (length between the two horns and the two eyes), width of the face (distance between the two eyes), width of the pelvis (distance between the outer tips of the hips), height at withers (vertical distance from the ground to the point of the withers), ear length (measure the length behind the ear from root to tip), height at withers (vertical distance from the ground to the point of the withers), height to the sacrum (vertical distance from the ground to the sacrum), chest depth (vertical distance from the sternum straps to the spine), muzzle circumference (perimeter taken a little above the nostrils and around the point where the dewlap meets the chin), round of the barrel (perimeter taken at the level of the front barrel), round of the hump (perimeter taken at the level of the hump), round of the hock (perimeter taken at the level of the hock), live weight (estimated by the barymetry method described by Doba (2016), Live Weight = 0.00016 × Thorasic Perimeter$^{2.8467}$; r2 = 0.9701; probability threshold of 0.00001).

Table 1. Sample size according to different factors

Factors	Modalities	Sample size
Sex	Cow	234
	Bull	87
Reproduction system	Controlled	52
	Non controlled	269
Localities	Banyo Bunji	50
	Banyo Centre	116
	Banyo Leswouroun	9
	Banyo Tiqué	67
	Banyo-Tibati Border	34
	Mayo Djinga	45
Coat colour	White	25
	Black	23
	Pie	139
	Red	134

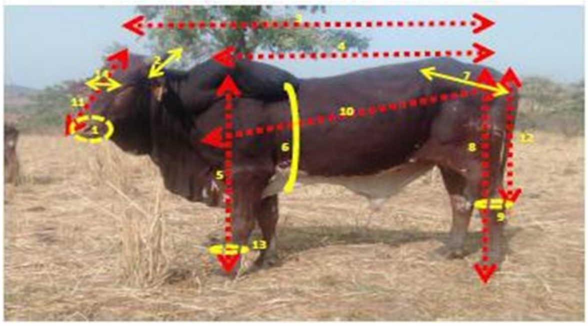

Figure 2. Biometric characteristics according to AU-IBAR (2015). 1: muzzle circumference, 2: length of the horn, 3: length of the body, 4: length of the trunk, 5: height at the withers, 6: chest circumference, 7: pelvis length, 8: height at the sacrum, 9: circumference hock, 10: scapulo-ischial length, 11: head length, 12: tail length, 13: barrel circumference, 14: face width.

In addition, to better appreciate the conformation of the animal, we used 10 biometric indices. These are: Scapulo-ischial index (ScI = scapulo-ischial length / Height at withers) Massiveness index (kg / cm) (MI = live weight / height at withers), Bones index (circumference of the anterior cannon / height at withers) ×100), Format index (FI = body length / height at withers), Cephalic index (CpI = (face width / face length × 100), Proportionality (Pr = height at withers / scapulo-ischial length) × 100), Thorasic development index (ThDeI = height at the withers / thoracic circumference), Surface index (cm^2) (SI = height at the withers × scapulo-ischial lengths), Dactylo Thoracic Index (DaThI = circumference of the anterior barrel / circumference of the thorax), Body Profile Index (PBI = height at withers / body length).

2.3. Statistical Analysis

The descriptive statistics (mean, standard deviation, coefficient of variation) as well as the General Linear Model were carried out using the SPSS.20 software while the XLSTAT-Pro version 2014.5.03 software was used to perform the principal component analysis (PCA). PCA reliability was established by the Kaiser-Meyer-Olkin Sampling Accuracy Test (KMO) and Bartlett's Sphericity Test. For a satisfactory factorial analysis, KMO > 0.50 is needed. A coefficient of variation of less than 15% is considered to indicate that the population is homogeneous, while a coefficient of more than 15% indicates that the values are relatively dispersed (Peter, 2020; Faria et al., 2010).

3. Results

3.1. Measurements of the Head, Trunk, Peripherals and Live Weight According To Sex, Reproductive System, Location and Coat

The descriptive statistics of the measurements of the head, trunk and peripheral are summarized in Tables 2, 3, 4, 5 and 6.

Table 2. Lengths of the head, forehead, horns and ears according to sex, breeding system, locality and coat

Sources of variation	N	Face length μ ± se (cm) (CV)	Face width μ ± se (cm) (CV)	Front length μ ± se (cm) (CV)	Horn length μ ± se (cm) (CV)	Ear length μ ± se (cm) (CV)
Sex		***	***	***	ns	***
cows	234	46.76±0.37ᵃ (0.09)	16.62±0.24ᵃ (0.14)	19.90±0.26ᵃ (0.09)	36.01±1.39 (0.32)	21.98±0.23ᵃ (0.32)
Bulls	87	48.49±0.57ᵇ (0.08)	18.61±0.37ᵇ (0.14)	20.75±0.40ᵇ (0.17)	32.89±2.12 (0.36)	23.34±0.35ᵇ (0.32)
Breeding system		***	***	*	*	***
Controlled	52	48.82±0.70ᵃ (0.06)	18.52±0.44ᵃ (0.17)	20.84±0.49ᵃ (0.18)	31.48±2.57ᵃ (0.33)	23.41±0.42ᵃ (0.11)
Uncontrolled	269	46.42±0.29ᵇ (0.09)	16.71±0.19ᵇ (0.14)	19.82±0.21ᵇ (0.11)	37.42±1.09ᵇ (0.33)	21.91±0.18ᵇ (0.10)
Localities		***	ns	***	***	***
Banyo Bunji	50	46.26±0.61ᶜ (0.06)	17.12±0.39 (0.09)	19.84±0.43ᵇᶜ (0.06)	41.83±2.27ᵃ (0.29)	22.23±0.37ᵇᶜ (0.09)
Banyo Centre	116	50.20±0.32ᵃ (0.08)	17.99±0.20 (0.19)	21.90±0.22ᵃ (0.18)	44.21±1.18ᵃ (0.32)	23.30±0.19ᵃ (0.11)
Banyo Leswouroun	9	45.98±1.21ᶜ (0.04)	17.45±0.77 (0.05)	19.39±0.85ᶜ (0.04)	19.70±4.46ᵃ (0.30)	21.60±0.74ᶜ (0.09)
Banyo Tiqué	67	46.89±0.57ᶜ (0.07)	18.00±0.36 (0.10)	20.98±0.40ᵇ (0.04)	35.53±2.09ᵃᵇ (0.29)	22.46±0.34ᵇᶜ (0.08)
Banyo-Tibati Border	34	47.35±0.72ᵇᶜ (0.07)	17.57±0.46 (0.08)	20.45±0.50ᵇᶜ (0.04)	36.09±2.64ᵃᵇ (0.24)	23.01±0.43ᵇ (0.08)
Mayo Djinga	45	49.06±0.65ᵇ (0.07)	17.56±0.42 (0.11)	19.41±0.46ᶜ (0.04)	29.32±2.41ᵇ (0.34)	23.35±0.40ᵇ (0.09)
Coat color		***	***	ns	***	***
White	25	46.16±0.85ᵃ (0.02)	15.84±0.51ᵃ (0.03)	19.84±0.55 (0.03)	38.96±2.73ᵃ (0.07)	21.32±0.47ᵃ (0.02)
Black	23	48.652±0.88ᵇ (0.02)	17.217±0.53ᵇ (0.03)	20.74±0.58 (0.03)	40.35±2.84ᵃᵇ (0.07)	22.91±0.49ᵇ (0.02)
Pie	139	46.871±0.36ᵃᵇ (0.01)	16.849±0.22ᵃᵇ (0.01)	20.30±0.23 (0.01)	41.51±1.16ᵇ(0.03)	22.14±0.20ᵃᵇ (0.01)
Red	134	47.269±0.37ᵃᵇ (0.01)	16.56±0.22ᵃᵇ (0.01)	20.25±0.24 (0.01)	41.25±1.18ᵃᵇ (0.03)	22.01±0.20ᵃᵇ (0.01)
Overall average	321	47.62±0.42 (0.09)	17.61±0.27 (0.16)	20.33±0.20 (0.15)	34.45±1.56 (0.34)	22.66±0.26 (0.11)

[a,b,c]Numbers assigned the same letter in the same column are statistically comparable. *P<0.05, ** P<0.01, *** P<0.001, ns= not significant, μ ± se= mean ± standard error, CV= coefficient of variation.

Table 3. Height at withers and Sacrum, chest depth, pelvis width and Thoracic circumference depending on sex, Breeding system, location and coat

Sources of variation	n	Height at withers μ ± se (cm) (CV)	Sacral height μ ± se (cm) (CV)	Chest depth μ ± se (cm) (CV)	Pelvis width μ± se (cm) (CV)	Thoracic circumference μ± se (cm) (CV)
Sex		*	**	***	**	***
Cows	234	128.48±0.95[a] (0.07)	134.07±0.68[a] (0.05)	66.76±0.57[a] (0.07)	37.71±0.29[a] (0.08)	162.10±1.30[a] (0.07)
Bulls	87	130.99±1.45[b] (0.05)	136.99±1.03[b] (0.05)	71.07±0.87[b] (0.10)	38.90±0.44[b] (0.06)	173.51±1.98[b] (0.10)
Breeding system		*	***	*	**	Ns
Controlled	52	131.63±1.75[a] (0.10)	137.53±1.25[a] (0.06)	70.02±1.06[a] (0.07)	39.08±0.53[a] (0.08)	169.06±2.40 (0.08)
Uncontrolled	269	127.83±0.74[b] (0.06)	133.53±0.53b (0.05)	67.82±0.45[b] (0.09)	37.53±0.22[b] (0.07)	166.55±1.02 (0.08)
Localities		ns	*	**	**	***
Banyo Bunji	50	127.11±1.55 (0.05)	132.99±1.11[c] (0.04)	67.65±0.93[bc] (0.07)	37.93±0.47[bc] (0.07)	160.75±2.12[c] (0.06)
Banyo Centre	116	131.71±0.80 (0.09)	136.12±0.57[a] (0.05)	70.48±0.48[a] (0.09)	38.28±0.24[ab] (0.08)	171.03±1.10[a] (0.08)
Banyo Leswouroun	9	128.76±3.05 (0.04)	135.35±2.18[bc] (0.03)	68.44±1.84[bc] (0.06)	38.46±0.93[abc] (0.05)	168.02±4.18[bc] (0.04)
Banyo Tiqué	67	129.21±1.43 (0.05)	134.93±1.02[bc] (0.05)	67.09±0.86[c] (0.08)	38.07±0.43[abc] (0.06)	167.46±1.96[bc] (0.09)
Banyo-Tibati Border	34	130.10±1.81 (0.04)	136.31±1.29[abc] (0.05)	70.96±1.09[ab] (0.08)	37.50±0.55[c] (0.05)	170.98±2.47[ab] (0.07)
Mayo Djinga	45	131.50±1.65 (0.05)	137.48±1.18[ab] (0.04)	68.87±0.99[bc] (0.06)	39.60±0.50[a] (0.04)	168.59±2.26[bc] (0.06)
Coat color		ns	ns	***	***	***
White	25	126.56±1.82 (0.01)	132.24±1.33 (0.01)	65.56±1.19[a] (0.02)	36.52±0.55[a] (0.02)	161.16±2.73[a] (0.02)
Black	23	127.52±1.8 (0.01)	134.00±1.39 (0.01)	68.65±1.24[b] (0.02)	38.17±0.58[b] (0.02)	164.78±2.84[bc] (0.02)
Pie	139	128.73±0.77 (0.01)	133.22±0.56 (0.00)	67.48±0.51[ab] (0.01)	37.72±0.24[b] (0.01)	164.91±1.16[bc] (0.01)
Red	134	128.40±0.79 (0.01)	134.08±0.58 (0.00)	67.38±0.52[ab] (0.01)	37.33±0.24[ab] (0.01)	165.36±1.18[c] (0.01)
Overall average	321	129.34±0.50 (0.07)	134.56± 0.37 (0.05)	67.37± 0.33 (0.09)	38.31±0.32 (0.08)	167.81±1.46 (0.08)

[a,b,c]Numbers assigned the same letter in the same column are statistically comparable. *P<0.05, ** P<0.01, *** P<0.001, ns= not significant, μ ± se= mean ± standard error, CV= coefficient of variation.

Table 4. Muzzle, barrel, shank and hump revolutions according to sex, breeding system, locality and coat

Sources of variation	n	Muzzle turn μ±es (cm) (CV)	barrel turn μ±es (cm) (CV)	shank turn μ±es (cm) (CV)	hump turn μ±es (cm) (CV)
Sex		***	***	***	***
Cows	234	43.25±0.39[a](0.08)	17.85±0.14[a](0.08)	37.62±0.31[a](0.08)	63.63±1.71[a](0.21)
Bulls	87	45.96±0.60[b](0.09)	19.36±0.22[b](0.08)	39.81±0.47[b](0.09)	83.11±2.60[b](0.25)
Breeding System		ns	ns	ns	**
Controlled	52	44.75±0.73 (0.11)	18.57±0.27 (0.10)	38.39±0.57 (0.09)	69.47±3.15[a](0. 35)
Noncontrolled	269	44.46±0.31 (0.08)	18.64±0.11 (0.09)	39.04±0.24 (0.09)	77.27±1.34[b](0.24)
Localities		**	***	***	***
Banyo Bunji	50	43.22±0.64[bc](0.08)	18.11±0.23[b](0.07)	37.61±0.50[b](0.07)	66.32±2.78[b] (0.26)
Banyo Centre	116	45.38±0.33[a] (0.10)	19.76±0.12[a](0.10)	41.40±0.26[a](0.09)	73.03±1.44[a] (0.31)
Banyo Leswouroun	9	42.86±1.27[c] (0.09)	18.66±0.46[b](0.06)	38.42±0.99[b](0.07)	78.56±5.48[a] (0.16)
Banyo Tiqué	67	45.19±0.59[ab](0.07)	18.37±0.22[b](0.06)	38.17±0.46[b](0.06)	77.61±2.57[a] (0.16)
Banyo-Tibati Border	34	45.32±0.75[ab](0.08)	18.46±0.27[b](0.05)	38.64±0.59[b](0.05)	77.00±3.24[a] (0.19)
Mayo Djinga	45	45.66±0.68[a] (0.08)	18.2±0.25[b] (0.06)	38.04±0.53[b](0.06)	67.68±2.96[b](0.21)
Coat color		ns	ns	ns	ns
White	25	44.28±0.78 (0.08)	17.92±0.35 (0.05)	37.92±0.69 (0.06)	72.24±3.66 (0.21)
Black	23	44.09±0.81 (0.09)	18.74±0.36 (0.06)	39.30±0.72 (0.06)	66.39±3.82 (0.21)
Pie	139	44.53±0.33 (0.10)	18.61±0.15 (0.06)	39.34±0.29 (0.07)	70.90±1.55 (0.21)
Red	134	43.99±0.34 (0.08)	18.49±0.15 (0.07)	38.82±0.30 (0.06)	71.54±1.58 (0.21)
Overall average	321	44.60±0.44 (0.09)	18.60±0.16(0.10)	38.72±0.35(0.09)	73.37±1.92(0.28)

[a,b,c]Numbers assigned the same letter in the same column are statistically comparable. *P<0.05, ** P<0.01, *** P<0.001, ns= not significant, μ ± se= mean ± standard error, CV= coefficient of variation.

Table 5. Body, trunk, scapulo-ischial, pelvis and tail lengths as a function of sex, breeding system, locality and coat

Sources of variation	n	Body length μ ± se (cm) (CV)	Trunk length μ ± se (cm) (CV)	Scapulo-ischial length μ ± se (cm) (CV)	Pelvis length μ ± se (cm) (CV)	Tail length μ ± se (cm) (CV)
Sex		***	***	***	***	*
Cows	234	182.26±1.81[a] (0.09)	127.68±1.13[a] (0.08)	134.89±1.18[a] (0.08)	41.98±0.27[a] (0.07)	99.26±0.78[a] (0.07)
Bulls	87	193.21±2.76[b] (0.11)	133.46±1.72[b] (0.09)	140.87±1.81[b] (0.09)	43.76±0.42[b] (0.05)	101.78±1.19[b] (0.08)
Breeding Systèm		***	***	ns	***	***
Controlled	52	195.62±3.35[a] (0.11)	134.84±2.08[a] (0.09)	139.86±2.19 (0.07)	43.70±0.51[a] (0.06)	103.15±1.44[a] (0.11)
Uncontrolled	269	179.86±1.42[b] (0.09)	126.30±0.89[b] (0.08)	135.90±0.93 (0.09)	42.04±0.21[b] (0.06)	97.89±0.61 [b] (0.06)
Localities		**	**	***	***	*
Banyo Bunji	50	185.86±2.96[ab] (0.08)	129.58±1.84[ab] (0.07)	131.93±1.93[b] (0.07)	42.04±0.45[b] (0.06)	102.76±1.27a (0.07)
Banyo Centre	116	180.88±1.53[ab] (0.13)	127.77±0.95[b] (0.10)	139.82±1.00[a] (0.09)	42.80±0.23[a] (0.08)	100.24±0.66a (0.09)
Banyo Leswouroun	9	185.69±5.82[b] (0.05)	129.96±3.63[a] (0.04)	137.97±3.81[ab] (0.07)	42.63±0.89[b] (0.04)	95.49±2.51b (0.09)
Banyo Tiqué	67	187.46±2.73[ab] (0.09)	128.19±1.70[a] (0.09)	139.63±1.79[a] (0.08)	42.98±0.41[ab] (0.05)	102.25±1.17a (0.06)
Banyo-Tibati Border	34	196.09±3.44[a] (0.07)	134.03±2.14[a] (0.05)	138.03±2.25[ab] (0.08)	42.48±0.52[b] (0.05)	102.89±1.48a (0.08)
Mayo Djinga	45	190.44±3.15[ab] (0.06)	133.91±1.96[a] (0.06)	139.92±2.06[a] (0.07)	44.27±0.48[a] (0.04)	99.50±1.35ab (0.05)
Coat color		ns	ns	ns	ns	ns
White	25	180.12±3.50 (0.07)	126.52±2.17 (0.07)	131.84±2.31 (0.09)	41.20±0.55 (0.06)	97.56±1.49 (0.05)
Black	23	184.26±3.65 (0.07)	127.74±2.27 (0.07)	134.35±2.41 (0.07)	42.65±0.57 (0.05)	100.65±1.55 (0.05)
Pie	139	178.58±1.49 (0.08)	126.08±0.92 (0.07)	137.22±0.98 (0.07)	42.07±0.23 (0.07)	98.59±0.63 (0.05)
Red	134	176.62±1.51 (0.08)	124.37±0.94 (0.09)	134.88±1.00 (0.09)	41.78±0.24 (0.06)	98.81±0.64 (0.05)
Overall average	321	187.74±2 .45 (0.10)	130.5±1.27 (0.09)	137.88±1.33 (0.09)	42.87±0.31 (0.07)	100.52±0.88 (0.08)

[a,b,c]Numbers assigned the same letter in the same column are statistically comparable. *P<0.05, ** P<0.01, *** P<0.001, ns= not significant, μ ± se= mean ± standard error, CV= coefficient of variation.

Table 6. Live weight according to sex, breeding system, locality and coat

Sources of variation	Live weight n	μ ± se (kg)	(CV)
Sex		***	
Cows	234	315.74±7.73[a]	(0.21)
Bulls	87	384.75±11.78[b]	(0.23)
Breeding system		ns	
Controlled	52	356.93±14.27	(0.22)
Uncontrolled	269	343.56±6.08	(0.24)
Localities		***	
Banyo Bunji	50	310.46±12.60[c]	(0.18)
Banyo Centre	116	370.59±6.55[a]	(0.24)
Banyo Leswouroun	9	348.80±24.80[bc]	(0.12)
Banyo Tiqué	67	350.03±11.65[bc]	(0.25)
Banyo-Tibati Border	34	368.08±14.68[b]	(0.21)
Mayo Djinga	45	353.49±13.42[bc]	(0.17)
Coat color		ns	
White	25	335.64±6.99	(0.19)
Black	23	335.24±6.86	(0.18)
Pie	139	332.73±16.87	(0.22)
Red	134	311.04±16.18	(0.18)
Overall average	321	350.24±8.70	(0.24)

[a,b,c]Numbers assigned the same letter in the same column are statistically comparable. *P<0.05, ** P<0.01, *** P<0.001, ns= not significant, μ ± se= mean ± standard error, CV= coefficient of variation.

The results show that this is a heterogeneous population (CV>15%) for the peripheral measurements (circumference of the hump, length of the horns) but also the live weight. With the exception of horn length, measurements were more pronounced in bulls than in cows, as were animals from controlled breeding systems. The sexual dimorphism being in favor of the males as well as the selection of the males would explain this observed variability. These measurements were significantly higher in animals from Banyo Center, the area teeming with breeders and dealers. The white coat color presented the smallest measurements, those of the other coat color (magpie, red and black) being statistically comparable. The tail goes completely through the hock, making the latter animals with long tails (wig under the hock).

3.2. Correlations between Measurements

Table 6 shows the correlation coefficients between the different metric characteristics as main variables but also those of sex and the reproductive system as additional variables. This table reveals three types of correlations: strong positive correlations (correlation coefficients between 0.8 and 1) recorded between the circumference of the shank and that of the barrel; the average positive correlations (correlation coefficients between 0.5 and 0.7) observed between the circumference of the muzzle, the live weight, scapulo-ischial length, the thoracic circumference, length of the forehead, the circumference of the hock, the circumference of the barrel, the length of the horn, the length of the face and the width of the face; but also the weak positive correlations (correlation coefficient less than 0.5) appearing mainly between the main variables, sex and the reproductive system. Figures 3 and 4 show the distribution of the main variables (in red) and additional variables (in blue) in the factorial plan (F1-F2).

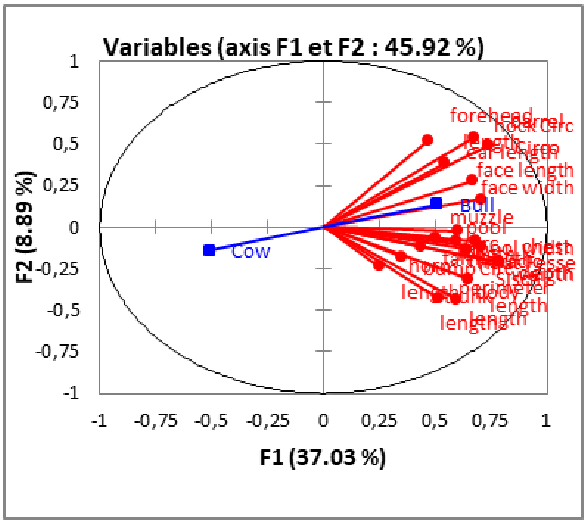

Figure 3. Correlation circle between body measurements.

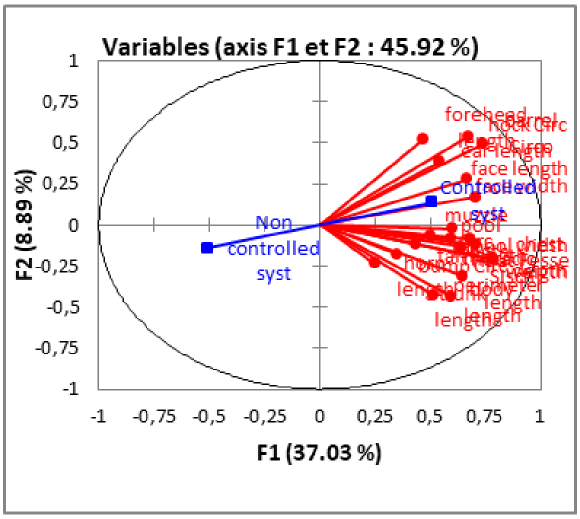

Figure 4. Correlation circle between body measurements.

Figures 3 and 4 show that the factors, sex and reproductive system, are well discriminated on the factorial axis F1 while the factors live weight, 6 measurements are better discriminated on the F2 axis. These figures show overall that the highest body measurements are observed in bulls and cattle from controlled breeding systems. These are therefore animals having a relatively larger format.

3.3. Biometric Index

A total of ten biometric indices were used to assess the general conformation of the animal as well as the development of the different regions. The latter are summarized in Table 7. The latter were globally significantly higher in males than in females.

3.4. Metric Variability

Principal component analysis was carried out in order to assess the individual contribution of the 20 quantitative characteristics in the morphometric variability observed within the population. The measurement of precision of the Kaiser-Meyer-Olkin sampling (0.84) as well as the Bartlett sphericity test (Khi^2 = 4451.08, ddl = 190, p-value <0.0001) were found to be very satisfactory (Table 9). Table 8 shows that the factorial axes F1 (37.03%), F2 (8.89%) and F3 (7.12%) contribute to 53.03% of the total phenotypic variability observed within the population. The KMO as well as the individual contribution (%) of the 20 variables to the F1 and F2 factorial axes are presented in Table 9.

Table 9 shows that the F1 axis, explaining at 37.03% the total phenotypic variability observed within the population, the strong contributions are those of the body weight (8.20%) and the thoracic circumference (8.30 %). Given the strong positive correlation that there is between body weight and chest circumference, this axis can be considered as that of growth performance. In addition, the F2 axis, explaining at 8.89% the total phenotypic variability observed in the 7 within the population, the strong contributions are those of the circumferences of the hock (16.58) and the barrel

(13.75%), the width of the forehead (15.39%) and the lengths of the body (10.70%) and trunk (10.41%). This axis can be considered as that of peripheral and body measurements.

3.5. Population Structure

The discriminant factor analysis (DFA) made it possible to detect 3 sub-populations below the base of the maximum likelihood.

3.5.1. Animals in subpopulation 1

The animals of this population (morphotype 1) are characterized by the predominance of magpie (47.5%) and monochrome (37.5%) coats. Drooping (30%) and erect (70%) bumps. More red coat and its derivatives (78.75) than black coat and its derivatives (20%). Clear muzzles, eyelids and hooves (56%). White-black horns (60%) in crescent (73.75%) and raised (72%). The limits of variation (at 95%) of the mean values of thoracic perimeter and height at the withers are between 181.86 and 185.36cm; 131.00 and 133.92cm respectively. Their live weight varies between 434.98 and 460.06kg. The circumference of the hock and the hump vary respectively between 40.39 and 41.91cm and between 75.59 and 85.53cm respectively. The length of the pelvis varies between 42.11 and 43.23 cm (Figure 5).

Table 7. Main biometric indices of the Gudali zebu

		Biometric index									
	n	MI μ±se (CV)	Pr μ±se (CV)	CpI μ±se (CV)	CPI μ±se (CV)	SI μ±se (CV)	FI μ±se (CV)	SI μ±se (CV)	BI μ±se (CV)	ThDeI μ±se (CV)	ThDaI μ±se (CV)
Sex		***	ns	***	**	***	**	ns	***	***	ns
Cows	234	2.46±0.05[a] (0.07)	95.53±0.94 (0.12)	35.65±0.49[a] (0.09)	0.70±0.00[a] (0.10)	1.73±0.02[a] (0.19)	1.42±0.01[a] (0.11)	1.05±0.01 (0.12)	0.14±0.00[a] (0.09)	0.79±0.00[a] (0.09)	0.11±0.00 (0.08)
Bulls	87	2.92±0.08[b] (0.09)	93.31±1.44 (0.12)	38.61±0.75[b] (0.07)	0.67±0.01[b] (0.10)	1.84±0.03[b] (0.22)	1.47±0.02[b] (0.11)	1.07±0.01 (0.14)	0.15±0.00[b] (0.08)	0.75±0.01[b] (0.10)	0.11±0.00 (0.09)
Total	321	2.69±0.06 (0.22)	94.42±1.06 (0.09)	37.13±0.55 (0.13)	0.69±0.01 (0.11)	1.79±0.02 (0.13)	1.44±0.01 (0.10)	1.06±0.01 (0.09)	0.14±0.00 (0.10)	0.77±0.00 (0.09)	0.11±0.00 (0.09)

Pr= proportionality, MI= massiveness index, CpI= cephalic index, PBI= body profile index, ScI=scapulo-ischial index, DaThI= dactylo-thoracic index, ThDaI= dactylo-thoracic index, BI= bones index

Table 8. Eigenvalue and phenotypic variance of the principal components in the analysis of the variability observed within the population

Principal components (F)	Value	Variance (%)	Cumulative variance
F 1	7.41	37.03	37.03
F 2	1.78	8.89	45.92
F 3	1.42	7.12	53.03
F 4	1.17	5.83	58.87
F 5	1.08	5.41	64.28
F 6	0.99	4.97	69.26
F 7	0.85	4.26	73.52
F 8	0.77	3.85	77.36
F 9	0.69	3.43	80.79
F 10	0.59	2.93	83.72
F 11	0.57	2.83	86.55
F 12	0.56	2.81	89.36
F 13	0.48	2.42	91.79
F 14	0.45	2.23	94.02
F 15	0.41	2.04	96.06
F 16	0.35	1.73	97.79
F 17	0.20	1.02	98.81
F 18	0.16	0.78	99.60
F 19	0.08	0.38	99.98
F 20	0.00	0.02	100.00

Table 9. KMO and contributions of the 20 variables (%) to the F1 and F2 factorial axes

Variables	KMO	F1	F2
Body weight	0.77	8.20	2.42
Circumference of muffle	0.96	4.86	0.02
Lorn length	0.77	0.84	3.15
Face length	0.94	6.01	4.45
Face width	0.93	6.66	1.61
Body length	0.76	4.71	10.70
Scapulo-Ischial length	0.93	5.59	5.45
Thoracic circumference	0.77	8.30	2.47
Trunk length	0.72	3.61	10.41
Height at withers	0.93	3.37	0.22
Sacral height	0.91	5.55	1.00
Barrel circumference	0.79	7.36	13.75
Hock circumference	0.79	6.11	16.58
Pelvis length	0.81	6.20	0.38
Pelvis width	0.78	4.72	0.39
Thoracic depth	0.92	6.77	0.79
Tail length	0.89	2.53	0.72
Forehead width	0.86	2.99	15.39
Hump circumference	0.78	1.62	1.74
Ear length	0.90	3.98	8.35

KMO= Kaiser-Meyer-Olkin sampling accuracy test

Figure 5. Cow of subpopulation 1

Figure 6. Cow of subpopulation 2

3.5.2. Animals in subpopulation 2

Animals of this population are characterized by their erect hump (97.45%), the magpie coat (35.66%) and simple (50.31%). The dominance of the red coat and its derivatives (65.60%) followed by speckling and stoat (12.10%). Predominantly black muzzles, eyelids and hooves (70%). Crescent horns (77.07%), low lyre (15.92%) and stump (3.18%) raised. The limits of variation (at 95%) of the mean values of thoracic perimeter and height at the withers are respectively between 152.83cm and 154.74cm; and between 124.35 and 127.69cm respectively. Their live weight varies between 265.40 and 274.55kg. The circumference of the hock and the hump vary respectively between 37.37 and 38.37cm and between 62.84 and 66.88cm. The pelvis length varying between 40.33 and 41.14 cm (Figure 6).

3.5.3. Animals of subpopulation 3

The animals of this population (morphotype 3) are generally identified by the red coat and its derivatives (67.85%) with a white list, but also animals with a matt white coat. a monochrome (46.43%) and pie (14.29%) pattern with erect bumps (82%). These animals have raised horns (86.90%) in a crescent (66.66%) or lyre (21.43%), black hooves (64.28%) but also black eyelids and muzzles (58.33%). The variation limits (at 95%) mean values of thoracic perimeter and height at the withers are respectively between 166.85 and 168.08; and between 127.4 and 130.08cm. Their average live weight varies between 339.4 and 346.58kg. The circumference of the hock and the hump vary between 38.44 and 39.78cm and between 69.18 and 77.16cm respectively. The length of the pelvis varies 42.11 and 43.23 cm (Figure 7).

Figure 7. Cow of subpopulation 3

3.6. Phylomorphometric Analysis

Discriminant factor analysis (DFA) made it possible to divide the 321 adult animals into the three (3) sub-populations (I, II and III) identifiable with two (2) phenotypes, suggesting an evolving population. In favor of this hypothesis, Paguem et al. (2020) reported, following the work of complete genomic characterization of five breeds from Cameroon (Gudali, White Fulani, Red Fulani, Namchi and Kapsiki), a similarity between the Gudali, White and Red Fulani, namely 163784 single nucleotide polymorphisms (SNPs) in common. In addition, phylogenetic analysis revealed that the Gudali breed was closer to the White Fulani breed than the other breeds (Red Fulani, Namchi and Kapsiki). The latter also report several genetic mutations. Figures 8 and 9 establish the relationship and the comparison between the different sub-populations.

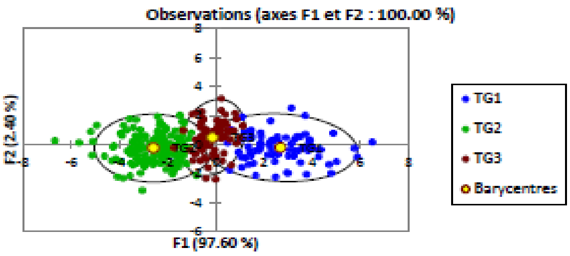

Figure 8. Population structure

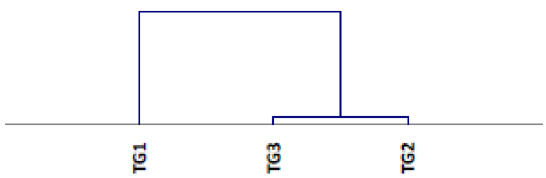

Figure 9. Dendrogram of sub-populations

4. Discussion

Overall measurements were higher in bulls. This result joins those of Pagot (1943) on the Azaouak zebus in Sudan, Coulomb (1976) on the N'Dama breed in Ivory Coast, Thys and Wandi (1970) on the Namchi breed in Cameroon, Ebangi and al. (2011) on the Namchi, Akouango et al. (2014) on the N'Dama, Yahaya breed et al. (2019) on the zebu Djelli, Kanh et al. (2019) on the N'Dama breed, Lhoste (1969) on the Gudali variety Ngaoundere and Ngono et al. (2019). The locality significantly influenced the different measurements. This result is in accordance with that found by Boma et al. (2018). Lhoste (1969) found in Gudali variety Ngaoundere cows a width of the head greater than that of the present study (18.61cm). It is probably due to the small size (13 bulls and 25 cows) used by this author. The height at the withers was 128.48cm and 130.99cm respectively in cows and bulls. This result is sufficiently close to that reported by Doba (2016), but however slightly higher than that found by Lhoste (1969), namely 123.2cm and 131.8cm respectively in Gudali variety Ngaoundere. The pelvis width was 38.90cm in the cows of the present study, against 50.06cm in the Gudali variety Ngaoundere cows selected at the Wakwa Research Center (Lhoste, 1969). The lengths of the pelvis and scapulo-Ischial were respectively 41.98cm and 134.89cm in cows, against 48.3cm and 145.2cm in Gudali variety Ngaoundere cows (Lhoste, 1969). It is likely that the observed differences are due to the variety and size effects of the population. The sex effect on the average adult weight was reported by Lhoste (1969) on the Gudali variety Ngaoundere zebu in station. He obtained 563kg in males against 335.4kg in females. The difference in weight observed between these two results would be justified by the fact that these are two varieties of Gudali: that of Ngaoundere in the station and that of Banyo in a peasant environment. In addition, the weight was estimated using the barymetric method while Lhoste (1969) carried out weighings on a weighbridge. The work on barymetry, carried out by Doba (2016) on the Gudali variety Ngaoundere and crosses zebu has shown weight superiority in favor of males. Either on average 416.12 ± 95.64 and 341.46 ± 51.97kg respectively in the Gudali bulls variety Ngaoundere and the cross, against 351.92 ± 52.85 and 358.85 ± 53.34kg respectively in Gudali variety Ngaoundere and crosses cows. These values, on the other hand, are well above those obtained in the present study.

Several other authors have reported the sex effect on adult weight in favor of males. These are Pagot (1943), Lhoste (1969), Coulomb (1976), Ebangi et al. (2011), Akouango (2014). The proportionality index was statistically comparable between the two sexes while the massiveness index was significantly influenced by sex, in favor of males. This conclusion is in harmony with that reported by Ngono and However, Ngono et al. (2019) found no significant effect of gender on surface area index and cephalic index as was the case in the present study. The factor axes F1 (37.03%), F2 (8.89%) and F3 (7.12%) contributed to 53.03% of the total phenotypic variability observed within the population. A percentage much lower than that reported by Ngono et al. (2019) on the White Fulani of North Cameroon, namely 73.45% for the first three main components. It is probable that the observed differences are due to the size of the population and to the breed effect (88White Fulani / 321Gudali variety Banyo). In Ivory Coast, N'goran's work et al. (2008) led to the conclusion according to which there is genetic diversity within this population. They also believe that this genetic diversity is due to the various uncontrolled crosses with other breeds. The measurements of animals in subpopulations 1, 2 and 3 are comparable to those of subpopulations 1, 2 and 3 found in Côte d'Ivoire by N'goran et al. (2008) for body length, thoracic perimeter, head length and live weight. However, these measurements were much higher than those found in Congo Brazzaville by Akouango et al. (2014) on the N'dama breed. The breed effect would explain the observed differences. Boma et al. (2018) also detected three subpopulations in local humpless cattle from Togo and attribute this diversity to interbreeding.

5. Conclusion

At the end of this study of which the main objective was the metric characterization of the Gudali zebu variety Banyo in the high Guinean savannah zone of Cameroon, the main results show that the different measurements were significantly influenced at varying degrees by sex, reproductive system and locality. Both males and animals from controlled reproduction systems have presented a significantly larger size. The results of the discriminant factor analyzes revealed the existence of three (03) subpopulations or morphotypes, grouped together on the basis of maximum likelihood. In perspective, it is desirable that this study extend to the other two varieties (Ngaoundere and Tignere) of Gudali by taking into account the zootechnical implication (reproduction, growth, production and carcass) of these different measurements.

Author Contributions

All authors have equal contribution and the authors reviewed and approved the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethical permission was taken from Cameroon Agricultural Research Institute for Development, Polyvalente Research Station of Bangangté (date; January 2020, numbered: 2020-1)

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DETERMINATION OF DIGESTIVE ENZYME ACTIVITY IN GILT-HEAD SEA BREAM (*SPARUS AURATA*) FEEDING WITH COMMERCIAL FEED

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Abstract: With the increase in the world population, aquaculture has a very important place in meeting the protein needs of humanity. Today, fish farming is developing rapidly depending on the increase in demand. Fish is one of the most important foodstuffs for human nutrition. Studies on the quality and characteristics of the feeds used gain importance with the increase in the knowledge about the nutrient sources of fish. It is known that there is a linear correlation between growth, productivity and feed efficiency in fish like another animal. At this point, the correct and effective use of feed, which is the most important input in production, is very important in terms of increasing the yield and product quality to be obtained from the product and reducing production costs. The identification of digestive enzyme activities is important in developing new feeding regimens and in ensuring optimal growing conditions. As most enzymes regulate the events of viability, the increase or decrease in their activity causes the degradation of the normal functions of fish and diseases in fishes. In this study, body weight and changes in digestive enzyme activity were investigated in sea bream feeding with commercial feed. For this purpose, a total of 3000 juveniles with an initial mean weight (IW) of 2.72±0.78 g were divided into 4 tanks (2000 L). After the six weeks final weight was 7.75 ± 0.67 at the end of experiment. While trypsin activity was increased until the end of the experiment, amylase activity was decreased. On the other hand, small decreases in lipase activity were observed throughout the experiment.

Keywords: Commercial feed, Enzyme activity, Lipase, Trypsin

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1. Introduction

The major aim of aquaculture applications is to ensure sustainable aquaculture production with high growth performance to encounter human food consumption (Navruz et al., 2020). Aquaculture is one of the growing food industries in the world. One of the biggest reasons for this growth is the increase in demand for fisheries (Michael et al., 2014). A lot of research has a grand extent on an improved information about feeding habits, nutritional requirements and digestive capabilities in different species on cultured marine fish. In this sense, a great number of studies on digestive enzymes of marine fish have been applied (Alarcon et al., 2001). The growth performance of fish rely on the enzyme activity of the digestive system and their capacity to digest protein, fat and carbohydrates (Shan et al., 2008). Gilthead sea bream (*Sparus aurata*) is one of the most delicious and valuable marine fish species. (Suzer et al., 2008). Its Latin name gets from the characteristic golden band among its eyes, live in marine waters as well as in the brackish waters of coastal lagoons. Gilthead sea bream is one of the key constituent of aquaculture. So, the aim of this work was to evaluate effect of commercial feed and study their effect a body weight and digestive enzyme activity on gilthead sea bream.

2. Material and Methods

The experiment was applied at Marine Research Station of Fisheries Faculty, University of Çukurova, Yumurtalık, Turkey. This study was carried out in 2016. Gilthead sea bream juveniles were supplied by Akuvatur Hatchery (Adana, Turkey). A total of 3000 juveniles with an initial mean weight (IW) of 2.72 ± 0.78 g were stocked into 4 tanks (2000 liters). Fish were hand-fed with a commercial diet (Çamlı Feed Ltd., Turkey, 2 mm; 49% crude protein, 19% crude fat, 12% moisture and 13% ash) three times daily at 08:00, 12:00 and 16:00 h during the six weeks. Also, in every two weeks interval fish weight measurements, 5 fish were taken from the each tank for enzyme analysis.

2.1. Enzyme analysis

Trypsin activity was assayed at using method of Holm et al. (1988). Amylase activity was measured for Métais and Bieth (1968), using 0.3% soluble starch dissolved in Na_2HPO_4 buffer pH 7.4 as substrate. Lipase activity (U/ml) was defined as the μmol of substrate hydrolyzed per min per ml of enzyme extract (Iijima et al., 1998).

3. Results

Variation of fish body weights observed at measurement periods (every two weeks) during the study is given in Figure 1. Final weight was 7.75 ± 0.67 gr. While the Trypsin activity was increased until the end of the experiment, amylase activity was decreased. After the six weeks, Trypsin activity was 1.55 ± 0.19 (U/ mg protein). Amylase activity was 36.42 ± 0.95 (U/mg protein). On the other hand, small decreases in lipase activity were observed throughout the experiment. Lipase activity was 53.66 ± 0.19 (U/ mg protein). The activity of amylase, lipase and trypsin enzymes in gilthead sea bream is shown in Figure 2.

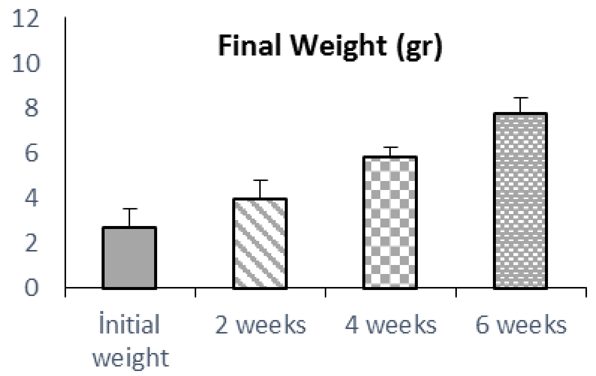

Figure 1. Changes in body weight (g) of sea bream juveniles.

4. Discussion

Digestive enzymes affect the digestion capacity of food by providing digestion. Therefore, the development of the fish is provided. The relationship between feeding frequency and feed use constitutes the basis of economical feeding in aquaculture. (Blier et al., 2008; Ling et al., 2010). Intestinal absorption ability of feed intake also effects nutrient utilization (Wen et al., 2009) Growth heterogeneity limits feed use and absorption in the intestinal. For example, production of Eurasian perch and yellow perch (P. flavescens) have a problem (P. flavescens) (Langeland et al., 2013). In this study, the objective was to compare digestive enzyme activities of gilthead sea bream (*Sparus aurata*) feeding with commercial feed to evaluate if differences in growth rate could be related to digestive enzyme activity. Growth capacity in fish can be controlled by environmental and physiological factors (Blier et al., 2008) In Atlantic cod (*Gadhus morhura*) (Lemieux et al., 1999) and Atlantic salmon (*Salmo salar*) (Torrissen and Shearer, 1992). Digestive enzymes play a key role in digesting nutrients and digestive capacity has been found to relation with the growth (Gisbert et al., 2004; Slack, 1995). Enzymes activities mirror digestive capacity and influence fish growth rate (Blier et al., 2008; Ling et al., 2010) Trypsin activity has been shown to have an effect on growth rate. The activity of digestive enzymes is considered as a significant indicator for fish growth and the level of digestive enzyme depends on the capacity of digestion absorption of nutrients which affects the development and the growth in fish (Wei et al., 2010). As digestion efficiency in fish depends on a good digestive functionality able to optimize the hydrolysis and absorption of nutrients (Kokou and Fountoulaki, 2018). Digestive enzymes played an important role in the hydrolysis of proteins, lipids and carbohydrates in the conversion of digested foods. These nutrients have been transported into the tissues and changed into material or energy for the growth (Furne et al., 2005). Similarly, present findings in our study supported that trypsine. Trypsin activity was increased with weight gain in study. Low amylase activity in carnivorous fish is the general assumption (Hidalgo et al., 1999; Krogdahl et al., 2004).

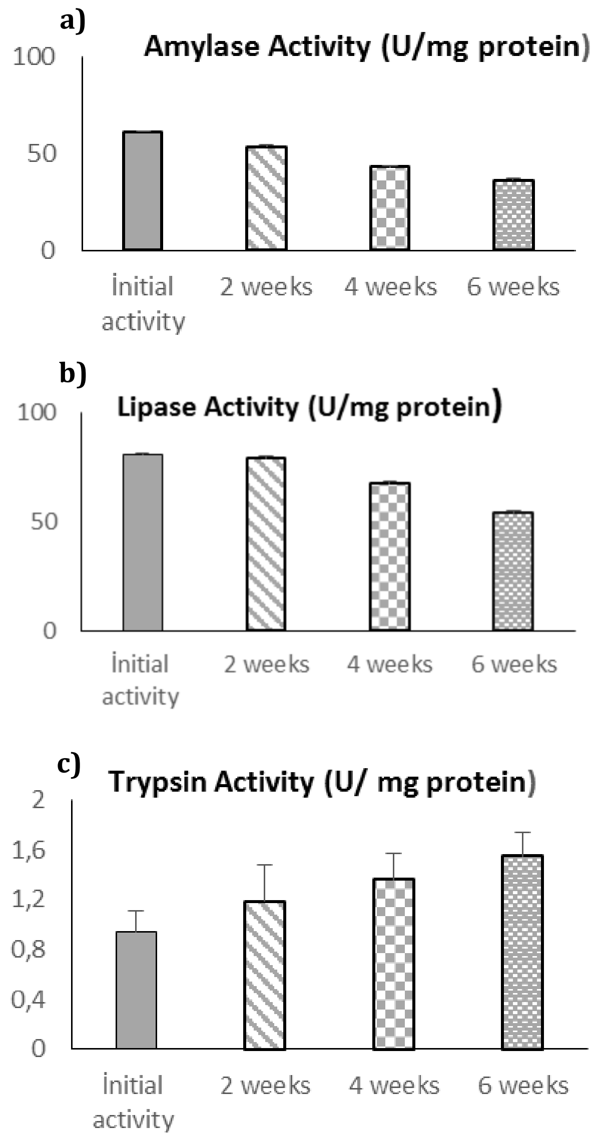

Figure 2. Changes in enzyme activities (U/ mg protein) of sea bream juveniles. a) Amylase, b) Trypsin and c) Lipase.

As a carnivorous species, gilthead sea bream has low amylase activities in the liver and intestine compared to carp (*Cyprinus carpio*) and gold fish (*Carassius carassius*) (Hidalgo et al., 1999). Amylase activity was decreased throughout the experiment. Small decreases in lipase activity were observed throughout the experiment. Aliyu-Paiko et al. (2010) and Li et al. (2012) stated that Lipases are inducible enzymes which could be stimulated by the dietary lipid content. Similarly, Ma (2014) described that fish fed showed the positive correlation between lipase activity and dietary lipid content.

5. Conclusion

The main objective of aquaculture practices is to provide sustainable aquaculture production with high growth performance to meet human food consumption. The present work presents the importance effect of using commercial feed on digestive enzyme in aquaculture to enhance growth which is an important aspect in eco sustainability of aquaculture.

Author Contributions

All task made by single author and the author reviewed and approved the manuscript.

Ethical Approval

A retrospective ethics permit is not required for the articles, which were produced from used master/doctorate or research studies before 2020.

Conflict of Interest

The author declared that there is no conflict of interest.

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Research Article
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SOME IMPORTANT CHEMICAL AND AROMA CHARACTERISTICS OF SOME NEW PEPPER CULTIVARS (*Capsicum annuum* L CV A30706 F1, *Capsicum annuum* L CV KILÇIK F1, *Capsicum annuum* L CV BITTER F1)

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Abstract: Obtaining agricultural products with better quality is closely related to the development of new cultivars. New cultivars are increasing gradually internationally as a result of the breeding programs. *Capsicum annuum* L. 'A30706 F1', *Capsicum annuum* L. 'Kılçık F1', *Capsicum annuum* L. 'Bitter F1' pepper cultivars are newly developed registered pepper cultivars in Turkey. A30706 F1 is a bitter and black coloured long pepper also called purple coloured, Kılçık F1 is a sweet long thin pepper and Bitter F1 is a hot long thin pepper. Among the cultivars while the highest amount of ascorbic acid (216.35 mg/100g) was determined in A30706 F1, the highest amount of phenolic compounds (63.69 mg GAE/100g) was determined in the Bitter F1 cultivar. Hexanal was found as major aroma component in A30706 F1 and Kılçık F1 pepper cultivars, which creates a sensory perception as fresh, cut grass. Hexanal and E-2-tetradecenal were found as major aroma components in Bitter F1 cultivar. E-2-tetradecenal creates a sensory perception as fatty, waxy, cheesy.

Keywords: Ascorbic acid, Pepper cultivars, Phenolic compounds, Volatile compounds

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1. Introduction

Plants have the capacity to synthesize, accumulate and spread aroma and scent molecules. These are commercially important in food, pharmacy, agriculture, chemical industries in terms of flavorings, drugs, pesticides, industrial feed stocks (Schwab, 2008). These aroma and scent molecules are also found in pepper.

After Columbus came to Europe with pepper seeds, pepper spread around the world with the help of the Spanish and Portuguese trade routes. It also spread from Europe to Africa, India, China, Japan and Korea with the help of spice routes. An obelisk from the Chavin culture in Peru has a black caiman carving and in its claws are the pods of peppers (Bosland and Votava, 2012). Today, pepper has many uses such as spices, industry, pharmacy, fresh consumption, flavoring agents and insecticides.

Ascorbic acid is an important antioxidant and has a protective role in various diseases (Domitrovic, 2006) and it is an essential component in the prevention of scurvy disease. Soluble solids used in sugar content estimation is an important criterion in all fruits and vegetables. The total amount of phenolic compounds in plants is related to UV protective, signaling compound, pigment formation, plant growth, defense mechanism (Lattanzio et al., 2006) and taste factors such as bitterness. The aroma composition is used in the food and pharmaceutical industries to give taste and smell.

Kocsis et al. (2002) carried out a study to determine the aroma composition of 11 sweet (*Capsicum annuum* L. 'Kalocsai-M-622', 'Szegedi-20', 'Kalocsai-801', 'Szegedi-80', 'Csa´ rda´ s', 'Folklo´ r', 'Reme´ny, Rubin', 'Ka´rmin', 'Zuhatag', 'Miha´ lyteleki') and 2 hot (*Capsicum annuum* L. 'Kalocsai-V-2' and 'Szegedi-178') Hungarian red pepper varieties. In the study (Kocsis et al. 2002) it was determined that hot varieties have more terpene, sesquiterpene and terpene derivative components than sweet varieties. Aroma and fragrance content of Kalocsai-V-2 (hot) variety were richer than Kalocsai-M-622 (sweet) variety. In this case, it is especially important to determine the aroma characteristics of the A30706 F1 cultivar, which is a hot pepper cultivar.

The highest amount of flavorings were 4-metoksi-fenol (3.69 ppm), etil hekzadekanoat (3.64 ppm), hekzanal (1.22 ppm) and izopulegol (1.01 ppm) in green pepper (*Capsicum annuum* L.) in study conducted by Jang et al. (2008). 2-methoxy-3-isobtylpyrazine (similar to bell pepper scent), nona-trans,cis-2.6-dienal (similar to cucumber scent), deca-2.4-dienals (similar to fried chicken scent), limonene (similar to citrus scent), methyl salicylate (similar to oil of wintergreen) compounds were determined at high levels in California green bell pepper (*Capsicum annuum* var. grossum, 'Sendt') with vacuum isolation analyze however furfural, benzaldehyde, heptan-2-one, hex-cis-3-enol, hept-trans-3-en-2-one, methyl salicylate compounds were at high levels with water solution analyze (Buttery et al., 1969).

Major essential oils in two pepper varieties (*Capsicum annuum* L. 'Twingo', *Capsicum annuum* L. 'No.1') were found as Benzaldehyde 20.9%), 2-Methoxy-3-isobutyl-pyrazine (20.4%), Z-B-Ocimene (13%), Dimethylbenzene (8.5%), Heptane-2-one (8.0%) in Twingo variety and as 2-Methoxy-3-isobutyl-pyrazine (12.7%), Linalool (8.3%), Z-β-Ocimene (6.2%), Nona-trans,cis-2,6-dienal (6.2%), Hexanal (5.6%) in No.1 variety (El-Ghorab et al., 2013).

The water soluble dry matter amount of peppers were 5.8% in the first group varieties (Ilıca 256, Acıkıl, Çorbacı, Manisa Yeşili, Çetinel and Demre), it was 5.3% in the second group varieties (table varieties consist of Çarliston, Kandil Dolma and industrial varieties consist of Üçburun, Yunan, Kale), it was 5.4% in third group varieties (California Wonder and Kapia) and forth group varieties (Jalepeno), it was 5.5% in fifth group varieties (use for pickle) and it was 5.1% in sixth group varieties (tomato pepper varieties) in study regarding morphological variability of some important pepper genotypes which were grown in Turkey (Duman and Düzyaman, 2004).

Sim and Sil (2008), determined the amount of phenolic substance in the pericarp of red pepper as 47.52 mg/g GAE in their study regarding antioxidant activities in the pericarp and seeds of red pepper (*Capsicum annuum* L.).

Phenolic, peroxidase and capsidiol levels in sweet pepper (*Capsicum annuum* L. 'Almuden') were determined as between 20-40 mg/100g in the green maturation period and 80-100 mg/100g in the red maturation period (Amor et al., 2008).

The subject of the study is the evaluation of three new pepper cultivars (*Capsicum annuum* L. 'A30706 F1', *Capsicum annuum* L. 'Kılçık F1', Capsicum annuum L. 'Bitter F1') in terms of ascorbic acid, soluble solids, amount of phenolic compounds and flavor composition.

2. Material and Methods

In this study 'A30706 F1', 'Kılçık F1' and 'Bitter F1' pepper cultivars (*Capsicum annuum* L.) were used as plant materials. A30706 F1 is a bitter and black coloured long pepper, Kılçık F1 is a sweet long thin pepper, Bitter F1 is a hot long thin pepper. This research was carried out at the experimental fields of Çanakkale Onsekiz Mart University's Faculty of Agriculture in Turkey in 2019.

2.1. Analyzing of Aroma Compounds in Pepper Cultivars

Aroma analyzes were performed using Shimadzu QP2010 Plus Gas Chromatography-Mass Spectrometer system located in Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Horticulture.

Methods reported by Vichi et al. (2007), Sabatini and Marsilio (2008), Reboredo-Rodriguez et al. (2013), Ekinci et al. (2016) ve Bozok et al. (2018) were modified and used for the identification of aroma components in pepper samples. The step of preparing samples for the analysis were as follows: 50 g sample of mushroom purees obtained with homogenizer was treated with 100 ml diethyl ether solvent in erlenmayer and the solvent was concentrated to 1 ml by centrifuge and concentrator. Operating conditions of GC / MS device are given below.

Carrier gas; Helium, column; DB-WAX® polyethylene glycol (PEG) (30 m x 0.25 mm x 0.25 µm), injection block temperature; 280°C, linear flow; 41cm/sec, pressure; 70.3 kPa, injection mode; split (1:50)

2.2. Oven Temperature Program

It was 1 minute at 40°C at the beginning, then 2 minutes at 200 °C with a speed of 4 °C/min and 10 minutes at 250 °C with a speed of 10 °C/min. Time of total analysis was 58 minutes. Detector; mass spectrometer (MS), library; Nist and Wiley, ion temperature; 250 °C, interfacial temperature: 230 °C, solvent cut time; 4 min, scanned mass range and scanning speed; 40-350 amu (m/z) and 666 amu/sec, ionization energy; 70 eV

2.3. Analyzing of Total Phenolics (mg GAE/100g) in Pepper Cultivars

Fruit juice (5 g) was supplemented with 5 ml methanol and centrifuged at 4000 rpm for 10 minutes. The samples were then supplemented with 2.5 ml 10% Folin-Ciocalteu and 2 ml 1 M Na2CO3 and kept in water bath at 45°C for 15 minutes. Samples were taken to Shimadzu UV-VIS spectrophotometer (UV-Vis Spectrophotometer, Shimadzu Corporation, Tokyo-Japan) and read at 765 nm absorbance value against 10% Folin-Ciocalteu. Results were expressed in total gallic acid equivalent (GAE) mg/100 g (Zheng and Wang, 2001).

2.4. Analyzing of Soluble Solids Content (%) in Pepper Cultivars

It was determined by measuring soluble solids content of fruit juices with using digital hand refractometer in marketable 36 fruits.

2.5. Analyzing of pH Value and Titratable Total Acidity (g/100g) in Pepper Cultivars

Amount of NaOH (0.1 N) that was spent when pH value was 8.1 was determined by using burette and WTW digital desktop pH meter. The amount of titratable acidity (g/100g) was calculated in terms of citric acid by formula (International Federation of Fruit Juice Producers, 1968).

2.6. Analyzing of Ascorbic Acid Content (mg/100g) in Pepper Cultivars

It was determined by method of Pearson and Churchill (1970) with using Shimadzu UV-VIS -1800 spectrophotometer. 175 ml of 0.4% Oxalic Acid was supplemented to 25 g fruit pulp and samples were filtered from Whatmann (No: 2) filter paper for ten minutes. L1 value was determined by reading of Oxalic acid/2.6 Diclorophenol indophenol: 1/10 solution in response to Oxalic acid/Pure water: 1/10 solution at 520 transmittance value. L2 value was determined by reading of filtered sample/2.6 Diclorophenol indophenol: 1/10: solution in response to Oxalic acid/Pure water: 1/10 solution at 520 transmittance value. In this way, ascorbic acid content was calculated by using the formulation.

2.7. Trial Design and Statistical Analysis of the Experiment

Experiment was set up based on completely randomised design with 3 replications consist of 30 plants in each replication in Çanakkale Onsekiz Mart University Dardanos farm which is located in Çanakkale Onsekiz Mart University Faculty of Agriculture. Seedlings planted with a distance of 0.33 meters within the row and 1 meter between the rows.

Samples were selected from 10 marked plants for each replication as 5 samples from each plant. A total of 50 peppers for each replication were crushed in a blender and stored until analyzed in a refrigerator at Çanakkale Onsekiz Mart University Faculty of Agriculture, Horticulture Laboratory, which can cool down to -80°C. For the vitamin C readings, samples were treated with 0.4% oxalic acid and stored in a refrigerator at +4° C until the analysis.

Before the experiment, soil samples were taken from ten different regions of the trial land at a depth (0-30 cm) that would represent the active root zone by simple random sampling method, representing the whole of the field (Crepin and Johnson, 1993) and fertilization was performed according to the results of the soil analysis. Irrigation was applied for every three days with using a drip irrigation system.

In the experiment, analysis of variance was performed using the SAS (9.1.3) computer package program for statistical analysis and LSD (P<0.05) test was used to compare the differences between the means of the data.

Biplot analysis was used to interpret the data of different aroma components in the aroma composition and the data were evaluated on the graph.

In the study, it was determined that the sum of PC1 (57%) and PC2 (43%) values in the biplot graph was 100%. It was decided that biplot analysis would be useful in terms of the visual advantage it provides in evaluating the variation of different aroma components according to the subjects. Biplot is a useful tool in information analysis and provides visual evaluation in large information matrices. Biplot can show distances between units and it can group units (Gabriel, 1971). Aroma compounds with major importance were included besides compounds which exist in all three cultivars in the biplot graph.

3. Results and Discussion

In the study, no significant difference was found (P<0.05) between the cultivars in terms of pH and soluble solids values. The titratable acidty values were found to be similar in Bitter F1 and Kılçık F1 cultivars and lower than A30706 F1 cultivar as shown in Table 1.

Table 1. The pH, titratable acidity, soluble solids values of pepper cultivars

	pH[a]	Titratable Acidity (g/100g)[a]	Soluble Solids (%)[a]
A30706 F1[b]	6.6	0.100 [A]	5.43
Bitter F1[b]	6.98	0.076 [B]	5.33
Kılçık F1[b]	6.9	0.073 [B]	5.83
LSD P<0.05[c]	NS	0.023	NS

[a]= pH, titratable acidity (g/100g), soluble solids (%) values [b]= pepper cultivars, [c]= LSD (P<0.05) value.

In the study examining the chemical properties of two Spanish pepper varieties (*Capsicum annuum* L. 'Fresno de la Vega', *Capsicum annuum* L. 'Benavente-Los Valles') at different maturity stages, the soluble solids, pH values were determined as 6.93, 4.98 in 'FresnodlaVega' and 6.73, 4.79 in 'BenaventeLos' (Bernardo et al., 2008).

Balkaya et al. (2009) determined the amount of water soluble dry matter between 5.2-8.0% when all populations were evaluated in their study on red conic pepper (*Capsicum annuum* 'Conoides Mill.') genotypes.

Soluble solids, titratable acidity (% citric asit) and pH contents of 'Arnoia' pepper variety were determined as 7%, 0.11, 4.6 (g/100g) respectively in red maturity stage (Martinez et al., 2007).

Phenolic compounds which related to taste properties such as bitterness were found similar in A30706 F1 and Kılçık F1 cultivars and lower than Bitter F1 cultivar when evaluated statistically (P<0.05). Ascorbic acid (vitamin C) values were statistically different (P<0.05) and the highest value was obtained in the A30706 F1 cultivar, followed by the ascorbic acid values of the Bitter F1 and Kılçık F1 cultivars, respectively as indicated in Table 2.

Table 2. The phenolic compounds and ascorbic acid values of pepper cultivars

	Phenolic Compounds (mg GAE/100g)[a]	Ascorbic Acid (mg/100g)[a]
A30706 F1[b]	47.72 [B]	216.35 [A]
Bitter F1[b]	63.69 [A]	205.20 [AB]
Kılçık F1[b]	50.21 [B]	194.87 [B]
LSD P<0.05[c]	58.85	21.45

[a]= Phenolic Compounds (mg GAE/100g), Ascorbic Acid (mg/100g) values, [b]= pepper cultivars, [c]= LSD (P<0.05) value.

The amount of ascorbic acid was determined between 64.9-15.2 mg/100g in the varieties (*Capsicum annuum* L. '730 F1', '1245 F1', 'Amazon F1', 'Serademre 8', 'Kusak 295 F1') examined in the study by Topuz and Ozdemir (2007), it was determined as 202.1 mg/100g in the cultivar (*Capsicum annuum* L. 'Caryca F1') examined in another study (Buckowska et al., 2016). Balkaya and Karaağaç (2009) determined ascorbic acid content between 53.0-155.0 mg/100g in red conical pepper genotypes (*Capsicum annuum* L. 'conoides Mill.') when all populations were evaluated.

Phenolic contents were found between 607-2724 mg/kg in study which include 29 pepper varieties grown in Turkey (Frary et al., 2008). Kolton et al. (2011) determined the amount of phenolic substance in sweet pepper (*Capsicum annuum* L. 'Spartacus') as 35.77 in the green maturity period and 56.44 mg/100g in the red maturity period.

3.1. Comparison of Pepper Cultivars (P<0.05) in terms of Aroma Components

It was determined that there was a significant difference (P<0.05) between aroma components.

The major aroma components were determined as hexanal in aldehydes (Table 3), hexanol in alcohols (Table 4), methyl salicylate in esters (Table 5), γ-muurolen in terpenes (Table 6).

Table 3. Comparison of pepper cultivars in terms of aldehyde group aroma compounds

Aroma Compounds[a]	A30706 F1[b]	Bitter F1[b]	Kılçık F1[b]	LSD P<0.05=[c]
Hexanal	26.36 A	14.50 C	18.85 B	4.0914
E-2-tetradecenal	14.76	14.42	12.52	N.I.
3-Phenyl butanal	4.31 AB	2.80 B	5.20 A	2.2898
Benzaldehyde	2.37 A	0.64 B	0 C	0.1888
E-2-Hexenal	1.42 A	0.76 B	0 C	0.2553
E-2-Heptenal	1.46 A	0.64 B	0 C	0.2584
2-Pentyl-2-nonenal	0	0.49	0	NS
Toplam Aldehyde (%)	50.68	34.24	36.57	

[a]= aroma compounds, [b]= pepper cultivars, [c]= LSD (P<0.05) value.

Table 4. Comparison of pepper cultivars in terms of alcohol group aroma compounds

Aroma Compounds[a]	A30706 F1[b]	Bitter F1[b]	Kılçık F1[b]	LSD P<0.05=[c]
Hexanol	10.73 A	6.74 B	11.43 A	3.4049
Z-3-Hexenol	2.19	1.42	2.44	NS
Heptanol	1.55 C	3.93 B	6.65 A	1.9458
2-Desycloxy Ethanol	2.32 B	3.74 A	1.61 B	0.8266
E-2-Hexenol	4.83 A	0.70 B	0 C	0.3065
Isohexanol	0	0.78	0	
3-methyl-3-butenol	0	0.92	0	
Total Alcohol (%)	21.62	18.23	22.13	

[a]= aroma compounds, [b]= pepper cultivars, [c]= LSD (P<0.05) value.

Table 5. Comparison of pepper cultivars in terms of ester group aroma compounds

Aroma Compounds[a]	A30706 F1[b]	Bitter F1[b]	Kılçık F1[b]	LSD P<0.05=[c]
Methyl salicylate	5.89 A	5.05 A	10.43 B	0.8537
Hexyl 2 methyl butyrate	3.81 A	2.44 B	4.49 A	0.8711
Ethyl Hexadecanoate	1.89 B	6.01 A	5.51 A	1.6278
2-Ethyl-3-Hydroxyhexyl butyrate	0 B	1.29 A	1.40 A	0.4401
2,2-Dimethyl-1,3-propanediol isobutanate	0 B	0.60 A	0.81 A	0.4377
Hexyl iso butyrate	0	0.91	0	
Ethyl decanoate	0	0.74	0	
Butyl iso butyrate	0	0.99	0	
Hexyl hexanoate	0	2.10	0	
Isobutyl isopentanoate	0	0.82	0	
Methyl tert-butylacetate	0	1.57	0	
Total Ester (%)	11.59	22.51	22.64	

[a]= aroma compounds, [b]= pepper cultivars, [c]= LSD (P<0.05) value.

Table 6. Comparison of pepper cultivars in terms of terpene group aroma components

Compound[a]	A30706 F1[b]	Bitter F1[b]	Kılçık F1[b]	LSD P<0.05=[c]
γ-Muurolen	5.12 A	4.88 A	2.38 B	1.8278
Z-Linalool oxide	1.46	2.68	2.46	N.I.
Total Terpene (%)	6.58	7.57	4.84	

[a]= aroma compounds, [b]= pepper cultivars, [c]= LSD (P<0.05) value.

A total of 20 aroma components from 5 component groups were detected in 'A30706 F1' pepper cultivar. In the first group consisting of aldehydes, there were 6 aldehyde components (Table 3). The second group consisting of alcohols included 5 alcohol components (Table 4). In the third group consisting of esters, there are 3 ester components (Table 5). In the fourth group consisting of terpenes, there were 2 terpene components (Table 6). In the other components group, there were 4 components (Table 7).

Table 7. Comparison of pepper cultivars in terms of other group aroma components

Compound[a]	A30706 F1[b]	Bitter F1[b]	Kılçık F1[b]	LSD P<0.05=[c]
Isobutyl methoxy pyrazine	2.34 [B]	2.74 [AB]	3.22 [A]	0.6062
Hexanoic acid (Caproic acid)	2.26	2.23	1.91	NS
Pentadecane	2.89 [C]	6.15 [A]	4.80 [B]	0.4766
Hexadecane	2.04 [B]	4.35 [A]	2.31 [B]	1.3584
Dodecan	0	0.86	0	
2-methyl butanoic acid	0 B	1.13 [A]	1.58 [A]	0.4661
Total Other Components (%)	9.53	17.46	13.82	

[a]= aroma compounds, [b]= pepper cultivars, [c]= LSD (P<0.05) value.

A total of 33 aroma components from 5 component groups were detected in 'Bitter F1' pepper cultivar. In the first group consisting of aldehydes, there were 7 aldehyde components (Table 3). The second group consisting of alcohols included 7 alcohol components (Table 4). The third group consisting of esters included 11 ester components (Table 5). In the fourth group consisting of terpenes, there were 2 terpene components (Table 6). In the other compounds group, there were 6 compounds.

A total of 19 aroma components from 5 component groups were detected in 'Kılçık F1' pepper cultivar. In the first group consisting of aldehydes, there were 3 aldehyde components (Table 3). The second group consisting of alcohols included 4 alcohol components (Table 4). In the third group consisting of esters, there were 5 ester components (Table 5). In the fourth group consisting of terpenes, there were 2 terpene components (Table 6). In the other compounds group, there were 5 compounds.

Aldehyde components were composed the major content in the aroma profile of all pepper cultivars. Among the aldehydes, hexanal was determined as the main aldehyde component in A30706 F1 and Kılçık F1 pepper cultivars. Hexanal and E-2-tetradecenal were the main aldehyde components in Bitter F1 pepper cultivar (Table 3). In a study (Ziino et al., 2009) conducted on different pepper varieties, hexanal was found as second major aldehyde component.

It was reported that hexanal component creates a fresh, cut grass sensory perception (Selli et al., 2014) and odor description of E-2-tetradecenal was specified as fatty, waxy, cheesy in study by Yüksel et al. (1998).

It was seen that aldehyde group aroma components were statistically significantly different (P<0.05) among the cultivars as stated in Table 3. The highest ratio of hexanal and 3-Phenyl butanal components was determined in A30706 F1 cultivar, followed by Kılçık F1 and Bitter F1 cultivars, respectively. There was no statistical difference (P<0.05) between the cultivars in terms of E-2-tetradecenal component.

Hexanol was the main alcohol component in all pepper cultivars ('A30706 F1', 'Kılçık F1', 'Bitter F1') (Table 4). Hexanol creates a mint and green grass sensory perception (Calin Sanchez et al., 2010).

According to statistical results (P<0.05) concerning alcohol group aroma components as mentioned in Table 4, it was determined that A30706 F1 and Kılçık F1 cultivars were similar in terms of hexanol component. The lowest hexanol ratio was determined in Bitter F1 cultivar. A30706 F1 and Kılçık F1 cultivars were found to be similar and lower than Bitter F1 in terms of 2-Desycloxy Ethanol component ratio. The highest ratio in heptanol component was determined in Kılçık F1 cultivar, followed by Bitter F1 and A30706 F1 cultivars, respectively. It has been reported that the heptanol component produces a musty, sweet, woody sensory perception (Feng et al., 2019). There was no statistical (P<0.05) difference between the cultivars in terms of Z-3-Hexenol component.

When the aroma components of the ester group were evaluated, it was determined that methyl salicylate component was the major component in all cultivars (Table 5).

It was determined that methyl salicylate caused peppermint (Niu et al., 2019) and oil of wintergreen (Buttery et al., 1969) sensory description. It was stated that hexyl 2-methyl butyrate has apple, grape fruit taste (Qin et al., 2017) and ethyl hexadecanoate component created soapy and oily sensory perception (Ding et al., 2015), respectively.

The ester group aroma contents of cultivars were significantly different (P<0.05) between cultivars as shown in Table 5. It was observed that the highest methyl salicylate ratio was in Kılçık F1 cultivar. It has been determined that the A30706 F1 and Bitter F1 cultivars have similar proportions of methyl salicylate component. It was determined that Hexyl 2 methyl butyrate component was found in highest value in Kılçık F1 cultivar, followed by A30706 F1 and Bitter F1 cultivars, respectively. In terms of Ethyl Hexadecanoate component, Kılçık F1 and Bitter F1 cultivars were found to be similar and higher than A30706 F1 when evaluated statistically (P<0.05). γ-Muurolen was determined as the main terpene component in A30706 F1 and Bitter F1 pepper cultivars (Table 6).

In the study carried out by Ziino et al. (2009) in fresh hot peppers (*Capsicum annuum* L.), it was reported that γ-muurolen, which is the major aroma component in the terpene group causes smell of herb, wood, spice (Ziino et al., 2009). Z-Linalool oxide component was determined as well as γ-Muurolen component in all pepper cultivars. The aroma of Z-Linalool oxide were described as floral, murici (Ferreira et al., 2016). Murici (*Byrsonima crassifolia* L., Malpighiaceae) is a fruit with a strong fruity and rancid cheese aroma (Rezende and Fraga, 2003).

Terpene group aroma components evaluated statistically (P<0.05) in Table 6. It was determined that A30706 F1 and Bitter F1 cultivars were similar in terms of γ-Muurolen component. The lowest γ-Muurolen ratio was determined in Kılçık F1 cultivar. There was no difference in terms of Z-Linalool oxide component between cultivars.

Among the other aroma components, pentadecane was determined as the main component in all ('A30706 F1', 'Kılçık F1', 'Bitter F1') pepper cultivars (Table 7). Pentadecane causes waxy sensory perception and was determined in pepper in various studies (Liu et al., 2010; Cirlini et al., 2019). Isobutyl methoxy pyrazine was determined as second main component in A30706 F1 and Kılçık F1 pepper cultivars. It creates sensory perception as green pepper. The hexanoic acid component, a 6-carbon saturated acid, is also known as caproic acid and generally causes sensory perception as sour, fatty, cheesy and sweat-like.

According to statistical results (P<0,05) concerning other aroma components as shown in Table 7, the highest ratio of Isobutyl methoxy pyrazine aroma component was obtained in Kılçık F1 cultivar, followed by Bitter F1 and A30706 F1, respectively. It was observed that the Pentadecane component was similar in Kılçık F1 and BitterF1 cultivars and higher than A30706F1. The highest hexadecane component ratio was determined in Bitter F1 cultivar. Similar ratios of hexadecane components were observed in A30706 F1 and Kılçık F1 cultivars. Aldehyde, Alcohol, Ester, Terpene and other group aroma components are shown in different colors on the biplot graph (Figure 1).

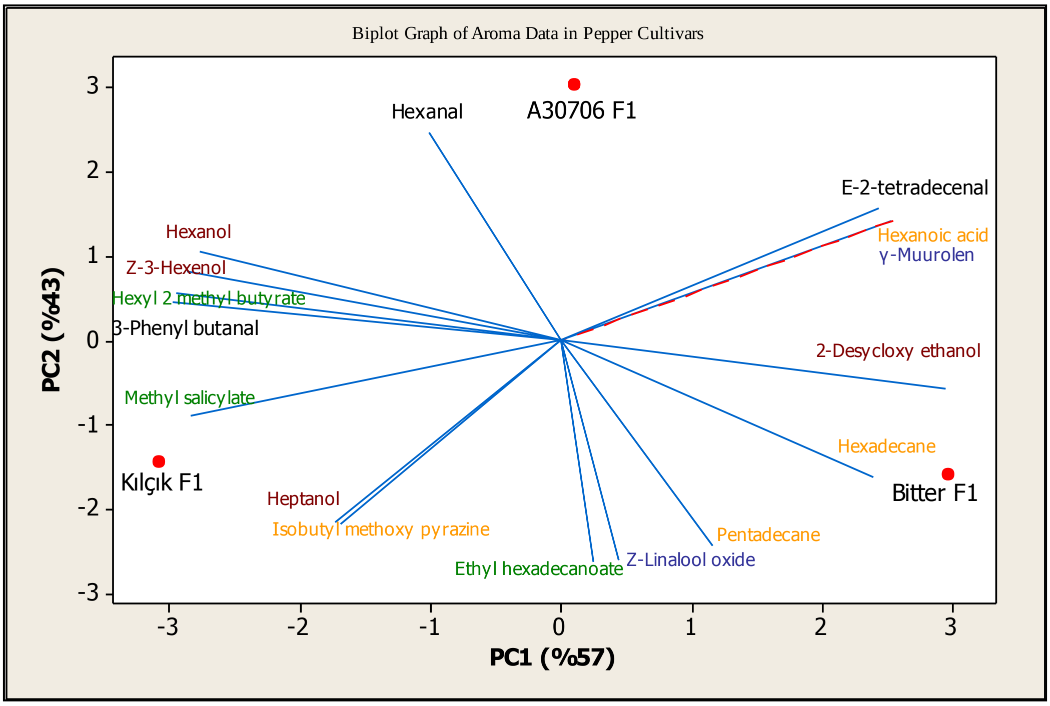

Figure 1. Biplot graph of aroma components according to pepper cultivars.

The fact that the E-2-tetradecenal, hexanoic acid, γ-muurolene compounds were in the same axis (PC1>0, PC2>0) only with the A30706 F1 cultivar showed that the A30706 F1 cultivar has higher values in terms of these compounds.

The fact that the hexanal compound was in positive axis with A30706 F1 cultivar (PC2>0) and in negative axis with other cultivars and was in a closer location to A30706 F1 than other cultivars showed that this compound was higher in A30706 F1 than other cultivars.

The presence of 2-decycloxyethanol, hexadecane, pentadecane, Z-linalooloxide, ethyl hexadecanoate compounds in the same axis (PC1>0, PC2<0) with only the Bitter F1 cultivar showed that these compounds were found in higher rates in the Bitter F1 cultivar than other cultivars.

The presence of methyl salicylate, heptanol, isobutyl methoxy pyrazine compounds in the same axis (PC1<0, PC2<0) with only the Kılçık F1 cultivar showed that these compounds were found in higher rates in the Kılçık F1 cultivar than other cultivars.

In the biplot graph; Hexanol, Z-3-hexenol, hexyl 2 methyl butyrate, 3-phenyl butanal compounds were found in positive direction (PC1<0) with Kılçık F1 cultivar and negative direction with other cultivars. At the same time, these compounds were located closer to Kılçık F1 cultivar than other cultivars. These two cases showed that the aforementioned compounds were found at higher rates in Kılçık F1 cultivar.

4. Conclusion

In the study, it was determined that the highest amount of ascorbic acid and titratable acidity was in the A30706 F1 cultivar (Table 1, Table 2). Ascorbic acid is a vitamin that the human body cannot produce, and pepper is known to be an important source of vitamin C. It is known that health problems such as scurvy disease and weakening of the immune system are seen in ascorbic acid deficiency.

It is seen that all three pepper cultivars in the study have a substantial amount of ascorbic acid. It was observed that the highest amount of phenolic compound was in Bitter F1 cultivar (Table 2). It is known that phenolic compounds give fruits and vegetables their peculiar bitter taste, odor and color. No significant differences (P<0.05) were found among the pepper cultivars in the study in terms of soluble solids and pH values.

It has been observed that Hexanal, E-2-tetradecenal, Hexanol, Methyl salicylate components were in major rate in the aroma composition (Table 3, Table 4, Table 5). Aroma composition is important in terms of flavor and fragrance in sectors such as agriculture, food, pharmacy.

The Hexanal component, which creates a sensory perception like fresh, cut grass has created a high rate (P<0.05) in A30706 F1 cultivar than other cultivars (Table 3).

E-2-tetradecenal component, which makes the perception of fatty, waxy, cheesy odor, was not significantly different between cultivars (P<0.05) but it was higher in A30706 F1 and Bitter F1, and lower in Kılçık F1 cultivar (Table 3). Hexanol compound, which gives mint and green grass scent, was statistically similar in A30706 F1 and Kılçık F1 cultivars (P<0.05). Hexanol compound was lower in Bitter F1 than other cultivars (P<0.05).

Methyl salicylate component can be synthesized from salicylic acid (Kalaivani et al., 2016) which is methyl ester of salicylic acid. Methyl salicylate makes sense of peppermint and oil of wintergreen sensory perception, was found statistically higher (P<0.05) in Kılçık F1 cultivar (Table 5), which is a sweet cultivar. Methyl salicylate was double times higher in Kılçık F1 cultivar than other cultivars.

The γ-muurolen component, which is not found in a major proportion in the aroma composition, causes herb, wood, spice sensory perception. Faustino et al. (2020), stated in his study that the γ-Muurolen compound was found in the essential oil nanoemulsion of Protium heptaphyllum resin and could have a larvicidal effect on *Aedes aegypti*.

The high rates of γ-Muurolen in bitter varieties A30706 F1 and Bitter F1 compared to Kılçık F1, which is a sweet variety, may be a reason for the self-protection mechanism of bitter varieties.

Author Contributions

TS; initiated the research idea, developed, analyzed and interpreted the data and wrote the manuscript. MAG; analyzed and organized the aroma components, suggested the research methods. MŞ; supervised the research, edited the manuscript. AA; organized the data and structured the paper.

Conflict of Interest

The author declared that there is no conflict of interest.

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Research Article
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IN VITRO MULTIPLE SHOOT INDUCTION FROM EMBRYONIC AXES OF ANNUAL HERBACEOUS LEGUME FABA BEAN (*Vicia faba* L.)

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Abstract: The faba bean (*Vicia faba* L.) is cultivated in the Mediterranean and Aegean regions only. It is a rich source of protein and an important source of food and feed for human and animal consumption. Faba beans have a narrow genetic base and their improvement through hybridization is not feasible because of high pollen self-incompatibility. This study was carried out using the embryonic axis of cv. Filiz99 and Eresen87 regenerated on MS medium containing 11 different combinations of BAP and NAA. The results showed 100% shoot regeneration frequency with maximum number of 3.3 and 3.5 shoots per explant on cv. Filiz99 and Eresen87 respectively. Regenerating shoots were rooted on 1 mg L–1 IAA. The in vitro regenerated shoots were continuously cultured for 3 weeks to acclimatize them. This approach could improve broad bean seed germination and subsequently regeneration. The results could also facilitate genetic transformation studies.

Keywords: Acclimatisation, BAP, NAA, Zygotic embryos

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1. Introduction

Faba bean (*V. faba* L.) is a herbaceous multipurpose herbaceous legume plant grown in many parts of the world (Duke 1981). It is economically important for food, fodder, and stabilization of soil particles in high wind erosion areas. It is widespread in North Africa, Europe, China, Central Asia, the USA, Canada, South America, and Australia. Worldwide Faba bean production for food and feed was 4.5 million t in 2012 (FAO, 2017). The 5 top (75% of world production) producing countries are the United Kingdom, France, Australia, Ethiopia, and China. It is an introduced crop in Turkey with plantations in the Mediterranean and Aegean regions of Turkey (Rahate et al., 2020). Almost no work on in vitro micropropagation of faba bean has been carried out in Turkey.

Tissue culture is generally used for cloning of superior genotypes and in breeding programs of herbaceous perennials (Karnoski, 1981; Boulay, 1987). There is a need to make attempts to regenerate these important herbaceous legumes through tissue culture. Cv. Filiz 99 and cv. Eresen 87 are two important varieties that are grown in Turkey for commercial food and feed production. Like other faba beans (Rowlands 1964), these are less genetically diversified cultivars and face problems of partial self-incompatibility that more often results in the collapse of their fertile ovules. This has also led to slow progress in broad bean breeding and varietal improvement activities in Turkey (Bond, 1987; Selva et al., 1989; Bahgat et al., 2009). Although a few reports on tissue culture of faba bean are available, there has been no report of tissue culture from Turkey and it is in fact an important issue. Faba beans are highly susceptible to biotic and abiotic stresses and have instability in yield that makes this crop less attractive (Bahgat et al., 2009). More often the problem of partial self-incompatibility results in the collapse of fertile ovules in broad bean resulting in genetically less diversified plants (Rowlands, 1964; Stoddard and Bond, 1987). This has also led to slow progress in broad bean breeding and varietal improvement activities (Bond, 1987; Selva et al., 1989; Bahgat et al., 2009).

Broad bean appears to be recalcitrant towards in vitro regeneration and tissue culture (Khalafalla and Hattori, 2000; Anwar, 2007; Anwar et al., 2010), due to the presence of inhibitors, high oxidative stress, and release of phenolic compounds (Böttinger et al., 2001). Due to instability among genotypes, most of the reported protocols are variety/cultıvar specific. Although there have been many reported studies of successful regeneration in faba bean (Shri and Davis, 1992; Rizvi and Singh, 2000; Polowick et al., 2004), most of the protocols are not repeatable. There is a need to overcome these problems to enhance shoot regeneration. Successful establishment of a reliable in vitro regeneration protocol could help in accelerated breeding. Establishing faster regeneration systems can be used in combination with traditional broad bean breeding techniques (Kuchuk, 2001).

The present communication aims to identify efficient, repeatable protocol for in vitro shoot multiplication from cv. Filiz 99 and cv. Eresen 87 of *V. Faba* in development of accelerated regeneration technology.

2. Material and Methods

2.1. Seed Source and Surface Sterilization

The widely cultivated in Turkey seeds of broad bean cv. Eresen 87 and Filiz 99 were obtained from the Aegean Field Crops Research Institute, Izmir, Turkey.

Cv. Eresen 87 has an average seed weight of 1.84-190 g, seed width of 1.74 -1.81 cm, seed length of 2.44 to 2.52 cm, and seed thickness of 0.61 to 0.65 cm. It has a thousand-grain weight of around 1350-1600 g with variable seed yield between 2000-5000 kg/ha depending on sowing time and environmental conditions. Cv. Eresen-87 is consumed both as a green vegetable and dry grains. The grains of the Eresen-87 variety used in the study are flat, light brown, black, and the weight of 100 is 135-160 g. Plant type is vertical, plant length is 90-107 cm, with 12-19 cm long beans. It is a medium early cultivar and tolerant to anthracnose and rust (Yaman, 1996, Pekşen and Artik, 2006; Alan and Geren, 2006).

The grain of cv Filiz-99 is flat with yellowish-brown color and black hilum. It has 100-grain seed weight of 115-125 g. It grows vertically with a plant height of 85-102 cm and bean length of 12-14 cm. cv. Filiz 99 has an average seed weight of 1.42 -1.51 g, seed width of 1.49 -1.58 cm, seed length of 2.00 to 2.13 cm, and seed thickness of 0.52 to 0.58 cm. It is harvested earlier and is moderately resistant to anthracnose and susceptible to chocolate dust disease (Pekşen and Artik, 2006; Alan and Geren, 2006).

Healthy and clean seeds were selected and subjected to surface sterilization using 60% commercial bleach (Ace®Istanbul, Turkey, containing 5% NaOCl) for 20 min followed by 3×5 min rinsing with sterile distilled water. These embryos were taken out from these seeds with soaking them in sterile water for 24 h at 24°C.

These were cultured on MS medium (Murashige and Skoog 1962) containing 0.00 (control), and 0.25 mg L^{-1} BAP+ 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25 mg L^{-1} NAA (11 different combinations).

All cultures were autoclaved for 20 min at 121˚C, using 105 kPA autoclave pressure. The pH was adjusted to 5.6-5.8 with1M NaOH or 1M HCl. All cultures were grown at 25±2˚C with a 16 h light photoperiod. Light was maintained at intensity of 45 µmol $m^{-2}s^{-1}$ by cool white fluorescent lamps.

2.2. Evaluation of seeds for SOD, CAT and POD Antioxidant Enzymes' Activity

These tests were performed using 500 mg of leaf tissues obtained from seedlings grown from non-water-soaked and water-soaked seeds. The leaf tissue were homogenized to prepare a crude enzyme extract in extraction buffer having 100 mM potassium phosphate buffer, pH 7.0 containing and 1% polyvinylpyrrolidone and 0.5% Triton X-100 using mortar and pestle chilled in liquid Nitrogen. It was followed by centrifuging this homogenate at 15000 rpm for 20 min at 4 °C. Thereafter, the resulting supernatant was used for each of the enzymatic assays as given with expression in milligrams of protein per minute (Chance and Maehly, 1955).

SOD (EC 1.15.1.1) activity was measured following Giannopolitis and Ries (1977) based on the inhibition of nitro blue tetrazolium (NBT) photoreduction. The reaction solution (3 mL) contained 50 mM NBT, 1.3 mM riboflavin, 13 mM methionine, 75 µM ethylenediaminetetraacetic acid (EDTA), 50 mM phosphate buffer (pH 7.8), and 20 to 50 mL of enzyme extract. The reaction solution was irradiated under fluorescent light at 75 µM.$m^{-2}s^{-1}$ for 15 min. The absorbance at 560 nm was measured against a blank (non-irradiated reaction solution). One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction.

CAT (EC 1.11.1.6) activity was measured following by measuring the decomposition of H_2O^2 as the decrease in absorbance at 240 nm. The reaction solution contained 50 mM phosphate buffer (pH 7.8) and 10 mM H^2O^2.

POD (EC 1.11.1.7) activity was measured following Chance and Maehly (Chance B., Maehly 1955) using 50 M pyrogallol, 50 mM H_2O_2, 1 mL of the 20 times diluted enzyme extract, and 5 mL of enzyme reaction solution containing phosphate buffer (pH 6.8). The assay mixture was incubated for 5 min at 25 °C, and the reaction was terminated by the addition of 0.5 mL of 5% (v/v) H_2SO_4. The spectrophotometer was used to measure purpurogallin production at 420 nm considering one unit of POD activity as the amount of purpurogallin formed per milligram of protein per minute.

The plant growth regulators, agar, and the chemicals used in this study were purchased from Sigma-Aldrich Co., St. Louis MO, and Duchefa Biochemie B.V., Haarlem, The Netherlands.

2.3. *In vitro* Rooting

Elongated and multiplied 2-3 cm long regenerated broad bean shoots were used for rooting. MS medium containing 1.0 mg L^{-1} Indole 3 acetic acid (IAA) was used for in vitro rooting. All cultures were kept at 25±2˚C in 16/8 h light/dark cycle in the growth chamber. Sixty (60) micro propagated shoots were used in each treatment divided into 12 replications containing 5 micropropagated shoots each (5 micropropagated shoots × 12 replications = 60 micropropagated shoots). Each experiment was replicated thrice. Data on frequencies of root induction, number of roots per plant, root length, number of shoot per explant and flower induction were recorded after three weeks of culture.

2.4. Ex vitro Acclimatization

The healthy plantlets of 6-8 cm length were taken out from the culture and washed in running tap water thoroughly so as to remove adhered agar-containing medium from the roots. Thereafter, plantlets were transferred to potting mixture containing (a) clay loam soil, (b) peat moss, (c) perlite and (d) clay loam soil: peat moss (1:1) soil mix.

The clay loam soil used in the experiment had 42% (w/w) clay and 28% (w/w) sand with 49% water saturation percentage, CEC of 31 cmol kg^{-1}, EC 1.25 dS m^{-1}, 0.05% (w/w) total salts, pH of 7.8, 5.14% (w/w) lime, 138.4 kg ha^{-1} phosphorus, potassium of 1744.4 kg ha^{-1}, organic matter of 1.01% (w/w), total nitrogen of 0.09 % and organic carbon of 0.81%.

Peat moss used in the study was prepared locally from leaves. It had a pH of 6.2 and EC of 0.15 dS m^{-1}, the porosity of about (63% v/w), which allowed for high water absorption and had a low bulk density of 0.01 mg m^{-3}.

The perlite used in the experiment had a bulk density of about 53 kg m^{-3} and contained (w/w) 71 % SiO_2, 11% Al_2O_3 , 4% Na_2O, 2% K_2O, 0.5% Fe_2O_3, 0.2% MgO, 0.5% CaO and 4% loss on ignition (chemical/combined water).

The plants were transferred to 2-liter plastic pots containing 1.65-liter soil and peat moss mix and covered with transparent bags. Each pot was given 10 ml water after every two days. During the second week of culture when the plants began to show signs of growth; transparent bags were gradually removed and the plants were watered weekly depending on the conditions of plants.

Greenhouse was maintained at 17±2ºC temperature and 69±2% relative humidity under 16 h light 8 h dark photoperiod.

2.5. Statistical Analysis

A total number of 60 explants were used for each treatment (regeneration and rooting) that were divided into equally distributed 12 replications. Data were subjected to one-way analysis of variance (ANOVA), and the post hoc tests were performed using Duncan's Multiple Range Test at 0.05 level of significance. The treatments were arranged in a completely randomized design.

3. Results

3.1. Antioxidant Enzyme Activities

Effects of hydropriming were evaluated by measuring SOD, CAT, and POD enzymatic activities on seedlings grown from non-water-soaked (control) and water-soaked seeds of cv. Eresen 87 and cv. Filiz 99 (Table 1). The results showed that SOD, CAT, and POD enzymatic activities of non-water-soaked seedlings (control) of both cultivars were higher compared to these activities on seedlings obtained after water soaking. Likewise, comparing two cultivars, these activities were significantly higher on cv. Filiz 99 irrespective of the treatment.

Table 1. Effects of the activity of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) on seedlings grown from non hydroprimed and hydroprimed seeds of faba bean cv. Eresen 87 and cv. Filiz 99

Treatments	Genotypes					
	SOD Activity ($units·mg^{-1}·protein·min^{-1}$)		CAT Activity ($units·mg^{-1}·protein·min^{-1}$)		POD Activity ($unit·min^{-1}·g^{-1}·FW$)	
	Eresen 87	Filiz 99	Eresen 87	Filiz 99	Eresen 87	Filiz 99
Control	131.2^{a}	139.6^{a}	20.4^{a}	22.2^{a}	9.7^{a}	10.6^{a}
Hydropriming	113.5^{b}	127.0^{b}	19.1^{b}	17.1^{b}	7.4^{b}	7.9^{b}

Means followed by a different letters within a column for each parameter are significantly different at the 0.05 level of probability by *t* test.

3.2. Isolation of Explant

It was very difficult to obtain mature embryos from non-water soaked surface sterilized seeds, where it was very difficult to detach cohering seed cotyledons to approach mature embryos. Therefore, cohering cotyledons were cut opened closer to embryos using sharp blade without giving damage to them. These embryos were cultured on a 1 × MS medium containing 11 different concentrations of BAP + NAA for regeneration. The embryos did not survive except a few developing protrusions on the surface of explants due to the fast development of oxidative stress-related necrosis arrested growth and regeneration.

Contrarily, Water-soaked mature embryos on cv. Filiz 99 regenerated variable number of shoots on embryonic axis. Explants began to induce shoot initials after 6-7 days of culture. All cultures on cv. Filiz 99 induced shoots without callusing (Figure 1a); whereas, all explants of cv. Eresen 87 induced callus at embryonic axes followed by shoot regeneration (Figure 1b).

3.3. Shoot Regeneration

Regeneration from embryos taken from water soaked seeds ranged 80.0 to 100.0% on each of cv. Filiz 99 and Eresen 87. Shoot regeneration frequency varied on cv. Filiz 99; it remained 93.3 to 100.0% on all concentrations of BAP + NAA except one concentration (0.25 mg L^{-1} BAP + 1.75 mg L^{-1} NAA) with 80% shoot regeneration percentage (Figure 2). Shoot regeneration frequency on cv. Eresen 87 remained 100.0% on 6 concentrations of BAP+NAA concentrations, 93.3% shoot regeneration was noted on 2 concentrations of BAP+NAA concentrations and 80.0% regeneration was noted on 3 concentrations of BAP+NAA.

3.4. Number of Shoots per Explant

The number of shoots per explant ranged from 1 to 3.5 on cv. Filiz 99 (Figure 3).

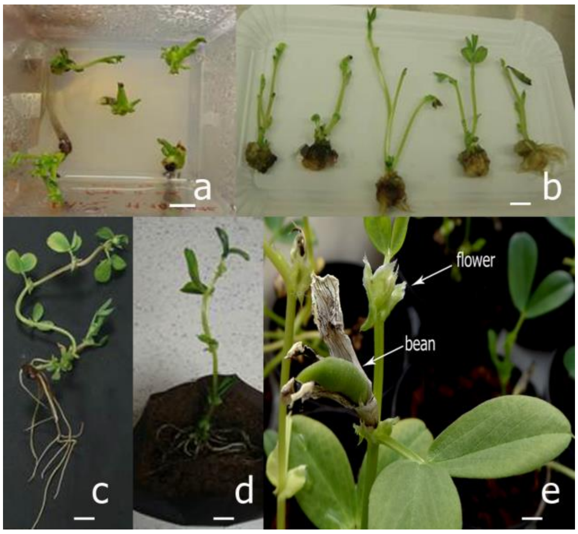

Figure 1. Shoot regeneration in broad bean from mature embryos obtained from water soaked (a) shoot regeneration on mature embryo explatsof cv. Eresen 87 (b) and cv. Filiz 99 (c) rooting of shoots obtained from cv. Ersen 87 (d) hardening of plants cv. Filiz 99 in plstic tubes (e) and their flowering and seed set.

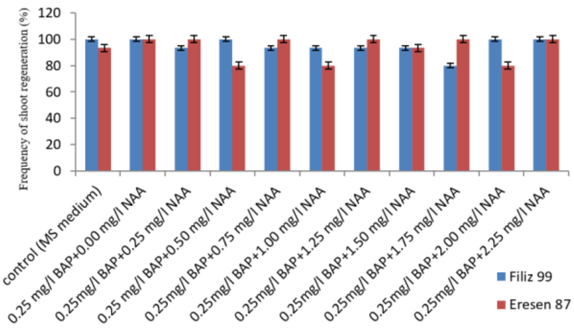

Figure 2. Comparison among average frequency of shoot regeneration percentage (%) of two broad bean varieties at different concentrations of NAA and BAP.

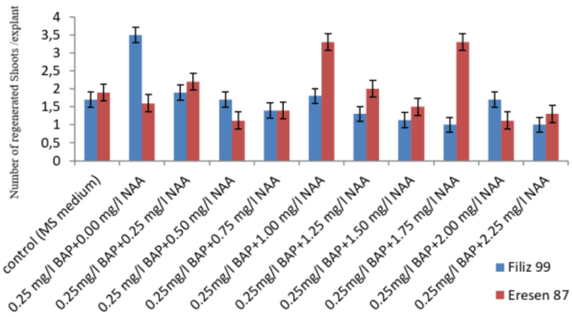

Figure 3. Comparison among average Number of regenerated shoots of two broad bean varieties at different concentrations of NAA and BAP.

The maximum number of shoots per explant was noted on MS medium containing 0.25 mg L^{-1} BAP. The rest of the cultures showed less than 1.9 shoots per explant. The number of shoots per explant showed inconsistency in two cultivars (Figure 3). The maximum number of shoots per explant on cv. Filiz 99 was recorded on MS medium containing 0.25 mg L^{-1} BAP. The maximum number of shoots on cv. Eresen 87 was noted on both MS medium containing 0.25 mg L^{-1} BAP + 1.00 mg L^{-1} NAA and 0.25 mg L^{-1} BAP + 1.75 mg L^{-1} NAA.

3. 5. Shoot Length

Shoot length per explant ranged 2.1 to 6.9 cm on cv. Filiz 99 and 1.7 – 6.5cm on cv. Eresen 87 (Figure 4). Maximum shoot length per explant for cv. Filiz 99 was 6.9 cm followed very closely by a shoot length of 6.4 cm on MS medium containing 0.25 mg L^{-1} BAP + 0.75 mg L^{-1} NAA and 0.25 mg L^{-1} BAP + 1.50 mg L^{-1} NAA (Figure 4). Minimum shoot length per explant for cv. Filiz 99 was noted on MS medium (control).

Shoot length on cv. Eresen 87 was inconsistent. Shoot length per explant ranged 1.7 – 6.5 cm on cv. Eresen 87. Maximum shoot length was noted on MS medium containing 0.25 mg L^{-1} BAP + 0.50 mg L^{-1} NAA. The rest of the concentrations of BAP+NAA never increased shoot length beyond 4.5 cm on any of the regeneration mediums. Minimum shoot length per explant was noted on MS medium containing 0.25 mg L^{-1} BAP + 0.25 mg L^{-1} NAA.

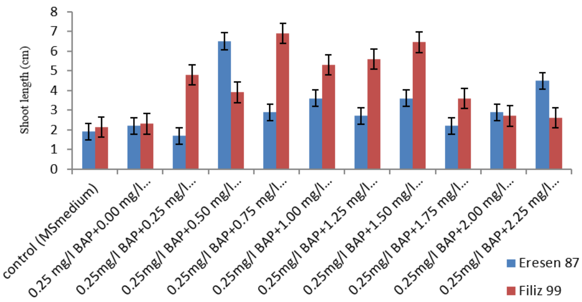

Figure 4. Comparison among average Length of regenerated shoots (cm) of two broad bean varieties at different concentrations of NAA and BAP.

3.6. Rooting and Acclimatization

The regenerating shoots were not difficult to root on MS medium containing 1 mg L^{-1} IAA. Profuse vigorous and strong root system with well-developed leaves was noted on all of the rooted shoots irrespective of the cultivar (Figure 1c). The plants had difficulty in acclimatization in clay loam soil, peat moss and perlite, and a mixture of perlite and clay loam.

A mixture of clay loam soil and peat moss not only helped in the development of a condition that was suitable for the growth and development of plants but induced a very suitable condition for the growth and development of plant roots (Figure 1d). There was no difficulty in the flowering of plants under shade house conditions. All acclimatized plants set seeds (Figure 1e).

4. Discussion

Broad bean is an important edible highly nutritious grain legume crop. It is prone to the number of biotic and abiotic stresses that affect its yield negatively. Broad bean has the problem of self-incompatibility and has very little genetic diversity. Poor genetic diversity is a hindrance in the development of new varieties (Bond, 1987; Selva et al., 1989). The study aimed to identify suitable strategies for the regeneration of broad bean and its acclimatization under *in vitro* conditions.

Water soaking changes the internal environment of cells through osmosis by dilution with water. It is assumed that during imbibing of water and oxygen during hydropriming, the broad bean (Messina, 1999; Davila et al., 2003; Urbano et al., 2003; Vidal-Valverde et al., 2003) carbohydrates, phenolic compounds (phenylpropanoid acids, flavonoids, flavones, flavanones, and catechins Myricetin, Daidzein, Apigenin and Quercetin) and inhibitors like tyramine in the surrounding water (Zaats et al., 1987; Bekkara et al., 1998). It is accepted that this imbibition, dislocation, and elimination from seeds aid in the germination of seeds and seedling growth (Paul and Chodhury, 1991). It is also assumed that explants taken from these seedlings could enhance regeneration (Zaats et al., 1987; Bekkara et al., 1998; El-Mergawi et al., 2014; Yildirim, 2019). It is understood that the seeds undergo stress after secretion of phenolic compounds etc. that leads to the generation of reactive oxygen species (ROS: Superoxide anion radicals, hydroxyl radicals, H_2O_2, alkoxy radicals, and singlet oxygen) from non-water soaked seedlings. Consequently, these lead to lipid peroxidation-linked membrane deterioration (Jiang et al., 2001; Siddiqui et al., 2012). Whereas, water soaking-based management ended up with exudation of these compounds and lowering of oxidation activities as confirmed by SOD, CAT, and POD enzymatic activities and helped in overcoming oxidative damage in agreement with (Karray-Bouraoui et al., 2010; Mane et al., 2011; El-Lethy et al., 2013). This stress management in this study is supposed to help in the induction of rapid and large regeneration in this study avoiding plant cell death and transforming the saved energy to regeneration under *in vitro* conditions (Khalafalla and Hattori, 2000; Anwar, 2007; Anwar et al., 2010). It is assumed that broad bean seeds' germination and growth are in agreement with Bekkara et al. (1998). The results of the present study further suggest that phenolics could be avoided to a large extent if the seeds are water-soaked for 24 hours. They noted 100% exudation of these compounds from water-soaked seeds incubated at 30 °C. Whereas, in the case of non-water-soaked seeds, oxidative stress lead secretion of phenolic compounds and inhibitors resulted in deleterious effects on broad bean cell walls causing oxidative burst lead cell deaths.

This is in agreement with Böttinger et al. (2001), who has suggested that the difficulties of indirect regeneration from broad bean due to the accumulation of phenolic compounds, lead to cell death. They have further suggested that accumulation of the phenolic compounds could be avoided by frequent culturing explants on regeneration medium for 1 to 2 weeks. Therefore, broad bean regeneration and establishment from tissue culture are considered very difficult in general terms (Böttinger et al., 2001). It helped in the continuous division of cells and resulted in high regeneration percentage (80 -100%) in this experiment. Therefore, the results confirmed that if the quantity of oxidants in plantlets is diluted or reduced, there is no need to use antioxidants like ascorbic acid, glutathione citric acid etc. as has been reported by Abdelwahd et al. (2008) and Klenotičova et al. (2013), for reducing browning in broad bean.

In general except for a few cultures cv. Eresen 87 showed more genetic stability over cv. Filiz 99 in the frequency of shoot regeneration percentage.

This study describes a procedure to regenerate shoots from zygotic embryos using 2 broad bean cultivars without induction of callus. Callus induction is not desirable when true-to-type plants are desired. Direct shoot regeneration was noted on embryonic nodes on MS medium containing 0.25 mg L^{-1} BAP + different concentrations of NAA on two cultivars used in the study. All shoots developed directly from the embryonic nodes. The number of shoots per explant ranged from 1 to 3.5 on cv. Filiz 99 1.12 to 3.3 on cv. Eresen 87 respectively. This study has an edge over previous studies in terms of time to induce shoots, their rooting, and acclimatization that was achieved in 75 days. Previously a shoot regeneration system has been reported by Griga et al. (1987) through indirect somatic embryogenesis and organogenesis by Taha and Francis, (1990), Tegederet al. (1995) and Böttinger et al. (2001). Bahgat et al. (2009) used shoot tip and epicotyl explants and found possibility of development of embryos over a period of 14 months. They found that somatic embryos which were derived from shoot tips of cv. Giza 2 readily developed to fertile plants, while the somatic embryos developing on cultivar 24 Hyto were arrested at the torpedo stage and did not produce plantlets. The results are not in agreement with Bahgat et al. (2008). They regenerated somatic embryos on two Egyptian broad bean cultivars Giza 2 & Hyto using 10 mg L^{-1} BAP+0.5 mg L^{-1} NAA followed by transfer of the callus to 1/2× B5 medium. It is assumed that a high concentration of BAP used in the study may have caused stress on the explants that led to induction of somatic embryogenesis. High doses or prolonged exposure to phytohormones can cause damage to the vascular and other tissues (Kuplemez and Yildirim, 2020).

The results of this study report a good shoot length of 2.13 to 6.9 cm on cv. Filiz 99 and 1.7 – 6.5cm on cv. Eresen 87 has not been reported in previous studies (Griga et al., 1987; Taha and Francis, 1990; Tegeder et al., 1995; Böttinger et al., 2001; Bahgat et al., 2008).

Desjardins et al., (1987) described rooting of faba bean as a difficult procedure. Schulze et al. (1985), obtained 20% rooting only. This study reports 100% rooting under *in vitro* conditions. The broad bean plants regenerated under in vitro conditions on sucrose-containing media faced difficulties during acclimatization. They grew under high humidity, low light intensity, and limited gas exchange (Desjardins et al., 1987).

To identify strategies for successful acclimatization of these plants these were grown on different types of soil mix.

It was found that clay loam soil was unsuitable for acclimatization because of high percentage of clay in the substrate that hindered growth and development of roots. Moreover, the broad bean grows well in soils with pH of 7.0 -7.5. The soil used in this study may had high percentage of clay and lime, higher pH and poor organic matter that may have unsuitable effects on growth and development of tissue cultured plants that are very prone to external environmental conditions.

pH of peat moss used in the study was 6.2 that was unsuitable for growth and development of broad bean that require pH of 7.0 to 7.5 for growth and development. Perlite has ability to absorb and accumulate large amount of water. Perlite has ability to hold 200 to 600 percent of its weight in water. Broad bean is a plant that lives on moderate amount of water throughout its life cycle. Growing of tissue cultured plants in water saturated soils could lead to oxidation and cell death of roots. This may had caused earlier death in plants.

A mixture of perlite and clay loam may have resulted in development of a soil that was hard with large amount of water and plants died due to over moist soils related suffocation.

A mixture of clay loam soil and peat moss not only helped in development of a condition that was suitable for growth and development of plants but induced a pH and environment that was very suitable for growth and development of plant roots. This in conjunction with surrounding temperature and humidity helped the plants to grow flourish and acclimatize easily. Establishment of reliable acclimatization of tissue cultured plantlets (Goncalves et al., 1998; Gürel et al., 2019) permitted saving of time. This can also help by use of tissue culture for breeding, transformation and functional genomic studies.

5. Conclusion

Traits that are difficult to obtain with traditional breeding methods, such as insect resistance, may not be transferred from one plant species to another with traditional plant breeding methods. The results of this study suggest that water soaking improves broad bean seed germination and subsequently regeneration. The results could facilitate inbreeding using the single seed descent method and genetic transformation. In addition, tissue culture studies are used to obtain plants from gene-transferred cells.

Author Contributions

All task made by single author and the author reviewed and approved the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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EFFECT OF SPECIES ON MACRO AND MICRO MINERAL COMPOSITION OF SOME SHRUB LEAVES WITH RESPECT TO SHEEP REQUIREMENTS

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Abstract: The purpose of the study was to evaluate the effect of species on the macro and mineral profiles of some shrub leaves used for small ruminant animals in Turkey. Species had a significant effect on the macro and micro mineral profiles of shrub laeves. Calcium (Ca) contents of forages varied from 5.6 to 21.2 g/kg DM, with highest being for *Rosa canina* and lowest for *Arbutus andrachne* and *Quercus coccifera*. Phosphorus (P) contents of shrub leaves ranged from 1.1 to 2.1 g/kg DM with highest being for *Arbutus unedo* and *Rosa canina* and the lowest for *Arbutus andrachne*. The magnesium (mg) content of forages ranged from 1.9 to 5.5 g/DM, with the highest being for *Pistacia lentiscus* and the lowest for *Quercus coccifera*. The potassium (K) content of forages ranged from 4.1 to 10.3 g/kg DM. The K contents of *Pistacia lentiscus* and *Arbutus unedo* were significantly higher than the other shrub leaves. The iron (Fe) content of shrub leaves ranged from 105.2 to 458.5 mg/kg DM. The Fe content of *Pistacia lentiscus* was significantly higher than the other shrub leaves. The zinc (Zn) contents of shrub leaves ranged from 15.5 to 36.0 mg/kg DM. The Zn content of *Quercus coccifera* was significantly higher than the other shrubs. Copper (cu) contents of forages varied widely from 3.0 to 6.7 mg/kg DM, with the highest being for *Arbutus unedo* and *Quercus coccifera*. The manganese (Mn) content of forages varied from 10.5 to 113.0 mg/kg DM, with the highest being for *Arbutus unedo* and the lowest for *Rosmarinus officinalis*. As a conclusion, shrubs' leaves have a significant amount of macro and micro minerals to support the growth and production of lamb and sheep, although the shrubs leaves' studied in the current experiment are not adequate to meet the dietary Cu requirement of lamb and sheep.

Keywords: Macro mineral, Micro mineral, Sheep, Shrubs

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1. Introduction

Tree and shrub leaves are very important resources in providing not only energy and protein but also minerals for small ruminant animals in most parts of the world (Theng et al., 2003; Kamalak et al., 2010; Ansah and Nagbila, 2011; Kaya and Kamalak, 2012; Atalay et al., 2017; Mboko et al., 2017; Ziblim et al., 2019). Although there is considerable information about the chemical compositions, digestibility, and metabolisable energy values of tree and shrub leaves (Kamalak et al., 2004; Ozkan and Sahin, 2006; Kilic et al., 2010), the information about the mineral contents of tree and shrub leaves is very limited. Macro and micro minerals may have an important role as structural functions in bones, as electrolytes in body fluids, as integral components of enzymes and other biologically important compounds (Bourne and Orr., 1988). Poor performance in ruminants may be caused by deficiency and excess of minerals (Ozkan et al., 2020)

Information about the macro and micro mineral compositions of shrub leaves from different species can be used to formulate the most accurate diet to achieve the optimum performance of grazing small ruminant animals and prevent diseases associated with mineral deficiency (Khan et al., 2007; Ozkan et al., 2016; Ozkan et al., 2020). Therefore, the aim of the current study was to determine the effect of species on the macro and mineral composition of some shrub leaves used for small ruminant animals in Turkey.

2. Material and Methods

2.1. Forage Samples

Leaves from *Arbutus andrachne*, *Arbutus unedo*, *Pistacia lentiscus*, *Quercus coccifera*, *Rhus typhina*, *Styrax officinalis*, *Glycyrrhiza glabra*, *Rosa canina*, and *Rosmarinus officinalis* were collected by hand from ten different shrubs in June, 2020, in Kahramanmaras, Turkey. The mean annual rainfall and temperature are 500 mm and 14.9 °C, respectively. The collected shrub leave samples were pooled and dried under sheds until they reached a constant weight. The soil of the study area is classified as Inceptisols, which was formed on a colluvial serpentine-limestone parent material (Yilmaz et al., 2000). Dried leave samples were ground using a laboratory mill with a 1 mm screen size for mineral analysis. Leave samples were subjected to a wet-ashing process with hydrogen peroxide, following three different steps. Firstly, the leave samples were kept at 145 °C at 75% microwave power for 5 minutes. Second, for 10 minutes, leave samples were kept at 80 oC at 90% microwave power. Finally, leave samples were kept at 100 °C with 40% microwave power for 10 minutes in a wet-ashing unit (speed wave MWS-2 Berghof products + Instruments Harresstr.1. 72800 Enien, Germany) resistant to 40 bar pressure (Mertens, 2005a). After wet-ashing, macro and micro mineral contents of leave samples were analyzed using Inductively Couple Plasma Optical Emission Spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (Mertens, 2005b). All mineral analysis was carried out in duplicate.

2.2. Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine the effect of species on the macro and mineral composition of shrub leave samples. Significance between individual means was identified using Tukey's multiple range tests. The mean differences were considered significant at P<0.05.

3. Result and Discussion

3.1. The Effect of Species on Macro Mineral Profile of Some Shrub Leaves

The macro mineral profiles of some shrub leaves are given in Table 1. Species had a significant effect on the macro mineral profiles of shrub leaves. The Ca content of forages varied from 5.6 to 21.2 g/kg DM, with the highest being for *Rosa canina* and the lowest for *Arbutus andrachne* and *Quercus coccifera*. NRC (1985) suggested that Ca contents should be in the range of 0.2 and 0.82% of DM to meet the requirements of lamb and sheep at gestation and lactation stages respectively. As can be seen from Table 1 the Ca contents of shrub leaves studied in the current experiment were higher than those suggested by NRC (1985) for lambs and sheep at gestation and lactation stages.

The P content of shrub leaves ranged from 1.1 to 2.1 g/kg DM, with the highest being for *Arbutus unedo* and *Rosa canina*, and the lowest for *Arbutus andrachne*. NRC (1985) recommended that P content of feedstuffs should be in the range of 0.16 and 0.38% of DM to meet the requirement of lamb and sheep at most production stages. As can be seen from Table 1 P contents of shrub leaves was significantly higher than those recommended by NRC (1985).

The Mg content of forages ranged from 1.9 to 5.5 g/DM, with the highest being for *Pistacia lentiscus*, and the lowest for *Quercus coccifera*. NRC (1985) recommended that the Mg content of feedstuffs be 0.12, 0.15 and 0.18 g/kg DM for growing lambs, pregnant, and lactating ewes, respectively. As can be seen in Table 1, the Mg contents of shrub leaves were higher than those suggested by NRC (1985) for growing lambs, pregnant, and lactating ewes.

The K content of forages ranged between 4.1 and 10.3 g/kg DM. The K contents of *Pistacia lentiscus* and *Arbutus unedo* were significantly higher than the other shrub leaves. Although the K content of diets for lamb growth should be more than 0.5% of DM, the K content of diets for lactating sheep should be in the range of 0.7-0.8 percent of DM (NRC 1985). As can be seen in Table 1, the K contents of shrub leaves were higher than those suggested by NRC (1985) for lamb and sheep.

3.2. The Effect of Species on Micro Mineral Profile of Some Shrub Leaves

The micro mineral profiles of shrub leaves are given in Table 2. Species also had a significant effect on the micro mineral profiles of shrub leaves. The Fe content of shrub leaves ranged from 105.2 to 458.5 mg/kg DM. The Fe content of *Pistacia lentiscus* was significantly higher than the other shrub leaves.

Table 1. Effect of species on the macro mineral composition (g/kg DM) of shrub leaves (n=2)

	Macro minerals			
Shrub species	Ca	P	Mg	K
Arbutus andrachne	5.8^{f}	1.3^{c}	3.1^{c}	5.9^{cd}
Arbutus unedo	11.2^{d}	2.0^{a}	2.9^{cd}	10.0^{a}
Pistacia lentiscus	5.6^{f}	1.0^{d}	5.5^{a}	10.3^{a}
Quercus coccifera	5.8^{f}	1.7^{b}	1.9^{e}	9.2^{ab}
Rhus typhina	17.0^{b}	1.6^{b}	2.1^{de}	7.8^{bc}
Styrax officinalis	13.1^{c}	1.7^{b}	4.0^{b}	7.0^{c}
Glycyrrhiza glabra	9.5d^{e}	1.6^{bc}	3.5^{bc}	4.1^{d}
Rosa canina	21.2^{a}	2.1^{a}	3.6^{bc}	4.2^{d}
Rosmarinus officinalis	8.7^{e}	1.7^{b}	4.3^{b}	4.8^{d}
SEM	0.515	0.061	0.238	0.476
P	<0.001	<0.001	<0.001	<0.001

^{abc}Different letters in same column indicate the statistical difference at P<0.05. SEM= standard error of mean.

Table 2. Effect of species on the micro mineral composition (g/kg DM) of shrub leaves (n=2)

	Micro minerals			
Shrub species	Fe	Zn	Cu	Mn
Arbutus andrachne	156.0^{d}	29.0^{b}	4.5b^{c}	17.2^{de}
Arbutus unedo	242.0^{b}	18.5^{c}	6.7^{a}	113.0^{a}
Pistacia lentiscus	458.5^{a}	32.0^{b}	5.0^{ab}	21.5^{d}
Quercus coccifera	120.7^{e}	36.0^{a}	6.7^{a}	27.7^{c}
Rhus typhina	105.2^{e}	16.2^{d}	5.2^{ab}	19.7^{de}
Styrax officinalis	162.5^{d}	15.5^{d}	3.0^{c}	15.7^{e}
Glycyrrhiza glabra	203.0^{c}	19.2^{cd}	4.2^{bc}	61.5^{b}
Rosa canina	230.2^{b}	18.0^{cd}	5.5^{ab}	29.2^{c}
Rosmarinus officinalis	116.0^{e}	21.2^{c}	3.0^{c}	10.5^{f}
SEM	6.044	0.993	0.471	1.252
P	<0.001	<0.001	<0.001	<0.001

^{abc}Different letters in same column indicate the statistical difference at P<0.05. SEM= standard error of mean.

NRC (1985) recommended that the Fe content of diets should be 30 mg/kg DM to meet the Fe requirements for all classes of sheep. On the other hand, a maximum tolerable level of the Fe has been indicated as 500 mg/kg DM (NRC 1980). As can be seen from Table 2, The Fe contents of shrub leaves studied in the current experiment were higher than the adequate level for sheep but lower than the maximum tolerable level for sheep.

The Zn content of shrub leaves ranged from 15.5 to 36.0 mg/kg DM. The Zn content of *Quercus coccifera* was significantly higher than the other shrubs. Although the Zn requirement of lamb for growth is 20 mg/kg DM, the Zn requirement of sheep at most production stages is 33 mg/kg DM (NRC 1985). Therefore, sheep or lambs consuming these shrubs are not likely to suffer from zinc deficiency.

The Cu content of forages varied widely from 3.0 to 6.7 mg/kg DM, with the highest being for *Arbutus unedo*, and *Quercus coccifera*. It is very difficult to determine the dietary Cu requirement of sheep since there are some factors affecting the dietary copper requirement of sheep. The amount of molybdenum in feedstuffs also effects the dietary Cu requirement of sheep. The high level of molybdenum in feedstuffs induces copper deficiency. There are also considerable differences among sheep breeds in terms of efficiency in absorbing Cu from feedstuffs. Even if it is not easy to give the exact dietary Cu requirement of sheep due to some factors affecting the dietary copper requirement of sheep, NRC (1985) recommended that the Cu content of diets should be 7-23 mg /kg DM to meet the Cu requirements for all classes of sheep. As can be seen from Table 2, the Cu content of shrub leaves were lower than those recommended by NRC (1985). Therefore, sheep should be supplemented with Cu when shrub leaves consists of most part of diets.

The Mn content of forages varied from 10.5 to 113.0 mg/kg DM, with the highest being for *Arbutus unedo*, and the lowest for *Rosmarinus officinalis*. Although the exact dietary requirement of Mn for sheep is not known, 20 mg/kg DM should be adequate for sheep at most production stages (NRC 1985). Although the Mn contents of most shrubs studied in the current experiment were adequate or considerably higher than the recommended level for sheep at most production stages (NRC 1985), the Mn contents of leaves of *Arbutus andrachne*, *Styrax officinalis*, and *Rosmarinus officinalis* were lower than that recommended by NRC (1985).

4. Conclusion

Species had a significant effect on the macro and micro mineral profiles of shrub leaves. Although shrubs leaves studied in the current experiment is not adequate to meet the dietary Cu requirement of lamb and sheep, shrubs leaves have a significant amount macro and micro minerals to support the growth and production of lamb and sheep.

Author Contributions

All authors have equal contribution and the authors reviewed and approved the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Approval

Ethical approval is not required, because this article does not contain any studies with human or animal subjects.

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COMPARISON OF CULTIVATED AND WILD RELATIVES OF SEVERAL FORAGE SPECIES IN MIXED RANGELAND BASED ON SOME NUTRITIONAL CHARACTERISTICS

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Abstract: Cultivated forage species may have higher nutrients contents (NC) and forage quality indicators (FQI) than their wild relatives. Nine forage samples collected five times from a mixed rangeland and an experimental field during two consecutive years was analysed for ash, crude protein (CP), ether extract (EE) and neutral detergent (NDF) and acid detergent (ADF) fibres. Then, their FQI such as digestible dry matter (DDM), dry matter intake (DMI), metabolizable energy (ME) and relative forage quality (RFQ) were calculated. Data were performed in a linear model with fixed effects (forage plant type [PT] and species [PS]) to NC and FQI, and subjected to hierarchical two-way clustering analysis. Cultivated and wilds relatives varied in CP (12.0-18.9% and 8.8-23.3%), ADF (20.9-33.1% and 39.3-73.5%) and NDF (37.2-61.6% and 26.7-46.1%) contents and ME (8.7-9.7 MJ kg^{-1} DM and 7.0-9.6 MJ kg^{-1} DM) and RFQ (98.8-186.7 and 74.6-161.7) values. There were interactions between PT and PS for all NC and FQI, except for CP and EE contents. In general, the CP, EE, ADF, ME and RFQ of cultivars were higher, whereas NDF was lower than those of the wilds. The most notable differences (NDF, ADF and RFQ) between the PT represented the differences in nutritional traits based on the clustering analysis. The cultivated and wilds relatives are comprised of NC and FQI that respond differently under same circumstances.

Keywords: Forage quality, Grassland, Nutritive value, Nutrient content, Plant breeding

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1. Introduction

Rangelands and pastures subjected to intensive management are consisted of simple mixtures of only grass (Gramineae) varieties and cultivars or grass and legume (Leguminosae) species (Hayes et al., 2013) but are not included other botanical families (OBF) or non-leguminous forbs (Elgersma et al., 2014). However, natural and semi-natural rangelands are characterized by a rich botanical composition (Dudek et al., 2020) due to a more excellent range of species belonging to grasses, legumes and OBF (Aydin et al., 2019). Grasses increase yield and stability of rangelands, whereas the legume and OBF species improve both their productivity and nutritional value or quality (Capstaff and Miller, 2018). Therefore, understanding nutritional value, as well as productivity of the range forages, is useful for determining the capacity and ability of rangelands to meet the needs of animals (Asaadi and Yazdi, 2011; Aydın et al., 2020). The botanical composition of grasslands helps to explain nutritional value and production levels (Michaud et al., 2012; Aydın et al., 2020), whereas plant functional traits enable to link morphological, physiological and phenological plant properties to their functions (Schellberg and Pontes, 2012).

Forage crops utilized in both grazing and the cut-and-carry system meets the physical and physiological requirements of domesticated ruminants (Capstaff and Miller, 2018). Indeed, these crops not merely maintain these animals, but also sustain the delivery of meat, milk and other products (Hayes et al., 2013; Lee, 2018). However, one of the greatest challenges to the efficient production of ruminants is the shortage of forage resources available throughout critical periods of their production cycle (Uzun and Ocak, 2019). In such cases, to enhance ruminant productivity, the high-yield and nutritious native forages that cultivated (hereafter cultivars) are used in grazing or the cut-and-carry system (Aydin et al., 2019). Indeed, for re-vegetation and rehabilitation, the cultivars are introduced into the rangelands that composed of their wild relatives (Algan et al., 2019).

The yield and quality of herbage and persistence in forages, the primary production traits, are critical for forage improvement in the various management systems and support feed conversion into unit quantities of an animal product as meat or milk (Hayes et al., 2013; Capstaff and Miller, 2018). The cultivation of forage plants has resulted in arguably the most desirable improvements such as dry matter (DM) yield and digestibility in forage species, such as *Medicago*, *Trifolium*, *Lolium*, and *Festuca* (Capstaff and Miller, 2018; Lee, 2018). Measuring the impacts of cultivars on high quality is very difficult since it needs laboratory analysis or animal feeding trials. Functional traits, such as digestible dry matter (DDM) and dry matter intake (DMI) that closely related to neutral detergent fibre (NDF) and acid detergent fibre (ADF) are easily measured predictor in quality of forage species (Bumb et al., 2016).

The information on whether cultivars may have higher nutrient content (NC) and forage quality indicators (FQI) than their wild relatives is scarce. Together with analysing distinct components of the wilds and the cultivars simultaneously may be useful to determine the impacts of species cultivation. Moreover, investigating the parallels and opposites between cultivars and wilds may help nutritional concepts and qualities of forages from biodiversity studies. Therefore, the objectives of this study were to determine similarities and differences between cultivars and wilds of several forage species that grown in field conditions and collected from rangeland, respectively and to provide to the farmers' useful recommendations on the best forage species to utilize.

2. Materials and Methods

2.1. Study Area and Species Selection

In this study, the third part of a research project (Aydın et al., 2018), the nine of forage species belong to cultivars and wild relatives (hereafter wilds) collected from an experimental field and the collected from the mountainous rangeland, respectively, (40°50′ to 41°51′ N and 37°08′ to 34°25′ E at nearly 1200 m above sea level) in Samsun province located in the middle Black Sea region of Turkey were used. The experimental field had a loam soil with sub-alkaline pH, normal saline, low-lime, organic matter, N and P, and high-K concentrations. The rangeland had a clay-loamy with sub-alkaline pH, low-saline, good organic matter, low-N and high-P and K contents (Tyler and Olsson, 2001). As was reported by Aydin et al. (2019), in the experimental area, winters are cool and damp, while summers are warm and damp for many years. The mean annual temperature and rainfall were 10.2°C (ranged from 3.1°C in winter to 16.7°C in summer) and 583.6 mm in the study period, respectively.

Forage plant species in the study were perennial desirable such as *Lotus corniculatus* L. (*L. corniculatus*), *Medicago sativa* L. (*M. sativa*), *Trifolium pratense* L. (*T. pratense*), *Trifolium repens* L. (*T. repens*), *Dactylis glomerata* L. (*D. glomerata*), *Festuca ovina* L. (*F. ovina*), *Lolium perenne* L. (*L. perenne*) *Cichorium intybus* L. (*C. intybus*) and *Sanguisorba minor* Scop. (*S. minor*). The cultivars represent legume, grass and OBF species of unknown genetic type but which reared in private or state farms and research centres (Kazak Tarım, Ankara, Turkey). The wilds were the most dominant and promising species for region rangelands (Uzun and Ocak, 2019; Aydın et al., 2020).

2.2. Collection and Laboratory Analyses of Forage Samples

Samples of the forage plant types, defined as cultivars and wilds were collected five times at the active growth stages (from before-flowering stage to after-flowering stage) of dominant species in the rangelands in two consecutive years (2016 and 2017). The herbage samples taken at each collection period were dried at 60°C for 48 h in an air-forced oven and then, stored at 4 °C until the proximate analysis. For proximate analysis, all samples were ground with a 1-mm screen and then, analysed for dry matter (DM, ID number: 2001.12), CP (978.04), ether extract (EE, 920.39) and ash (930.05) as reported by AOAC (2005) procedures. The NDF and ADF contents of the samples were also determined using the ANKOM A200/220 (ANKOM Technology Corp., Fairport, NY, USA) fibre analyser filter bag technique (Van Soest et al., 1991). Then, their FQI such as DDM (equation 1), DMI (equation 2), metabolizable energy (ME, equation 3), relative feed value (RFV, equation 4) and relative forage quality (RFQ, equation 5) were calculated as described by Rohweder et al. (1978).

$$\text{DDM (\%)} = 88.9 - 0.779 \times (\text{ADF, \% of DM}) \quad (1)$$

$$\text{DMI (\% of body weight, BW)} = 120/\ (\text{NDF, \% of DM}) \quad (2)$$

$$\text{ME (MJ kg}^{-1}\text{ DM)} = 0.17\%\ \text{DDM} - 2.0 \quad (3)$$

(Belyea et al., 1993)

$$\text{RFV} = (\text{DDM} \times \text{DMI})/1.29 \quad (4)$$

$$\text{RFQ} = (\text{DMI}_{\text{legume or grass}}, \text{\% of BW}) \times (\text{TDN}_{\text{legume or grass}}, \text{\% of DM})/1.23 \quad (5)$$

The $\text{DMI}_{\text{legume or grass}}$ and total digestible nutrients (TDN) were calculated separately for legume and grass species as described by Undersander et al. (2010). Ten nutritional characteristics chosen for this study were among the most common agronomic metrics (Lee, 2018; Aydin et al., 2019).

2.3. Statistical Analysis

Data was performed by adjusting one linear model (equation 6) with fixed effects forage plant type (cultivars and wilds) and plant species (nine species) to the nutritional traits (NC and FQI). Analyses of variance were performed with GLM procedure of SPSS Statistics (Windows version of SPSS, release 21.0, SPSS Inc., Chicago, IL, USA).

$$Y_{ijk} = \mu + PT_i + PS_j + PTPS_{ij} + e_{ijk} \quad (6)$$

Where: Y_{ijk} is the nutritional traits of k species, of i PT (plant type) and *j* PS (plant species); μ is the mean value; PT*i* is an effect of plant type; PS*j* is the effect of plant species; PTPS*ij* is the interaction of the plant type and plant species factors, and *eijk* is the error value and then means were compared by Tukey's range test. The experimental unit for the cultivars and the wilds were the parcel and collection repetitions, respectively. The total number of samples was 65: two plant types × nine plant species × three or five (collection repetitions) × three (analytical replicates). The replication values of each trait for each species belonging to the wilds and cultivars were subjected to hierarchical two-way clustering analysis using the JMP statistical program (SAS Institute Inc.USA). Thus, both plant type (as defined cultivated or wild type) and nutritional traits (as defined NC or FQI) were clustered according to similarity measures to simultaneously identify the associations between species and nutritional traits (Amiri and Shariff, 2012).

3. Results

Legumes contained higher CP, ash, DDM, DMI, ME, RFV and RFQ, and lower ADF and NDF than grasses (Table 1). Compared to the OBF species, legumes had higher CP and lower DMI, RFV and RFQ values, whereas grasses had lower DDM, RFV and RFQ, and higher CP, ash, ADF and NDF.

Table 1. The mean, standard error and 95% confidence intervals of the studied variables of each forage family

Variable[1]	CP	EE	Ash	ADF	NDF	DDM	DMI	ME	RFV	RFQ
Legumes (n=30)										
Mean	18.7	2.4	9.5	39.6	36.2	64.3	2.79	8.9	140.5	141.8
Standard error	0.63	0.13	0.24	1.71	1.17	0.61	0.065	0.10	4.49	5.25
95% confidence interval										
Lower bound	17.4	2.1	9.0	36.1	33.8	63.0	2.66	8.7	131.3	131.0
Upper bound	19.9	2.6	10.0	43.1	38.6	65.5	2.93	9.1	149.7	152.5
Grasses (n=22)										
Mean	11.7	2.5	8.2	53.2	44.2	62.2	1.87	8.6	91.4	100.0
Standard error	0.61	0.20	0.36	4.80	2.48	0.91	0.055	0.16	3.99	3.08
95% confidence interval										
Lower bound	10.5	2.1	7.4	43.2	39.1	60.3	1.76	8.3	83.1	93.6
Upper bound	13.0	2.9	8.9	63.2	49.4	64.1	1.99	8.9	99.7	106.4
Other botanical families (n=13)										
Mean	11.1	2.5	10.1	39.7	37.9	64.1	2.84	8.9	148.4	147.6
Standard error	0.71	0.22	0.54	4.14	2.63	2.08	0.179	0.35	13.34	13.76
95% confidence interval										
Lower bound	9.5	2.0	8.9	30.7	32.2	59.6	2.49	8.1	119.4	117.6
Upper bound	12.6	2.9	11.3	48.7	43.6	68.6	3.27	9.7	177.9	177.5

CP= crude protein, EE= ether extract, ADF= acid detergent fibre, NDF= neutral detergent fibre, DDM= digestible dry matter, DMI – dry mater intake, ME= metabolizable energy, RFV= relative feed value, RFQ= relative forage quality.

[1]Unit of CP, EE, Ash, ADF, NDF and DDM is % of dry matter, while DMI and ME is % of body weight and MJ/kg DM, respectively.

There were significant differences between the wilds and cultivars in terms of the NC (Table 1) and FQI (Table 2) variables. The content of CP was affected by the plant type and plant species factors, while EE content was only affected by plant type (P < 0.001, Table 2). The wilds had lower CP (14.9% vs 14.3%) and EE (2.8% vs 2.2%) levels compared to the cultivars. The CP content of *M. sativa* was higher than those of other species (P < 0.05), except for *T. repens*. The CP contents of *L. corniculatus* and *T. pratense* were higher (P < 0.05) than those of the grasses (*D. glomerata*, *F. ovina*, and *L. perenne*) and the OBF species (*C. intybus* and *S. minor*). The grass and OBF species had similar values in CP content.

There were interactions between two factors for all NC (Table 2) and FQI (Table 3), except for CP and EE contents. Except for the cultivar *T. repens*, cultivar *C. intybus* had the highest ash content among the cultivars (P < 0.05), but did not differ from all wilds. The ADF content of the wild *D. glomerata*, *F. ovina*, and *L. perenne* and *C. intybus* had higher than those of the wild *L. corniculatus*, *T. pratense* and *S. minor*, and all cultivars (P < 0.05). The cultivar *L. perenne* had a lower ADL content compared to all wilds (P < 0.05). In terms of the NDF content, the wild *C. intybus* had higher (P < 0.05) value than the wild *S. minor*. The NDF contents of cultivar *F. ovina* and *D. glomerata* were higher than those of the other species, except for the cultivar *T. pratense* and the wild *C. intybus* (P < 0.05).

The wild *S. minor* had a higher DDM value compared to the DMM values of the wild *D. glomerata*, *F. ovina* and *C. intybus* (P < 0.05). These three wild types had lower DDM values compared to the cultivar *T. repens*, *L. perenne* and *S. minor* (P < 0.05). Except for the cultivar *T. repens,* the DMI of the cultivar grasses was lower than those of the cultivar legume and OBF species (P < 0.05). The DMI of the wild *D. glomerata* and *F. ovina* were lower than all wild legumes and the wild *S. minor* (P < 0.05). The cultivar legumes had a higher DMI level compared to the wild *T. pratense*, *C. intybus* and all wild grasses (P < 0.05). The ME of the cultivar *T. repens*, *L. perenne*, *S. minor* and the wild *S. minor* were higher than those of the wild *D. glomerata*, *F. ovina* and *C. intybus* (P < 0.05). The RFV of the cultivar *T. repens* was similar with cultivar *L. perenne*, wild *M. sativa* and wild *T. repens*, whereas the RFQ of the cultivar *T. repens* were higher than those of the wild *M. sativa*, *T. pratense*, *T. repens* and *C. intybus* as well as all grasses (P < 0.05).

Table 2. Nutrients contents (% of dry matter) of cultivated and wild types of some forage species belonging to grass, legume and other botanical families

Plant type	Plant species	CP	EE	Ash	ADF	NDF
Cultivars						
	L. corniculatus	17.4	2.7	8.2b	28.8dfe	40.2cde
	M. sativa	18.9	2.1	8.7b	32.4c-f	43.4bcd
	T. pratense	15.7	2.4	8.3b	33.1c-f	46.6abc
	T. repens	18.0	2.5	9.6ab	26.6ef	37.4cde
	D. glomerata	13.4	3.9	9.3b	31.0dfe	57.9ab
	F. ovina	12.0	3.1	7.3b	30.6dfe	61.6a
	L. perenne	14.6	2.6	8.9b	20.9f	41.6cde
	C. intybus	12.3	2.7	10.3a	30.0dfe	40.1cde
	S. minor	11.9	3.0	9.3b	26.3ef	42.4cd
Wilds						
	L. corniculatus	17.7	2.9	9.5ab	41.4bcd	29.9de
	M. sativa	23.3	2.5	9.7ab	45.1ab	32.1cde
	T. pratense	16.4	2.2	10.1ab	49.5b	34.5cde
	T. repens	19.6	2.0	10.8ab	45.5ab	32.6cde
	D. glomerata	11.3	1.9	8.8b	71.1a	38.6cde
	F. ovina	9.7	2.1	7.1b	73.5a	39.0cde
	L. perenne	10.7	1.9	7.8b	68.2a	35.1cde
	C. intybus	8.8	2.3	9.9ab	63.3a	46.1abc
	S. minor	11.2	2.0	8.6b	39.3b-e	26.e
Plant type						
Cultivars		14.9a	2.8a	9.2	45.7	28.9
Wilds		14.3b	2.2b	9.1	25.0	55.2
Plant species						
L. corniculatus		17.6b	2.8	8.9	35.0	35.1
M. sativa		21.6a	2.3	9.3	36.2	40.4
T. pratense		16.2b	2.3	9.4	39.0	43.3
T. repens		19.0a	2.2	10.3	34.3	38.4
D. glomerata		12.1c	2.6	9.0	45.9	56.0
F. ovina		10.7c	2.5	7.2	48.7	55.1
L. perenne		12.4c	2.2	8.3	37.9	48.0
C. intybus		10.6c	2.5	11.5	43.2	46.7
S. minor		11.5c	2.4	8.9	33.4	33.8
SEM		0.59	0.01	0.22	2.11	1.20
Main effect of						
Plant type		<0.001	<0.001	<0.001	<0.001	<0.001
Plant type		<0.001	0.888	<0.001	<0.001	<0.001
Plant type × Plant type		0.111	0.062	0.040	<0.001	0.001

[a,c,d] Means of the same column followed by different letters differ significantly (P < 0.05).

CP= crude protein, EE= ether extract, ADF= acid detergent fibre, NDF= neutral detergent fibre, SEM= standard error of mean.

Table 3. Forage quality indicators of cultivated and wild-types of some forage species belonging to grass, legumes and other botanical families

Plant type	Plant species	DDM[1]	DMI	ME	RFV	RFQ
Cultivars						
	L. corniculatus	66.5ab	3.11ab	9.3ab	162.3ab	175.5abc
	M. sativa	63.7ab	2.83abc	8.8ab	140.4abc	151.2a-e
	T. pratense	63.1ab	2.66a-e	8.7ab	131.7a-e	141.3a-f
	T. repens	68.2a	3.31a	9.6a	176.3a	186.7a
	D. glomerata	64.8ab	2.09d-g	9.0ab	105.4c-f	106.6e-h
	F. ovina	64.4ab	1.96efg	9.0ab	98.9c-f	98.5e-h
	L. perenne	69.0a	2.34c-g	9.7a	125.2a-f	125.5c-h
	C. intybus	65.5ab	3.19ab	9.1ab	165.7ab	166.9a-d
	S. minor	68.4a	3.20ab	9.6a	175.3ab	182.4ab
Wilds						
	L. corniculatus	65.7ab	2.93abc	9.2ab	149.1abc	143.4a-f
	M. sativa	63.9ab	2.70a-d	8.9ab	134.7a-d	129.4b-g
	T. pratense	62.1abc	2.48b-f	8.5abc	120.6b-f	115.8d-h
	T. repens	63.5ab	2.66a-e	8.8ab	131.8a-e	126.5c-h
	D. glomerata	58.8bc	1.70g	8.0bc	77.9ef	84.7gh
	F. ovina	58.5bc	1.63g	7.9bc	74.1f	93.4fgh
	L. perenne	61.5abc	1.76fg	8.5abc	83.9def	102.9e-h
	C. intybus	53.0c	1.90fg	7.0c	78.1ef	74.6 h
	S. minor	68.1a	3.13ab	9.6a	168.0ab	161.7a-d
Plant type						
Cultivars		66.0	2.74	9.2	142.4	148.3
Wilds		61.7	2.32	8.5	113.1	114.7
Plant species						
L. corniculatus		66.1	3.02	9.2	155.7	159.4
M. sativa		63.8	2.75	8.9	136.9	137.6
T. pratense		62.5	2.55	8.6	124.8	125.4
T. repens		65.2	2.91	9.1	148.4	149.1
D. glomerata		61.1	1.85	8.4	88.3	92.9
F. ovina		61.0	1.77	8.4	84.7	95.5
L. perenne		64.8	2.01	9.0	101.6	112.6
C. intybus		59.3	2.55	8.1	121.9	120.8
S. minor		68.2	3.16	9.6	171.1	170.6
SEM		0.59	0.075	0.10	4.70	4.54
Main effect of						
Plant type		<0.001	<0.001	<0.001	<0.001	<0.001
Plant species		<0.001	<0.001	<0.001	<0.001	<0.001
Plant type × Plant species		0.013	0.004	0.013	0.014	0.009

[a,c,d] Means of the same column followed by different letters differ significantly (P < 0.05). DDM= digestible dry matter, DMI= dry matter intake, ME= Metabolizable energy, RFV= relative feed value, RFQ= Relative forage quality.

[1]Unit of DDM, DMI, and ME is % of dry matter, % of body weight, and MJ/kg DM, respectively.

Both cultivars and wilds separated into two main clusters (named as I and II) according to the similarity of the nutritional traits (Figure 1). In terms of the families and the number of subset and species, there was no similarity among the species within the clusters (I and II) of both cultivars and the wilds. The Cluster I of the cultivars had two species (*F. ovina* and *D. glomerata*), whereas that of the wilds had two sub-groups that had one (*C. intybus*) and three (*L. perenne*, *F. ovina*, and *D. glomerata*) species, respectively. Cluster II of both cultivars and wilds showed two subsets (II_1 and II_2). The II_1 and II_2 subsets of the cultivars included two (*T. pratense* and *M. sativa*) and five (*L. perenne*, *C. intybus*, *S. minor*, *L. corniculatus* and *T. repens*) species, respectively. Despite that, the II_1 and II_2 subsets of the wilds included one (*S. minor*) and four (*T. pratense*, *L. corniculatus*, *T. repens* and *M. sativa*) species, respectively. Based on the dendrogram, the most similar species pairs were *T. repens* – *L. corniculatus*, *S. minor* – *C. intybus*, *M. sativa* – *T. pratense* and *D. glomerata* – *F. ovina* in the cultivars, whereas *M. sativa* – *T. repens* and *D. glomerata* – *F. ovina* in the wilds. The plant type clustered into two sets that included similar NC and FQI. However, the most similar pairs were RFV - RFQ and ADF - NDF in the cultivars, while RFV - RFQ and ADF - DDM in the wilds.

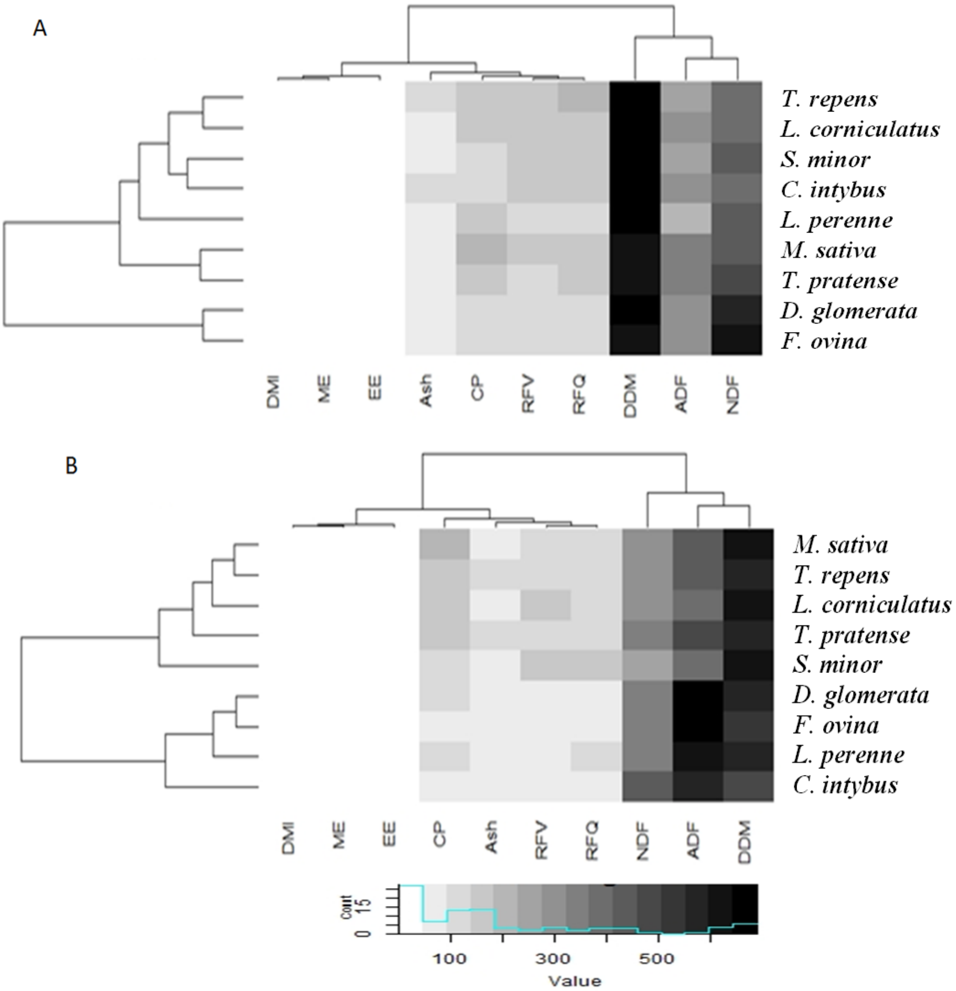

Figure 1. Dendrograms that derived from a two-way clustering analysis of the nine cultivated forage species (A) and their wild relatives (B) together with their nutritional traits. The horizontal and vertical dendrogram is the clusters of plant species and nutritional traits, respectively, according to similarities in the studied parameters. The intensity of colour histogram indicates the lowest (light white) and the highest (dark black) strength of similarity. CP= crude protein, EE= ether extract, ADF= acid detergent fibre, NDF= neutral detergent fibre, DDM= digestible dry matter, DMI= dry matter intake, ME= metabolizable energy, RFV= relative feed value, RFQ= relative forage quality.

4. Discussion

The results of the present study indicated that the cultivars and the wilds comprised of components that respond differently to ecological processes under the same circumstances. Indeed, the cultivars resulted in a remarkable higher nutritive value relative to the wilds. These results confirmed that the quality, one of the primary production traits (Hayes et al., 2013) for forage species, is improved by cultivation of forage species (Lee, 2018). All nutritional traits of both cultivars and wilds were within the range reported in the literature (Asaadi and Yazdi, 2011; Bidgoli et al., 2013; Lee, 2018; Aydin et al., 2019). In agreement with Asaadi and Yazdi (2011), therefore, adequate nutrients were available in rangelands that included the studied wilds. The most notable was that the ADF content, the RFV, and RFQ values of cultivars were a mean of 30% higher and the NDF content was 48% lower than those of the wilds.

These findings might be related to the fact that the legume and OBF cultivars generally had lower ADF and NDF, and higher ash concentrations than their wilds and all grasses (Elgersma et al., 2014; Aydin et al., 2019). These changes in nutritional traits that represented the quality of the forages are very critical in terms of digestible nutrients and energy provision to the ruminants (Hayes et al., 2013). Available study results (Dudek et al., 2020) indicate that differences in the nutrient content of the forage species depend on the difference between years in terms of season (Fan et al., 2020), and, in particular, the amount of precipitation (Gulwa et al., 2017). Unfortunately, we did not estimate the year × species or type interaction in the present study because the samples collected in each year were mixed at the end or were not analysed separately.

Because the cultivars such as *M. sativa*, *L. corniculatus*, *D. glomerata*, *S. minor*, and CI are highly valuable and palatable species for ruminants (Aydın et al., 2020), these cultivars are introduced within the degraded rangeland for re-vegetation and restoration purposes (Schröder and Prasse, 2013; Uzun et al., 2015; Aşcı, 2016; Algan et al., 2019). Based on our results, the use of mixtures of the legume and OBF cultivars (*L. corniculatus*, *M. sativa*, *T. pratense*, *S. minor* and *C. intybus*) for that aim may cause the combined beneficial impacts for the rangelands (Kemp et al., 2010; Hutton et al., 2011) due to the higher CP and lower ADF and NDF contents in these cultivars than companion grasses (Elgersma et al., 2014). Grazing such rangelands may help to boost the productivity of animals and to diminish costs (Algan et al., 2018). However, it should remember that the potential of rangeland improvement by the cultivars depends upon whether they are good competitors to the present vegetation in stressful environmental factors (Schröder and Prasse, 2013).

The productivity of the OBF species and their use for livestock is much lower than the grass and legume species (Elgersma et al., 2014). As reported previously (Elgersma et al., 2014; Aydin et al., 2019), the wild *S. minor* and *C. intybus* generally showed lower FQI values due to CP, ADF, and NDF contents. The results of the present study indicated that the OBF species cultivated relatively new compared to the grass and legume forage caused significant improvements in the FQI of the OBF cultivars. The differences between the cultivars and the wilds may be due to leaf form, structure and leaf to stem ratio (Onoda et al., 2017; Lee, 2018; Aydin et al., 2019). The increased NC and FQI in the cultivars may, also, be due to the impacts of fertilizer applied in the field conditions (Onoda et al., 2017; Lee, 2018; Algan et al., 2019). Indeed, the ME and RFQ values of the OBF cultivars were higher than the grass cultivars, but not the legumes. The results of two-way clustering analysis confirmed the significant differences among nutritional traits of the cultivars and the wilds. These findings supported the idea that the *C. intybus* and *S. minor* provide a suitable balance between CP and energy, as well as minerals and thus, these species may cause high-animal productivities in harsh environments (Asaadi and Yazdi, 2011). Indeed, plant type containing high-CP and ash as well as low-ADF and NDF representing high-digestibility are more nutritious (Arzani et al., 2010; Lee, 2018).

Due to the interaction effect between plant type and plant species factors, there was substantial variation between the cultivars and the wilds. This interaction suggested that the cultivars may differ in their rates of response to cultivation pressures and also, the responses of species in terms of nutritional traits may not always be equally strong, as described previously (Capstaff and Miller, 2018; Pfeiffer et al., 2018). Indeed, variation in CP and EE values within and between the studied species was less compared with ADF and NDF values. In the present study, grass species had a lower feeding value due to insufficient CP content and unsatisfactory fibre digest for ruminants compared to the legumes and the OFB species (Amiri and Shariff, 2012; Algan et al., 2019; Aydin et al., 2019). The fact that grass species had a lower ME and RFQ compared to the species from legume and the other families could be associated with their cell wall and contents (Aydin et al., 2019). Therefore, both types of grass species may limit animal productivity. However, the NC and FQI results of the studied wilds indicated that these species were sufficient in guaranteeing the roughages required by grazing ruminants (Amiri and Shariff, 2012; Elgersma et al., 2014).

The results of the hierarchical two-way clustering analysis showed that the wilds and the cultivars were comprised of nutritional traits that respond differently under the same circumstances. The colour histogram of the dendrogram showed that low-NC and FQI were rare in the cultivars or common in the wilds. These clustering groups agree with the results of previous studies (Amiri and Shariff, 2012; Aydin et al., 2019) related to the rangeland forages. The clusters of families in both the cultivars and wilds were probably related to the NC and FQI of the species belonging to each family (Arzani et al., 2010; Amiri and Shariff, 2012; Aydin et al., 2019). Although the significant variations within and between the forage plant species, the results of clustering analysis confirmed that ADF and NDF contents of forages were the best quality indicator (Ruckle et al., 2017; Lee, 2018; Aydin et al., 2019).

5. Conclusion

The results of the present study indicate that, under normal circumstances, the cultivars resulted in a remarkable higher nutritive value relative to the wilds. Also, the results divulge that legume (*L. corniculatus*, *M. sativa*, *T. pratense* and *T. repens*) and probably the OBF species (*C. intybus* and *S. minor*) are highly acceptable to enhance the productivity of ruminants compared to grasses (*D. glomerata*, *F. ovina* and *L. perenne*). The information reported herein may help to improve the animal diet based on the wilds and to farmers for increasing productivity of the small ruminants.

Author Contributions

IA; supervision project administration and resources. IA and NO; funding acquisition, conceptualization, methodology and writing-original draft preparation. BP and NO; investigation, data curation and formal analysis. NO; writing-review and editing, All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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Research Article
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SEED WATER UPTAKE RELATES GERMINATION AND EMERGENCE POTENTIAL OF WHITE COATED FRENCH BEAN CULTIVARS

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Abstract: Water uptake was related to germination (%), seedling emergence (%), mean germination time (h), and electrical conductivity (μScm^{-1}g^{-1}) of nine French bean seed cultivars. Normal germination percentages were ranged between 43 and 99%, seedling emergence 45 and 88%, RE was between 43 and 100%, electrical conductivity 48.5 and 144.1 (μScm^{-1}g^{-1}), mean germination time (0.95 and 1.82 d). Seed water uptake at 25°C varied 20.43 and 50.89% after 2 h and 32.47 and 56.36% after 6 h. Seed water uptake (%) after 2 and 6 h imbibition was significantly correlated with normal germination percentages (R^2=0.82, P<0.01, 0.77, P < 0.05), normal seedling emergence (R^2=0.76, P < 0.05, R^2=0.93, P<0.001), mean germination time (R^2=0.77, P < 0.05, R^2=0.66, P < 0.05), and electrical conductivity (R^2=0.73, P < 0.05, R^2=0.83, P<0.01). Results were interpreted by prediction of water uptake in relation to seed germination and emergence.

Keywords: Beans, Seed quality, Imbibition, Seedling emergence, Mean germination time

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1. Introduction

Germination is a triphasic physiological process (Nonogaki et al., 2010). In the first phase, fast imbibition (increase in seed weight), a stable period in the second and fast imbibition end up with radicle emergence in the third. This extent of this process is related to various seed characteristics such as seed coat colour (Abdullah et al., 1991, Legesse and Powell, 1992, Zhang et al., 2006), seed ageing (Balesevic-Tubic et al., 2005, Zhang et al., 2006), seed maturation (Legesse and Powell, 1992, Shephard and Naylor, 1996), imbibition temperature and seed coat damage (Powell et al., 1986). In legumes, imbibition is a very influential stage of seed quality, in these species, fast imbibition damages cell structures and causes high solute leakage (i.e electrical conductivity). Solute leakage causes fungi infection around the seed, seedbed and seed death occurs. This is the basic reason why electrical conductivity is used as a seed vigour test in legume species (Matthews and Powell, 2011). Death due to the fast water uptake is also named, imbibitional chilling injury when sown at low temperature (10°C≥), low seed moisture content (i.e., 10%≥) and seed coat damages (cracks on the surface) accelerate the amount of solute leakage and reduce stand establishment in the field i.e., peas, beans, soybeans (Powell et al., 1986, Demir, 1996). Sowing was done in wet and cold soil conditions in northern Europe. However, unlike northern, the Mediterranean region, legumes are not necessarily sown in cold and wet soils. Legumes in such areas are commonly sown as a second crop following wheat and barley harvest where the soil temperature is reasonably high and soil is not wet at all i.e., end of June. So, when seed moisture is raised to i.e., 13%≤ and soil temperatures are high then imbibitional injury is less likely to occur (Demir, 1996) since seed does not take water very fast. Moreover, the temperature may not accelerate the negative effect. Normally, a temperature of >30°C during the day or >20°C at night causes seeds to germinate. Therefore, planting beans in the field should be limited to temperate climates or delayed until the soil is warm enough for satisfactory emergence in cool climates (Kigel et al., 2015).

This work was designed to test the relationship between seed water uptake and germination and seedling emergence when seed moisture is above 12%≤ and optimum soil temperature in bean seeds. This could be a fast discriminative feature in between high and low-quality seed lots regarding germination and seedling emergence potential.

2. Material and Methods

Nine different white-coated French bean (*Phaseolus* *vulgaris* L. cvs Algan, Turna, Burçin, Seçil, Özlem, Albus, Suzan, Gentile, Gaudi) cultivars were obtained from different lots. Seeds were washed with distilled water and dried on paper towels at room temperature, and ventilated until they regained their original moisture content. Initial seed moisture content was determined by using the high-temperature oven method (ISTA, 2020). Seeds were kept at 100 % relative humidity over a night (16 h) before germination and emergence tests. Seed weight was calculated with four replicates of 10 seeds and mean was taken as seed weight for the cultivar. Seeds with cracks on the seed coat were eliminated from the lots. Germination test was conducted on three replicates of 50 seeds in between wet towel papers for 8 days at 25°C (ISTA, 2020) in the dark. Towel papers were placed in plastic bags and placed into the incubator. Total (2 mm radicle emergence) and normal (well-developed seedlings) germination percentages were evaluated after 8 days. Seed water uptake was determined in three replicates of 10 seeds in each lot seeds were weighed and placed on top of 90 mm diameter Petri dishes (wetted, 5ml of distilled water of Whatman papers) at room temperature. Then seeds were weighed after 2 and 6 hours and water uptake were determined as %, compared to the initial weight.

The mean germination time (MGT) was calculated by using the formula (equation 1);

$$MGT = \frac{\sum n.t}{\sum n} \quad (1)$$

where, n= number of seeds newly emerged (2 mm radicle emerged) at time t, t= days from sowing, and ∑n= final germination.

Seeds (three replicates of 50 seeds/lot) were sown 4 cm deep in a mixture of garden soil/peat moss (Plantaflour, Germany), in seedling trays (32x16x6 cm) in an unheated glasshouse. Daily minimum and maximum temperatures varied between 9°C and 29°C respectively. The number of emerged seedlings (unfolding cotyledons on the surface) was calculated after 16 days.

Electrical conductivity measurements were done on two replicates of 10 weighed (0.001 g) seeds of each lot in 50 ml distilled water after 4 hours of soaking at 20°C in dark. The results were expressed as $\mu Scm^{-1}g^{-1}$.

Significant differences between experimental groups were assessed with one-way ANOVA, using SPSS statistical program. Determination of coefficient (R^2) values and regression equations were determined to assess the prediction potential of seed water uptake.

3. Results and Discussion

Total germination percentages were ranged from 97 and 100%, normal germination was between 43 and 99%. Differences in normal germination percentages were significant ($P < 0.05$) but this was not the case in total germination. All seed lots were germinated fast, MGT was 0.95 and 1.82 days. The cultivar lot 4 had the lowest normal germination even though it had 100 % total germination. The highest total and normal germination were obtained from lot 6 which was the smallest cultivar with seed weight 0.9g/10 seeds. Seeds affect the germination percentage at the time of germination; legumes affect production grain (Adebisi et al., 2013). Usually, small seeds germinate faster because large seeds need more water absorption than small seeds and consequently more time to germinate. A similar result was found by Shahi et al. (2015) showed that the germination rate index of large size wheat seeds is lower than that of small and medium seeds. The seed weights of the other 9 cultivars varied between 2.3 and 4.8 g/10 seeds. Moisture content was ranged between 13.0 and 15.7% among the lots (Table 1). Seedling emergence percentages showed significant differences among the lots. Electrical conductivity values ranged between 48.5 and 144.1 $\mu S\ cm^{-1}g^{-1}$ seed. Seed water uptake was the lowest in lot 6 as 20.43 %, 32.47 %, the highest in lot 9 as 50.89% and 56.36, after 2 and 6 h of imbibition (Table 2). Seed water uptake after 2 and 6 hours was related significantly ($P < 0.05$ and $P < 0.01$) to normal germination (Figure 1), mean germination time (Figure 2) seedling emergence (Figure 3) and electrical conductivity readings (Figure 4). Seed water uptake was also related to normal seedling emergence percentages and electrical conductivity readings at $P < 0.05$ – $P < 0.01$ level (Figures 2 and 4).

Table 1. Total germination (2 mm radicle emergence, TG, %), normal germination (NG, %), mean germination time (MGT, h), 10 seed weight (SW, g) and seed moisture content (MC, %) of white French bean seed cultivars

	Seed lots								
Criterion	1	2	3	4	5	6	7	8	9
TG (%)	100	97	100	100	100	100	100	100	100
NG (%)	63	67	65	61	61	43	79	60	99
MGT (h)	1.66	1.16	1.24	1.37	1.31	1.82	1.42	1.40	0.95
SW (g)	4.2	2.3	3.7	4.4	4.8	4.7	3.5	3.2	0.9
MC (%)	14.6	14.6	15.3	13.5	14.7	15.7	13.7	13.9	13.0

Table 2. Seedling emergence (%), mean emergence time (d), electrical conductivity at 6th hours (EC), and seed water uptake (SWU) at 2 and 6th hours in white French bean seed lots

Seed lots	SE (%)		EC (µScm⁻¹g⁻¹)		SWU (%)			
					2 h		6 h	
1	63	c	77.6	f	24.51	d	34.67	ef
2	81	ab	97.1	g	25.55	d	47.25	bc
3	63	c	67.2	e	23.23	d	35.99	ef
4	77	abc	65.4	d	30.64	c	43.23	cd
5	45	d	48.5	a	22.67	d	33.06	f
6	79	abc	59.8	b	20.43	d	32.47	f
7	80	abc	99.6	h	43.82	b	50.07	b
8	68	bc	61.4	c	24.97	d	39.28	de
9	88.0	a	144.1	ı	50.89	a	56.36	a

The same letters in a column do not differ significantly at P < 0.05. Letters are started to be given from the shortest value at EC.

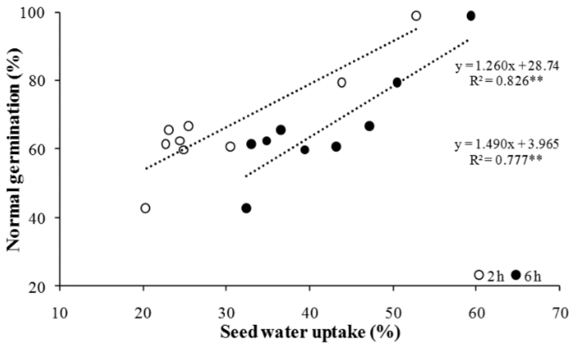

Figure 1. The relationship between normal germination (%) and seed water uptake (%) at in white seed lots of French bean cultivars. %, 2h (○) and 6h (●) and electrical conductivity (EC, µScm⁻¹g⁻¹).

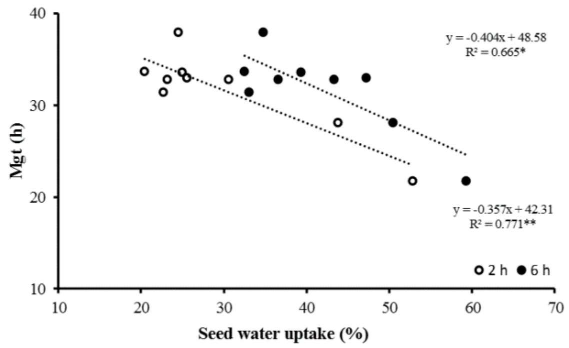

Figure 2. The relationship between mean germination time (h) and seed water uptake (%) at in white seed lots of French bean cultivars. %, 2h (○) and 6h (●) and electrical conductivity (EC, µScm⁻¹g⁻¹).

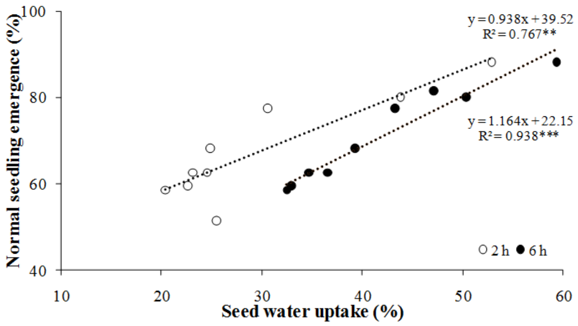

Figure 3. The relationship between normal seedling emergence (%) and seed water uptake (%) at in white seed lots of French bean cultivars. %, 2h (○) and 6h (●) and electrical conductivity (EC, µScm⁻¹g⁻¹).

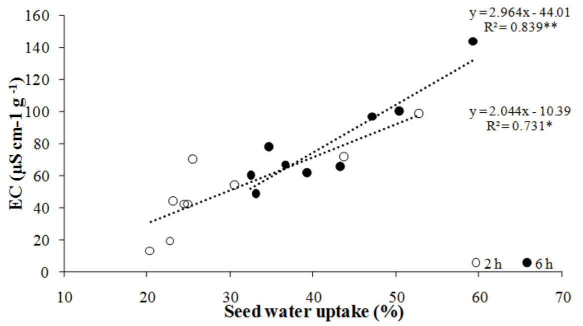

Figure 4. The relationship between seed water uptake values in white lots of French bean cultivars. %, 2h (○) and 6h (●) and electrical conductivity (EC, µScm⁻¹g⁻¹).

Various seed characteristics were related to water uptake Balesevic-Tubic et al. (2005) found that the ageing process affected sunflower seeds' imbibition rate. Imbibition rate was faster in seeds which stored in ambient conditions than those at 4°C. They concluded that the sorptive characteristics of seed may be influenced by changes in cellular level by ageing. In our work, the seed lots with the lowest normal germination did not imbibe faster than highly germinated ones like lots 3 and 5. This obviously showed that ageing itself in this work was not a factor in the imbibition rate. In sorghum seeds, less mature seeds (pale colour) had lower density and were imbibed faster than mature (dark-coloured) seeds. The faster imbibition was accompanied by high electrolyte leakage which was related to low germination in the final stage. They concluded that the physical proportion of the seed pericarp is influential in governing water uptake and leakage (Shephard and Naylor, 1996) in sorghum. In our work, the seed lots were commercially available ones so the difference in maturity among the lots were not known.

Faster imbibition in dwarf white French bean cultivars resulted in higher electrolyte leakage and lower normal seedling emergence (Powell et al., 1986). They concluded that adherence loss of seed coat in white-coated cultivars, induces imbibition injury, unlike the coloured ones. In our work, we used only white cultivars. We were not able to compare white and coloured cultivars. While our results are controversial with these conclusions.

Basically, faster seed water uptake did not associate with lower germination, emergence and germination time (Figures 1 and 2). The reason for that may be the seed growing/testing conditions. We raised seed moisture above 13% and sow seeds to relatively high temperatures (Demir, 1996), while Powell et al. (1986) tested emergence initially at 4°C (6h) then 20 °C. Moreover, the seed moisture content in their work was 8-9 %, lower than our samples. Moreover, in our work seeds were not subjected to chilling during imbibition. It was proposed that low temperature during early imbibition increased the occurrence of damage in cell walls in peas (Powell and Matthews, 1978).

Bean cultivation in a Mediterranean climate, in some regions, overlaps summer months i.e., mid of June. In the southern part of Turkey, beans are sown after barley and wheat were harvested at the beginning of summer. So, the soil temperature is reasonably high and dry. In such cases, fast water uptake by seeds is positively related to normal germination, seedling emergence and electrolytic leakage. That appears to be that imbibitional injury may be strictly related to cold/wet sowing environments (Powell and Mathews, 1978; Powell, 2006). This may be one reason why our results do not agree with earlier reports in white French beans. Kolasinska et al. (2000) found that soil temperature at sowing appeared to be the most important environmental factor influencing field emergence and a factor that can distinguish the potential for a seed lot to emerge from the field. However, further investigations are necessary to confirm that by using a larger number of seed lots.

Fast and effective germination prediction has value in seed testing. Our work indicates that seed water uptake of 2 or 6 h can give a pre-testing indication of normal germination percentages. Faster imbibition resulted in high normal germination (Figure 1). Leftover seeds may also be selected according to their water uptake regarding normal germination potential. Higher seed water uptake was associated with faster germination i.e., lower mean germination time.

As a result, fast water uptake in white French beans is an indication of higher normal germination and better seedling emergence potential in the field. However, we tested this at optimum temperatures. Results may be different at lower sowing conditions which require further investigations.

Author Contributions

SE initiated the research idea, developed, organized, analyzed, collected and interpreted the data. GO analyzed the data, produced the figures. ID supervised the research, structured the paper and edited the manuscript. All authors reviewed and approved the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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Research Article
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GGE-BIPLOT ANALYSIS OF DURUM WHEAT YIELD TRIALS

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Abstract: Durum wheat (*Triticum durum* L.) is a vital crop in the rain-fed areas of Turkey. In this study, the grain yield of twenty durum wheat genotypes (sixteen advanced lines with four checks) tested across 8 rain-fed environments during the 2008-2009 cropping season was evaluated using GGE (Genotype (G) Main Effect + Genotype by Environment Interaction (GEI)) Biplot Approach. Environment (E) captured most (83 %) of the grain yield (GY) variation, while the portions attributed to G and GEI were only 6 and 11 %, respectively. In addition, most of the testing Es were highly correlated. According to GGE-Biplot analysis, 'Which won where' pattern partitioned the testing Es into three mega-environments (ME): the first ME with six Es with G13 (the highest yielder) as the winning genotype; the second ME encompassed one environment (E1, Konya) with G4 (the lowest yielder) as the winning genotype, and the last ME represented by one location (E6, Altintas) with G10 (the higher yielder) as the winning genotype. GGE-Biplot analysis showed that although the Durum Wheat Yield Trials were conducted in many environments, outcomes alike can be obtained from one or two representatives of each ME. On the other hand, no correlation of these MEs with their geographic location was observed. In conclusion, the presence of cross-over GEI underscores that efforts should be given to identify specifically adapted genotypes rather than broadly adapted ones tested on multi-environment trials (METs).

Keywords: Durum wheat, Genotype by environment interaction, GGE-Biplot, Grain yield

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1. Introduction

Multi-environment trial (MET) plays an essential role in plant breeding. The main goal in plant breeding is to select new cultivars agronomically superior (i.e., high grain yielder) over commonly grown cultivars (Rakshit et al., 2012; Li and He, 2021). However, little attention is given to the interaction of genotypes with unpredictable target environments. In this context, METs can help understand genotypes' performance in various environments by measuring the stability of genotypes (Gs) across environments (Es) (Tekdal et al., 2017; Scapim et al., 2000). But, MET data are rarely used to their full potential, even though data on most plant traits have been collected. Furthermore, in the analysis of such data, primarily genotypes are selected based on G main effects, while GEI (genotype by environment interaction) is ignored (Yan and Tinker, 2006; Kendal, 2019).

Various statistical models have been suggested to analyze the complexity of the GEI (Yan and Kang, 2003). One of those models, the biplot methodology, depicts the complex GEI in a simple, graphical fashion (Gabriel, 1971). Two types of Biplot, AMMI (Additive Main-effect and Multiplicative Interaction) Biplot (Crossa, 1990; Gauch, 1992) and GGE (Genotype + Genotype by Environment Interaction) Biplot (Yan et al., 2000; Yan and Kang, 2003), are the most commonly used to understand GEI comprehensively. Both G and GEI should simultaneously be included in a model to evaluate genotypes (Yan and Tinker, 2006; Sabaghnia et al., 2008). The G + GEI (GGE) biplot discards E main effects and merges G main effects with the GEI dataset (Yan et al., 2000). It dissects the GEI pattern in the data and delineates 'which-won-where' and mega-environments (Yan et al., 2007; Yan, 2019; Yan et al., 2021).

So far, GGE-biplot analysis has been applied to many crops such as soybean (Yan and Rajcan, 2002), rice (Samonte et al., 2005), bread wheat (Kaya et al., 2006; Roozeboom et al., 2008; Akcura et al., 2011; Nehe et al., 2019), barley (Dehghani et al., 2006; Mohammadi et al., 2009), peanuts (Putto et al., 2008), lentils (Sabaghnia et al., 2008), corn (İlker et al., 2009), oats (Yan et al., 2010) and sorghum (Rao et al., 2011). However, despite reports on GGE-biplot analysis in selecting superior genotypes or test environments in such crops, its application to durum wheat METs in Turkey is insufficient (Tekdal et al., 2017; Kendal, 2019; Mohammadi et al., 2021). Genotypes (i.e., breeding lines) are routinely tested to select ones adaptable to Turkey's Winter Durum Wheat Zone. The target environments (i.e., Winter Durum Wheat Zone) are distributed across latitudes and altitudes with various climatic conditions, representing durum wheat-growing areas in the Central Anatolian Region and Transition Regions of Turkey. Therefore, to show the usefulness of the GGE-Biplot method in dissecting the complex GEI in MET data, we analyzed the GYs of 16 improved lines with four checks tested in eight rainfed environments.

2. Material and Methods

2.1. Field Trials

During the 2008-2009 cropping season, 20 winter durum wheat genotypes were tested in eight rainfed sites (Konya, Çumra, Eskişehir, Ulaş, Emirdağ, Altındaş, Esenboğa, and Malya), representing the Central Anatolia Region (CAR) and Transition Regions (TZs) of Turkey (Tables 1 and 2). The experiment was set up in a randomized block design with three replications. Sowing was done with an experiment drill in 1.2 m × 7 m plots (6 rows with 20 cm spacing apart). The sowing rate was 550 seeds m^{-2}. Fertilizers were applied as 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} during planting and 50 kg N ha^{-1} just before the stem elongation stage (Zadoks Stage 30). Harvest was done by a plot-combine in 1.2 m × 5 m plots. Grain yield (GY) was expressed as tonnes per hectare (t ha^{-1}).

2.2. Statistical Analysis

In the analysis of variance (ANOVA), the E and blocking effects were accepted as random, while the G effect was accepted as fixed. ANOVA was applied to GY data combined over the years. Gs and Es were descending ranged based on LSD (Least Significant Difference) test. Gs performances, their stabilities, and the ideal G were determined by the GGE-Biplot analysis method. Also, following the same process, the distinctive and representative abilities of the Es and the ideal E were determined. Again, thanks to the related procedure, ME and which-won-where patterns were determined for Es and Gs. ANOVA, LSD test and GGE-biplot analysis were conducted using GENSTAT 12 (Yan et al., 2000; Yan et al., 2001; Yan, 2002).

Table 1. Genotypes

Code	Pedigree	Yield (t ha^{-1})
G1	1-KOBAK2916*61-130/3/GOKALA//BR180/ WLS/4/ B24SYRIAN-2	3.02 ^{eg}
G2	2-KOBAK2916*61-130/3/GOKALA//BR180/WLS/4/ B24SYRIAN-2	2.75 ^{j}
G3	KND1149//68111/WARD/3/RICCYA(WINTER)/BERK	3.01 ^{eg}
G4	1-ALTINTAS/3/ZF/LDS//185-1/3/61-130/LDS	2.58 ^{k}
G5	KIZILTAN	2.81 ^{ij}
G6	2-ALTINTAS/3/ZF/LDS//185-1/3/61-130/LDS	2.86 ^{gh}
G7	3-ALTINTAS/3/ZF/LDS//185-1/3/61-130/LDS	3.00 ^{gh}
G8	073-44/BERKMEN 469 WINTER	2.83 ^{hj}
G9	TA=TRANSVAAL AFRIKCA/BERK469/GERARDO516	2.97 ^{gh}
G10	KUNDURU	3.25 ^{bd}
G11	AKBUG."S"/RUGBY NEW.N.DURUM/BD2777//SARI BUG.	2.97 ^{gh}
G12	1-61-130/UVY162/64140/WARD	3.10 ^{cf}
G13	HARA456/4/61-130/414-44//68111/WARD/3/69T02/69T11/ ZF7113	3.75 ^{a}
G14	2-61-130/UVY162/64140/WARD	3.29 ^{b}
G15	MIRZABEY	3.26 ^{bc}
G16	3-61-130/UVY162/64140/WARD	3.32 ^{b}
G17	CKM79/KOBAK/LEEDS//6783	3.18 ^{be}
G18	WALNOVA GE 598(ITALIA)//YUMA/FATO"S"/3/ TWWOH84-32	3.08 ^{df}
G19	BERK469//68140/WARD/CKM79"S"	3.06 ^{ef}
G20	ALTINTAS	3.09 ^{cf}
	Mean	3.06
	LSD (0.05)	0.17

Genotypes were descending ranged based on LSD (Least Significant Difference) test.

Table 2. Environments

Code	Environment	Yield (t ha^{-1})	Precipitation (mm)	Latitude	Longitude	Altitude (m)
E1	Konya	1.86 ^{h}	320	37°51' N	32°33' E	1029
E2	Cumra	4.47 ^{a}	285	37°35' N	32°38' E	1012
E3	Eskisehir	2.91 ^{e}	371	39°48' N	30°27' E	813
E4	Ulas	2.23 ^{f}	353	39°16' N	36°46' E	1472
E5	Emirdag	4.29 ^{b}	416	39° 4' N	31°21' E	959
E6	Altindas	3.47 ^{c}	560	39° 3' N	30° 6' E	1019
E7	Esenboga	2.11 ^{g}	402	40° 7' N	32°59' E	942
E8	Malya	3.16 ^{d}	310	39°16' N	34°18' E	1157
	Mean	3.06				
	LSD (0.05)	0.11				

Environments were descending ranged based on LSD (Least Significant Difference) test.

3. Results

3.1. Analysis of Variance

Analysis of variance (ANOVA) revealed that E and G main effects and GEI were significant (P<0.001, Table 3). As is typical of most METs, GY was significantly affected by E, accounting for 83% of the total variation (G+E+GEI). However, the effect of GEI was greater than that of Gs. The Gs ratio of 17% over (G+GEI) suggested the possible presence of multiple durum wheat mega-environments (MEs) in the CAR and TZs of Turkey, where the genotype rank was different from other MEs. Partitioning the G + GEI by GGE biplot analysis showed that the first two principal components (PC1 and PC2) were factors, accounting for 70% of the total sum of squares of G+GEI (Figure 1).

Table 3. Analysis of variance for grain yield

Source	df	SS	MS	F	Model	Explained (%)
Environment (E)	7	404.19	57.74	184.04***	Random	83
Replication (E)	16	5.02	0.31			
Genotype (G)	19	28.30	1.49	3.86***	Fixed	6
G x E Interaction	133	51.29	0.39	4.00***	Random	11
Error	304	29.28	0.10			
Total	479	518.08				100
$CV_{(\%)}$ = 10.14	R^2 = 0.94	Mean = 3.06 t ha^{-1}				

***= significant at the 0.001 probability level.

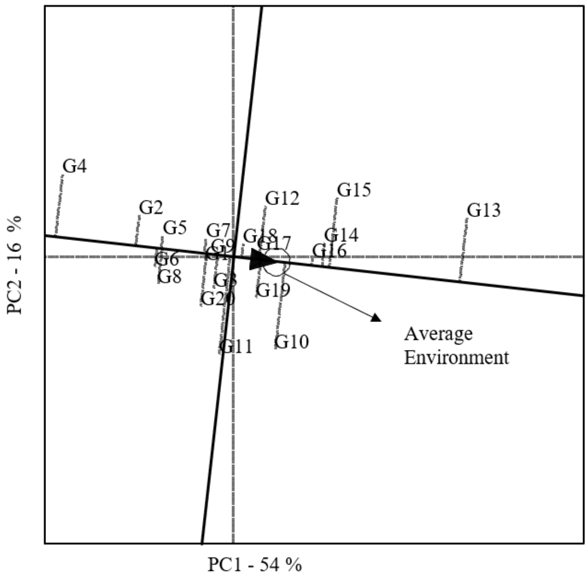

Figure 1. Comparing genotype performance vs. stability. G stands for genotypes.

3.2. Genotype and Environment Means

The average GYs of genotypes over environments ranged from 2.58 t ha^{-1} for G4 to 3.75 t ha^{-1} for G13, with an overall average of 3.06 t ha^{-1} (Table 1), whereas the average GYs for environments varied between 1.86 t ha^{-1} in E1 (Konya) and 4.47 t ha^{-1} in E2 (Çumra) (Table 2). Average GY in the GEI data matrix (data not given) ranged from 1.40 t ha^{-1} for the G4 genotype in E7 (Esenboğa) to 5.74 t ha^{-1} for G13 in E5 (Emirdağ). The lowest and highest yielding genotypes were originated from Turkey's Winter Durum Wheat Breeding Program.

3.3. Genotype Performance and Stability

The genotype performance and stability were depicted by the GGE biplot (Figure 1). Also, it can be evaluated together with the average environmental coordination (AEC) method (Yan, 2001; Yan, 2002). Accordingly, G16 and G14 were the best performers in terms of GY, followed by G17 and G18 (Figure 1). On the other hand, G2 and G4 were the worst yielders. It can be observed that G15, G12, G19, and G10 were the least stable for GY with higher projections from the AEC abscissa. In contrast, G1 and G9 were relatively stable, if not higher grain yielders. G13 was the highest grain yielder but not stable.

Furthermore, Figure 2 showed the genotype ranking for GY in terms of the 'ideal genotype', indicating the best performer with stability across environments (Yan and Tinker, 2006). Our study revealed that G13 followed by G16, G14, and G15 was close to the ideal genotype. Those were high yielders within all genotypes tested, but G13 and G15 were not stable (Table 1).

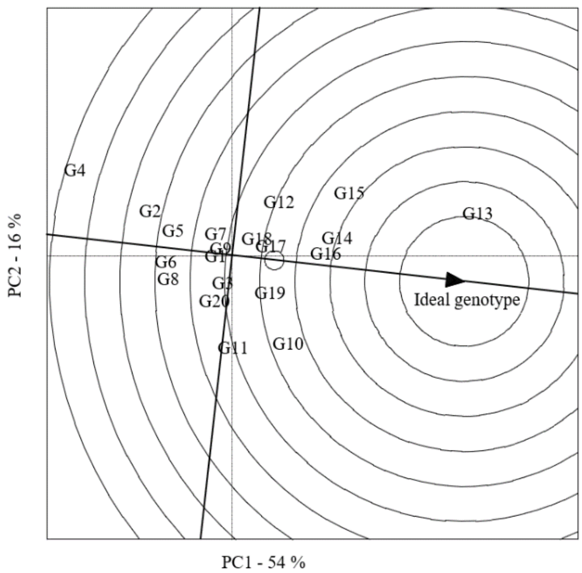

Figure 2. Detecting ideal genotype. G stands for genotypes.

3.4. Comparison of Environments

Most environments were highly correlated for GY, except E1 and E6 (Figure 3). E1 consistently showed inverse relationships with the remaining environments, as the vector showed wide-angle. However, E2, E3, E5, and E8 were not associated with close right angles to E6 and E4 (Yan and Tinker, 2006).

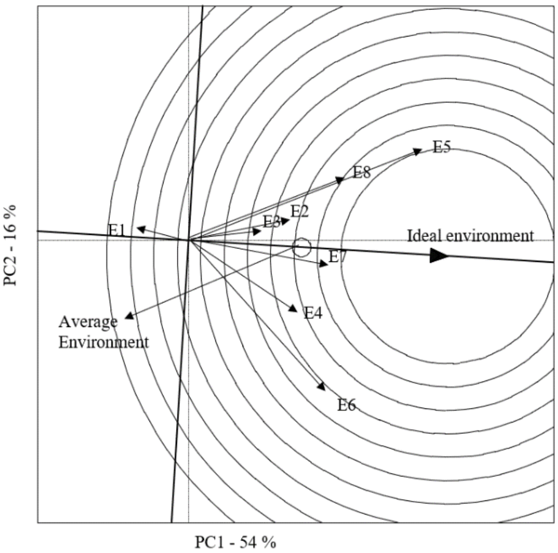

Figure 3. Detecting environment representativeness, its discriminative ability, and ideal environment. E stands for environments.

The vector length of an environment represents its discrimination ability (Yan et al., 2000). Therefore, E5 and E6 were the most discriminatory environments. The angle between an environment and AEC represents the representativeness of the environment: the larger the angle, the less representative the environment (Yan et al., 2000). Therefore, E7 was the most representative while E6 was the least representative. An ideal environment should both distinguish genotypes and represent the average environment. According to the definition of an ideal environment, there was no ideal environment between them (Figure 3). However, E7 as the most representative environment and E5 as the most discriminatory environment could be considered ideal if combined.

3.5. Which-Won-Where Pattern and Mega-Environment Detection

The which-won-where biplot is established by combining the furthest (i.e., vertex) genotypes that form a polygon (Yan, 2001). Genotypes at the polygon's vertices are either the best or the poorest in one or more environments. The genotype at the vertex of the polygon performs best in the environment entering the sectors. The which-won-where biplot for GY is presented in Figure 4. Biplot demonstrated the presence of the cross-over GEI and mega-environments (MEs) for GY. The biplot (Figure 4) was substantially illustrative as it could distinguish environments more effectively and the polygon (trapezoid) was well distributed (Yan, 2002; Yan and Tinker, 2006). The trapezoidal polygon had four genotypes, namely G13, G4, G11, and G10 (Figure 4). G13 genotype performed best in 1E2 (Cumra), E3 (Eskişehir), E4 (Ulas), E5 (Emirdağ), E7 (Esenboğa) and E8 (Malya), while G4 performed best in E1 (Konya) and G10 in E6 (Altintas). The vertex genotype G11 did not perform well in any test environments. The equality lines effectively divided the biplot into four sectors, holding all environments. Thus, the test environments were divided into three MEs: the first ME1 with 1E2 (Cumra), E3 (Eskişehir), E4 (Ulas), E5 (Emirdağ), E7 (Esenboğa), and E8 (Malya), with G13 as the winning genotype. The second ME included E1 (Konya) with G4 as the winning genotype, while the last ME was represented by E6 (Altıntaş) with G10 as the winner. Sector 4 did not have any ME as none of the test environments were engaged. On the other hand, there was no correlation between environments in an ME in terms of geographic location, precipitation pattern, and altitude (Table 2 and Figure 4).

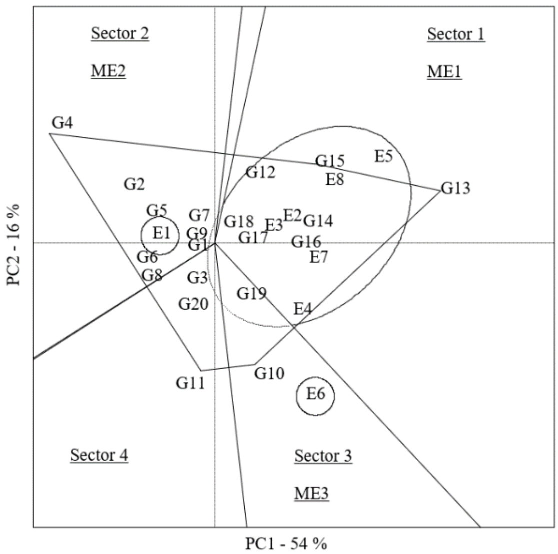

Figure 4. Detecting mega-environments and which-won-where pattern. G and E stand for genotypes and environments.

4. Discussion

The GGE biplot has been used in only a few studies to analyze the MET data of durum wheat in Turkey (Tekdal et al., 2017; Kendal, 2019). In our research, E contributed 83% of the total variation in the data, whereas G and GEI contributed less. Gauch and Zobel (1997) reported that E usually accounted for approximately 80% of the total variation in MET data. In the MET data of bread wheat, Kaya et al. (2006) reported that the variation described by E was as high as 81%. A similar trend was reported by Dehghani et al. (2006) for barley MET in Iran. Putto et al. (2008) revealed that 50-80% of the total variation attributed to E, while the main effect of G contributed 15-46% of the total variation. In our study, GEI explained a higher proportion of variation than G alone. Compared with G, the higher ratio of GEI indicated the possible presence of different MEs in the test Es (Yan and Hunt, 2002; Mohammadi et al., 2009; Akcura et al., 2011; Rakshit et al., 2012; Kendal, 2019).

In our study, the first two PC explained 70% of the variability for the GY data of durum wheat METs. Also, G, together with GEI, accounted for 17% of the total variation for GY. Thus, the GGE-biplot can be interpreted as a useful visualization of variation in MET data (Gauch and Zobel, 1997). Furthermore, visual representation of PC1 and PC2 revealed the variation in the dataset. Thus, it was clear that five of the nine high grain yielders (G13, G15, G12, G10, and G19) were unstable, while eight of the eleven low yielders (G1, G3, G9, G7, G6, G5, G8, and G2) were stable. This can be explained by the fact that a different gene cluster governs the trait of interest, and the effect of E on the expression of that gene cluster varies significantly (Li and He, 2021).

The GGE-biplot allows the detection of genotypes close to the ideal genotype. Accordingly, a process alike can be applied to an ideal environment. G13 was most relative to the ideal genotype, followed by G16, G14, and G15. However, the G13 was the highest yielder but less stable. G13, which was closest to the ideal genotype, showed the best performance in E5 (Emirdağ), while it reached nearly the average yield in E6 (Altıntaş) and was behind the average yield at the E1 (Konya). In addition, G13 exhibited different GY values across test environments, indicating the cross-over GEIs (Nehe et al., 2019). A similar observation was reported in various crops (Dehghani et al., 2006; Kaya et al., 2006; Sabaghnia et al., 2008; Dehghani et al., 2008; İlker et al., 2009; Rakshit et al., 2012; Kendal, 2019).

Our study showed that all test environments except E1 and E6 were closely related, and most of them (i.e., E2, E3, E4, E7, and E8) were also close to the average environment. In other words, the discriminating ability and representativeness of test environments could be easily detected. Thus, E5 and E6 with higher vector lengths were more distinctive than E8 and E7. Therefore, environments close to the average environment, such as E7, E2, and E3, were the most representative and suitable test environments for selecting widely adapted genotypes. On the other hand, the distinctive and non-representative E6 helped determine specifically adapted genotypes. Therefore, a specifically adapted genotype to a particular environment could be conveniently described by employing this type of graphical representation (Plavsin et al., 2021).

Furthermore, closer relationships between test environments showed that the same information could be obtained from fewer environments. Thus, similar environments could be eliminated from the future METs for durum wheat in Turkey. It is vital in allocating scarce resources while setting up METs most appropriately (Yan et al., 2021). The presence of wide angles between environment vectors, indicating strong negative correlations between test environments, emphasizes the presence of solid crossover GEIs for GY in some environments (Yan and Tinker, 2006; Yan, 2019). They noted that genotypes that perform better in one environment would underperform in another. At the same time, closer relationships between the test environments indicate the absence of crossover GEIs, suggesting genotype sequencing does not vary from one environment to another. A mixture of cross-over and non-cross-over GEI types is typical in MET data (Kaya et al., 2006; Fan et al., 2007; Sabaghnia et al., 2008; Rao et al., 2011; Rakshit et al., 2012; Yan, 2019; Yan et al., 2021). It could be possible because some genotypes were more sensitive to changes in the growing environment, while others would be stable in response to the environment.

The 'which-won-where' biplot model may be associated with cross-over GEI, ME differentiation, specific adaptation (Gauch and Zobel, 1997; Yan et al., 2000; Yan and Tinker, 2006; Putto et al., 2008, Rao et al., 2011; Nehe et al., 2019; Li and He, 2021; Plavsin et al., 2021). Based on the biplot analysis, test environments were divided into three MEs. It has been suggested that although testing genotypes has been conducted over many environments, a similar conclusion can be drawn from one or two representatives of ME. By doing so, the testing cost of genotypes over environments can be significantly reduced. However, the Biplot model needs to be validated in multi-year and environmental trials conducted on durum wheat (Yan et al., 2021).

5. Conclusion

The specific adaptation of the genotypes tested in this research suggests that it requires more emphasis than broader adaptation in durum wheat breeding (DWB). In this context, participatory DWB gains more importance than the current research station-oriented breeding program. 'Which-won-where' analysis has shown the existence of MEs, and most geographically different environments can produce similar outcome. Therefore, to effectively run ME with limited resources, discriminatory environments that cover representative environments can be included, rather than broadly expanding the trials onto relevant environments. Following a similar analysis, durum wheat breeders in other regions need to identify MEs and allocate test environments accordingly. The presence of cross-over GEI indicates that the current procedure does not realistically depict the actual situation. Instead, efforts are needed to identify environment-specific genotypes from multi-year and environmental data as this will take into account the stability parameter of the genotypes, so they should be taken into account for their release.

Author Contributions

All task made by single author and the author reviewed and approved the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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Research Article
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VARIATION OF EPIPHYTIC FLORA AFFECTING SILAGE QUALITY IN PURE AND MIXED MUNG BEAN AND SWEET SORGHUM

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Abstract: This study was carried out to determine the microorganism population affecting silage quality of sweet sorghum and mung bean cultivated with different sowing patterns as mixture or sole crop. Twin row (20×55 cm row spacing), narrow row (55 cm row spacing) and conventional row (75 cm row spacing) were used as mixture sowing patterns. The mixtures were formed based on the plant densities and alternative row numbers of sweet sorghum and mung bean. Sowing was made on alternation rows of 1 row mung bean plus 1 row sweet sorghum (R1:1) and 1 row mung bean plus 2 rows sweet sorghum (R1:2). In pure and mixed cultivations, the plant density of sorghum was 14 plants m^{-2} while the plant densities of mung bean were 14, 21 and 28 plants m^{-2}. The experiment was planned as two-factors (sowing patterns and mixtures) and was arranged in randomized blocks according to the split plot design with 3 replications. Pure and mixed plants were harvested when the sweet sorghum plant reached the dough stage. Lactic acid bacteria, enterobacteria and yeast and mold populations in the plant epiphytic flora were investigated under experimental factors. There were significant effects of the main factors and their interactions on the plant epiphytic microorganisms. According to the results obtained from the current study, mixed cultivation of sweet sorghum and mung bean in conventional row pattern improved the desired lactic acid bacteria population for silage quality, while reducing the undesirable enterobacteria and yeast and mold population for silage quality. It was determined that the (R1:2) MB14+SS14 mixed cultivation system was the most suitable mixture in conventional row pattern in terms of high lactic acid bacteria population.

Keywords: Sowing patterns, Intercropping, Lactic acid bacteria, Mung bean, Silage, Sweet sorghum

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1. Introduction

The product formed as a result of fermentation of forage plants in an oxygen-free environment is silage (Kizilsimsek et al., 2017). The most important advantage of silage production is that it produces a stable feed containing high energy and digestible nutrients by providing a high dry matter conservation compared to dry forages (Ertekin et al., 2022). Silage making has many advantages over other roughage storage methods. For example, nutrient loss in dry storage can be higher than silage (Ertekin and Kızılşimşek, 2020). However, the factors affecting the fermentation quality in silage production depend on the chemical composition and dry matter content of the ensiled plants. In addition to these features, the epiphytic (natural) flora of the plants entering the silo is an important factor (Kung et al., 2018). Microorganisms found in this epiphytic flora are divided into desirable and undesirable microorganisms (Kung, 2010). Lactic acid bacteria represent desirable microorganisms, while enterobacteria, yeast and molds are in the undesirable class (Santos et al., 2015). These microorganisms can cause a wide variety of end products to occur in the silo (Kung and Shaver, 2001).

Knowing the microbial population of the forage plant with sufficient chemical composition and dry matter content can help to obtain a healthy silage (Kung et al., 2018). For example, the insufficient lactic acid bacteria population of an ensiled forage plant may delay fermentation and increase nutrient loss in the silo (Kızılşimşek et al., 2016). It is a popular method to inoculate forage plants with lactic acid bacteria before ensiling when insufficient lactic acid bacteria population is detected in the natural flora of forage plants or when there is a high presence of undesirable microorganisms (Ertekin and Kızılşimşek, 2020). Therefore, it is of great importance to know the microbial population in the natural flora of the ensiled plants.

In this study, it was aimed to examine the microbial population in the epiphytic flora of sweet sorghum and mung beans grown with different sowing patterns and mixed growing systems and to facilitate the storage of forage by ensiling.

2. Material and Methods

2.1. Material

In this study, the ERDURMUŞ sweet sorghum cultivar registered by the Western Mediterranean Agricultural Research Institute Directorate in 2018 and the mung bean population obtained from Uzbekistan through a commercial company engaged in horticultural seeds were used as plant material.

2.2. Soil and Climatic Characteristics of the Experimental Field

This study was carried out at Hatay Mustafa Kemal University, Faculty of Agriculture, Field Crops Department, Telgaliş Research and Application Field (36°15'13.56"N 36°30'7.96"E, altitude 96 m) for two years in 2019 and 2020 under second crop production conditions. The soil of the experiment area is clay-loam and the total salt content is low and slightly alkaline. Lime and phosphorus content is moderate and organic carbon content is quite low. The climate data of the region were given in Figure 1. The rainfall values of the growing periods of 2019 and 2020 were considerably lower than the long-term averages. On the other hand, the opposite situation occurred in temperature values.

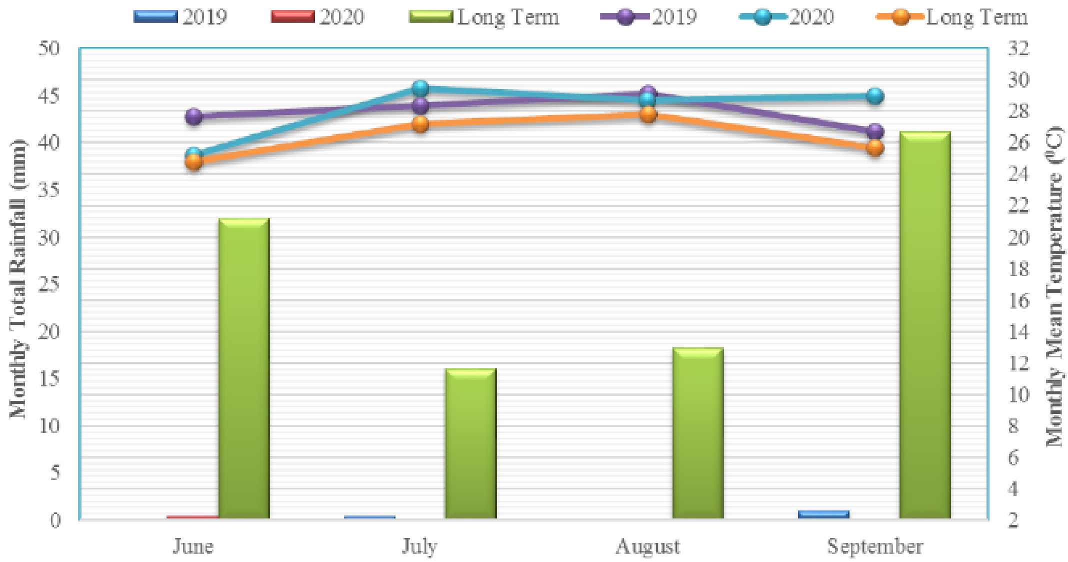

Figure 1. Some climatic data of the experimental field.

2.3. Cultivation Techniques, Experimental Factors and Harvest

The study was planned as two-factor and was carried out in randomized blocks according to the split plot design with three replications. The trial was established on June 20 in 2019 and on June 23 in 2020 under the second crop production conditions. The main factors of the experiment were sowing patterns and the sub-factors were mixed cultivation systems. Twin row (20×55 cm row spacing), narrow row (55 cm row spacing) and conventional row (75 cm row spacing) were used as sowing patterns (Figure 2).

Figure 2. An image taken with a drone from the trial area.

The mixtures were formed based on the plant densities and alternative row numbers of sweet sorghum and mung bean. Sowing was done on alternation rows of 1 row mung bean plus 1 row sweet sorghum (R1:1) and 1 row mung bean plus 2 rows sweet sorghum (R1:2). The plant density of sweet sorghum was included in the mixtures as 14 plants m^{-2} (SS14) and the plant densities of mung bean as 14 plants m^{-2} (MB14), 21 plants m^{-2} (MB21) and 28 plants m^{-2} (MB28).

In-row distances calculated according to sowing patterns of plant species were taken into account while sowing. Before planting, 5 kg da^{-1} NPK were applied and mixed into the soil basally. When the plants reached a height of 40-50 cm (approximately 30 days after emergence), a deep hoe was made by hand for weed control and soil aeration in the entire experimental area. In both years, 2 days after hoeing, 5 kg da^{-1} N as urea was applied and irrigated at field capacity. Harvest was done on September 20 in 2019, and on September 23 in 2020, about 90 days after emergence, when sweet sorghum plants reached the dough stage and mung bean plants reached 50% pod forming stage. Side rows and 0.5 m lengths from the beginning of each row were removed from all plots as a side effect and the plants were cut manually with the help of a sickle. The plant species harvested from the mixtures were weighed separately and their fresh weights were recorded. Fresh weight ratios of mixtures were calculated based on the fresh weights of the plant species obtained from the plots. 250 g samples were taken to determine the microbial population and transported from the field to the laboratory by cold chain.

2.4. Method

According to Yan et al. (2019), 20 g of fresh sample from each treatment was homogeneously blended in 180 mL of sterile Ringer's solution for 60 seconds with the help of a blender (Arçelik K8130 MV). Then the obtained samples were filtered through Whatman no 54 filter paper. In the dilution series (from 10-1 to 10-10) made from these samples, Lactic acid bacteria (LAB), enterobacteria and yeast and mold populations were determined using the MRS (DE MAN, RAGOSA, SHARPE) agar, VRB-G (Violed Red Bile Glucose) and MEA (Malt Extract Agar), respectively (Figure 3).

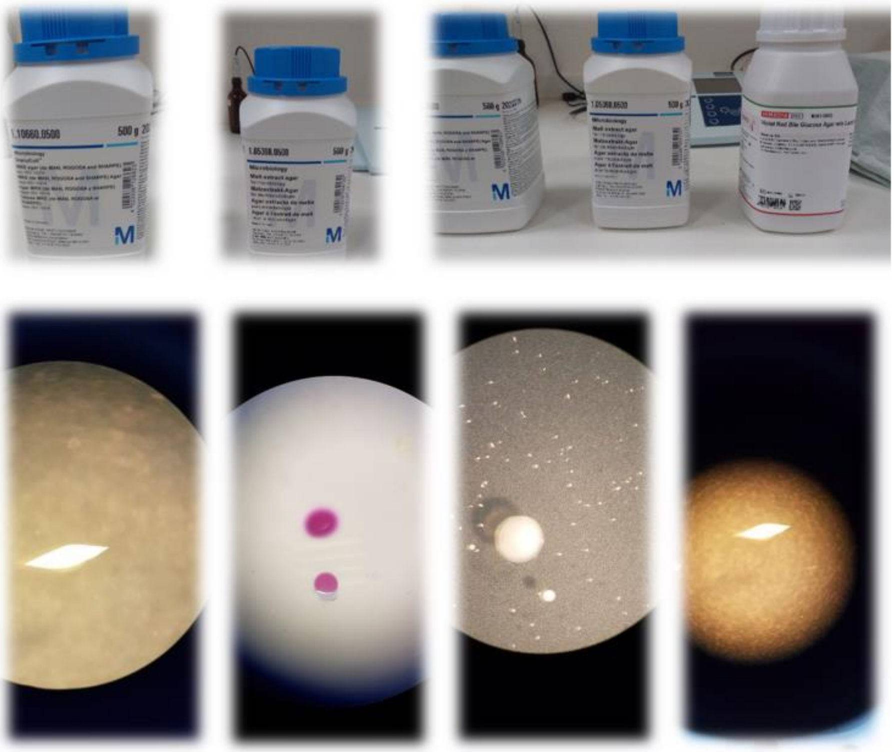

Figure 3. The media used to determine the microorganism population and the counted microorganism colonies.

For this purpose, the filtrates obtained from fresh materials according to a certain procedure were inoculated to agar media sterilized in an autoclave (WiseClave WAC-80) and kept in a water bath (WiseCircu WCB-22) under a sterile cabinet on agar media kept in a water bath (WiseCircu WCB-22). Pouring of the agar media and inoculating of the microorganism were made into disposable sterile plastic petri dishes. MRS and MEA media prepared to determine the LAB and yeast and mold numbers, respectively, were incubated in anaerobic conditions at 37 °C for 48 hours in a climate cabinet (Devpet Esde series). In addition, samples containing VRB-G prepared to determine the number of enterobacteria were incubated at 33 °C for 18 hours. A maximum of 300 colonies were counted in each petri dish.

2.5. Statistical Analysis

Microorganism population data obtained from present study were subjected to analysis of variance according to split-split plots in randomized block design with trial factors and year effect. As a result of variance analysis, microorganism populations that were found to be important ($P < 0.05$) statistically were grouped by Tukey pairwise test (Genç and Soysal, 2018).

3. Results and Discussion

In this study, lactic acid bacteria, enterobacteria and yeast and mold populations in the epiphytic flora of mung beans and sweet sorghum plants grown with different sowing patterns and intercropping systems and affecting silage quality were investigated. The effects of years, sowing patterns (SP), mixtures (M) and SP×M interaction on lactic acid bacteria population were found to be significant (Table 1). Lactic acid bacteria count results of the years, sowing patterns and mixtures were given in Table 1. In 2019 and 2020, lactic acid bacteria were 3.04 $\log_{10}$cfu g^{-1} DM and 2.96 $\log_{10}$cfu g^{-1} DM, respectively. The number of lactic acid bacteria was determined between 2.84 $\log_{10}$cfu g^{-1} DM and 3.30 $\log_{10}$cfu g^{-1} DM in sowing pattern treatments. The highest plant lactic acid bacteria were obtained from conventional row cultivation. The lowest plant lactic acid bacteria was obtained from twin row and this treatment was statistically in the same group with narrow row. The number of lactic acid bacteria in the mixtures varied between 2.03 $\log_{10}$cfu g^{-1} DM and 3.53 $\log_{10}$cfu g^{-1} DM. While the highest lactic acid bacteria were detected in the SS14 system, the lowest was obtained from the MB21 system.

According to interactions (SP×M), the number of plant lactic acid bacteria varied between 1.80 $\log_{10}$cfu g^{-1} DM and 4.03 log_{10}cfu g^{-1} DM (Figure 4). The highest lactic acid bacteria count was detected in the (R1:2) MB14+SS14 mixed system of conventional row cultivation. The lowest lactic acid bacteria were obtained from MB21 treatment of twin row. The lactic acid bacteria population obtained from the current study was lower in pure mung bean cultivation compared to pure sweet sorghum cultivation. A very high lactic acid population was detected in the epiphytic flora of pure sweet sorghum, and this situation was positively reflected in the number of lactic acid bacteria in the epiphytic flora of intercropping systems. The lactic acid bacteria of the intercropping systems were higher than those of the pure mung bean systems. Plants host many different microorganisms in their epiphytic flora and these microorganisms directly affects silage quality (Kung and Shaver, 2001). Wang et al. (2019) reported that lactic acid bacteria were predominant in alfalfa+sweet corn mixtures and pure sweet corn compared to pure alfalfa. Wang et al. (2017) found that even in maize harvest residues, the number of natural lactic acid bacteria was twice as high as in alfalfa and common vetch legume species. Similarly, the results regarding lactic acid bacteria counts obtained from this study were similar to the information highlighted above.

While the effects of years, Y×SP interaction, mixtures and SP×M interaction on plant enterobacteria numbers were significant, the effect of sowing patterns was insignificant (Table 1). The plant enthorobacteria numbers were determined as 4.93 log_{10}cfu g^{-1} DM in 2019, and 5.27 log_{10}cfu g^{-1} DM in 2020. Plant enterobacteria numbers varied between 4.87 log_{10}cfu g^{-1} DM and 5.39 log_{10}cfu g^{-1} DM in the interactions of years and sowing patterns (Figure 5). The highest number of enterobacteria was detected in narrow row in 2020 while the lowest was determined in the same sowing pattern in 2019 (Figure 5). The number of plant enterobacteria in the mixtures varied between 4.87 log_{10}cfu g^{-1} DM and 5.48 log_{10}cfu g^{-1} DM (Table 1). The highest number of plant enterobacteria was determined in the SS14 treatment. The lowest plant enterobacteria count was obtained from MB14 and MB21 treatments.

Table 1. Microbial changes in plant epiphytic flora according to trial years and treatments (sowing patterns and mixtures)

Treatments	Microbial Populations		
Years (Y)	Lactic acid bacteria	Enterobacteria	Yeast and Mold
2019	3.04±0.07 ^{a}	4.93±0.05 ^{b}	4.66±0.04 ^{b}
2020	2.96±0.07 ^{b}	5.27±0.05 ^{a}	4.79±0.04 ^{a}
P values (Y)	0.0046**	0.0120*	0.0007***
Sowing patterns (SP)			
Conventional row	3.30±0.07 ^{a}	5.11±0.05	4.48±0.06 ^{b}
Narrow row	2.88±0.07 ^{b}	5.13±0.08	4.86±0.05 ^{a}
Twin row	2.84±0.09 ^{b}	5.05±0.07	4.84±0.04 ^{a}
P values (SP)	< 0.0001***	0.2340^{ns}	< 0.0001***
P values (Y×SP)	0.6556^{ns}	0.0261*	0.4392^{ns}
Mixtures (M)			
MB14	2.37±0.06 ^{e}	4.87±0.08 ^{e}	4.28±0.04 ^{f}
MB21	2.03±0.08 ^{f}	4.87±0.10 ^{e}	4.42±0.08 ^{ef}
MB28	2.43±0.09 ^{e}	5.32±0.13 ^{ab}	4.78±0.07 ^{cd}
SS14	3.53±0.16 ^{a}	5.48±0.06 ^{a}	5.09±0.06 ^{a}
(R1:1)MB14+SS14	3.42±0.08 ^{abc}	5.16±0.07 ^{bcd}	5.05±0.08 ^{ab}
(R1:1)MB21+SS14	3.01±0.09 ^{d}	5.13±0.09 $^{b-e}$	4.75±0.09 ^{cd}
(R1:1)MB28+SS14	3.47±0.07 ^{ab}	5.08±0.08 $^{b-e}$	4.84±0.10 ^{bc}
(R1:2)MB14+SS14	3.21±0.06 ^{bcd}	4.98±0.13 ^{cde}	4.82±0.09 ^{bcd}
(R1:2)MB21+SS14	3.42±0.13 ^{abc}	4.90±0.22 ^{de}	4.58±0.12 ^{de}
(R1:2)MB28+SS14	3.16±0.10 ^{cd}	5.19±0.08 ^{abc}	4.65±0.05 ^{cde}
P values (M)	< 0.0001***	< 0.0001***	< 0.0001***
P values (Y×M)	0.9272^{ns}	0.7118^{ns}	0.2937^{ns}
P values (SP×M)	< 0.0001***	< 0.0001***	< 0.0001***
p values (Y×SP×M)	0.9822^{ns}	0.8093^{ns}	0.7460^{ns}
CV	7.77	4.00	4.16

^{a,b}Mean values with different superscripts in the same column indicate a significant difference ($P < 0.05$).

According to the SP×M interaction, enterobacteria numbers varied between 4.43 log_{10}cfu g^{-1} DM and 6.22 log_{10}cfu g^{-1} DM (Figure 6). The highest number of plant enterobacteria was determined in MB28 system of narrow row cultivation. The lowest plant enterobacteria count was obtained from MB28 application in twin row cultivation. The plant enterobacteria numbers obtained from the present study were generally close to each other. Plant enterobacteria counts in pure sweet sorghum plots were higher than other treatments. Fresh plants contain many different microorganisms in their epiphytic (natural) flora, which can lead to the formation of a wide variety of end products in the silo (Kung and Shaver, 2001). Enterobacteria can sometimes cause ethanol production during the first 48 hours of fermentation in the silo, which is an undesirable feature in this case (Kung et al., 2018). It has been reported that the number of enterobacteria in the plant varies according to the growing conditions (Kung et al., 2018). This study showed that the enterobacteria count in sweet sorghum epiphytic flora was higher than in epiphytic flora of mung bean.

The effects of years, sowing patterns, mixtures and SP×M interactions on yeast and mold populations in plant epiphytic flora were significant ($P < 0.001$). While the number of yeast and molds was 4.66 log_{10}cfu g^{-1} DM in 2019, it became 4.79 log_{10}cfu g^{-1} DM in 2020 (Table 1). Among the sowing patterns, plant yeast and mold numbers were determined between 4.48 log_{10}cfu g^{-1} DM and 4.86 log_{10}cfu g^{-1} DM. The highest plant yeast and mold counts were obtained from narrow row and the lowest value was in conventional row. In addition, there was no statistical difference between narrow row and twin row planting treatments (Table 1). The number of plant yeasts and molds in the mixtures varied between 4.28 log_{10}cfu g^{-1} DM and 5.09 log_{10}cfu g^{-1} DM. The highest plant yeast and mold counts were obtained from the SS14 application. The lowest number of plant yeast and mold was determined in MB14 treatment (Table 1). Depending on the interaction (SP×M), the number of plant yeasts and molds varied between 3.89 log_{10}cfu g^{-1} DM and 5.20 log_{10}cfu g^{-1} DM. The highest plant yeast and mold numbers were obtained from (R1:1) MB14+SS14 treatment in double row. The lowest plant yeast and mold numbers were found in MB14 application in conventional row cultivation (Figure 7).

Although the plant yeast and mold numbers obtained from this study were generally close to each other but the statistical differences occurred within the applications. Yeast and mold density in plant epiphytic flora can sometimes result in high ethanol content in the silo, which can limit the aerobic stability of silages (Kung et al., 2018). Therefore, high yeast and mold populations in the natural flora of the plant are an undesirable feature. Wang et al. (2017) determined that the number of natural plant yeasts in corn harvest residues, which is a forage crop, was higher than in the legume type, similar to current study.

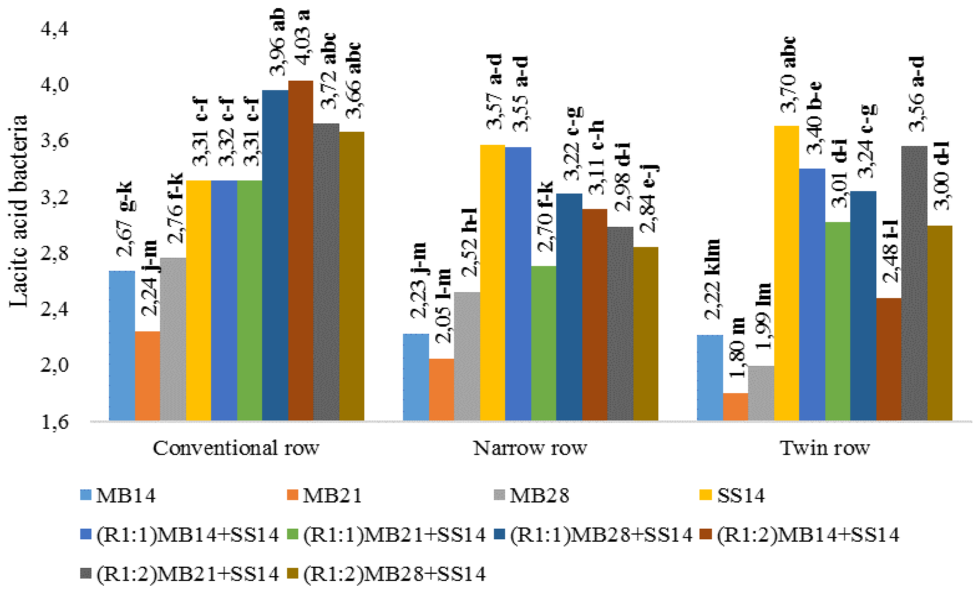

Figure 4. Lactic acid bacteria changes according to sowing pattern × mixture interactions.

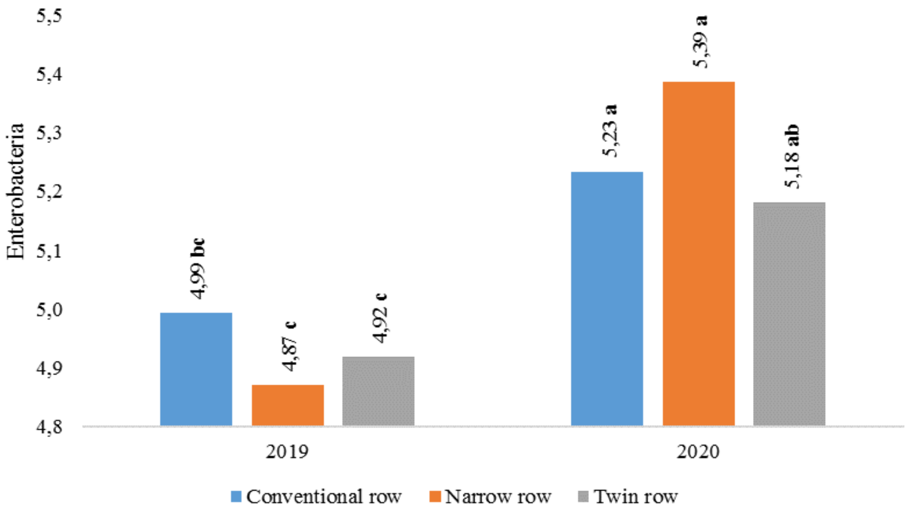

Figure 5. Entrerobacteria changes according to year × sowing pattern interactions.

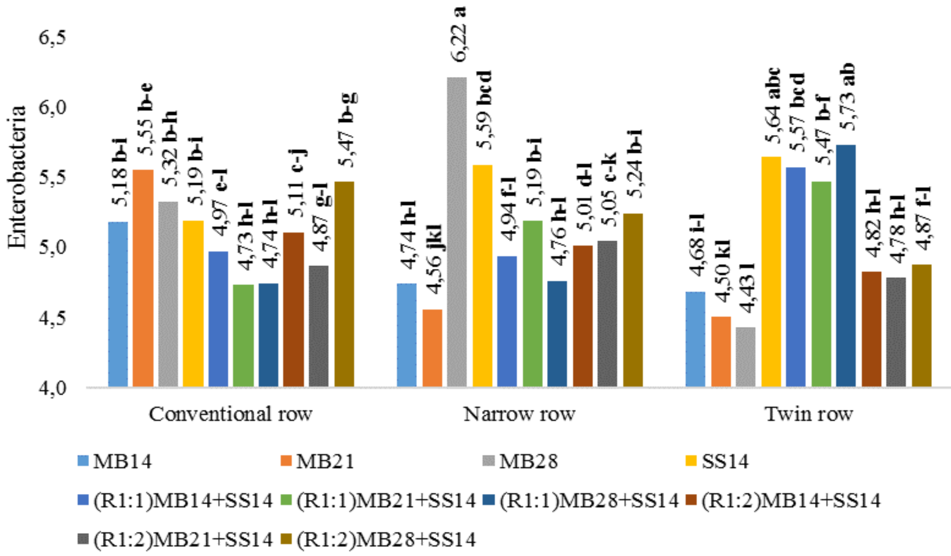

Figure 6. Entrerobacteria changes according to sowing pattern × mixture interactions.

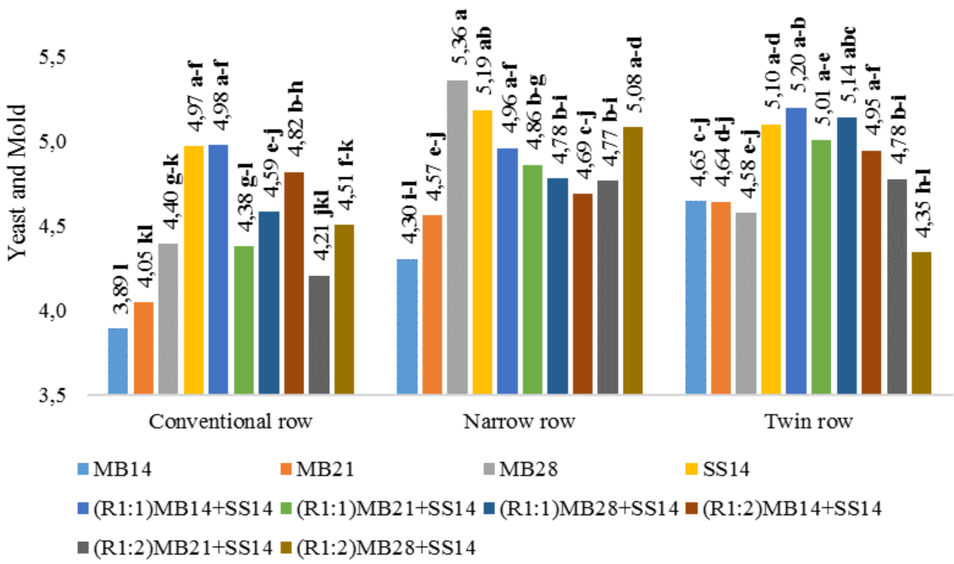

Figure 7. Yeast and mold changes according to sowing pattern × mixture interactions.

4. Conclusion

In this study, the change of microorganisms (lactic acid bacteria, enterobacteria, and yeast and molds) were investigated in mung bean and sweet sorghum grown with different sowing patterns and intercropping systems. The population of lactic acid bacteria, which has a positive effect on silage quality, was found to be higher in intercropping systems of conventional row method than others. On the other hand, enterobacteria, yeast and mold numbers, which are in the group of undesirable microorganisms for silage quality, were found to be lower in conventional row cultivation than others. Results from this study showed that intercropping systems of sweet sorghum and mung bean in conventional row method improved the population of lactic acid bacteria which is beneficial for silage quality.

Author Contributions

İ.E. and Ş.Y. wrote the manuscript and conceived the perspective, read, and approved the final manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Research Article
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PHYSICAL CHANGES OF SOME COLORED TABLE GRAPE VARIETIES DURING RIPENING

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Abstract: In this study, it was aimed to determine the physical changes of seven different colored table grape varieties in the samples taken at different periods until the ripening time. The cultivars used in the study are Alphonse Lavallée, Royal, Tekirdağ Çekirdeksizi, Michael Palieri, Karaerik, Bilecik İrikara and Horoz Karası. Grape samples were harvested for four weeks (20.08.2019, 27.08.2019, 02.09.2019 and 09.09.2019) every week for approximately one month until ripening. Cluster weight, bunch length and width, berry weight, berry width-length and berry hardness values were taken from the harvested grapes. Horoz Karası variety attracted attention with its cluster weight (550.86 g) and cluster width (15.01 cm) characteristics. The Michael Palieri variety stood out with its berry weight (8.92 g) and the Alphonse Lavallée variety with its berry hardness (0.94). Physical characteristics of all cultivars from fall to maturity differed according to both periods and cultivars. While the varieties and periods used in the study serve the literature, they are also an infrastructure for different studies with more varieties, different locations and different periods.

Keywords: Berry weight, Berry width, Berry hardness, Table grape

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1. Introduction

Considering the world agricultural activities, it can be stated that grape is the third most valuable product after tomato and potato with its economic value of 67.8 billion dollars in 2016 (Alston and Sambucci, 2019). According to the Food and Agriculture Organization of the United Naitons (FAO) data, viticulture reached a value of 167.90 billion dollars in 2018 and it is estimated that it will reach a value of 254.29 billion dollars by 2024. From the perspective of producers, viticulture activities are seen as an important source of income. Concentration of trade, increasing competitiveness in global markets has been an important goal for entrepreneurs and policy makers (Seccia et al., 2015).

Considering the statistics in recent years, it is seen that grape has increased despite the decrease in the vineyard areas. The reason for this is the positive effect of conscious cultural and chemical practices applied in viticulture on yield and quality per unit area. In particular, the development of technology and the reduction of vineyard areas have led to an increase in efforts to increase productivity. Various studies and researches are carried out on increasing productivity (Bahar et al., 2006; Sabır et al., 2010; Topuz, 2016).

Most of the grape in Turkey is used for table and raisin, and some for wine. The share of fresh grape production in our entire fruit production is % 50 (TUİK, 2019). The potential of table grape production should be recognized and factors such as domestic market, foreign market and producer requests should be taken into consideration (Kiracı et al., 2009; Söyler et al., 2019). In addition, grapes, which have an important place in human nutrition, have so many benefits for human health. Natural nutrition methods are recommended against increasing diseases in recent years. The fact that grapes are rich in antioxidant substances increases its importance even more. Therefore, it is necessary to increase the consumption of grapes (Cabaroğlu and Yılmaztekin, 2006).

One of the factors that determine the quality of grapes is ripening. If viticulture is to be carried out economically in a region, it is very important to know the optimum maturity of the variety to be grown. The ripening of grapes is affected by climatic conditions. Temperature, rains and sunshine duration for each variety to mature are variety specific (Winkler et al., 1974).

The climatic conditions of the Tokat province in Turkey; Being suitable for viticulture, it also enables the cultivation of commercially important table grapes. In the study, it was aimed to determine the physical changes that occur for 4 weeks until the harvest time in standard colored table grape varieties adapted to the conditions of Tokat province in Turkey.

2. Material and Methods

2.1. Material

This study was carried out in 2017 in the vineyard of Central Black Sea Transitional Zone Agricultural

Research Institute (40° 32' 17.20" N, 36° 45' 09.53" E). The planting density of the vineyard is 3.0 x 1.75 m. A midwire cordon support system is used in the vineyard. Grape varieties used as material in the study were grafted onto 1103 paulsen American rootstock and were planted with a double-arm cultivation system with a stem height of 70 cm.

2.2. Methods

Grape samples were harvested approximately 1 month before the ripening time of the varieties, every week and for a total of four weeks (20.08.2019, 27.08.2019, 02.09.2019 and 09.09.2019). Necessary processes in the harvested grapes were carried out in the laboratories of Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Horticulture in Turkey. Grape cultivars were harvested on 20 and 28 August, on 2 and 9 September, between 08:00 and 10:00 in the morning. In each harvest period, 10 clusters were taken from one replication and brought to the laboratory in ice containers. Analyzes of physical properties were made. The analyzes made are as follows;

2.2.1. Cluster weight

Samples of 3 clusters of each variety and replication harvested on the same days for four weeks were brought to the laboratory. The cluster weight was determined by weighing the samples with a precision balance (DENSI PC-100W model with 0.01 precision).

2.2.2. Cluster length and width (cm)

The length and width of the cluster, whose weight was taken, were measured with a ruler.

2.2.3. Physical properties of the berry

With 10 berry taken from each bunch (4-4-2), a total of 100 berry weight was taken. The width (mm) and length (mm) of 10 randomly selected berries from the granulated samples of each replication were measured with the help of caliper. The fruit flesh firmness of the same berries was measured with a precision scale (0.01 g) and a hardness meter (PCE. SLJ-B) with a 1.54 mm piercing tip.

2.2.4. Statistical analysis

The study was carried out according to the divided plot design with 3 replications and 6 vines in each replication. After the obtained data were subjected to analysis of variance, LSD (0.05) test was used to compare the means (Genç and Soysal, 2018). All the data of the cultivars during the harvest period were separately evaluated (random blocks) and analyzed.

3. Results and Discussion

When the varieties were examined among themselves, the differences in physical properties other than berry width and berry size were found to be statistically significant. The highest values in terms of cluster weight and cluster width were obtained in Horoz Karası (550.86 g; 15.01 cm) cultivar. The highest value in cluster length was obtained in Karaerik variety (22.61 cm). In terms of berry weight, Michele Palieri (8.92 g) stood out. Finally, when the berry hardness was examined, it was found that Alphonse Lavallée (0.94) had the highest value (Table 1).

When the physical properties were examined in terms of periods, the differences in all physical properties except berry width and berry weight were found to be statistically significant. When the characteristics that show differences are examined, the 4th period (438.68 g) comes to the fore in terms of cluster weight, while the 2nd period (398.96 g) follows it, and the 1st and 3rd periods (395.33; 376.20 g) are in the same group. In terms of cluster width, the 2nd and 3rd periods are in the same statistical group with the highest values (13.30; 13.19 cm) and the order changes as 4th period (12.62 cm) and 1st period (11.99 cm). In cluster length, the 4th period (21.04 cm) came to the fore, followed by the 2nd period (20.41 cm), the 1st period and the 3rd period (19.32; 18.83 cm) lastly took place in the same group. In terms of berry size, the 2nd, 3rd and 4th periods (23.38; 23.83; 23.78 mm) were in the same statistical group with the highest values. Berry hardness value was included in the same statistical group with the highest values in the 1st and 3rd periods (0.92; 0.85) (Table. 2).

Table 1. Cluster weight (g), cluster width (cm), berry width-length (mm), berry hardness values of the cultivars*

Cultivar	Cluster weight (g)	Cluster width (cm)	Cluster length (cm)	Berry weight (g)	Berry width (mm)	Berry width (mm)	Berry harness
Alphonse Lavallée	352.42^{cd}	13.06^{b}	19.67^{bc}	7.52^{b}	22.22	22.84	0.94^{a}
Bilecik İrikara	389.70^{bc}	10.90^{c}	17.98^{cd}	3.95^{d}	17.6	18.37	0.85^{ab}
Horoz Karası	550.86^{a}	15.01^{a}	20.13^{b}	8.90^{a}	36.76	30.63	0.93^{a}
Karaerik	372.28^{cd}	12.18^{b}	22.61^{a}	5.48^{c}	19.47	21.64	0.74^{bc}
Michele Palieri	441.34^{b}	12.89^{b}	22.33^{a}	8.92^{a}	23.21	26.03	0.84^{ab}
Royal	381.32^{cd}	13.06^{b}	19.11^{bcd}	8.12^{b}	23.14	23.75	0.78^{bc}
TÇ	328.14^{d}	12.35^{b}	17.46^{d}	4.96^{c}	18.81	20.27	0.66^{c}
LSD	57.14	1.24	2.11	0.66	N.S	N.S	0.12

*Mean values with different superscripts in the same effects indicate a significant difference (P<0.05).

TÇ= Tekirdağ çekirdeksiz

Table 2. Cluster weight (g), cluster width (cm), berry width-length (mm), berry hardness values of the periods*

Period	Cluster weight (g)	Cluster width (cm)	Cluster length (cm)	Berry weight (g)	Berry width (mm)	Berry length (mm)	Berry hardness
1	376.20^{b}	11.99^{b}	19.32^{b}	6.66	20.55	22.45^{b}	0.92^{a}
2	398.96^{ab}	13.30^{a}	20.41^{ab}	6.75	20.72	23.38^{a}	0.75^{b}
3	395.33^{b}	13.19^{a}	18.83^{b}	7.15	20.86	23.83^{a}	0.85^{a}
4	438.68^{a}	12.62^{ab}	21.04^{a}	6.78	29.99	23.78^{a}	0.74^{b}
LSD	43.19	0.94	1.6	N.S	N.S	0.62	0.08

*Mean values with different superscripts in the same effects indicate a significant difference (P<0.05).

When the physical properties of the cluster and the berry were examined in terms of the interaction of the variety X period, only the differences in the cluster weight and cluster width were found to be statistically significant. The highest value in terms of cluster weight was obtained from the 2nd period of Horoz Karası (649.49 g), while the lowest value was obtained from the 4th period of Tekirdağ Çekirdeksiz (279.40 g). In terms of cluster width, Horoz Karası 2nd period stood out again (17.56 cm), while the lowest value was obtained in Bilecik İrikara 1st period (9.67 cm). The results of the cluster weight, cluster width and other physical properties are as in Table 3.

Grape is not a climacteric fruit and is consumed when it is harvested. In determining the maturity of table and wine grapes, physical properties such as appearance of the fruit, skin color, berry size, presence of firm and spilled berries, and stem rupture resistance are taken into account along with chemical properties (Kara and Gerçekcioğlu, 1993). The characteristics (shape, color, width, length) of the cluster, which is the structure formed by the combination of grape berries, are also physical criteria for grapes.

Table 3. Cluster weight (g), cluster width-length (cm), berry width-length (mm), berry hardness values of cultivar X period interaction*

Cultivar	Period	Berry weight (g)	Berry width (mm)	Berry length (mm)	Berry harness	Cluster weight (g)	Cluster width (cm)	Cluster length (cm)
Alphonse Lavallée	1	7.14	21.81	21.98	0.91	403.42^{e-I}	12.78^{b-F}	20.67
Alphonse Lavallée	2	7.95	22.49	23.44	0.82	347.89^{g-K}	13.06^{b-F}	20.67
Alphonse Lavallée	3	7.67	22.35	23.09	0.89	334.11^{h-K}	13.67^{b-E}	17.22
Alphonse Lavallée	4	7.33	22.24	22.85	1.14	324.27^{h-K}	12.72^{b-F}	20.11
Bilecik İrikara	1	3.00	16.16	16.75	1.00	283.94^{j-K}	9.67^{h}	15.17
Bilecik İrikara	2	4.22	18.03	18.60	0.77	368.47^{e-K}	10.89^{f-G-H}	17.44
Bilecik İrikara	3	4.44	17.85	19.03	0.84	472.69^{c-F}	12.22^{d-G}	19.89
Bilecik İrikara	4	4.13	18.34	19.09	0.77	433.69^{d-H}	10.80^{f-G-H}	19.42
Horoz Karası	1	9.01	21.51	29.85	0.95	470.78^{c-F}	12.47^{c-F}	18.39
Horoz Karası	2	9.06	20.95	30.78	0.77	649.49^{a}	17.56^{a}	22.67
Horoz Karası	3	8.61	20.49	30.47	1.11	476.80^{c-D-E}	14.89^{b-C}	18.78
Horoz Karası	4	8.91	84.08	31.41	0.90	606.36^{a-B}	15.11^{a-B}	20.67
Karaerik	1	4.75	18.29	20.08	0.88	308.20$^{ı-J-K}$	10.78^{f-G-H}	22.33
Karaerik	2	5.37	19.38	21.42	0.79	382.47^{e-K}	13.28^{b-F}	23.67
Karaerik	3	5.71	19.73	22.20	0.67	455.24^{c-G}	13.22^{b-F}	20.56
Karaerik	4	6.10	20.47	22.84	0.62	343.20^{g-K}	11.44^{e-H}	23.89
Michele Palieri	1	9.27	23.88	25.07	0.99	432.09^{d-H}	11.61^{e-H}	20.78
Michele Palieri	2	7.64	22.15	25.40	0.69	376.51^{e-K}	13.11^{b-F}	22.33
Michele Palieri	3	9.61	22.94	26.87	0.87	394.02^{e-J}	12.45^{c-G}	20.00
Michele Palieri	4	9.15	23.87	26.79	0.79	562.73^{a-B-C}	14.39^{b-D}	26.22
Royal	1	8.24	23.10	23.39	0.91	359.44^{f-K}	12.83^{b-F}	19.00
Royal	2	8.13	23.32	24.15	0.81	319.73^{h-K}	13.00^{b-F}	18.33
Royal	3	8.53	23.31	23.90	0.87	324.99^{h-K}	12.44^{c-G}	18.00
Royal	4	7.56	22.82	23.58	0.52	521.13^{b-C-D}	13.94^{b-E}	21.11
TÇ	1	5.21	19.10	20.01	0.79	375.52^{e-K}	13.78^{b-E}	18.89
TÇ	2	4.84	18.70	19.90	0.62	348.15^{g-K}	12.22^{d-G}	17.78
TÇ	3	5.49	19.36	21.26	0.74	309.49$^{ı-J-K}$	13.44^{b-E}	17.33
TÇ	4	4.31	18.10	19.93	0.48	279.40^{k}	9.94^{g-H}	15.83
	LSD	N.S	N.S	N.S	N.S	114.31	2.51	N.S

*Mean values with different superscripts in the same effects indicate a significant difference (P<0.05).
TÇ= Tekirdağ çekirdeksiz

Parameters such as cluster weight and cluster size vary according to grape varieties. It is not only the variety that affects the cluster structure and properties, but also; Many factors such as ecological conditions, presence of buds and its condition on the shoot, cultural processes applied to the vine also come into play as influencing factors (Çelik et al., 1998; Çelik, 2011; Kamiloğlu and Üstün, 2014).

There is an increase in weight and volume in the period from berry setting to ripening. Although this increase varies according to cultivar characteristics, just like in cluster characteristics, it is also closely related to factors such as pruning, precipitation, sun exposure, light, soil characteristics, spraying, and plant growth regulators (Ağaoğlu, 2002).

In a study conducted by Cangi et al. (2011) with wine varieties in Kazova region, the averages of cluster weights of Gewürztraminer, Pinot Noir, Syrah and Narince varieties differed between 2007 and 2008. This difference was attributed to the difference between cultivars and years. The fact that the findings of the cluster weights in the study made a statistical difference between both cultivars and periods shows parallelism with this study. In another study, the weights of clusters in different parts of the vine were examined in Cardinal and Amasya grape varieties in Çanakkale ecology, and as a result of the research, it was concluded that the averages of the two varieties varied according to the places. In this case, it is a proof that not only the variety but also the cluster characteristics can vary even in the same vine. Verigo, Horoz Karası, Altoni Red, Ergin Çekirdeksizi, Perlette and Italia cultivars were used in another study in which three-year (2004, 2005, 2007) data were obtained on some table varieties in KKTC ecological conditions. Different varieties have come to the fore every year in terms of cluster weight. This shows that the period (year) difference is effective in cluster weight as in our study. While the Horoz Karası variety appeared as the variety with the highest cluster weight average (50.86 g) in the study, this study also showed a parallel value with the study, with an average weight of 468-736 g (Tangolar et al., 2007). In the study carried out with Boğazkere, Chardonay, Emir, Merlot, Narince, Öküzgözü, Riesling varieties in Kazova region, it was reported that the berry size increased until the harvest period and this varied according to the cultivars (Şen, 2008). In the study, similar to this study, the differences in berry weight were important in terms of varieties.

In a study conducted by Aydın (2015) on the determination of some chemical contents of grape varieties grown in Amasya at different maturity periods, the averages of 100-berry weights taken in three different periods (one week before the harvest, one week before the harvest and one week after the harvest), respectively; It has been reported that it is in the red pointed fragrant grape variety with 774. 56 g, 838. 44 g and 861. 63 g and it varies according to the varieties in parallel with the study.

4. Conclusion

Considering the effect of sampling periods in the study on cluster characteristics, cluster weights increased towards maturation. Cluster width reached its highest values in the 2nd and 3rd periods. The highest value of cluster length is the fourth period. Considering the effects of the periods on the grain properties, the effects of grain weight and grain width were found to be insignificant. Berry size value found its highest value in the 2nd, 3rd and 4th periods. Berry hardness changed according to the periods and the highest values were determined in the 1st and 3rd periods.

In the study, the physical properties of all cultivars from mole to ripening differed according to both periods and cultivars. In recent years, it is known that people's perception of the food they consume focuses on quality rather than quantity. We can minimize quality losses by harvesting the best quality grapes at the right time. While the varieties and periods used in the study serve the literature, they are also an infrastructure for different studies with more varieties, different locations and different periods.

Author Contributions

All authors have equal contribution and all authors reviewed and approved the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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CHEMICAL COMPOSITION, METABOLISABLE ENERGY, ORGANIC MATTER DIGESTIBILITY AND METHANE PRODUCTION OF SOME TANNIN CONTAINING FORAGES

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Abstract: The aim of the current experiment was to evaluate the chemical composition, gas, methane production, metabolisable energy (ME), organic matter digestibility (OMD) of some tannin containing hays. There are significant variations among hays in terms of the chemical composition. Crude protein contents of hays ranged from 14.3 to 23.5% with the highest being for Marrubium supinum hay and lowest for Anthyllis circinata hay. Neutral detergent fiber contents of hays ranged from 40.6 to 57.7% with the highest being for Polygonum aviculare hay and lowest for Scorpinus muricatus hay. Acid detergent fiber contents of hays ranged from 22.5 to 32.9% with the highest being for Lotus corniculatus hay and lowest for Scorpinus muricatus hay. Condensed tannin contents of hays ranged from 0.7 to 7.3% with the highest being for Polygonum aviculare hay and lowest for Marrubium supinum hay. Gas production of tannin containing hays ranged from 77.5 and 105.5 ml/0.5 g DM with the highest being for Anthyllis circinata and Scorpinus muricatus, and lowest for Marrubium supinum. Metabolisable energy content of legume hays varied between 7.6 and 9.1 MJ/kg DM with the highest being for Scorpinus muricatus hay and lowest for Cichorium intybus, Bituminaria bituminosa and Marrubium supinum hays. Organic matter digestibility of legume hays varied between 58.2 and 72.4% with the highest being for Scorpinus muricatus hay and lowest for Bituminaria bituminosa hay. The tannin containing hays investigated in the current experiment will provide not only protein but also fiber for ruminant animals. In addition they had low anti-methanogenic potential. The current experiment will provide information for the nutritionist to prepare well balanced diets for ruminants animals. However further in vivo experiments are required to determine the feed intake and anti-methanogenic potential of hays.

Keywords: Forage, Chemical composition, Tannin, Digestibility, Metabolisable energy, Methane emission

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1. Introduction

Forage will provide not only nutrients but also fiber for ruminant animal to meet their requirement. There are some forages in pasture which contains considerable amount of condensed tannin which may have potential on mitigation of enteric methane production when they are consumed by ruminant animals. Although there are a lot of studies involved in chemical composition of forages there is a lack of information about condensed tannin and anti-methanogenic potential of forages. *In vitro* gas production technique is widely used to evaluate forages in terms of potential nutritive value, metabolisable energy and organic matter digestibility for ruminant animals (Kamalak et al., 2004; Kamalak et al., 2005; Ozturk et al., 2006; Kamalak et al., 2010; Kamalak and Canbolat, 2010; Kamalak et al., 2011; Ozkan et al., 2017; Atalay et al., 2018; Boga et al., 2020; Kamalak et al., 2021). It is well known that some anti-nutritive factors such as tannin and saponin decrease the enteric methane production from ruminant animals. Therefore the aim of the current experiment was to evaluate the chemical composition, ME, OMD, gas and anti-methanogenic potential of tannin containing forages using in vitro gas production technique.

2. Material and Methods

2.1. Tannin Containing Hays

Hays obtained from 3 replicate plots established in the experiment field at flowering stage from7 different plant species namely, *Anthyllis circinata*, *Cichorium intybus*, *Scorpinus muricatus*, *Lotus corniculatus*, *Bituminaria bituminosa*, *Polygonum aviculare*, *Marrubium supinum* in 2019 in Turkey were dried in 65 °C until a constant weight. Hay samples were then milled to pass a 1 mm sieve for chemical analysis and *in vitro* gas production assay.

2.2. Chemical Analysis of Hays

Dry matter (DM), crude ash (CA), crude protein (CP) and ether extract (EE) contents of hay samples were analyzed according to AOAC (2005). Neutral detergent fiber (NDF) and ADF contents of hay samples using the method described by Van Soest and Wine (1967) and Van Soest

(1963) respectively. Condensed tannin contents of forages were determined by the Butanol –HCL method (Makkar, 1995). All chemical analyses were carried out in triplicate.

2.3. *In Vitro* Gas Production of Hays

Approximately 500 mg of hay samples were incubated in 100 mL calibrated glass syringes in triplicate for 24 h in a water bath set at 39 °C with buffered rumen fluid of three fistulated for anaerobic fermentation. Rumen fluid used in vitro gas production was obtained from slaughter house in Kahramanmaras. In vitro gas production trial of hay samples was carried out according to the method described by Menke et al (1979).

Metabolisable energy (ME, MJ/kg DM) and organic matter digestibility (OMD) of hay samples were determined using equations suggested by Menke and Steingass (1988) (equation 1 and 2).

$$ME\ (MJ/kg\ DM) = 2.20 + 0.1357GP + 0.057CP + 0.002859EE^2 \quad (1)$$

$$OMD\ (\%) = 14.51 + 0.88490GP + 0.448CP + 0.686CA \quad (2)$$

Where; GP= 24 h net gas production (ml/200 mg), CP= Crude protein (%), EE= Ether extract (%), CA= Ash content (%), Methane content (%) of total gas produced after 24 hour fermentation were determined using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al., 2008). The amount of methane (ml) was calculated using the formula given below (equation 3).

$$\text{Methane production (mL)} = \text{Total gas production (mL)} \times \text{Percentage of methane (\%)} \quad (3)$$

2.4. Statistical Analysis

The effect of species on chemical composition gas production, methane production, ME and OMD of tannin containing hays. Differences between means were identified by Tukey test (Genç and Soysal, 2018). Mean differences were considered significant at $P<0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

3. Results and Discussion

The effects of species on the chemical composition of tannin containing hays were given in Table 1. Species had a significant effect on the chemical composition of tannin containing hays.

Table 1. Species had a significant effect on the chemical composition of tannin containing hays.

Hays	DM	CA	CP	EE	NDF	ADF	CT
Anthyllis circinata	92.8^{bc}	10.4^{d}	14.3^{g}	3.5^{b}	49.6^{c}	28.2^{e}	1.3^{bc}
Cichorium intybus	93.7^{b}	17.3^{a}	18.1^{e}	3.5^{b}	56.7^{a}	32.1^{b}	1.3^{bc}
Scorpinus muricatus	91.6^{c}	13.3^{b}	20.2^{c}	4.7^{ab}	40.6^{d}	22.5^{f}	1.6^{bc}
Lotus corniculatus	94.4^{a}	7.7^{e}	19.4^{d}	4.2^{ab}	51.1^{b}	32.9^{a}	2.3^{b}
Bituminaria bituminosa	89.0^{d}	6.8^{f}	15.2^{f}	4.2^{ab}	42.2^{d}	29.6^{d}	1.1^{c}
Polygonum aviculare	94.8^{a}	12.4^{c}	21.4^{b}	4.9^{a}	57.7^{a}	30.7^{c}	7.3^{a}
Marrubium supinum	93.2^{b}	12.0^{c}	23.5^{a}	4.9^{a}	44.8^{cd}	27.1^{e}	0.7^{c}
SEM	0.353	0.194	0.207	0.347	1.611	0.493	0.326
P	***	***	***	***	***	***	***

^{ab}Column means with common superscripts do not differ (P>0.05), SEM= standard error mean, DM= dry matter (%), CA= crude ash (%), CP= crude protein (%), EE= Ether extract (%), NDF= neutral detergent fiber (%), ADF= acid detergent fiber (%), CT= condensed tannin (%).

Crude ash contents of hays ranged from 6.8 to 17.3% with the highest being for Cichorium intybus hay and lowest for Bituminaria bituminosa hay. Crude protein contents of hays ranged from 14.3 to 23.5% with the highest being for Marrubium supinum hay and lowest for Anthyllis circinata hay. CP contents of forages used ruminant diets should be higher than 8% of DM to meet maintenance requirement (Norton, 1994). In addition, CP contents of forages used ruminant diets should not be less than 10% to avoid low dry matter intake (Ranjhnan, 2001). As can be seen from Table 1 hays investigated in the current study had a CP contents that higher than those requested for maintenance and proper food intake, which can be used as a protein supplement for poor quality forages to improve productivity of ruminant animals.

Ether extract contents of hays ranged from 3.5 to 4.9% with the highest being for *Polygonum aviculare* and *Marrubium supinum* hays, and lowest for *Anthyllis circinata* and *Cichorium intybus* hay. Neutral detergent fiber contents of hays ranged from 40.6 to 57.7% with the highest being for *Polygonum aviculare* hay and lowest for *Scorpinus muricatus* hay. Acid detergent fiber contents of hays ranged from 22.5 to 32.9% with the highest being for *Lotus corniculatus* hay and lowest for *Scorpinus muricatus* hay. Condensed tannin contents of hays ranged from 0.7 to 7.3% with the highest being for *Polygonum aviculare* hay and lowest for *Marrubium supinum* hay. Except for *Polygonum aviculare*, the CT contents of forages investigated is not likely detrimental on the digestibility and animal performance.

Yusuf and Muritala (2013) suggested that wide variation in chemical composition can be expected among forages even if they were grown in the same environmental conditions and harvested at the similar maturity due to the inherent characteristics of forages associated with ability to extract and accumulate nutrients from soil and fix nitrogen from atmosphere. Some of differences among forages in terms of chemical composition may be associated with differences in leaf: stem ratio, which may results in differences in chemical composition, especially in NDF and CP contents of forages.

As can be seen Table 1, forages with high cell contents investigated in the current experiment will provide not only CP but also fiber for ruminant animals. NRC (1989) recommends that dairy cow ration should contain of 25% NDF of DM with 75% of the NDF from forages whereas feed intake of dairy cattle decreased with increasing NDF content of diets ranging from 22.5 to 45.8% (Arelovich et al., 2008).

The gas production, methane production, metabolisable energy and organic matter digestibility of tannin containing hays were given in Table 2. Species had a significant effect on the gas production, methane production, ME and OMD of tannin containing hays. Gas production of tannin containing hays ranged from 77.5 and 105.5 ml/0.5 g DM with the highest being for *Anthyllis circinata* and *Scorpinus muricatus*, and lowest for *Marrubium supinum*. The differences among hays in terms of gas production might be associated to compositional differences of hays, especially cell contents and CT contents. The extent of total gas production depends on the available carbohydrate for fermentation of rumen micro-organism (Blümmel and Orskov, 1993). However, the presence of secondary metabolites such as tannin and saponin in hay may affect the extent of gas produced during fermentation (Kondo et al., 2014; Jayanegara et al., 2014).

Generally, the percentage methane of usual feeds such as hay, concentrate or mixture of hay and concentrate range from 16 to 20%. Feedstufs can be classified in terms of anti-methanogenic potential using percentage of methane production after 24 h anaerobic fermentation (Lopez et al., 2010) According to this classification, most of hay samples had a low anti-methanogenic potential since the percentage of methane fell into the range of >11% and ≤14%. Metabolisable energy content of legume hays varied between 7.6 and 9.1 MJ/kg DM with the highest being for *Scorpinus muricatus* hay and lowest for *Cichorium intybus*, *Bituminaria bituminosa* and *Marrubium supinum* hays. Organic matter digestibility of legume hays varied between 58.2 and 72.4% with the highest being for *Scorpinus muricatus* hay and lowest for *Bituminaria bituminosa* hay.

Table 2. The gas production, methane production, metabolisable energy and organic matter digestibility of tannin containing hays

Hays	Gas	CH_4 (%)	CH_4 (ml)	ME	OMD
Anthyllis circinata	105.5^{a}	14.9^{b}	12.7^{c}	8.7^{ab}	66.6^{c}
Cichorium intybus	80.75^{d}	12.7^{c}	14.1^{b}	7.6^{c}	65.5^{bc}
Scorpinus muricatus	105.2^{a}	14.1^{bc}	12.0^{c}	9.1^{a}	72.4^{a}
Lotus corniculatus	100.7^{ab}	16.8^{a}	14.9^{b}	8.8^{ab}	66.5^{bc}
Bituminaria bituminosa	86.2^{cd}	14.2^{bc}	14.8^{b}	7.8^{c}	58.2^{d}
Polygonum aviculare	92.2^{bc}	13.4^{bc}	13.0^{c}	8.5^{b}	68.2^{b}
Marrubium supinum	77.5^{d}	14.0^{bc}	16.2^{a}	7.8^{c}	64.1^{c}
SEM	0.328	0.337	0.501	0.179	1.115
P	***	***	***	***	***

^{ab}Column means with common superscripts do not differ (P>0.05), SEM= Standard error mean, GP= gas production (ml), CH_4= methane production, ME= metabolisable energy (MJ/kg DM) , OMD= organic matter digestibility(%).

4. Conclusion

There are significant variation among hay samples in terms of chemical composition and potential nutritive value. The tannin containing hays investigated in the current experiment will provide not only protein but also fiber for ruminant animals. In addition they had low anti-methanogenic potential. The current experiment will provide information for the nutritionist to prepare well balanced diets for ruminants animals. However further in vivo experiments are required to determine the feed intake and anti-methanogenic potential of hays.

Author Contributions

All authors have equal contribution and the authors reviewed and approved the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Approval

Ethical approval is not required, because this article does not contain any studies with human or animal subjects.

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EFFECTS OF TEMPERATURE AND PRECIPITATION ON TEA YIELD IN TURKEY

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Abstract: The study aims to determine the changes in annual tea yield in Turkey between 1975 and 2019 and analyse the yield relationship with temperature and precipitation conditions. Within the scope of the study, statistical relationships between monthly temperature and precipitation data and annual tea yield were examined. In addition, the annual changes and trends in the yearly tea yield were revealed. As a result of the study, a statistically significant positive relationship was determined between annual average, maximum and minimum temperatures and annual tea yield. On the other hand, the relationship between total yearly precipitation and annual tea yield was insignificant. Relationships between temperature and yield are more substantial in summer. A statistically significant increasing trend was defined in tea yield during the 1975-2019 period in the research area. There was a considerable increase in annual average temperatures in the same period but an insignificant increase in precipitation. In the light of these, an increase in tea yield due to temperature rises can be expected in the region by looking at climate change scenarios.

Keywords: Turkey, Climate change, Tea yield, Temperature, Precipitation

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1. Introduction

Known as the second most consumed beverage in the world, tea "*Camellia sinensis* (L.)" is a plant made from the young leaves and buds of the tea plant belonging to the Theaceae family (Üstün and Demirci, 2013). India, China, Sri Lanka, Kenya, Turkey, Indonesia, Bangladesh, Argentina, Malawi are the countries that produce the most tea in the world (Koday, 2014). According to FAO statistics, tea farming areas in the world reached 4193 thousand hectares in 2018; dry tea production was 6338 thousand tons in 2018 and Turkey, which ranks 7th in terms of the breadth of tea agricultural lands, ranked 5th in dry tea production. According to the 2017-2018 data of the Indian Tea Board, Turkey ranks first in the world in tea consumption per capita with 3.2 kilograms per year (FAOSTAT, 2018; ÇAY-KUR, 2019). On the other hand, Turkey ranks 31st in world tea exports and 25th in world tea imports (Republic of Turkey Ministry of Agriculture and Forestry, 2020). In Turkey, the tea plant is grown in the Eastern Black Sea Region, starting from the Soviet Union border and extending to Fatsa in the west (Horuz and Korkmaz, 2006).

Bhagat et al. (2010) discuss that climate has always been considered important in crop production. The tea plant, which has a vast ecological amplitude, can be grown in various climate conditions. The tea plant, which requires plenty of precipitation, can grow in acidic soils and have a tree's appearance. It is also an evergreen plant that can grow from 1 meter to 18 meters depending on its type. The yield of the tea plant, which has an economic life of 50-60 years, varies depending on climatic conditions and pruning-care conditions. (Kurt and Hacıoğlu, 2013). Micro-climatic conditions influence tea cultivation even for optimum development (Rahman et al., 2017). It shows that temperature is the main environmental variable affecting shoot extension; low temperatures significantly reduce yields, especially in the cold season (Tanton, 1982).

As stated in RCP4.5 and RCP8.5 scenarios, prepared by Akçakaya et al. (2015), for the geography of MGM including Turkey, according to the climate projections made using the GFDLESM2M global model data, it is predicted that the temperatures will increase in all of Turkey and the precipitation will decrease for Turkey in the period of 2016-2099. Although many effects of global climate change are expected, one of the most important effects is agriculture (Mendelsohn, 2008). Global climate variability could explain more than 30% of the inter-annual variability of crop yields (Ray et al., 2015; An et al., 2020). The variability in temperature has a decisive role specifically in the yield of horticultural crops (Wheeler et al., 2000; Ustaoğlu and Karaca, 2010), and the beginning, end and length of the growing period are related to temperature (Chmielewski, 1992). The effects of climate change on agriculture due to the increase in greenhouse gases in the atmosphere are expected to differ on a regional basis depending on the type of product, temperature rise and changes in precipitation regime (Rosenzweig and Hillel, 1995; McCarl at al., 2001; Tubiello at al., 2002).

It is very important to examine the spatial and temporal differences in tea yield and determine the effects of temperature and precipitation conditions on yield, due to its importance for the economy of the region and Turkey, and the fondness of Turkish people for its consumption. In this context, the aim of the study: 1) Is to reveal the statistical relationships between temperature and precipitation conditions and tea yield in the 1975-2019 period. 2) To determine the inter-year changes and trends in tea yield for 1975-2019. 3) To make inferences about the effects of possible temperature and precipitation changes on yield according to climate change scenarios by analysing the changes observed in temperature and precipitation conditions during the 1975-2019 period.

2. Material and Methods

2.1. Materials

The field of study covers Giresun, Trabzon, Rize, and Artvin provinces, which meet 99.9% of Turkey's tea production according to 2019 agricultural statistics published by TURKSTAT (Table 1 and Figure 1). Since the tea production in Ordu, where very little production is made and the fluctuation from year to year is very high, was included in Giresun in the data sources before 2004, it was also included in the Giresun data in this study.

Table 1. General information about agricultural land and tea production (HGM, 2014; TURKSTAT, 2020)*

Provinces	TF (decare)	AA (decare)	TPA (decare)	AA/ PA (%)	TPA/AA (%)	TPA/PTA (%)	TP in 2019 (ton)
Ordu	5861000	2528301	95	43.1	0.1	0.0	41
Giresun	7025000	1418759	20312	20.2	0.3	1.4	30710
Trabzon	4628000	944251	186069	20.4	4.0	19.7	325031
Rize	3835000	560847	552866	14.6	14.4	98.6	905650
Artvin	7393000	300466	89459	4.1	1.2	29.8	146016

*TURKSTAT (2020) and HGM (2014).

TF= total field, AA= agricultural area, TPA= tea production area, PA= province area, PTA= province total area, TP= tea production (ton)

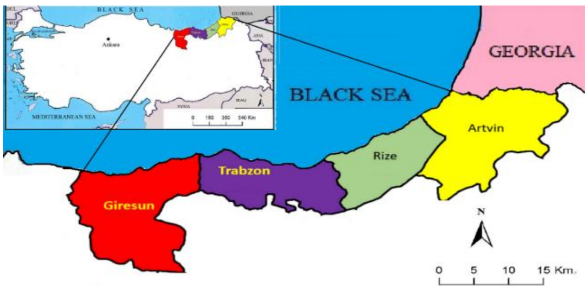

Figure 1. Location of the research area.

In the research area, the rainiest season of the year is autumn in Giresun, Trabzon and Rize, while it is winter in Artvin. The annual total precipitation for the 1975-2019 period varies between 825.3 mm (Trabzon) and 1291.3 mm (Giresun). Annual average temperatures are between 12.3 °C (Artvin) and 14.8 °C (Giresun). The annual temperature difference is the highest in Artvin (17.3 °C) and the least in Giresun (16.4 °C). While the coldest month is February in Giresun, Trabzon and Rize, it is January in Artvin. The hottest month is August in all provinces (Table 2 and Figure 2).

2.2. Data Collection

This study used monthly total precipitation, monthly average temperature, monthly average maximum temperature, and monthly average minimum temperature data of Giresun, Trabzon, Rize and Artvin meteorological stations. The data were obtained from the Turkish State Meteorological Service. Also, it was extracted monthly temperature and precipitation records from the climate dataset gridded at 0.5 intervals from KNMI Climate Explorer (http://climexp.knmi.nl) for each province. These data were found to be compatible with the data of Turkish State Meteorological Service.

The data on tea production areas and tea production amounts covering the period 1975-2003 were taken from the State Institute of Statistics agricultural statistics and the General Directorate of Tea Enterprises (ÇAY-KUR). The data for the period 2014-2109 were obtained from the website of the Turkish Statistical Institute. Since reliable data on the annual tea yield were not available on a provincial basis before 1975, the analysis included 1975 and later.

2.3. Statistical analyses

The analysis of the data was conducted in three phases. Firstly, statistical relationships between monthly temperature and precipitation data and annual tea yield were examined. Pearson Correlation Coefficient and simple linear regression analysis method were used to determine the relationships. The mathematical correlation of the relationship between two or more variables is examined by regression analysis, and the direction and degree of the relationship is questioned by correlation analysis (Ersöz and Ersöz, 2020). Linear regression tests the relationship between X and Y variables and whether a linear trend exists (Karabulut and Cosun, 2009).

Table 2. Distribution of precipitation in the period 1975-2019 (Edited from the data of the Turkish State Meteorological Service)

Station	Total annual precipitation	Seasonal precipitation totals (mm) and percentages							
		Winter		Spring		Summer		Autumn	
		mm	%	mm	%	mm	%	mm	%
Giresun	1291.3	340.4	26.4	245.3	19.0	248.9	19.3	456.8	35.4
Trabzon	825.3	222.3	26.9	172.7	20.9	136.4	16.5	293.8	35.6
Rize	2247.0	634.2	28.2	334.1	14.9	469.4	20.9	809.4	36.0
Artvin	709.4	248.9	35.1	171.9	24.2	110.4	15.6	178.3	25.1

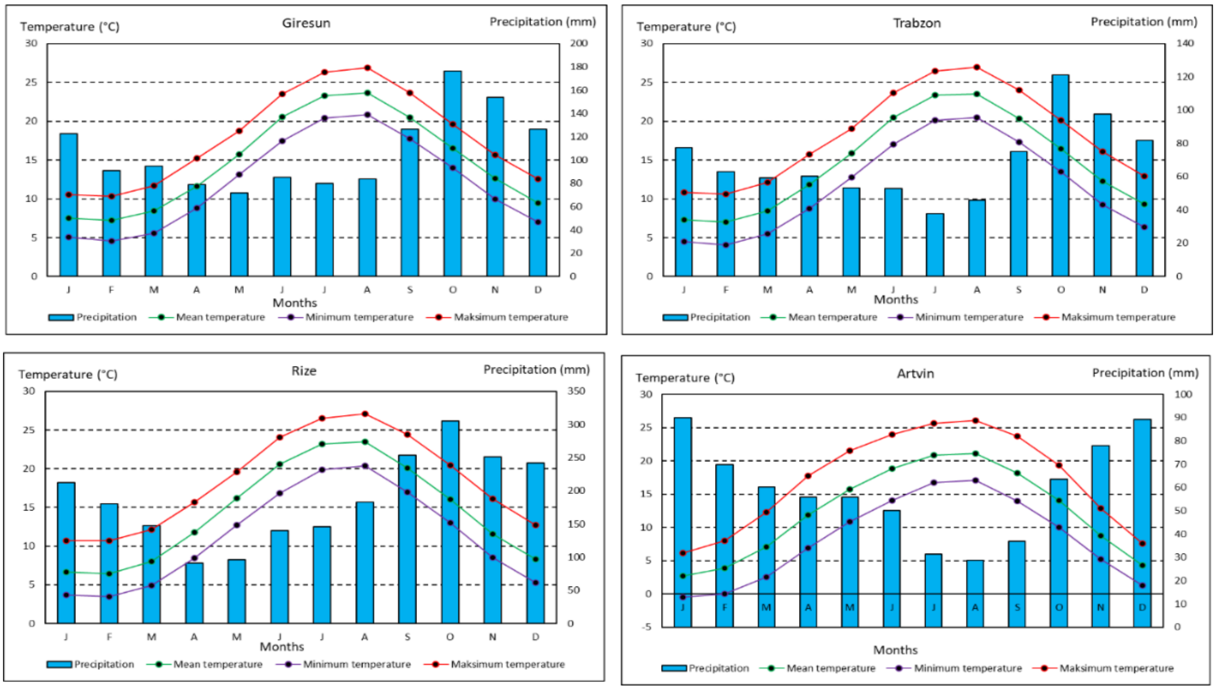

Figure 2. Temperature and Precipitation Charts for the Period 1975-2019 (From the Data of the Turkish State Meteorological Service).

The data of monthly total precipitation, monthly average temperature, monthly average minimum, and maximum temperature from October of the previous year, which is called the biological year (Fritts, 1976), to October of the year in which the tea harvest is made, is the independent variable. The data on annual yield values obtained per decare is the dependent variable. The aim is to reveal the effect of the temperature and precipitation conditions of October, November and December of the previous year on the yield in the harvest year (İrdem, 2021). The correlation coefficients obtained were significant at 0.95, 0.99 and 0.999 confidence levels. The differences between the months and provinces in the results were evaluated. The regression results were also used to explain the temperature and precipitation parameters analysis in the annual tea yield. The IBM SPSS 22.0 package program (SPSS, 2013) was used in the analysis.

In the second stage, changes, and trends in annual tea yield over the years were revealed. The annual tea yields of the provinces studied in the 1975-2019 period were calculated. The tea production amounts of each year between 1975 and 2019 were proportioned to the tea planting areas and the number of kilograms of yield per acre was calculated. While examining the temporal changes in the annual tea yield, the yield differences between the provinces were also emphasized, and the periods when the tea yield was high and low in the 1975-2019 period were determined. Trends in tea yield were demonstrated by simple linear regression testing.

The changes observed in temperature and precipitation in the research area during the 1975-2019 period were analysed in the last stage. According to possible climate change scenarios, the changes that could occur in tea production were evaluated. This study used 1971-2000 reference period and 2016-2099 future period climate change projections prepared by Akçakaya et al. (2015) and analyzed by Gürkan et al. (2016). This projection used GFDLESM2M global model data and regcm4.3.4 regional climate model according to RCP4.5 and RCP8.5 scenarios for geography, including Turkey.

3. Results and Discussion

3.1. Relationship between Average Temperatures and Tea Yield

In the field of research, strong positive relationships were determined between the average temperatures of

September of the previous year and the tea yield. The correlation coefficient is significant at the confidence level of 0.999 in all provinces. According to the regression analysis results, the change in average temperatures in September of the previous year in Giresun explains 22% of the change in tea yield in Giresun, 24% in Trabzon, 30% in Rize, and 21% in Artvin between years. In October and November of the previous year, a significant positive relationship between average temperatures and tea yields in Giresun at the confidence level of 0.99 is noted. There is no significant relationship in any of the provinces for December of the previous year (Table 3).

In the January-April period, the effect of average temperatures on tea yield is most evident in February. There is a significant positive correlation at 0.999 trust level in Artvin and 0.99 trust level in Giresun this month. The change in average temperatures in Giresun in February explains 21% of the difference in tea yield between years. April is the month with the lowest correlation. It is clear that the correlation between average temperatures and tea yields has become increasingly evident in May, and especially in the June-August period, the correlation coefficient obtained in all provinces was significant at the confidence level of 0.999. It is important that the power of the average temperatures between May and August in Rize, which meets most of the Turkey's tea production, in explaining the change in tea yield, is at the highest values among 4 provinces.

The effect of annual average temperatures on tea yield is generally more evident than the effect of monthly average temperatures. It is significant at a confidence level of 0.999 in all provinces. According to the regression analysis results, the change in average annual temperatures in Giresun explains 44% of the change in tea yield between years, 40% in Trabzon, and 49% in Rize and Artvin (Table 3).

Table 3. Correlation of average monthly mean temperatures with tea yield

Provinces	Giresun		Trabzon		Rize		Artvin	
Months	r	R^2	r	R^2	r	R^2	r	R^2
September	0.47***	0.22	0.49***	0.24	0.55***	0.30	0.46***	0.21
October	0.39**	0.16	0.24	0.06	0.40*	0.16	0.29	0.08
November	0.39**	0.15	0.20	0.04	0.31	0.10	0.30*	0.09
December	0.21	0.05	0.21	0.05	0.24	0.06	0.17	0.03
January	0.30*	0.09	0.24	0.06	0.32*	0.10	0.27	0.08
February	0.38**	0.13	0.25	0.06	0.27	0.08	0.46***	0.21
March	0.31*	0.09	0.13	0.02	0.23	0.05	0.32*	0.10
April	0.04	0.00	-0.03	0.00	-0.02	0.00	0.11	0.01
May	0.57***	0.33	0.41**	0.17	0.51***	0.26	0.36*	0.11
June	0.67***	0.45	0.52***	0.27	0.59***	0.35	0.56***	0.32
July	0.47***	0.22	0.54***	0.29	0.57***	0.33	0.42***	0.18
August	0.54***	0.29	0.68***	0.46	0.68***	0.46	0.62***	0.38
Annual	0.67***	0.44	0.63***	0.40	0.70***	0.49	0.70***	0.49

*P≤0.05, **P≤0.01, ***P≤0.001, r= correlation coefficient.

3.2. Relationship of tea Yield with Average Minimum Temperatures

In the research area, strong positive relations were found between the average minimum temperatures of September of the previous year and the tea yield in all provinces except Giresun. Although the correlation value obtained for Giresun is significant at the confidence level of 0.95, the correlation coefficient obtained for this month in other provinces is significant at the confidence level of 0.999. According to the regression analysis results, while the change in average minimum temperatures in September of the previous year in Giresun explains 11% of the change in tea yield between years, it explains 23% of it in Trabzon, 24% in Rize, and 27% in Artvin. In October of the previous year, there was a significant positive correlation at the confidence level of 0.95 in Giresun and Artvin, and 0.99 in Rize. There is no significant relationship between the average minimum temperatures and tea yield in provinces other than Artvin for November and December of the previous year. The correlation value obtained for November in Artvin is significant at the confidence level of 0.95 (Table 4).

The province where the effect of average minimum temperatures on tea yield in the January-April period is most evident is Artvin. In Artvin, a significant positive correlation is observed at the level of 0.95 in January and March, and at 0.999 in February.

Table 4. Correlation of average monthly minimum temperatures with tea yield

Provinces	Giresun		Trabzon		Rize		Artvin	
Months	r	R^2	r	R^2	r	R^2	r	R^2
September	0.33*	0.11	0.48***	0.23	0.49***	0.24	0.52***	0.27
October	0.33*	0.11	0.28	0.08	0.43**	0.18	0.39*	0.15
November	0.25	0.07	0.14	0.02	0.27	0.07	0.40*	0.16
December	0.24	0.06	0.19	0.04	0.20	0.04	0.30	0.09
January	0.32*	0.10	0.25	0.06	0.29	0.08	0.40*	0.16
February	0.27	0.07	0.15	0.02	0.22	0.05	0.45***	0.20
March	0.13	0.02	0.07	0.01	0.19	0.04	0.35*	0.13
April	-0.06	0.00	-0.05	0.00	0.02	0.01	0.15	0.02
May	0.47***	0.22	0.29	0.08	0.51***	0.26	0.46***	0.21
June	0.53***	0.28	0.44**	0.19	0.57***	0.33	0.58***	0.33
July	0.38*	0.15	0.49***	0.24	0.52***	0.27	0.46***	0.21
August	0.50***	0.25	0.71***	0.50	0.68***	0.46	0.63***	0.40
Annual	0.53***	0.28	0.59***	0.34	0.68***	0.46	0.74***	0.54

*P≤0.05, **P≤0.01, ***P≤0.001, r= correlation coefficient.

April is the month with the lowest correlation for average minimum temperatures and average temperatures. Similarly, for the average minimum temperatures, it is observed that the correlation becomes more evident with May. Although the correlation value obtained for all provinces in the May-August period is significant at different confidence levels, it is examined that the relationship is even stronger especially in Artvin and Rize.

The effect of annual average minimum temperatures on tea yield is quite consistent with average temperatures. The correlation value obtained in all provinces is significant at the confidence level of 0.999. According to the regression analysis results, the change in annual average minimum temperatures in Giresun explains 28% of the variation in tea yield between years, 34% in Trabzon, 46 in Rize and 54% in Artvin (Table 4).

3.3. Relationship of Tea Yield with Average Maximum Temperatures

In the research area, significant positive relations were found between the average maximum temperatures of September of the previous year and tea yield except Giresun. Especially in Trabzon and Rize, correlations are very prominent. The change in average maximum temperatures in September of the last year in Trabzon explains 23% of the difference in tea yield over the years, while in Rize, it explains 38%. In October of the previous year, the significant relationship was only in Rize at the confidence level of 0.99. There is no significant correlation between average maximum temperatures and tea yield in November and December of the previous year (Table 5).

Rize is the province where the effect of average maximum temperatures on tea yield in the January-April period is most evident. In Rize, there was a significant positive correlation at 0.95 confidence levels in January, February, and March. There is almost no correlation between tea yield and average maximum temperatures in April. Although it is seen that the correlation becomes more evident in May, there are not as strong correlations as in average temperatures and average minimum temperatures. It is seen that the relationship is even stronger in May-August, especially in Trabzon and Rize, compared to other provinces (Table 5).

Although the effect of average annual maximum temperatures on tea yields is significant at 0.95 confidence level in Giresun, the correlation value is much more remarkable in other provinces. The correlation value obtained for other provinces is significant at the confidence level of 0.999. According to the regression analysis results, the change in average annual maximum temperatures in Giresun explains 16% of tea yield variation between years. In Trabzon, it explains 39%, 52% in Rize, and 29% in Artvin (Table 5).

3.4. Relationship between Total Precipitation and Tea Yield

The relations between total monthly precipitation and tea yield remain extremely low compared to temperature-yield relationship. The correlation coefficient obtained only in March in Trabzon is statistically significant at the confidence level of 0.95. The change in total precipitation in Trabzon in March explains 12% of tea yield changes between years. None of the correlation values found in other provinces and months are statistically significant. While there was a positive correlation between tea yield and monthly total precipitation for some months in some provinces, negative correlations were also found in another province for the same month. For example, while there is a positive correlation between the total precipitation in April and tea yield in Trabzon and Rize, there is a negative correlation in Giresun and Artvin. Although the positive effect of annual total precipitation is not statistically significant in provinces except Artvin, the obtained correlation values are close to the significance limit for a confidence level of 0.95. The change in annual total precipitation in Giresun explains 7% of the variation in tea yield between years, 6% in Trabzon, and 9 in Rize. In Artvin, on the other hand, a negative correlation was found between annual total precipitation and tea yield. However, the point to be noted here is that the negative correlation between annual total precipitation and tea yield in 2010 and 2011 also reduced the long-term average correlation. While the correlation value between the annual total precipitation and tea yield in Artvin for the 1975-2019 period was -0.14, this value was found to be 0.40 for the 1975-2009 period, which corresponds to a significant positive relationship at the confidence level of 0.95. For other provinces, the 1975-2009 is stronger than the 1975-2019 period correlation (see Table 6).

3.5. Changes and Trends in Tea Yield over the Years

When the areal distribution of annual tea yield is examined, it has been determined that the highest yield on decare basis is Rize with 1187 kilograms and the lowest yield is Giresun with 798 kilograms, according to the average of 1975-2019 period (Figure 3).

Table 5. Correlation of average monthly maximum temperatures with tea yield

Provinces	Giresun		Trabzon		Rize		Artvin	
Months	r	R^2	r	R^2	r	R^2	r	R^2
September	0.20	0.04	0.48***	0.23	0.62***	0.38	0.33*	0.11
October	0.13	0.02	0.22	0.05	0.41**	0.16	0.13	0.02
November	0.14	0.02	0.25	0.06	0.29	0.08	0.24	0.06
December	0.17	0.03	0.28	0.08	0.30	0.09	0.17	0.03
January	0.22	0.05	0.25	0.06	0.38*	0.14	0.21	0.05
February	0.27	0.07	0.35*	0.12	0.37*	0.14	0.37*	0.14
March	0.15	0.02	0.20	0.04	0.32*	0.10	0.17	0.03
April	-0.10	0.01	-0.05	0.00	0.07	0.01	0.02	0.00
May	0.43**	0.18	0.42**	0.18	0.49***	0.24	0.22	0.05
June	0.40*	0.16	0.55***	0.30	0.65***	0.42	0.32*	0.10
July	0.36*	0.13	0.50***	0.25	0.62***	0.38	0.32*	0.10
August	0.37*	0.14	0.62***	0.39	0.69**	0.48	0.52**	0.27
Annual	0.40*	0.16	0.62***	0.39	0.72***	0.52	0.54***	0.29

*P≤0.05, **P≤0.01, ***P≤0.001, r= correlation coefficient.

Table 6. Correlation of total monthly precipitation with tea yield

Provinces	Giresun		Trabzon		Rize		Artvin	
Months	r	R^2	r	R^2	r	R^2	r	R^2
September	0.24	0.06	0.04	0.00	0.29	0.09	0.28	0.08
October	0.05	0.00	0.23	0.05	0.12	0.02	0.07	0.01
November	-0.03	0.00	-0.12	0.02	0.01	0.00	-0.02	0.00
December	0.28	0.08	-0.03	0.00	-0.10	0.01	-0.10	0.01
January	0.24	0.06	0.14	0.02	-0.06	0.00	-0.17	0.03
February	-0.19	0.04	-0.04	0.00	0.00	0.00	-0.22	0.05
March	0.27	0.07	0.35*	0.12	0.27	0.07	0.08	0.01
April	-0.23	0.05	0.21	0.04	0.06	0.00	-0.19	0.04
May	0.07	0.01	0.17	0.03	-0.03	0.00	0.09	0.01
June	0.10	0.01	-0.05	0.00	0.10	0.01	0.14	0.02
July	0.14	0.02	-0.13	0.02	0.23	0.05	-0.08	0.01
August	-0.25	0.06	0.04	0.00	0.06	0.00	-0.04	0.00
Annual	0.27	0.07	0.25	0.06	0.30	0.09	-0.14	0.02

*P≤0.05, r= correlation coefficient.

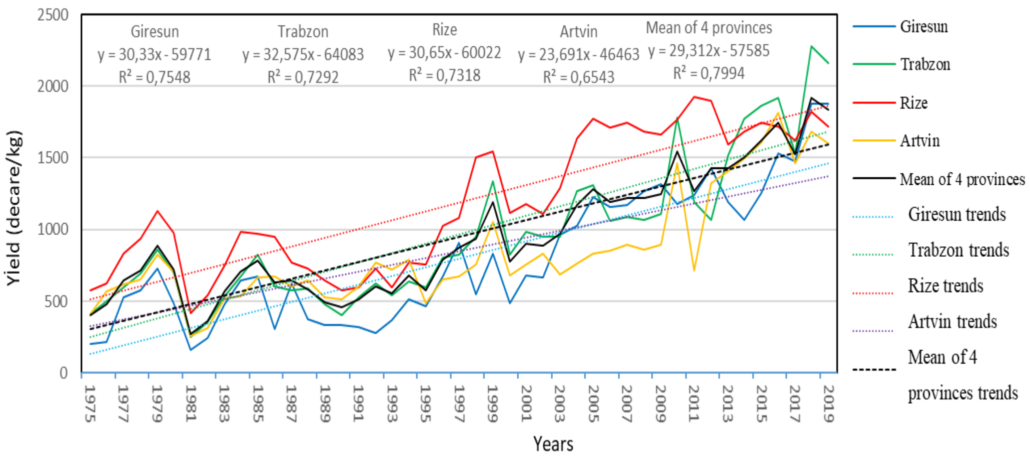

Figure 3. Changes and trends in tea yield in the research area between years (State Institute of Statistics agricultural statistics; ÇAY-KUR Annual Activity Reports; TURKSTAT, 2020).

Table 7. Results of the linear trend test applied to tea yield

	Provinces	a	b	t	r (β)	R^2	Results
Tea yield	Giresun	99.958	30.330	11.505	0.87***	0.755	Increasing trend.
	Trabzon	219.75	32.575	10.761	0.85***	0.729	Increasing trend.
	Rize	481.84	30.65	10.833	0.86***	0.732	Increasing trend.
	Artvin	302.87	23.691	9.021	0.81***	0.654	Increasing trend.

**P≤0.001, r= correlation coefficient.

The average yield of the four provinces in the field of study for 1975-2019 is 950 kg. The highest average yield was achieved in 2018 with 1916 kilograms, while the lowest average was obtained in 1981 with 272 kilograms. In 2018, the total tea production of these four provinces was 1480534 tons, while in 1981, production decreased to 192218 tons. In the period 2004-2019, the yield was over 1000 kg. On the other hand, in the 1975-2004 period, it is seen that the yield exceeded 1000 kilograms only in 1999. It is also noteworthy that since 2014, the yield has exceeded 1500 kilograms. In contrast, in 1975, 1976, 1981, 1982, 1989, and 1990, the average yield of four provinces decreased to 500 kg (Figure 3). When the temporal changes observed in tea yield are evaluated, a fluctuating course draws attention, but an increasing trend is also observed. According to linear trend analysis, the increases are significant at the confidence level of 0.999 in all provinces. When the analyzed 45-year period is divided into 15-year slices, the average yield in the 1975-1989 period as 593.1 kg, the average yield in the 1990-2004 period as 792.4 kg, and the average yield in the 2005-2019 period as 1465.3 kg reveals the extent of the yield increase (Table 7 and Figure 3).

Tea yield generally increases in years when the annual average temperatures increase. Especially in 1979 and 2010, the positive relationship between the increase in yield and temperature is quite evident. However, although the temperature has increased, it has been observed that the yield has decreased in some years. In 1981, for example, tea yields dropped significantly despite increasing average annual temperatures (Figure 4). When the relationship of this extreme decrease in yield with temperature and precipitation parameters is examined in detail, it is seen that the winter temperatures in the mentioned year, especially in January, are well above the average, and the actual precipitation is well below the average. For example, while the long-term average of January precipitation in Rize was 212 mm, only 130 mm precipitation fell in 1981. In January, the average temperature was 6.6 °C for many years, while in 1981, the average temperature was 8.5 °C. The long-term average of winter precipitation was 633 mm for many years.

In contrast, 439 mm precipitation fell in the winter of 1981. The average winter temperature was 7.1 °C. On the contrary, in 1981, the average winter temperature was 9.3 °C. In addition, the total precipitation for the June-July period of 1981 was only about half of the long-term average (while the long-term average was 289 mm, in 1981 it was 150 mm). All these adverse conditions may influence the sudden decrease in tea yield. It is seen that tea yields are generally high when the total annual rainfall increases in the field of study. However, in 2010 and 2011, there is a situation contrary to this generalization. The average yield of 4 provinces increased in 2010. As opposed to this, the annual total precipitation of these 4 provinces decreased. In 2011, however, while the average yield of 4 provinces decreased, the annual total precipitation of these 4 provinces increased. Figure 4 shows that the strong correlation between temperature conditions and tea yield eliminates precipitation's positive or negative effect in these two years.

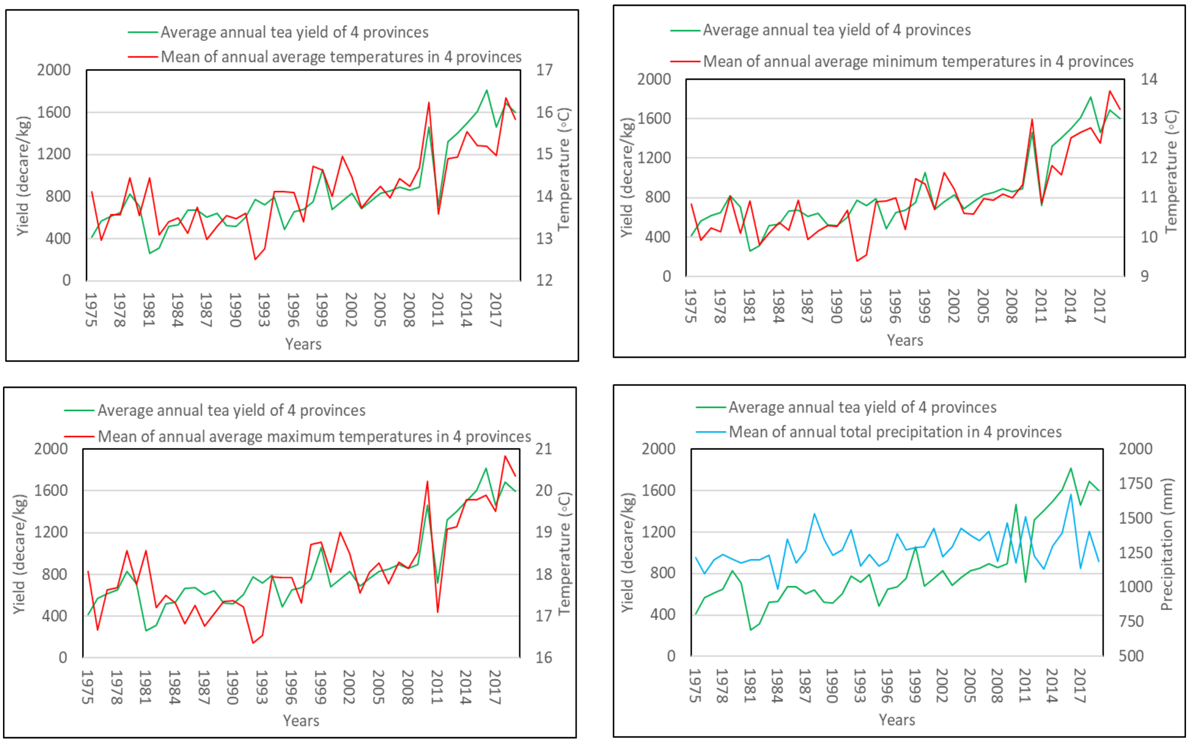

Figure 4. Harmony of trends in temperature and precipitation with trends in yield (Climate data is obtained from the Turkish State Meteorological Service).

3.6. Possible Yield Changes Based on Climate Change Scenarios

Turkish State Meteorological Service has created climate projections for Turkey and its region. According to the RCP4.5 and RCP8.5 scenarios for the geography, including Turkey, projections for the 1971-2000 reference period and the future 2016-2099 were produced using GFDLESM2M global model data. From the regional climate model data with a resolution of 20 km, projection outputs of temperature and precipitation parameters for the periods 2016-2040, 2041-2070, 2071-2099 were viewed seasonally. (Gürkan et al., 2016). According to the projection results prepared by (Akçakaya et al., 2015) and analysed in the study conducted by Gürkan et al. (2016), in both scenarios (RCP4.5 and RCP8.5), increases in temperatures are expected in all basins, thus also in the Eastern Black Sea Region, throughout the entire period (2016-2099). According to the RCP4.5 scenario; it is predicted that the increase in the Eastern Black Sea basin will generally be around 0.5°C-1°C in the first period and will increase to 1.5°C-2°C in the 2041-2070 and 2071-2099 periods. According to the RCP8.5 scenario, average temperatures in the Eastern Black Sea Region are generally around 0.5°C-1°C in the first period, 1.5°C-2°C in the 2041-2070 period and is predicted to increase over 2.5°C in the 2071-2099 period. While a decrease is foreseen in total precipitation in all periods in Turkey, an increase of 5-10% is expected in the first two periods in the Eastern Black Sea basin based on RCP4.5 scenario. A decrease of 5-10% is predicted in the third period. When RCP8.5 scenario is considered, a decrease is expected in total precipitation in all periods throughout Turkey. In the first period, an increase of 5-10% is expected in the Eastern Black Sea Region. However, a decrease of 5-10% is anticipated in the second period and a decrease of 15%-20% in the third period.

According to the linear trend model, there was a significant increase in the research area in 1975-2019 at the average, average maximum, and average minimum temperatures of 0.999 confidence level (Table 8).

On the other hand, the increase in tea yield in the years when temperatures increase is also noted (see Figure 4). In this respect, the rise in tea yield likely continues due to the temperature increases predicted by climate change scenarios. Yet, as in 1981, extreme hot winters or other possible temperature extremes will harm yield.

In the period 1975-2019, there is a significant increase in the total annual rainfall at the level of 0.95 confidence in Rize and Trabzon. The rise in Giresun is also at the confidence level limit of 0.95, although in Artvin, a trend has not been detected (see Table 8). Considering the relationship between tea yield and total annual rainfall, it is also essential to reveal the effect of changes in precipitation on tea yield. Although climate change scenarios related to precipitation are not as regular as temperature, the expected reductions in the post-2040 period according to the RCP8.5 scenario may adversely affect the yield. The tea plant will inevitably be negatively affected, primarily due to the expected decrease in summer precipitation.

Table 8. Results of the linear trend test applied to temperature and precipitation

	Provinces	a	b	t	r	R^2	Results
Total annual precipitation	Giresun	100.46	0.311	2.012	0.29	0.086	no trend
	Trabzon	64.213	0.243	2.523	0.36*	0.129	increasing trend
	Rize	177.5	0.496	2.023	0.30*	0.087	increasing trend
	Artvin	58.946	0.009	0.070	0.01	0.001	no trend
Annual mean temperature	Giresun	13.790	0.043	5.679	0.66***	0.429	increasing trend
	Trabzon	13.884	0.045	6.361	0.70***	0.485	increasing trend
	Rize	13.199	0.061	8.162	0.78***	0.608	increasing trend
	Artvin	11.277	0.043	5.289	0.63***	0.394	increasing trend
Annual mean max. temperature	Giresun	17.096	0.045	4.462	0.56***	0.316	increasing trend
	Trabzon	17.073	0.064	6.924	0.726***	0.527	increasing trend
	Rize	16.935	0.072	8.847	0.80***	0.645	increasing trend
	Artvin	16.196	0.047	4.227	0.54***	0.294	increasing trend
Annual mean min. temperature	Giresun	10.994	0.056	6.939	0.73***	0.528	increasing trend
	Trabzon	10.719	0.053	6.995	0.73***	0.532	increasing trend
	Rize	10.115	0.056	7.695	0.76***	0.579	increasing trend
	Artvin	6.867	0.067	8.224	078***	0.611	increasing trend

*P≤0.05, **P≤0.01, ***P≤0.001, r= correlation coefficient.

4. Discussion

This study determined that changes in temperature conditions were much more effective on tea yield than changes in precipitation.

Similarly, Lobell et al. (2011) found that temperature change was more effective than the change in rainfall in their study, which investigated the impact of climate trends on corn, rice, wheat, and soybean production globally for 1980-2008. Eaqually, İrdem (2021) examined the effects of temperature and precipitation on hazelnut yield in Turkey during 1993-2019 and observed that temperature change affected the yield more than the change in precipitation.

Ali et al. (2014) state that temperature is the most critical microclimatic parameter for tea leaf production. Despite this, the relationship between temperature and tea leaf production was not detected as significant. There is also a moderately positive relationship between tea leaf production and precipitation. While substantial positive relationships between temperature and yield were found in this study, positive relationships between precipitation and yield were not significant.

It is examined that the correlation of tea yield with average maximum temperatures was positively significant. However, Carr and Stephens (1992) note that it is difficult to distinguish the effects of maximum temperatures on tea yield from other factors, whereas air or leaf temperatures often exceeding 35 °C are likely to reduce shoot growth rates and even photosynthesis.

Long rainy periods negatively affect tea yield due to the decrease in sunlight and the reduction of photosynthesis of the leaves (Wijeratne et al., 2007; Esham and Garforth, 2013; Duncan et al., 2016). Similarly, Rahman et al. (2017) put forward that there may be a decrease in yield due to lack of sunlight in years when the annual total precipitation is too high. It is also suggested that excessive water may adversely affect tea production due to increased soil saturation and failure of absorption. The year with the least annual total precipitation of the four provinces analyzed in this study was 1674 mm in 2016, and the average yield of four areas in this year was 1747 kg. The year with the least annual precipitation was 985 mm in 1984, while the yield was 710 kg.

There was an increase in temperatures and precipitation in the research area from 1975-2019. It is also observed that tea yields increased in parallel during the same period. Gürkan et al. (2016) present that temperature and precipitation will continue to rise in the Eastern Black Sea basin from 2016-2040. Yet, one could not claim that tea yields will continue to increase in the future. According to Nowogrodzki (2019), climate change reduces the yield of tea plants by changing precipitation levels, rising temperatures, changing the timing of seasons, encouraging insect pests, and causing soil erosion and puddles, especially with heavy rains.

Biggs et al. (2018) state that increased crop stress caused by changes in precipitation and temperature conditions affects harvest quality and timing. In contrast to this, Yurt (1991) argues that high temperature increases the rate of tea. Based on this, tea quality can be expected to increase in the future depending on the increase in average temperatures.

In the studies conducted on a day-to-day basis, Bütüner (2019) determined that the fruits' growth, development and maturation are fast in the periods when the number of sunny days is high. For instance, he argues that the most extended harvest period in the 2003-2016 period was in 2008, which is also related to temperature. However, the study determined that the yield in 2008 was lower than in 2007 and 2009 when the harvest time was shorter. In that case, climate change may cause changes in harvest times, but at this point, the effect of summer temperature and precipitation on yield is more remarkable.

5. Conclusion

As a result of the study, a significant positive relationship was found between annual average, maximum and minimum temperatures, and annual tea yield. Still, no significant correlation with total yearly precipitation was detected. The relations between temperature and yield are more substantial in summer, and relationships between the monthly precipitation and its yield are more irregular. In 1975-2019, a significant increase in tea yield was determined, more vital in the last 15 years.

There was a significant increase in annual average temperatures and no significant increase in precipitation in research between 1975 and 2019. According to climate change scenarios, it can be expected that the temperature and precipitation increases predicted for the 2016-2040 period in the Eastern Black Sea basin will lead to increases in tea yield. However, when the damage to the tea shoots from heavy rains and the expected increases in harmful insects due to the temperature rise is taken into account, there may even be decreases in yield and quality.

In the following stages, the results of this study can be supported by the analyses considering the factors affecting tea yield other than temperature and precipitation (climatic parameters such as wind, humidity, sunbathing times, and the effects of methods and techniques used in agriculture, etc.).

Author Contributions

All task made by single author and the author reviewed and approved the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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Research Article
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A STUDY ON THE DETERMINATION OF MECHANIC HARVEST PROPERTIES OF SOME SWEET CHERRY VARIETIES

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Abstract: Cherry is a hand-harvested fruit due to various difficulties and constraints. This condition results in the use of labor at a high rate. Turkey's annual cherry production is at the level of 724 thousand tons. Various tools or machines that have been developed for cherry harvest have the potential to contribute greatly to the production in this area. The share of the labor required for harvesting in cherry production in the total labor requirement is around 70%. In this research, it was aimed to collect the necessary data to mechanize the cherry harvest by determining the physico-mechanical properties of cherry fruit. As a result of the present study, several physical, biological, and mechanical properties of four sweet cherry variety (0900 Ziraat, Starks Gold, Merton Late, Lambert) were determined and compared in terms of fruit mass, net fruit weight, tensile force, weight, thickness, length, width, sphericity, surface area, volume of fruit, and also weight, width, length, sphericity of seed, tensile force of stalk, stalk length, and weight. Tensile force of fruit, tensile force of stalk and weight of the fruit of 0900 Ziraat variety were found 2.579 N, 7.041 N, 9.592 g, respectively. After the evaluation of the obtained data, it was determined that all four cherry varieties examined were suitable for mechanical harvesting. However, the most suitable variety for mechanical harvesting was found as '0900 Ziraat'.

Keywords: Sweet cherry, Fruit harvesting, Mechanization, Tensile force

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1. Introduction

Cherry (*Purunus avium* L.) belongs to Rosaceae family, Prunoideae subfamily, Prunus genus in botany. The origin of the cherry is known as the region between the Southern Caucasus, the Caspian Sea, and Northeast Anatolia. It spread east and west from these gene centers and covered a large area on Earth. Accordingly, our country is one of the origin centers of cherries (Başkaya, 2011). There are around 1500 cherry variety in the world, and this number is increasing day by day with ongoing breeding studies. Besides, the same varieties were named with different names, and different varieties were named with the same names in terms of regions (Çakaryıldırım, 2003).

Cherry cultivated areas and the amount of cherry produced accordingly increase every year in Turkey. Our production, which was 230000 tons from 29000 ha in 2000, increased from 67046 ha to 417905 tonnes in 2010 and from 82729 ha to 724944 tonnes in 2020 (Anonymous, 2022).

According to 2020 World cherry production data, Turkey ranks first in terms of cherry production and cultivated area (Table 1; Anonymous, 2022). Of the 664224 tons of cherries produced in Turkey in 2019, 80508 tons were exported. This amount constitutes 12.1% of the total production. 69% of the exported cherries were sold to EU member countries (Anonymous, 2020; Anonymous, 2021). '0900 Ziraat' variety is the cherry variety with the highest production volume in Turkey and is an important export product (Erogul, 2018).

Table 1. The highest cherry produced countries in the world (Anonymous, 2022)

Rankings	Country	Production (metric ton)	Cultivated Area (ha)
1	Turkey	724944	82729
2	USA	294900	34400
3	Chile	255471	39645
4	Uzbekistan	185068	12718
5	Iran	164080	24033

Harvesting of cherry fruit is still mostly done by hand both in the world and in Turkey. Therefore, harvesting is one of the areas where the labor requirement per unit area is high in fruit growing and hand-harvesting constitutes 30-60% of the total production cost (Moser, 1989; Gezer, 2001). When the situation of mechanical fruit harvesting in Turkey is examined, it is seen that the process with the highest labor requirement per unit area is harvesting. When we look at the share of the labor required for harvest in the total labor requirement, sour cherry, and cherry production ranks first with 70% (Gezer, 2001). Like cherries, sour cherry harvest costs account for 30-60% of the total production cost (Yılmaz and Gökduman, 2020). Kocabiyik et al. (2009) determined the human energy cost, work success, and some physico-mechanical properties of fruits in apple, peach, apricot, cherry, and plum harvest. As a result, they determined the highest human energy input for cherry harvest. Pırlak and Güleryüz (2000) reported that the fruit harvest is 100-250 times higher than the grain harvest in terms of labor and approximately 40 times higher in terms of production costs.

Erdoğan (1988) evaluated the human labor needs in horticultural agriculture in terms of harvest mechanization. According to the results of the study, human labor needs in strawberry and cherry harvesting were found to be higher than other fruits. Pırlak and Güleryüz (2000) examined the mechanical harvesting of fruit species and concluded that the human labor required for manual cherry harvesting is high. This process constitutes 40-80% of the working time in production.

Knowing the biological properties of agricultural products are necessary and important in the design, construction, operation, control, determination of yields, analysis and evaluation of the quality of the products. Knowing these properties is beneficial not only for engineers but also for food scientists, processors, plant-breeding designers and specialists (Mohsenin, 1986). For these reasons, studies have been carried out by many researchers to determine the physico-mechanical and biological properties of different fruits. Research has been carried out for many fruits such as apricot (Altıkat and Temiz, 2019), apple (Polat et al., 2020), plum (Alnıak Sezer and Çetin, 2021), black berry (Çalışır and Aydın, 2004), sour cherry and cherry (Özgüven et al., 2001; Vursavuş et al., 2006; Kocabıyık et al., 2009; Pérez-Sánchez et al., 2010; Göksel and Aksoy, 2014; İkinci and Bolat, 2015; Taşova and Güzel, 2017; Krumov and Christov, 2018; Sarısu et al., 2019). However, it should not be forgotten that there are many variety of each fruit and that even the same variety vary according to the climate, soil, and cultivation method of the region in which they are grown (Altikat and Temiz, 2019). Due to that, it is important to conduct studies on the samples of fruit variety in different regions, to have a large amount of data on the characteristics of that fruit variety, and to create a more reliable infrastructure for studies on mechanical harvesting. In this study, it was aimed to determine the physico-mechanical properties of four cherry variety grown in Tekirdağ, to collect the data necessary for the mechanization of cherry harvest and to determine the suitability of these variety for mechanical harvesting.

2. Material and Methods

2.1. Cherry Varieties

The cherry fruits used for the research were obtained from the trees in the orchard of Tekirdağ Viticulture Research Institute (40.970860 N, 27.472279 E). Four different cherry varieties (0900 Ziraat, Starks Gold, Merton Late, and Lambert) were used in the study (Figure 1).

Figure 1. Photos of garden and cherry varieties used in the experiments.

For each cherry variety, measurements and calculations were made on a total of 103 samples from randomly selected trees, in order to determine the properties of cherries for mechanical harvesting and to determine their suitability for mechanical harvesting. These are weight, thickness, width, height, sphericity, surface area, volume, density, net fruit weight, tensile force of the fruit from the stalk, seed weight, width, thickness, height, sphericity, surface area, and tensile force of the fruit stalks from the branch, weight, length, thickness, and number of the stalks. In addition, pH measurements and color analyzes were performed for the examined varieties (Vursavuş et al., 2006; Kocabıyık et al., 2009; Pérez-Sánchez et al., 2010; İkinci and Bolat, 2015; Sarısu et al., 2019).

The thickness, width, height of the fruits, and the length and thickness of the fruit stalks were measured with a digital caliper (Mitutoyo) with an accuracy of 0.01 mm, and the weights of the fruits and stalks were measured with a precision balance (AND - GF 600) with an accuracy of 0.001 g. The tensile force of the fruit (from the stalk) and the tensile force of the fruit stalk (from the branch) were made with a 1 gr precision hand dynamometer (Lutron FG 5020), pH measurements were analyzed by pH meters (Hanna Instruments pH 211), and color measurements were obtained from a colorimeter (HunterLab D25LT - Reston, VA). Sphericity, net fruit weight, surface area, and density values were calculated from the obtained measurement results. Additionally, net fruit weight was calculated by subtracting the weight of the fruit stalk and seed from the total fruit weight.

2.2. Statistical Analysis

Analysis of variance (ANOVA) was applied to the data obtained from the study by using the statistical package program (Statistica, 1999), and the differences between the group means were determined with the Duncan Multiple Comparison Test (Genç and Soysal, 2018).

2.3. The Tensile Force of the Fruit from the Stalk (F_{T-f}) and the Stalk from the Branch (F_{T-s})

To determine the tensile force of the fruit (from the stalk), an equipment was made on the tip of the hand dynamometer to pull the fruit from the stalk. With the help of this equipment, the samples were pulled in the direction of the fruit stalk axis and the tensile forces (F_{T-f})

were determined as N (Newton). After the fruits were plucked from their stalks, the remaining stalks were pulled from the branch with the help of an equipment and the tensile force (F_{T-s}) was measured. Fruits and stalks which tensile forces were measured were numbered for further measurements.

2.4. Fruit Sizes and Sphericity

The size measurements of the fruits were made with a digital caliper and the sphericity values were calculated by placing the values in Equation 1 given below (Moser, 1989; Vursavuş et al., 2006; Yılmaz and Gökduman, 2020):

$$\phi = (xyz)^{1/3}/z \quad (1)$$

where;

ϕ = Sphericity (%)

x: Height of fruit (mm)

y: Thickness of the fruit (mm)

z: Width of the fruit (mm)

3. Results and Discussion

3.1. pH Values

The juices of the fruits were squeezed, and pH measurements were made in three replications for each cherry variety, and the averages are given in Table 2. Numerically the highest pH was measured in Lambert, and the lowest pH was measured in 0900 Ziraat variety. The results are in line with the work done by Vursavuş et al. (2006), Göksel and Aksoy (2014), Sarısu et al. (2019), Eroğul and Özmen (2020).

3.2. Color Measurements

L* (lightness), a* (redness), b* (yellowness) values were measured with HunterLab colorimeter. The measurements were made from randomly selected 3 samples for each variety and their mean values are summarized in Table 3.

According to the results of the study, the variety with the highest brightness (L*) and yellowness (b*) values was 'Starks Gold', and the variety with the highest redness (a*) value was '0900 Ziraat'.

Table 2. pH analysis results of cherry varieties

Varieties	pH
0900 Ziraat	3.86
Starks Gold	3.95
Merton Late	3.97
Lambert	4.24

Table 3. Color analysis results of cherry varieties

Varieties	L*	a*	b*
0900 Ziraat	18.25	9.25	2.66
Starks Gold	60.32	0.55	28.19
Merton Late	15.86	6.93	1.28
Lambert	13.83	1.56	0.23

Findings for the variety 'Ziraat 0900' are in agreement with Vursavuş et al. (2006). There are differences between the values measured in the study and the values obtained by Göksel and Aksoy (2014), Sarısu et al. (2019), Eroğul and Özmen (2020). It is thought that these differences may be caused by cultivation and environmental conditions.

The data obtained by measuring the parameters of fruit, seeds, and stalks of four different cherry varieties were statistically analyzed and the results were summarized in Table 4, 5, and 6.

3.3. Fruit Parameters Measurements

Measured and calculated fruit parameters of investigated cherry variety are shown in Table 4.

When the tensile forces of the fruits pulling from the stalk were examined, the highest value (2.579 N) was measured in 0900 Ziraat variety, the lowest value (1.530 N) was measured in Lambert variety and the difference was statistically significant ($P < 0.05$). When the weights of the fruits were examined, all variety were found to be statistically different from each other, the highest value was found in 0900 Ziraat with 9.592 g, and the lowest value was determined in Lambert variety with 3.684 g.

Table 4. Results of investigated fruit parameters

Fruit parameters	Cherry varieties			
	0900 Ziraat	Starks Gold	Merton Late	Lambert
Tensile force of fruit (from stalk) (N)	2.579±0.117^{a}	2.187±0.872^{b}	2.246±0.664^{b}	1.530±0.803^{c}
Weight (g)	9.592±1.095^{a}	6.000±0.948^{c}	7.130±1.592^{b}	3.684±0.732^{d}
Width (mm)	26.351±1.384^{a}	22.526±1.474^{c}	23.424±1.885^{b}	15.917±1.133^{d}
Thickness (mm)	23.213±1.201^{a}	19.858±1.236^{b}	20.143±1.719^{b}	17.557±1.496^{c}
Length (mm)	24.957±1.121^{a}	21.267±1.096^{b}	20.850±1.550^{c}	16.467±1.208^{d}
Sphericity (%)	0.939±0.022^{b}	0.939±0.022^{b}	0.912±0.025^{c}	1.042±0.032^{a}
Surface area (cm^2)	19.228±1.712^{a}	14.049±1.514^{b}	14.402±2.188^{b}	8.672±1.260^{c}
Volume (cm^3)	9.850±1.544^{a}	5.728±1.066^{c}	6.966±1.621^{b}	3.528±0.760^{d}
Density (g/cm^3)	0.984±0.097^{c}	1.061±0.122^{a}	1.027±0.059^{b}	1.063±0.181^{a}
Net fruit weight (without seed) (g)	9.223±1.090^{a}	5.623±0.927^{c}	6.794±1.567^{b}	3.403±0.717^{d}

$^{a-d}$Mean values with different superscripts in the same row indicate a significant difference ($P<0.05$), n=103.

Table 5. Results of investigated seed parameters

Seed parameters	Cherry varieties			
	Ziraat 0900	Starks Gold	Merton Late	Lambert
Weight (g)	0.369±0.041[a]	0.377±0.042[a]	0.336±0.046[b]	0.284±0.044[c]
Width (mm)	11.126±0.421[a]	10.332±0.440[c]	10.581±0.429[b]	9.473±0.506[d]
Thickness (mm)	7.153±0.323[b]	6.988±0.291[c]	7.360±0.373[a]	6.823±0.390[d]
Length (mm)	9.240±0.342[a]	8.901±0.385[b]	8.976±0.372[b]	8.300±0.353[c]
Sphericity (%)	0.810±0.023[c]	0.834±0.022[b]	0.837±0.029[b]	0.856±0.024[a]
Surface area (cm^2)	2.547±0.155[a]	2.330±0.159[c]	2.462±0.147[b]	2.067±0.188[d]

[a-d]Mean values with different superscripts in the same row indicate a significant difference (P<0.05), n= 103.

Table 6. Results of investigated stalk parameters

Stalk parameters	Cherry varieties			
	Ziraat 0900	Starks Gold	Merton Late	Lambert
Tensile force of stalk (from branch) (N)	7.041±2.793[ab]	6.355±2.391[b]	7.669±2.577[a]	7.522±2.567[a]
Number of stalks in cluster (unit)	1.320±0.546[c]	2.350±0.667[a]	1.728±0.795[b]	1.728±0.795[b]
Weight (g)	0.317±0.024[b]	0.106±0.016[c]	0.176±0.060[a]	0.176±0.063[a]
Length (mm)	55.680±5.549[a]	41.630±4.381[b]	54.534±5.917[a]	54.534±6.105[a]
Thickness (mm)	1.113±0.143[b]	1.196±0.086[c]	1.156±0.163[a]	1.164±0.157[a]

[a-d]Mean values with different superscripts in the same row indicate a significant difference (P<0.05), n= 103.

The sphericity values of the fruits were calculated, the highest value (104%) was calculated for Lambert variety, the lowest value (91%) was calculated for Merton Late variety and the difference was statistically significant (P<0.05). The surface area values of the fruits were calculated, the highest value (19.228 cm^2) was found for 0900 Ziraat variety, the lowest value (8.672 cm^2) was found for Lambert variety and the difference was statistically significant (P<0.05). When the net fruit weights were examined, all variety were found to be statistically different from each other, the highest value was found in 0900 Ziraat with 9.223 g, and the lowest value was determined in Lambert variety with 3.403 g. The results regarding fruit sizes are consistent with the findings obtained in previous studies (Vursavuş et al., 2006; Delice et al., 2012; Sarısu et al., 2019; Eroğul and Özmen, 2020).

3.4. Fruit Seed Parameters Measurements

When fruit seed weights were examined, the highest values were measured in 0900 Ziraat and Starks Gold, the lowest value was obtained from in Lambert variety and the differences were found to be statistically significant. The measurement results were given in Table 5. In addition, the sphericity values of the fruit seeds were calculated, the highest value (85%) was in Lambert variety, the lowest value (81%) was in 0900 Ziraat variety and the differences compared to other variety was found to be statistically significant (P<0.05). The results are in line with the values measured by İkinci and Bolat (2015).

3.5. Fruit Stalk Parameters

The tensile force of the cherry stalks from the branch was measured, and there was no statistical difference between the 0900 Ziraat variety and the other examined varieties. However, the highest values were found in Merton Late and Lambert varieties, and the lowest values were found in Starks Gold varieties. Measured and calculated values are summarized in Table 6.

The number of cherry stalks in cluster was evaluated, and there was no statistical difference between Merton Late and Lambert variety (P>0.05). The highest value was measured in Starks Gold with 2.350 units, and the lowest value was measured in 0900 Ziraat variety with 1.320 units (P<0.05). The lengths of the cherry stalks were determined, and there was no statistical difference between Merton Late, 0900 Ziraat, and Lambert varieties. Starks Gold variety was found to have the shortest stalk length statistically. Similarly, as a result of stalk thickness measurements, the lowest value was found in Starks Gold variety. Merton Late and Lambert were measured as the varieties with the highest stalk thicknesses. The obtained results are similar to other studies on the subject (İkinci and Bolat, 2015; Sarısu et al., 2019).

For 0900 Ziraat variety, the tensile force of the fruit while pulling from the branch was found to be 2.579 N, and the tensile force of the fruit stalk from the branch was found to be 7.041 N. Therefore, if the cherry is picked by pulling from the fruit, the fruit will be separated from the stalk first, and the stalk will remain on the branch. When it is desired to collect the cherries without a stalk, they can be picked by applying a lower force, but since the upper part of the fruit whose stalk is broken off will be opened, the endurance time will be reduced. In addition, when stalkless picking is preferred, pulling down from the top of the fruit rather than squeezing from the sides will cause less damage.

If cherries are to be picked with a stalk, they should be picked by pulling from the stalk, not from the fruit. Since the thickness of the stalks is an average of 1.157 mm, a design should be made to hold the fruit tightly so that the stalk does not slip while pulling. In addition, the stalk length, which was found to be 51.59 mm on average, is sufficient for the handle to be held by an apparatus.

Since the difference between the tensile force values of the fruits from the stalks is statistically significant, it will be beneficial for the tensile force to be adjustable rather than fixed in the designs to be made. The difference between the weight, width, thickness, height, and volume values of the cherries was also found to be statistically significant. For this reason, the system should be designed to be able to change and adjust according to different properties of fruit varieties when necessary. According to the results of the research conducted by Krumov and Christov (2018), cherry fruits should be transported with appropriate methods and the variety are suitable for mechanized harvesting. In the study conducted by Peterson and Wolford (2001) on mechanization, they stated that the cherries harvested by the machine they developed were damaged only 2-6% more than the traditionally harvested cherries, and the ratio of marketable cherries were found as 85-92%. Eroğul and Özmen (2020) reported that 0900 Ziraat variety is the most suitable variety after storage, shelf-life properties and stands out in terms of some quality characteristics.

3.6. Ratio of Fruit Mass to Tensile Force (M/F$_T$)

Moser (1989) stated that if the ratio of fruit mass to tensile force (M/F$_T$) is equal or greater than 1, the fruit is machine harvestable. The relationship between the tensile force of the product and the mass of the product is very important in the design of the harvesting units of the harvesters, especially in terms of the selection of the harvesting method. Table 7 summarizes the fruit mass to tensile force ratio results of the research.

The results indicates that all variety were suitable for machine harvesting (M/F$_T$>1) in the case of harvesting the fruit without a stalk, and 0900 Ziraat variety was suitable for harvesting with a stalk. When the results were examined, it was determined that 0900 Ziraat was the most suitable variety for machine harvesting in the case of picking cherries with or without stalks. The results found are in agreement with similar studies (Kocabıyık et al., 2009). Between the examined varieties, least suitable variety for machine harvesting is Lambert variety (Figure 2).

Table 7. Ratio of fruit mass to tensile force (M/F$_T$) results

	Symbol	Unit	Cherry varieties			
			0900 Ziraat	Starks Gold	Merton Late	Lambert
Tensile force of fruit (from stalk)	F_{T-f}	N	2.579	2.187	2.246	1.530
Tensile force of stalk (from branch)	F_{T-s}	N	7.041	6.355	7.669	7.522
Weight	M	g	9.592	6.000	7.13	3.684
According to tensile force of fruit (from stalk)	M / F_{T-f}		3.719	2.744	3.175	2.408
According to tensile force of stalk (from branch)	M / F_{T-s}		1.362	0.944	0.930	0.490

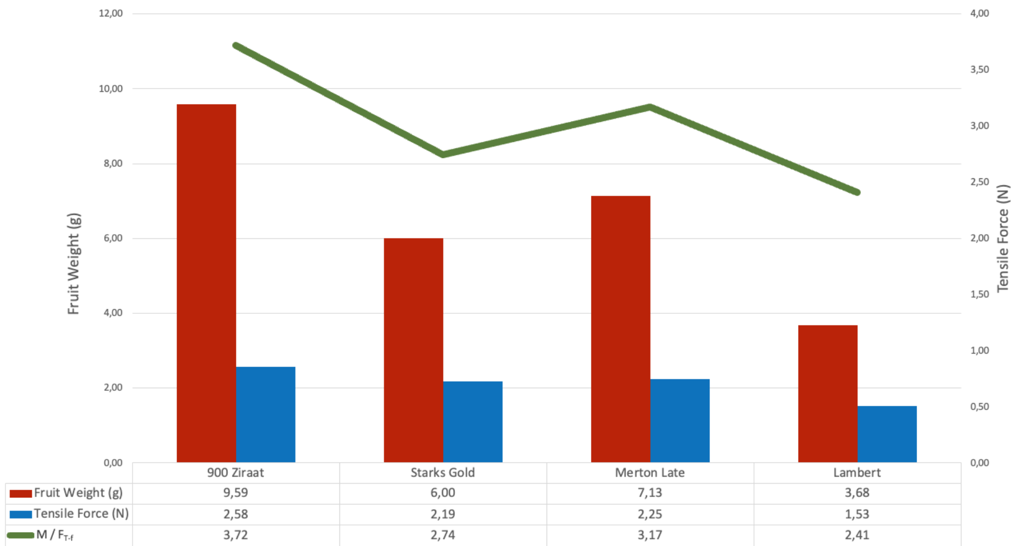

Figure 2. M/F_{T-f} values for the cherry varieties.

4. Conclusion

In the research, the physico-mechanical properties of four cherry varieties grown in Tekirdag were determined and the data were measured to mechanize the cherry harvest. As a result of the evaluation and analysis of the obtained data, it was determined that all four cherry varieties were suitable for mechanical harvesting. However, it was determined that the most suitable variety for mechanical harvesting was ‘0900 Ziraat’. However, it would be beneficial to conduct similar studies in different regions for different cherry varieties, and thus to collect more results and high diversity of data on mechanization.

Author Contributions

E.O.: initiated the research idea, developed, organized, analyzed and interpreted the data and wrote the manuscript. S.A.: supervised the research, suggested the research methods, structured the paper and edited the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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EVALUATION OF SOME ADVANCED BREAD WHEAT (*Triticum aestivum L.*) LINES FOR AGRONOMIC TRAITS UNDER KIRKLARELİ AND TEKİRDAĞ CONDITIONS

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Abstract: This study was carried out to determine grain yield and yield components of five bread wheat genotypes and 20 advanced lines at Kırklareli and Tekirdağ locations in 2017-2018 cropping year. The experiments were arranged in a randomized complete block design with four replications. In the study, grain yield (GY), plant height (PH), spike length (SL), number of spikelets/spike (SS), number of grains/spike (KS), grain weight/spike (KWS), test weight (TW) and thousand kernel weight (TKW) were investigated. Bread wheat genotypes were found statistically significant for all investigated traits according to the data obtained from two locations. According to the results of two locations the investigated traits such as GY ranked from 423.8 to 572.5 kg da^{-1}, PH 85.3 to 116.2 cm, SL 8.8 to 12.2 cm, SS 16.5 to 21.6, KS 39.4 to 65.6, KWS 1.30 to 2.91 g, TW 65.9 to 75.6 and TKW 32.6 to 44.7 g. Relationship between GY and SS, KS and KWS were found positive and significant. In addition, relationship between PH and SL (r=0.39*), SS and KS (r=0.39*), SS and KWS (r=0.42*), TW and KS (r=0.42), TW and KWS (r=0.44*), KS and KWS (r=0.64**) were significant and positive. The highest grain yield was obtained from SME9 bread wheat advanced line at both Kırklareli and Tekirdağ, which was concluded as promising.

Keywords: Bread wheat, Advanced line, Yield, Yield components

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1. Introduction

Wheat is a grain that is essential in human nutrition, industry, and animal feed, while also having strategic importance in many countries' trade. Wheat is the primary source of nutrition for over a third of the world's population. Wheat also provides 19% of the calories and 21% of the protein consumed by the world's population (Ali, 2017; Bordoni et al., 2017; Akan et al., 2021).

Supplying the production needs for appropriate and balanced nutrition has become a major issue today and in the next years as a result of the rapid increase in population in the world and in Turkey, as well as the narrowing of production areas. In order to overcome this problem, it is important to develop genotypes that are suitable for regional conditions, have a high yield, and have the desired quality traits. (Bayram and et al., 2017; Koc and Akgun, 2018).

Wheat production is influenced by a variety of factors, including yield and quality traits, genetic structure of genotypes, climate and soil type, cultivation practices, and biotic and abiotic stress factors. (Dogan and Kendal, 2012; Kızılgeci et al., 2017).

Wheat cultivation areas in our country fell by 25.9% between 2001 (9.3 million ha) and 2020 (6.9 million ha). While 19 million tons were produced in 2001, 20.5 million tons were produced in 2020 (TUIK, 2022). Despite the decrease in production areas, the production of high yielding and disease resistant varieties that adapt to ecological conditions, and also the dissemination of the produced varieties to farmers, has played an important role in maintaining and increasing production levels.

In this study, five commercial varieties commonly produced in our country and 20 advanced bread wheat lines were evaluated in terms of yield and yield components under Kırklareli and Tekirdağ ecological conditions.

2. Materials and Methods

This research was conducted out in Kırklareli and Tekirdağ locations during 2017-2018 cropping years. Five cultivars (Lucilla, Rumeli, Glosa, Esperia, and Aslı) and 20 advanced bread wheat lines were used as plant materials in the experiment. The research was arranged in a randomized complete block design with four

replications. Sowing was done in the first week of November in both growing seasons, and it was done manually in 5 m long plots with 20 cm row spacing and 6 rows with 500 seeds per m^2. In the experiment, the plot sizes were 6 m^2 for both sowing and harvesting in the trial (6 m × 1 m). Weed control was done manually in the trial plots and no application was made for diseases and pests.
With sowing, 5 kg da^{-1} of nitrogen and 5 kg da^{-1} of phosphorus were applied and top dressing was divided into two and applied as 9 kg da^{-1} N during tillering and 6 kg da^{-1} N during jointing. Harvest was done in the first week of July in both growing seasons. In the study, grain yield (GY), plant height (PH), spike length (SL), number of spikelets/spike (SS), number of grains per spike (KS), grain weight per spike (KWS), test weight (TW) and thousand kernel weight (TKW) were investigated.
The data obtained from two locations were subjected to variance analysis and Duncan multiple comparison test was applied to compare the means. (JMP 15.1 SAS Institute Inc, 2020). While correlation analyzes were performed using the JMP program, visualization was made using the ggplot2 package in R software (Wickham, 2009).

3. Results and Discussion

While genotype and location were both found to be statistically significant in terms of grain yield in the wheat genotypes. However, the genotype × location interaction was found to be statistically insignificant (Table 1).

Table 1. Means and values related to grain yield and plant height

Genotypes	Grain Yield (kg da^{-1})			Plant Height (cm)		
	Kırklareli	Tekirdağ	Mean	Kırklareli	Tekirdağ	Mean
Lucilla	477.5b-e	570.8ab	524.1a-c	88.7k-m	89.0ı-l	88.8jk
Rumeli	497.9a-c	515.8a-f	506.8b-d	100.5ef	96.5e-h	98.5fg
Glosa	457.9b-f	481.6c-g	469.7c-g	89.7j-m	88.0j-l	88.8jk
Esperia	434.6c-f	493.3b-g	463.9d-g	85.2m	87.7kl	86.5k
Aslı	497.5a-c	495.6b-g	496.5b-e	95.0f-j	98.2d-f	96.6gh
SME1	438.7c-f	440.0e-h	439.3fg	98.7fg	98.5de	98.6fg
SME2	462.9b-f	471.8c-g	467.3d-g	100.2ef	104.0b	102.1ef
SME3	461.2b-f	519.6a-e	490.4b-f	110.2b-d	103.5bc	106.8c
SME4	442.0c-f	435.1f-h	438.6fg	104.8de	97.2d-g	101.0ef
SME5	447.5c-f	484.8c-g	466.1d-g	119.5a	113.0a	116.2a
SME6	440.8c-f	501.0b-g	470.9c-g	111.0bc	102.0b-d	106.5cd
SME7	440.8c-f	452.7d-h	446.7e-g	109.2b-d	113.0a	111.1b
SME8	420.4d-f	522.7a-d	471.5c-g	114.7ab	113.5a	114.1ab
SME9	561.2a	583.7a	572.5a	87.7lm	85.5l	86.6k
SME10	445.4c-f	477.9c-g	461.6d-g	96.2f-ı	91.0ı-k	93.6hı
SME11	436.6c-f	434.2gh	435.4g	105.8c-e	100.0b-e	102.8de
SME12	399.1f	482.9c-g	441.0fg	97.5f-h	96.7e-h	97.1gh
SME13	403.3ef	513.3a-g	458.3d-g	91.7ı-l	92.3h-k	92.0ıj
SME14	465.8b-f	537.9a-c	501.8b-d	85.5m	85.2l	85.3k
SME15	462.5b-f	487.1c-g	474.7c-g	93.8g-k	93.5f-ı	93.6hı
SME16	480.4b-d	468.7c-g	474.5c-g	105.3de	100.0b-e	102.6e
SME17	479.2b-d	454.6d-h	466.9d-g	98.7fg	98.8c-e	98.7fg
SME18	432.4c-f	472.3c-g	452.3d-g	92.2h-l	100.0b-e	96.1gh
SME19	531.6ab	545.2a-c	538.4ab	95.7f-ı	92.7g-j	94.2hı
SME20	462.1b-f	385.6h	423.8g	95.7f-ı	93.0g-ı	94.3hı
Location	459.2b	489.1a	474.1	98.9a	97.3b	98.1
Genotype (G)	*	**	**	**	**	**
Location (L)		**			**	
G x L		ns			**	

** significant at 1%, * significant at 5%, ns= not significant

While wheat genotype grain yields ranged from 399.1 to 561.2 kg da^{-1} in Kırklareli and 38.6 to 583.7 kg da^{-1} in Tekirdağ, the average grain yield was determined to be 474.1 kg da^{-1}. The highest grain yield was obtained from the SME9 genotype in both locations and according to the location averages. It has been reported that the yield is affected by many factors such as the genetic potential of the genotype, breeding techniques, and ecological conditions. In previous studies, Bayram et al. (2017), 213.5-756.8 kg da^{-1}, Gungor and Dumlupinar, (2019a), 515.2-790.7 kg da^{-1}, Koc and Akgun, (2018), 722.6-1003.3 kg da^{-1}, Karaman et al. (2021), 186.3-813.0 kg da^{-1} and Sagır and Kara (2021) determined a variation between 147.3-401.3 kg da^{-1}.

The average values for plant height are given in Table 1. The difference in plant height among genotype, location, and genotype × location was found to be statistically significant in the study. Plant heights ranged from 85.2 to 119.5 cm in Kırklareli and from 85.2 to 113.5 cm in Tekirdağ. The SME5 and SME8 genotypes had the longest plant height, while SME9, Esperia and SME14 genotypes had the shortest plant height. The average plant height was found to be 98.9 cm in Krklareli, 97.3 cm in Tekirdağ. The plant height in wheat varies genetic performance, climate and soil structure, and cultural practices according to reports. Mut et al. (2017) reported 60.2-80.3 cm, Gungor and Dumlupinar, (2019a) 80.7-112 cm, and Demirel et al. (2021), 52.16-96.66 cm.

In terms of spike length, differences between genotype, locations, and genotype × location interaction were found to be statistically significant. The spike lengths of genotypes were determined to be 8.6-11.6 cm in Kırklareli and 9.0-13.2 cm in Tekirdağ, with the average value of the locations as 10.4 cm (Table 2). The genotypes with the biggest spike length values were SME11 and SME17, whereas the genotype with the shortest spike length was Aslı cultivar. The spike length was found 8.87-11.10 cm by Aydogan and Soylu, (2017) and 4.65-11.9 cm by Demirel et al. (2021).

Table 2. Means and values related to spike length and no spikelets/spike

Genotypes	Spike Length (cm)			Spikelets/Spike (numbers)		
	Kırklareli	Tekirdağ	Mean	Kırklareli	Tekirdağ	Mean
Lucilla	10.2de	9.7kl	9.9h-j	19.7b-d	20.2c-f	20.0c-h
Rumeli	9.6e-g	9.6kl	9.6ıj	20.5ab	20.5c-e	20.5b-d
Glosa	9.4fg	9.7kl	9.5j	18.0e-g	20.7b-d	19.3e-k
Esperia	9.7e-g	10.1ı-k	9.9h-j	20.0b-d	20.7b-d	20.3b-e
Aslı	8.6h	9.0l	8.8k	19.0c-f	19.5d-g	19.2f-k
SME1	9.5fg	9.5kl	9.4j	17.7fg	19.0e-h	18.3kl
SME2	10.9bc	11.8cd	11.3b	19.2b-e	19.0e-h	19.1g-k
SME3	9.9d-g	10.1ı-k	10.0h-j	19.0c-f	19.0e-h	19.0h-l
SME4	10.2de	11.8cd	11.0b-d	20.0b-d	22.2ab	21.1ab
SME5	11.1a-c	10.9d-ı	11.0b-d	21.5a	21.7a-c	21.6a
SME6	11.0a-c	11.2c-f	11.1b-d	19.7b-d	19.2d-h	19.5d-j
SME7	11.1a-c	12.0bc	11.5b	20.5ab	17.8hı	19.1g-k
SME8	10.9bc	11.5c-e	11.2bc	20.2a-c	19.5d-g	19.8d-h
SME9	10.0d-f	11.3c-f	10.6d-g	20.0b-d	20.7b-d	20.3b-e
SME10	10.5cd	10.9e-j	10.7c-f	18.2e-g	19.2d-h	18.7ı-l
SME11	11.3ab	13.2a	12.2a	17.5g	22.7a	20.1b-g
SME12	9.4fg	11.1d-g	10.3e-h	18.7d-g	18.7f-ı	18.7ı-l
SME13	9.7e-g	10.6f-j	10.2f-h	17.7fg	18.2g-ı	18.0l
SME14	9.7e-g	10.3g-k	10.0h-j	18.7d-g	18.2g-ı	18.5j-l
SME15	9.7e-g	10.2h-k	8.9h-j	15.7h	17.3ı	16.5m
SME16	9.9d-g	11.6c-e	10.8c-e	18.7d-g	20.7b-d	19.7d-ı
SME17	11.6a	12.8ab	12.2a	20.5ab	20.5c-e	20.5b-d
SME18	9.3g	10.8e-j	10.1g-ı	19.2b-e	21.5a-c	20.3b-e
SME19	10.5cd	11.0d-h	10.9c-e	20.0b-d	20.5c-e	20.2b-f
SME20	9.4fg	10.0jk	9.7h-j	19.7b-d	22.2ab	21.0a-c
Location	10.1b	10.8a	10.4	19.2b	20.0a	19.6
Genotype (G)	**	**	**	**	**	**
Location (L)		**			**	
G x L		**			**	

** significant at 1%

In terms of the number of spikelets per spike, the interaction of genotype, location, and genotype × location was found to be statistically significant. It ranged from 15.7 to 21.5 in Kırklareli and from 17.3 to 22.7 in Tekirdağ. When the two locations were combined, the average number of spikelets per spike was found to be 19.6. The largest number of spikelets per spike was determined in the advanced line SME5, while the lowest number of spikelets per spike was determined in the advanced line SME15 based on the mean data of two locations. In other researches, the number of spikelets was reported as 16.5-21.2 by Gungor and Dumlupinar (2019a), 12.1-16.3 by Sagır and Kara (2021), and 18.15-22.13 by Akan et al. (2021).

According to the combined analysis in terms of the number of grains per spike in wheat genotypes; Genotype, location and genotype × location interaction were found to be statistically significant. The average number of grains per spike (55.5) in Tekirdağ location was higher than the average (52.4) in Kırklareli location. While the number of grains per spike was found to be 35.2-68.0 in Tekirdağ location, the average grain number per spike was determined as 40.3-71.7 in Kırklareli location. The highest number of grains per spike was obtained in variety Rumeli in Kırklareli location, advanced line SME4 in Tekirdağ location, the lowest grain number in advanced lines SME15 in Kırklareli location and SME1 in Tekirdağ location. The average number of grains per spike obtained by combining the two locations was found to be 53.9 (Table 3). Aydogan and Soylu, (2017), 31.2-44.9, Bayram et al. (2017), 13.7-26.6, Gungor and Dumlupinar, (2019a), 16.5-21.2, Demirel et al. (2021), determined between 11.3-31.33 with a high variation.

Table 3. Means and values related to no grains/spike and grain weight/spike

Genotypes	Grains/Spike (grains)			Grain weight/Spike (g)		
	Kırklareli	Tekirdağ	Mean	Kırklareli	Tekirdağ	Mean
Lucilla	62.8b	61.5a-d	62.1a-c	2.48b-d	2.76a-d	2.62a-d
Rumeli	71.7a	59.5a-f	65.6a	3.06a	2.53b-f	2.80ab
Glosa	47.0e-g	53.7b-h	50.3g-ı	2.09e-ı	2.61a-e	2.35d-ı
Esperia	58.2bc	62.2a-d	60.2a-d	2.72b	2.11e-g	2.41c-g
Aslı	51.7c-e	44.0hı	47.8hı	2.33c-f	2.01fg	2.17f-j
SME1	43.5fg	35.2ı	39.4j	1.26l	1.34h	1.30k
SME2	45.0e-g	51.5d-h	48.2hı	1.95h-j	1.94g	1.95j
SME3	52.2c-e	52.7c-h	52.5e-h	2.20e-h	2.19e-g	2.19f-j
SME4	51.2c-e	68.0a	59.6a-d	2.25d-g	2.83a-c	2.54b-e
SME5	62.5b	64.5ab	63.5ab	2.03g-j	2.74a-d	2.39c-h
SME6	49.5d-f	44.7g-ı	47.1hı	2.03g-j	1.90g	1.97j
SME7	61.5b	61.7a-d	61.6a-c	2.05f-j	2.39c-g	2.22f-j
SME8	52.2c-e	50.0e-h	51.1f-ı	2.48b-d	2.40c-g	2.44c-f
SME9	49.0d-f	52.8c-h	50.8g-ı	1.86ı-k	2.28d-g	2.07ıj
SME10	57.7bc	60.5a-e	59.1a-e	2.58bc	2.31c-g	2.45c-f
SME11	49.5d-f	63.3a-c	56.3c-g	2.67b	3.14a	2.91a
SME12	46.0e-g	61.5a-d	53.7d-h	1.64k	2.54b-f	2.09h-j
SME13	42.2fg	55.7b-g	49.0hı	2.11e-ı	2.14e-g	2.12g-j
SME14	61.2b	54.3b-h	57.7b-f	2.28d-g	2.02fg	2.15f-j
SME15	40.3g	48.5f-h	44.3ıj	1.81jk	2.33c-g	2.07ıj
SME16	51.5c-e	61.8a-d	56.6c-g	2.29d-g	3.05ab	2.67a-c
SME17	56.3b-d	56.0b-g	56.1c-g	2.13e-ı	2.19e-g	2.16f-j
SME18	46.7e-g	58.2a-f	52.5e-h	2.03g-j	2.55b-f	2.29e-ı
SME19	56.0b-d	43.8hı	49.8g-ı	2.36c-e	2.31c-g	2.33d-ı
SME20	45.7e-g	62.0a-d	53.8d-h	2.29d-g	2.81a-d	2.55b-e
Location	52.4b	55.5a	53.9	2.20b	2.38a	2.29
Genotype (G)	**	**	**	**	**	**
Location (L)		**			**	
G x L		**			**	

** significant at 1%

The average grain weight per spike are given in Table 3. In terms of grain weight per spike, significant differences were found between genotype, location, and genotype × location. The grain weight per spike was determined to be 1.3-2.91 g and the average grain weight per spike was determined to be 2.29 g based on the average of the two locations. The grain weight per spike was found to vary between 1.26-3.06 g at the Kırklareli location and 1.34-3.14 g in the Tekirdağ location. Variety Rumeli in Krklareli location and advanced line SME11 in Tekirdağ location had the highest value. In both locations, advanced line SME1 had the lowest value. In previous works, Aydogan and Soylu (2017), 1.33-2.07, Altındal and Akgun (2017), 0.76-1.94 g, Gungor and Dumlupinar (2019a), 0.93-2.25 g, Subası and Ayrancı (2021), 0.669-1.981 g reported.

In terms of test weight, genotype, location, and genotype × location were found to be statistically significant in the study (Table 4). The test weight in Kırklareli was found to be between 67.6-76.0 kg hl^{-1}, whereas in Tekirdağ it was found to be between 63.4-75.1 kg hl^{-1}. In Kırklareli, genotypes SME16 (76.0 kg hl^{-1}) and SME19 (75.4 kg hl^{-1}) had the highest value, while advanced line SME16 (75.1 kg hl^{-1}) and cultivar Lucilla (74.2 kg hl^{-1}) had the highest value in Tekirdağ. The SME20 genotype had the lowest value in Kırklareli, whereas the SME1 genotype had the lowest value in Tekirdağ. The test weight was determined to be 71.7 kg hl^{-1} as average of the two locations. In previous studies, it is stated to be varied between 73.32 to 84.91 kg hl^{-1} (Aydogan and Soylu, 2017; Mut et al., 2017; Gungor and Dumlupinar, 2019a; Karaman et al., 2021).

Table 4. Means and values related to test weight and thousand kernel weight

Genotypes	Test Weight (kg hl^{-1})			Thousand kernel weight (g)		
	Kırklareli	Tekirdağ	Mean	Kırklareli	Tekirdağ	Mean
Lucilla	75.0b-d	74.2ab	74.6b	36.3ı-k	41.2b	38.7de
Rumeli	75.0b-d	72.1c	73.5c	37.6e-g	38.5de	38.1fg
Glosa	73.2fg	71.8cd	72.5e-g	39.4c	40.0c	39.7b
Esperia	75.0b-d	70.5g-ı	72.7ef	37.2f-h	38.8d	38.0fg
Aslı	71.4j	70.9d-ı	71.2ıj	35.7kl	37.0g-ı	36.4k
SME1	68.5kl	63.4p	65.9n	34.3m	34.7jk	34.5l
SME2	72.9gh	71.2c-h	72.0gh	37.5e-g	37.8e-g	37.6g-ı
SME3	72.6g-ı	68.4m-o	70.5kl	37.4fg	38.8d	38.1fg
SME4	69.1k	70.9e-ı	70.0l	37.9ef	37.8e-g	37.9f-h
SME5	74.7b-d	71.1d-h	72.9de	36.9g-ı	37.4f-ı	37.2ıj
SME6	73.0f-h	68.3no	70.7jk	36.6h-j	36.8hı	36.7jk
SME7	74.4de	71.1d-h	72.7ef	34.8m	35.4j	35.1l
SME8	75.2bc	71.7c-e	73.4cd	38.7cd	37.8e-g	38.2ef
SME9	71.4j	71.5c-f	71.4ı	34.4m	38.4de	36.4k
SME10	71.9ıj	68.6m-o	70.2kl	38.2de	37.9d-g	38.1fg
SME11	74.4de	70.1ı-k	72.2fg	34.3m	32.3m	33.3m
SME12	74.6cd	73.4b	74.0c	35.0lm	34.0kl	34.5l
SME13	72.4hı	67.8o	70.1kl	39.2c	33.5l	36.4k
SME14	73.7ef	69.6j-l	71.6hı	34.4m	30.7n	32.6n
SME15	71.2j	68.9l-n	70.0l	44.9a	44.4a	44.7a
SME16	76.0a	75.1a	75.6a	41.4b	36.5ı	38.9cd
SME17	74.5c-e	71.3c-g	72.9de	36.2ı-k	38.1d-f	37.2ıj
SME18	68.3lm	69.2k-m	68.7m	38.0d-f	40.6bc	39.3b-d
SME19	75.4ab	70.3h-j	72.9de	41.2b	37.7e-h	39.5bc
SME20	67.6m	70.8f-ı	69.2m	35.8j-l	38.9d	37.4hı
Location	72.9a	70.5b	71.7	37.3	37.4	37.4
Genotype (G)	**	**	**	**	**	**
Location (L)		**			ns	
G x L		**			**	

** significant at 1%, ns= not significant

While genotype and genotype × loction interaction were both found to be statistically significant in terms of thousand grain weight in the wheat genotypes. However, the location was found to be statistically insignificant (Table 4). The thousand-grain weight of wheat genotypes in the Kırklareli location ranged from 34.3 to 44.9 g. The SME15 genotype had the highest thousand grain weight, whereas the SME11 genotype had the lowest. The thousand grain weight at Tekirdağ location ranged from 30.7 to 44.4 g. The SME15 genotype had the highest thousand grain weight, while the SME14 genotype had the lowest. When two locations were combined, the average thousand grain weight was found to be 32.6-44.7 g. The greatest thousand grain weight was determined in the SME15 genotype, while the lowest thousand grain weight was determined in the SME14 genotype, based on the average of two sites (Table 4). Aydogan and Soylu (2017) stated that the weight of a thousand grains varied between 28.0-35.2 g, Gungor and Dumlupinar (2019a), 35.8-47.2 g, Mut et al. (2017), 29.2-38.4 g, Karaman et al. (2021), 23.51-46.71 g, and Sagır and Kara (2021) claimed that it ranged between 28.0-35.2 g.

According to correlation analyses of yield and yield components examined at two locations in bread wheat genotypes, there was no significant relationship between grain yield and the rest of investigated traits. Plant height and spike length (r=0.39**), number of spikelets/spike and number of grains/spike (r=0.39*), and grain weight/spike (r=0.42*) were found to be positive and significant. It was found that test weight and the number of grains/spike (r=0.39*) and grain weight per spike (r=0.44*) had a significant and positive relationship. There was a positive correlation between the number of grains per spike and the grain weight per spike (r=0.64**) (Figure 1). Boru et al. (2019) found a positive and significant relationship between grain yield and ear length (r=0.666*), grain number per spike (r=575*), and grain weight per spike (0.825*), as well as a negative and significant relationship between ear length and grain number per spike (0.578*) and kernel weight per ear (r=0.586*). In their study on Bolu ecological conditions, Gungor and Dumlupinar (2019b) found that grain yield was positively correlated with plant height (r=0.755**), heading time (r=0.118), spike length (r=0.141), number of spikes per spike (r=0.210), number of grains per spike (r=0.223), 1000 grain weight (r=0.015), and gluten ratio (=0.274).

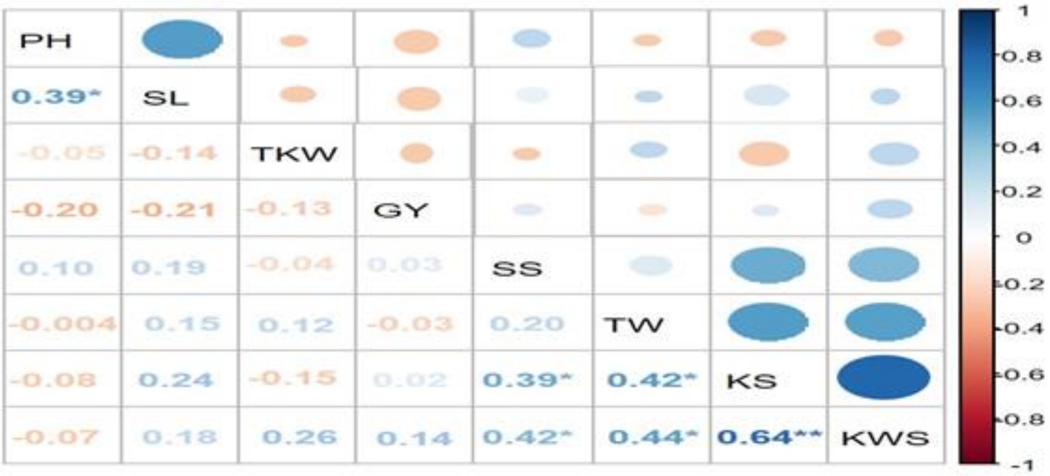

Figure 1. Correlation of yield and yield components in wheat genotypes.

4. Conclusion

Wheat production and economy have an important role for our country. This study was carried out under the ecological conditions of Kırklareli and Tekirdağ in Thrace Region. In this study, registered varieties and newly developed bread wheat lines were evaluated in terms of yield and yield components at two locations. It is determined that, SME9 line was found to be promising as high yielding at both locations. Thus, it is concluded that it might be appropriate to evaluate at more locations.

Author Contributions

All authors had equal contributions and all authors reviewed and approved the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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SOCIO-ECONOMIC STRUCTURE OF CATTLE ENTERPRISES IN NORTHEAST ANATOLIA REGION: AN EXAMPLE OF İSPİR COUNTY OF ERZURUM PROVINCE

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Abstract: This study was carried out to reveal the current situation regarding the socio-economic characteristics of cattle enterprises in İspir county of Erzurum province. For this purpose, a face-to-face survey was conducted with 394 cattle farm owners determined using the random sampling method. The data obtained were interpreted using frequency analysis. The enterprises were mostly small-scale family types that had less than 20 (69.3%) animals. The number of cattle in the enterprises was classified as less than 11, 11-20, 21-30, 31-40, and more than 40 heads. Additionally, educational status of the owner of the enterprises were grouped as illiterate, literate, Primary School graduate, Secondary School graduate and High School graduate. The average age of the breeders was determined to be 55.2 years, with the majority between 50-70 years. The level of education of the breeders was low and the majority of them were primary school graduates (68.8%). More than half of the enterprise owners (58.4%) had more than 30 years of experience in cattle breeding. Apart from the owner, the number of people who cared for the animals was usually 2 people (43.7%) or 3 people (33.5%), and the person who cared for the animals was generally a family member. Only 27.9% of the enterprises were members of a union and 49.3% of the member enterprises preferred the Agricultural Credit Cooperative. As a result; the high average age of the population engaged in animal husbandry in the county makes it necessary to clear the way for young entrepreneurs with various supports and to prevent migration to the cities. In addition, carrying out various training and incentive activities to eliminate the disadvantages such as the low level of education of the breeders and membership of a union will make important contributions to the development of the Country's livestock sector.

Keywords: Cattle breeders, Farmers' experience, İspir, Socio-economic structure

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1. Introduction

The livestock sector has a very important and strategic place in the economy of the countries in terms of animal products, the added value and employment it creates. Livestock activities are a continuous production branch that can be performed throughout the year (Koçyiğit et al., 2022). Despite the arithmetic increase in food production in the world in recent years, the world population rised geometrically. This situation makes it difficult for people in many countries to meet their nutritional needs, especially protein, and causes malnutrition problems (Özsağlıcak and Yanar, 2021).

Animal husbandry is the most significant source of livelihood for the rural population in the Northeast Anatolian Region. Today, Erzurum and its counties maintain their feature of being one of the most important centers of Turkey in the development of animal husbandry with its large meadow and pasture areas. Rural areas have preceded as the centers of agricultural production for many years, and solutions for rural development problems in these places have been tried to be solved. However, with the mechanization in agriculture and the industrialization in urban areas unemployment rate has increased in rural areas, and migration from these areas to the cities has started (Yalçın and Kara, 2016). With the migration, young population decreased and a demographic structure with elderly people was remained in rural areas. As a result of this cycle, a significant part of the enterprises in rural areas continued their animal husbandry activities with traditional methods, remained in the small family enterprise model, and sustained animal production far from today's economic principles and requirements. In such rural areas, the provision of public services has become difficult, structural problems and low productivity have continued in livestock enterprises, and inadequacies in organization and product marketing have been emerged. Rural areas, where public services cannot be provided adequately, have lost their attractiveness and therefore the interest of new entrepreneurs in rural areas has decreased (Şahin, 2015). This situation has become an important obstacle for agriculture and livestock sector in sustainable and balanced development. Although Turkey's geographical and socio-economic characteristics have an important potential for animal product production, animal husbandry could not reach the desired level in the country due to the reasons stated above. While, the share of animal husbandry in agricultural production in developed countries is over 50%, this rate has remained around 25-30% in Turkey (Tapkı et al., 2018).

Although Erzurum is in the Eastern Anatolia Region, İspir county is in the Black Sea Region. The county is located at the intersection of North East Anatolia and East Black Sea Region. The county is in a transition place between continental and maritime climates, and mainly continental climate characteristics are observed in the region. This characteristic of the climate leads to the formation of different climates in terms of geographical conditions in the north and south of the county. Compared to other counties of Erzurum, the winter months are milder in İspir county (Anonymous, 2021). These climatic conditions have direct or indirect effects on the socio-economic structure of the county.

The population of İspir county is 14 955 as of 2021, 49.6% of the total population is men and 50.4% are women. According to TUIK data; The ratio of the population in the 15-64 age group, which is called the working age, is 59.4% (30.3% Male, 29.1% Female), the population ratio in the 0-14 age group, which is defined as the child age group, is 14.6% and the population ratio of those who are 65 years old and older is 25.9% (Figure 1).

The population of working age in the county has decreased numerically by 10.7% in the last 10 years (Anonymous, 2022a). In recent years, the demographic structure has changed dramatically in the county where the young population has decreased and the elderly population constitutes the majority (Figure 1).

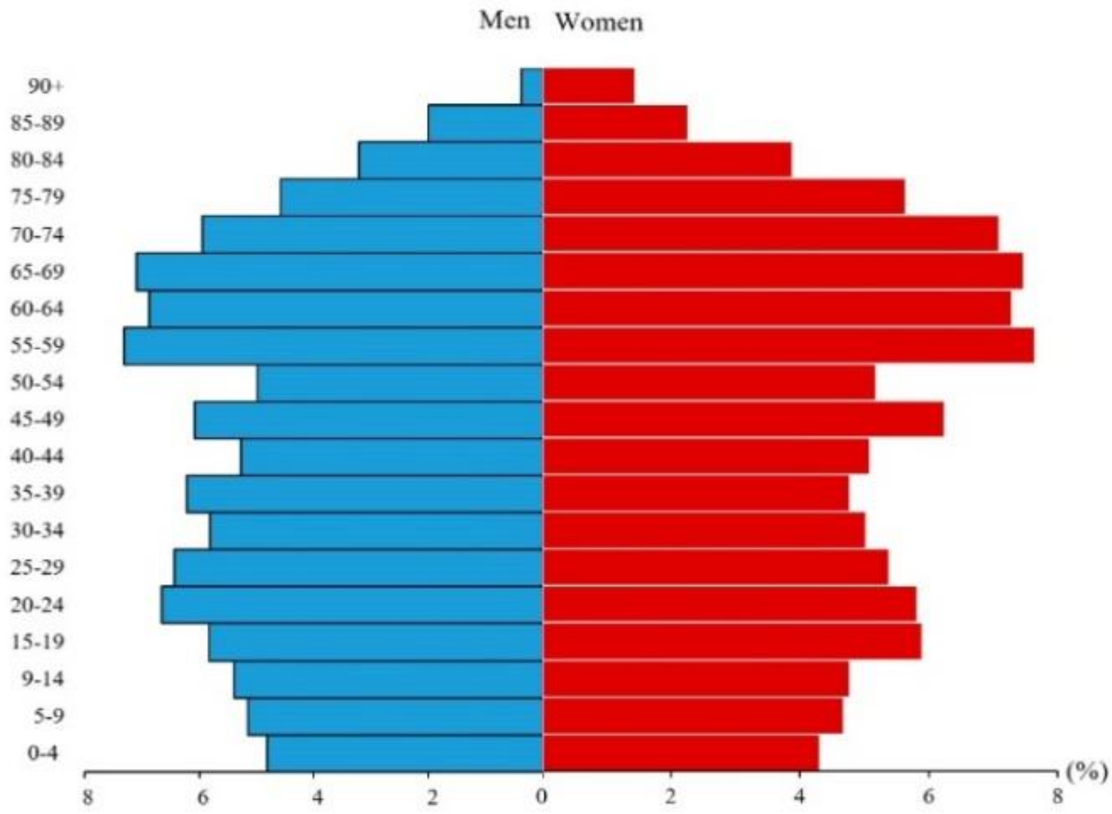

Figure 1. 2021 population pyramid of İspir county (Anonymous, 2022a).

According to 2021 TUIK data, there are 23102 cattle in the county. The cattle presence constitutes 2.7% of the total cattle population in Erzurum province. There was a 7.3% decrease in the number of cattle in 2021 compared to the previous year. High-yielding European breeds constitute 19.4% of the total cattle presence of the county, while crossbreds 77.3% and indigenous breeds 3.4% (Anonymous, 2022b). The number of lactating cows constitutes 37.7% of the total cattle population of the county. Annual milk yield per milked cow was 3.7 tons/head in high-yielding European breeds, 2.9 tons/head in crossbreds and 1.3 tons/head in domestic breeds. The annual milk yield per cow is close to the Turkey averages (Anonymous, 2022c). However, although the annual milk yield has increased over the years both in Turkey and in the county, the annual milk yield per cow is quite low when compared to EU countries with an annual milk yield of over 6.0 tons/head.

Cattle enterprises in the county are generally small-scale family-type. Providing support to small-scale enterprises is highly important for the development of the agricultural economy, as well as sustainable development policies. In order to ensure the development of the agricultural sector, in addition to other structural problems, economic and social issues in enterprises should also be taken into consideration. Socio-economic problems such as low agricultural income, poverty risk, low market power, weak market integration, quality of education and health services and other cultural issues are among the most important problems for enterprises. Therefore, improvements in these areas seem to be of great importance. Ensuring a balance in terms of economic and social situation in enterprises will enable them to adapt to future changes and continue their activities in the long run. This study was carried out to determine the socio-economic profile of cattle farms in İspir county of Erzurum province, to reveal the existing problems related to animal husbandry and to offer solutions for the realization of sustainable activities.

2. Material and Methods

2.1. Animal Materials

The study was carried out on the owners of randomly selected dairy cattle enterprises located in İspir county of Erzurum province. A face-to-face survey was conducted on 394 individuals, and data obtained from the questionnaire comprised the material of the present study. The enterprises were visited and the current situation was tried to be revealed through observation together with survey questions. Since the variance is unknown as well as the population is limited and there are qualitative variables dependent on probability, the method whose formula is given in equation 1 was utilized for the determination of the sample size of the research (Arıkan 2007).

$$n = \frac{N.t^2.p.q}{(N-1).D^2 + t^2.p.q} \quad (1)$$

In this formula; n= minimum number of necessary samples, N= population size, D= acceptable or desired sampling error (5%), t= table value (t=1.96 for α= 0.05), p= the rate to be calculated (0.5) and q=1-p.

With the formula given above, the estimated sample size was calculated to be as approximately 325 (equation 2).

$$n = \frac{2107.(1.96)^2.0.5.(1-0.5)}{(2107-1).(0.05)^2 + (1.96)^2.0.5.(1-0.5)} = 325 \quad (2)$$

After obtaining the number of samples, the number of surveys was increased by 21.23%. The final number of surveys to be carried out in the villages of the İspir county of Erzurum province was determined as 394.

2.2. Statistical Analysis

The data obtained from the survey work were transferred to Excel 2010 computer program before statistical analysis was performed. The number of cattle in the enterprises was classified as less than 11, 11-20, 21-30, 31-40, and more than 40 heads. Additionally, educational status of the owner of the enterprises were grouped as illiterate, literate, Primary School graduate, Secondary School graduate and High School graduate. Chi-square analysis available in SPSS statistics program was used to determine the effects of the number of cattle and the educational status of the owners of the enterprises in the enterprises on the structural characteristics of cattle barns in the enterprises (SPSS 2011).

3. Results and Discussion

The distribution of the enterprises according to the number of animals in the county are presented in Figure 2. Cattle enterprises in İspir county are generally small-scale. In these enterprises, the percentage of holdings with 0-10 animals is the highest (39.6%), followed by enterprises with 11-20 and 21-30 animals (29.7% and 14.2%, respectively). Enterprises with less than 20 animals in the county constitute 69.3% of all cattle enterprises. Similar results were reported in various studies conducted in Turkey (Özder and Özder, 2008; Şeker et al., 2012; Savaş and Yenice, 2016; Şahin and Karadağ Gürsoy, 2016).

Similarly, Ayenew et al. (2011) reported the average number of cattle in farms in Ethiopia as 21.8 heads in urban (Urban) regions and 8.1 heads in rural (Peri-urban) regions. The number of milking cows in the enterprises were reported as 2 heads in 52.5% of the enterprises in Tanzania (Mzingula, 2019), 1-5 heads in %37.6 of the enterprises in West Kenya (Amimo et al., 2011), 6.7 heads in average in Bangladesh (Datta et al., 2019), and 10-50 heads in more than half (50.7%) of the enterprises in Nigeria (Saleh, 2018). On the other hand, in a study conducted in Cameroon, it was reported that 37.4% of the enterprises had 50-100 cattle (Mingoas Kilekoung et al., 2014). In another study conducted in the Northern Benin province of West Africa, it was determined that the average herd size was 45 heads, but the number of cattle in 41.0% of the enterprises was less than 25 heads (Houessou et al., 2019).

The average age of dairy cattle breeders in İspir county of Erzurum was determined as 55.2 years old and the majority of breeders in the county were between the ages of 51-70 (Figure 3). While the age group of 50-60 years was in the first place with a share of 27.2%, it was followed by the groups of 61-70, 41-50, 31-40, >70, <30, respectively. While the average age of enterprise owners having 0-10 animals was 62.5 years, the average age of the breeders having 41 heads and above animals was 49.2 years.

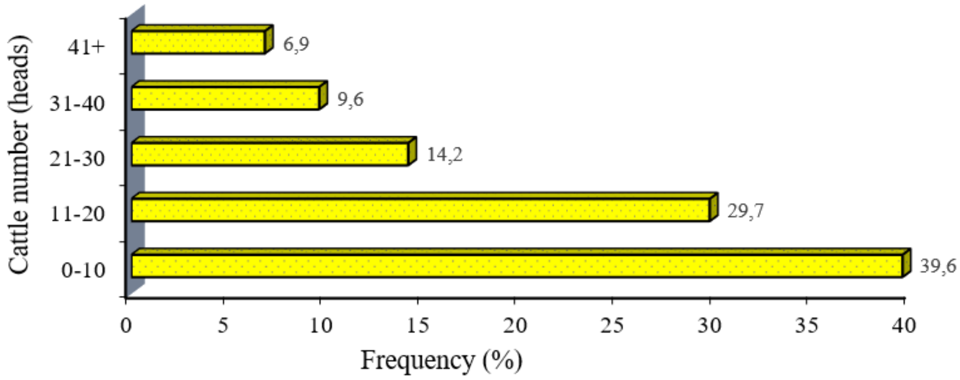

Figure 2. Distribution of the enterprises according to the number of cattle.

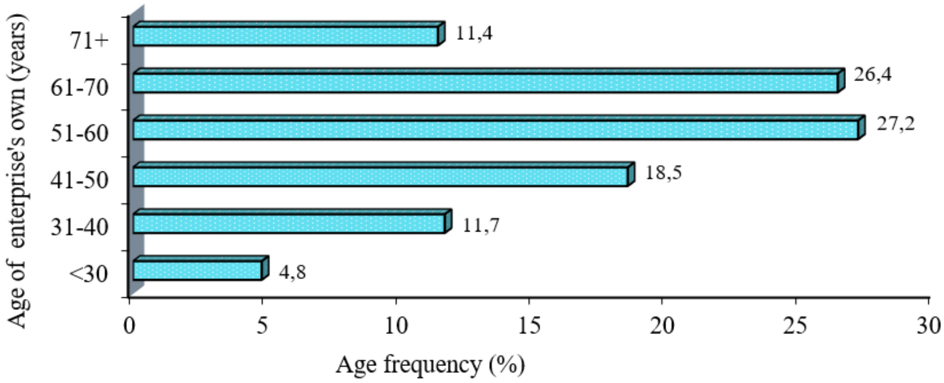

Figure 3. Age groups of cattle breeders in İspir county.

Similarly to the findings of the present study, it was reported that the average age of breeders in Tanzania was 52 years, and the majority (43.3%) of the enterprise owners were between the ages of 40-59 (Mzingula, 2019), while in Western Kenya, the majority of the respondents (38.8%) were between 46-60 years of age (Amimo et al., 2011). In studies conducted in South Africa, Grobler et al. (2008) reported that 60% of the breeders were in the 50-70 age group, while Van den Berg (2013) reported that the majority of the breeders were older than 61 years. On the other hand, the average age of cattle breeders in Azerbaijan and Georgia was reported to be 33.4 and 41.3 years, respectively (Neudert et al., 2020). The average age of breeders was 48 years in Nigeria (Saleh, 2018), 47 years in Finland (Sahlström et al., 2014), while the majority of the cattle breeders (59.1%) in Cameroon was between 26-45 years old (Mingoas Kilekoung et al., 2014). In different studies conducted in Turkey, it was reported that the age of breeders was between 41-47 years (Demir et al., 2014; Bakan and Aydın, 2016; Şahin and Karadağ Gürsoy, 2016; Tapkı et al. al., 2018; Mat and Cevger, 2020; Paksoy and Bulut, 2020). Considering these data, it can be said that the dairy cattle breeding sector in İspir county have an older population structure.

There is a strong relationship between the level of education and the yield obtained in livestock enterprises. It can be said that the level of education is generally high in enterprises where farming is carried out more consciously (Şahin and Karadağ Gürsoy, 2016). The education level of dairy cattle enterprise owners in İspir county was considerably low (Figure 4). Among cattle breeders in the county, primary school graduates represent the highest population with 68.8%, followed by literate 17.3%, secondary school graduates (5.8%), high school graduates (5.6%) and illiterate 2.5%. In addition, there were no breeders with a university or college degree in the county.

Similarly, it was reported that the majority of the breeders were primary school graduates (51.7%), while the percentage of high school and university graduates were 5.9% and 0.8%, respectively, in the Çatak, Erciş and Özalp countries of Van province, (Terin and Ateş, 2010). Furthermore, a big majority of the breeders (75.4%) in Edirne province were primary school graduates, while 21% were secondary school graduates, 3.5% were high school graduates, and there was no breeder with a college degree (Özder and Özder, 2008). In Giresun province, the percentage of primary school graduates was 54.2%, high school graduates was 9.1%, and the percentage of the breeders without any education was 19.3% (Tugay and Bakır, 2009). On the other hand, the percentage of high school and university graduate breeders was reported as 15.0% and 14.0% in Tekirdağ province (Soyak et al., 2007), 21.0% and 1.0% in Kahramanmaraş province (Kaygısız et al., 2010), 18.4% and 2.4% in Muş province (Şeker et al., 2012), 17.7% and 3.4% in Kars province (Tilki et al., 2013), and 20.8% and

1.0% in Çayırlı county of Erzincan province (Özyürek et al., 2014).

Similar results were reported in studies conducted abroad, Amimo et al. (2011) determined that 36.1% of breeders in western Kenya did not receive formal education, while 23% were primary school graduates and 26% were secondary school graduates. The proportion of farmers who graduated from primary school was reported to be the majority in Tanzania (83.3%) (Mzingula, 2019) and almost half in Cameroon (42.7%) (Mingoas Kilekoung et al., 2014). Furthermore, Ayenew et al. (2011) reported that 27.7% of cattle farm owners in Ethiopia were illiterate, 25.5% could read and write, and 19.6% had higher education. In the northern Benin province of West Africa, the majority of the farm owners were reported to be illiterate (Houessou et al., 2019). However, the graduation rates of at least one school and college graduates in Azerbaijan and Georgia were reported to be 73.4%-18.3% and 47.7%-43.2%, respectively (Neudert et al., 2020). On the other hand, the percentages of breeders who were graduated from at least one official school and college graduates in Azerbaijan and Georgia were reported as 73.4%-18.3% and 47.7%-43.2%, respectively (Neudert et al., 2020). Duguma et al. (2012) stated that 35.2% of farmers in Ethiopia had a college degree, while Saleh (2018) stated that 75.6% of them had a university degree in Nigeria.

Advanced age and lower education level of cattle enterprise owners are the main reasons for not be able to following and adopting new technological developments as well as not spending enough time and energy to improve their own or employees' skills and to eliminate the lack of knowledge. This situation can create a significant obstacle to the success of agricultural activities and the development of the livestock sector.

The breeders with an experience of 31-40 years in cattle farming represented the highest share in the county (40.4%) (Figure 5). This group was followed by the farmers with an experience of 21-30 years (27.9%), 41 years and more (18.0%), 11-20 years (10.4%) and 0-10 (3.3%).

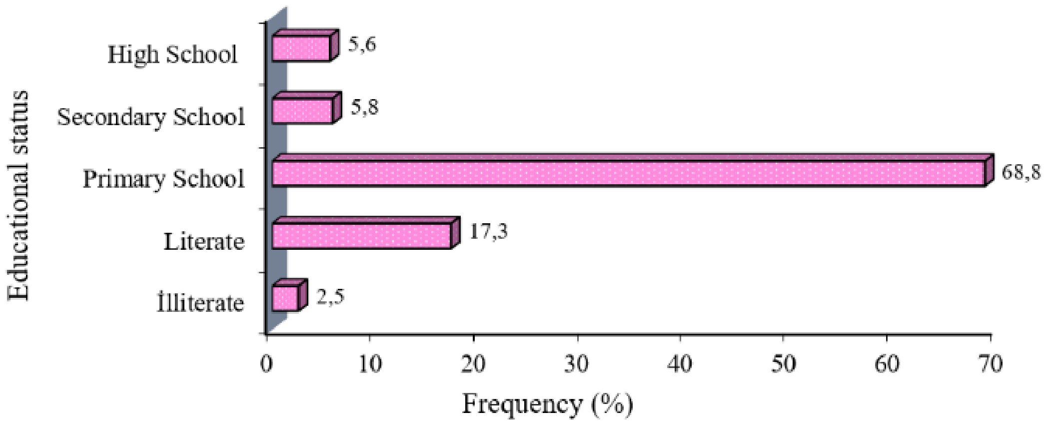

Figure 4. Educational status of cattle breeders.

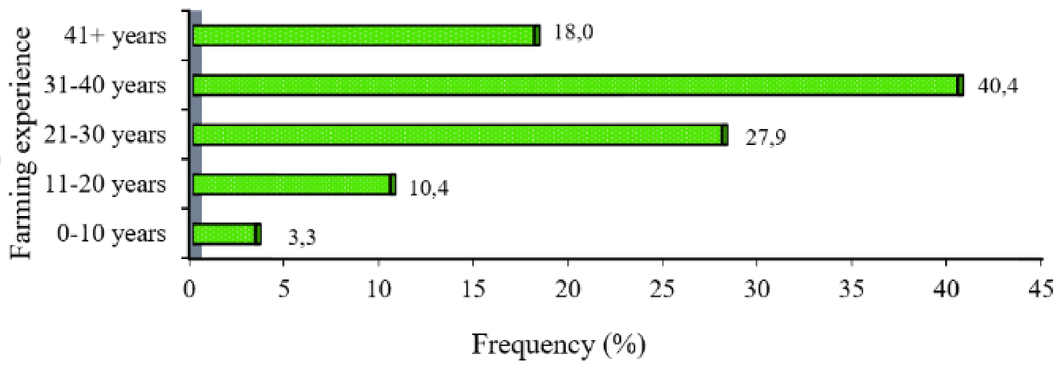

Figure 5. Experience of cattle breeders.

In similar studies, the duration of experience of cattle breeders were reported as 16-30 years (63.0%) by Tugay and Bakır (2006), >21 years (71.2%) by Terin and Ateş (2010), and 14-36 years (47.0%) by Kılıç and Aydın Eryılmaz (2020). Average experience duration on the other hand was reported as 30.2% by Tilki et al. (2013), 15.2% by Kutlar et al. (2013), 24.3 years by Bakan and Aydın (2016), and 16.2 years in Eastern Mediterranean by Yılmaz et al. (2020).

The average duration of experience was reported between 16-35 years in Bangladesh (Datta et al., 2019), 22 years in Finland (Sahlström et al., 2014), and 8 years in Uganda (Ahikiriza et al., 2021). Majority of the breeders (50.0%) had over 15 years of cattle breeding experience in Ethiopia (Duguma et al., 2012). Furthermore, 41.4% of cattle breeders have more than 21 years of experience (Van den Berg, 2013). Cattle breeders in Thailand were reported to have 10.57-14.23 years of experience in average (Rhone et al., 2008). In Nigeria, on the other hand, 62% of cattle farmers had 1-10 years of cattle breeding experience.

The duration of experience of cattle breeders was higher in İspir county compared to the results of similar studies. However, although this situation may seem advantageous, since majority of the farm owners in the county are elderly and have low education levels, this situation disadvantageous in terms of the sustainability of cattle breeding activity in the county.

The distribution of the surveyed enterprises having another economic activity other than cattle breeding and the branch of their activity was presented in Figure 6. It was determined that 41.6% of the breeders had another occupation in addition to cattle farming. Majority of the respondents worked in other businesses (57.0%), while others were occupied in the private sector (24.2%) or in a public institution (18.8%) apart from cattle breeding.

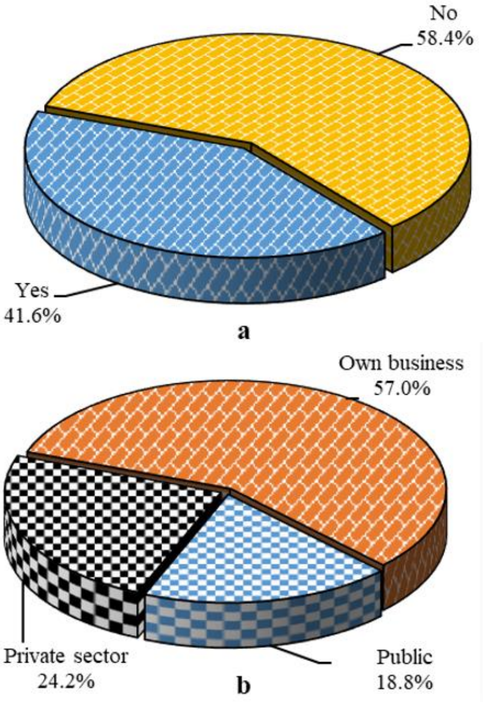

Figure 6. The status of the breeders to have another occupation (a) and the sector they work in (b).

The cattle breeders having another occupation other than cattle breeding was reported as 48.0%, 37.0%, and 29.8% in Muş province (Şeker et al., 2012), Sivas province (Hozman and Akçay, 2016) and Narman county of Erzurum province (Koçyiğit et al., 2018), respectively. On the other hand, Koçyiğit et al. (2016) reported that the percentage of cattle breeders having additional economic activity in Hınıs county of Erzurum province was considerably low (17.0%). Also, Duguma et al. (2012) reported that 25.9% of cattle farmers in Ethiopia were at the same time government workers, 25.9% were retired, 20.4% were traders, 11.1% were housewives and only 16.7% were full-time farmers.

Most of the enterprises that carry out dairy cattle breeding in Turkey also perform other agriculture and livestock activities (Bakan and Aydın, 2016; Hozman and Akçay, 2016; Savaş and Yenice, 2016). In the present study, it was determined that the percentages of enterprise owners having additional occupation was higher than other studies.

The average family population in the enterprises in the county was determined as 4.6 people. The average family size varies between 3.9 and 5.4 people by enterprise groups. It was observed that as the number of animals in the farms increases, the average number of individuals in the family increases in as well. The total number of family members in the enterprises mostly consists of 3 (30.7%), 4 (21.6%) or 5 people (22.2%) (Figure 7).

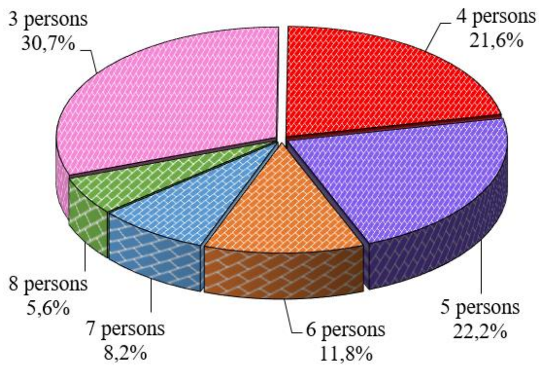

Figure 7. The number of family members in the enterprises.

The average number of family members in the cattle enterprises were reported as 4.84, 4.8, between 3-5 people, 3.8 people and 3-5 people by Yılmaz et al. (2020), Yılmaz et al. (2014), Tugay and Bakır (2009), Kutlar et al. (2013) and Kaygısız et al. (2010) respectively. Results of these study were in accordance with the present study's findings. On the other hand, in many studies, the average number of family members was reported to be higher than in this study. Güler et al. (2016), Ünalan et al. (2013), Tilki et al. (2013), Terin and Ateş (2010), Öztürk and Karkacıer (2008), Gürel and Akay (2008) and Şahin et al. (2001) reported that the average number of family members in cattle enterprises in their studies was 6.0, 5.2, 7.2, 9.0, 6.2, 5.3, and 6.1 people, respectively.

Similarly to the research findings, the average number of family members in cattle enterprises in Azerbaijan and Georgia was reported to be 4.8 and 3.6, respectively (Neudert et al., 2020). On the other hand, the average number of family members in enterprises in Ethiopia was 6.0 people in urban (Urban) regions and 7.1 people in rural (peri-urban) regions (Ayenew et al., 2011). Moreover, the same number was reported as 8 people in

West Kenya (Amimo et al., 2011), and 8.7 people in Uganda (Ahikiriza et al., 2021). Mzingula (2019) reported that 64.1% of the families of cattle enterprises in Tanzania had 5-8 members, while Saleh (2018) reported that 60% of the families in Nigeria had 1 to 20 members.

The distribution of the number of people working on the cattle farms in the İspir county is presented in Figure 8. It was determined that mostly 2 (43.7%) or 3 people (33.5%) worked in cattle enterprises in the county. Similarly, Güler et al. (2016) reported that mostly 2 or 3 people were working in cattle enterprises in Hınıs county. In another study, Daş et al. (2014) reported that the number of employees in cattle enterprises was between 3-5 people. In another study conducted in the United States, it was reported that at least two people worked full-time in enterprises, and, in addition, one or two people per enterprise worked part-time (Dou et al., 2001).

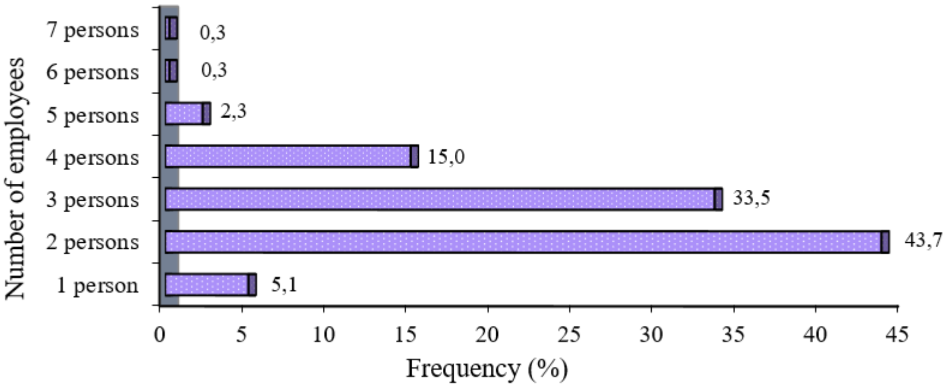

Figure 8. Number of employees working in the cattle enterprises.

Dairy cattle farming is one of the agricultural activities that requires a high level of labour and care. Asked to the breeders, “Is there anyone else who takes care of the animals?” and the majority of the breeders answered yes (97.7%) to the question. It was stated that the person taking care of the animals was a member of the family in 96.6% of the enterprises. The percentages of those employing workers for this job was found to be considerably low (0.5%) (Figure 9).

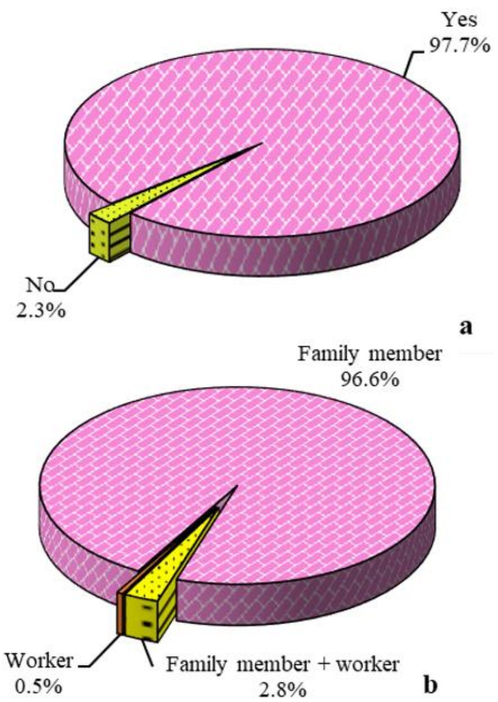

Figure 9. Is there anyone else who takes care of the animals, (a) is this person family member or (b) worker?

In the similar conducted on the subject, the people who take care of the animals in the enterprises were generally family members as in this study (Gürel and Akay, 2008; Ünalan et al., 2013; Güler et al., 2016).

In a study conducted by Demir et al. (2014), it was reported that in 23.5% of the enterprises in Kars province an average of 1.6 (min.1; max.4) workers/shepherds were hired, while the remaining enterprises use only family labour. On the other hand, in another study conducted in Uganda, it was determined that the average number of people hired permanently in the cattle enterprises was 1.4 people in small-scale enterprises, and 3.8 people in large enterprises (Ahikiriza et al., 2021). Furthermore, Goonewardene et al. (1995) reported that the number of workers who are members of the family for cattle enterprises in Alberta was 1 person at 43%, 2 persons at 28%, and 3 persons at 13% of the enterprises.

Of all the surveyed enterprise owners, 72.1% were not member of any organization related to agriculture. It was determined that 49.3% of the enterprises that were members of a union (27.9%) were members of the Agricultural Credit Cooperative, 27.4% of them were members of the Village Cooperative, and 7.5% were members of the Cattle Breeders' Central Association (Figure 10).

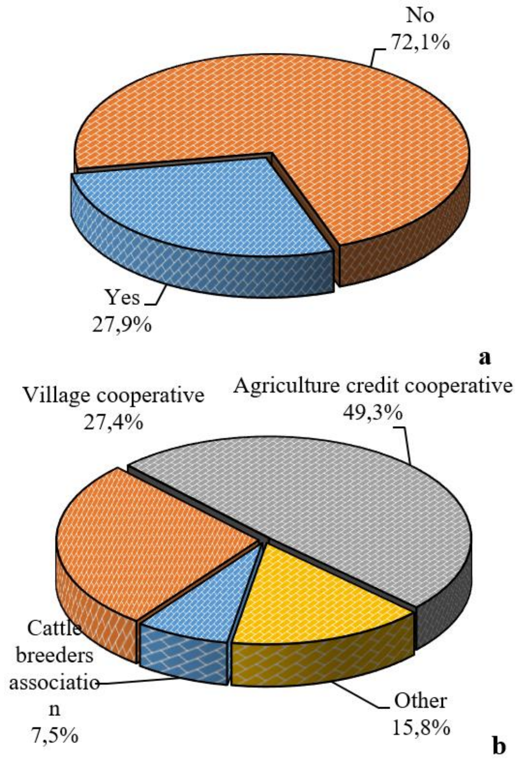

Figure 10. The status of the breeders being a member of a union (a) and the proportions of the unions they are members of (b).

Livestock organizations were important for providing services to farmers and producers in every field they need, facilitate their access to markets and enable small enterprises to contact with each other. In particular, the organization of small enterprises provides many opportunities in the development and implementation of new projects, as well as helping enterprises strengthen their own position. However, the percentage of enterprises that are a member of an organization or a union is considerably low in Turkey (Tilki et al., 2013; Bakan and Aydın, 2016; Savaş and Yenice, 2016; Şahin and Karadağ Gürsoy, 2016). Similarly, Neves et al. (2021) stated that only 11.4% of farmers in Brazil were associated with a cooperative. On the other hand, the proportion of being a member of a cooperative in Russia was reported to be between 29% and 56% (Yanbykh et al., 2019).

4. Conclusion

In this study, the socio-economic characteristics of the cattle farms in the İspir county of Erzurum province were examined. It was determined that the majority of the breeders in the county were in the age range of 50-70 years, and the average age was 55.2 years, enterprises in the county were mostly small-scale family enterprises. The education level of the breeders was low and most (68.8%) were primary school graduates. In addition, more than half of the enterprises had more than 30 years of experience in cattle breeding. However, although the high experience appears to be an advantage, considering the age and level of education of the farm owners, this situation becomes a disadvantage in terms of the sustainability of the cattle farms in the county. For this reason, the active participation of young and educated entrepreneurs in agricultural activities is vitally important both for the future of agriculture and for sustainable animal husbandry.

In the county, 72.1% of the enterprises were determined not to be members of any agricultural organization, and farmers cannot receive adequate services in other areas they need, especially in veterinary services, feed and material supply, and marketing issues. In addition, the insufficient number of members of the existing unions also causes the union activities to not be carried out effectively. In order to overcome these deficiencies, unions should work more actively and farmers' interest in unions, cooperatives, or associations should be increased. Demand for animal products due to growth and urbanization of the human population will continue to provide a key opportunity for economic growth in small-scale enterprises in this sector. In the next century, small enterprises will be critical for food production and food security. Small-scale enterprises in the region should be informed and encouraged to take advantage of these opportunities. As a result; developments in the livestock sector should be well evaluated, young entrepreneurs should be left the field open, and migration should be prevented. Government institutions, civil societies and organizations should make serious supports and investments so that the farmers can take advantage of these opportunities and get the maximum benefit.

Author Contributions

All authors have equal contribution and the authors reviewed and approved the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Approval

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The study has been approved by Atatürk University Faculty of Agriculture Ethics Committee Chairmanship that ensuring compliance with EC Directive 86/609/EEC for animal experiments (Approve number: E-38813508-000-2200006932).

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Research Article
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SEMIPARAMETRIC REGRESSION MODELS AND APPLICABILITY IN AGRICULTURE

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Abstract: Parametric regression models assume that the dependent variable is a linear relationship with the independent variables and the form of the relationship is known. Nonparametric regression methods are applied in cases where the relationship type is not known or assumptions cannot be provided. However, when there is more than one independent variable, some of the independent variables may be in a linear relationship with the dependent variable, while some may be in a nonlinear relationship. In order to model these variables, semiparametric regression models, which are a combination of parametric and nonparametric regression methods, are used. In this study parametric, nonparametric and semiparametric regression models, parametric estimates, fit statistical values of the models, confidence intervals and standard error values were calculated. As a result of the analysis, the parameters of the milking unit and the quarantine area among the parametric variables, the operation area, the ventilation area, the number of ventilation, the quarantine area, the infirmary area, the manure pit and the distance to the center among the non-parametric variables were found to be statistically very important (P<0.01). As a result, it was concluded that the correct definition of the variables (parametric and non-parametric) that are effective in determining the operating cost of agricultural enterprises and consequently the sales price, and the selection of the appropriate model are extremely important and that semiparametric models can be used easily in this field.

Keywords: Semiparametric, Regression, Agricultural businesses

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1. Introduction

Regression statistically analyzes the functional effect of independent variables on the dependent variable, based on a given or obtained data set. Regression analysis is an important method that is widely used in determining the relationship between variables. Regression analysis, which dates back to the 19th century, examines the conditional distribution of the dependent variable for certain values of the independent variables. It is used in many fields such as science, medicine, engineering and social sciences to determine and predict the relationships between variables (Aytaç, 1991; Alpar, 2003).

Linear regression analysis is examined on the assumptions that independent variables affect the dependent variable linearly and that the dependent variable has a normal distribution. Many theoretical and practical studies have been carried out for linear regression analysis, and the results of these studies provide a theoretical and practical basis for examining more complex regression models. When certain conditions are met, linear regression analysis yields appropriate results in solving practical estimation problems. However, in most estimation problems, some of the independent variables do not affect the dependent variable linearly. Thus, the need to examine regression models that are not fully linear and contain more complex correlations arises. Thus, regression analysis is examined in two different groups as parametric and non-parametric regression (Begun et al., 1983; Aneiros-Pérez, 2008).

The most important feature of parametric regression analysis is that the shape of the regression function is known beforehand. In addition, it is required to provide assumptions such as constant error variances for all values of the independent variable, normal distribution of error terms, no autocorrelation between error terms, and no multicollinearity between independent variables. If the assumptions are not provided, the results of the estimations made for the regression function cause misinterpretations. Thus, in case the assumptions of the model created by parametric regression analysis are not met, some adjustments can be made to provide assumptions. Thus, estimations can be made since necessary assumptions are provided (Buckley et al., 1988; Berry, 1993; Yatchew, 2003).

In non-parametric regression analysis, the shape of the function is not known beforehand. As in parametric regression analysis, important assumptions are not required. The only assumption is that the mean of the error terms is zero and the variance is a finite number. Therefore, there is flexibility in determining the relationship between variables.

Semiparametric regression method is also called "partial linear regression models" because of the combination of parametric and non-parametric regression function and additive. If the independent variables are unrelated in the semiparametric regression model, the coefficients of the parametric variables of the model are estimated by applying the least squares method and the partial regression functions of the non-parametric variables are estimated by non-parametric methods such as spline. (Newey, 1989). While some assumptions are needed in parametric and non-parametric methods, research continues even if the assumptions are not fulfilled in the semiparametric regression method (Shi, 2009; Toprak, 2015).

In this study, three different regression methods as parametric, non-parametric and semiparametric regression methods, smoothing method in regression, roughness penalty approach and spline correction techniques together with estimation methods used in smoothing parameter are explained. Afterwards, semiparametric regression, which is the main subject of the study, was discussed and two different approaches, partial spline and backfitting algorithm, were examined in the estimation of the model. In addition, inferences regarding the semiparametric regression model were applied for both parametric and non-parametric regression methods.

2. Material and Methods

2.1. Material

The data used in this thesis belong to 60 agricultural livestock enterprises in Kahramanmaraş. The variables that are thought to be effective on the price of the barns, the price of the farm, the presence of the milking unit, the presence of the quarantine zone, the shelter area, the ventilation area, the number of ventilation, the presence of the quarantine area, the presence of the infirmary area, the presence of the manure pit, the presence of the birth unit and the distance to the city center are discussed. While some variables were included in the model in parametric form, some variables were included in non-parametric form.

In practice, semiparametric regression model, which is an additive model, was used to evaluate parametric and non-parametric variables. In the statistical evaluations, SAS 9.4 package program was used.

2.2. Methods

2.2.1. Regression analysis

Regression analysis is an analysis method used to measure the relationship between two or more variables that have a cause-effect relationship between them. In this analysis method, if the analysis is made using a single variable, it is called univariate regression, and if more than one variable is used, it is called multivariate regression analysis. Regression analysis is used to apply the existence of the relationship between the variables, the strength of the relationship if there is a relationship, and to make predictions or estimations about the subject by using this relationship. In the regression, one of the variables is considered as dependent and the others as independent variable (Hurvich and Tsai, 1989; Omay, 2007).

2.2.2. Parametric regression method

The parameter is the mean, ratio, variance, etc. that belong to the population. Parametric regression is to show the mean relationship between dependent and independent variables with a mathematical function and to express the parameters in this function clearly. Parametric regression assumes that the regression function is represented as a linear function of the arguments $x_1, x_2, \dots, x_q$. $E\ (y \mid X)$ explains the functional relationship of the mean distribution of y with X when the conditional expected value X is known (equation 1 and 2) (Speckman, 1988; Schimek, 2000).

$$E\ (y \mid X) = \boldsymbol{X}\beta \tag{1}$$

or

$$y = \boldsymbol{X}\beta + \varepsilon_i \tag{2}$$

shown in the form.

2.2.3. Non-parametric regression method

Non-parametric regression, simple non-parametric regression model, one of which is the dependent variable (y) and the independent variable (x) whose relationship with the dependent variable is unknown (equation 3),

$$y_i = f(x_i) + \varepsilon_i, \qquad i = 1,2, \dots, n \tag{3}$$

shown in the form. The main purpose of the nonparametric regression method is to estimate the unknown mean function $f(x_i)$ rather than estimating the parameters.

Although there are no limiting assumptions in the non-parametric regression method, it may have some features. It is difficult to make predictions when the number of independent variables is large. In addition, the resulting graphics are shown in a complex structure. As a result of these situations, the “dimensionality problem” arises. At the same time, it is difficult to handle discrete independent variables with non-parametric regression method and to interpret the effects of the y dependent variable with the increase in the number of independent variables. These difficulties can be eliminated by applying the semi-parametric regression method (Tezcan, 2011).

2.2.4. Semiparametric regression method

The most important advantage of non-parametric regression models is the absence of any assumptions about the functional form of the relationship between the dependent variable and the independent variables in regression models. The flexibility provided by non-parametric regression models makes this model applicable. However, it is very difficult to calculate the smoothing process in this model. In addition, as the number of independent variables increases, the reliability of non-parametric estimates decreases gradually due to the size problem. Thus, when the functional form of the relationship is not known in the regression model, it may result in the absence of an important interpretation of the data in parametric and non-parametric regression models.

In order to overcome these problems, semiparametric regression model (semi-parametric regression model), which is regression models consisting of some parametric and some non-parametric variables, is applied (Schennach, 2004).

Semiparametric regression models are examined as a special case of additive models that generalize standard regression methods and provide an appropriate interpretation of the effect of each variable. Semiparametric regression model, where some of the variables are parametric and some of them are non-parametric variables (equation 4);

$$y_i = \alpha + f_1(x_1) + \cdots + f_j(x_j) + x_{j+1}\beta_1 + \cdots + x_k\beta_k + \varepsilon \qquad (4)$$

shown in the form. The j variables in the semiparametric regression model have a non-linear effect on the dependent variable y and show the non-parametric part of the model. Other variables have a linear effect on the y dependent variable and show the parametric part of the model. In addition, there may be discrete variables such as dummy variables in the parametric part of the model. In the non-parametric part of the model, when there is more than one variable, the inconveniences of the non-parametric model will also be valid for these models. In order to eliminate these problems, the variables in the non-parametric part of the model are added to the model and a new model is created (Zhongyi and Baocheng, 2001).

2.2.5. Estimation of semiparametric regression models

Iterative algorithms are used in the estimation of semiparametric regression models and additive models. There are many algorithms developed for the estimation of these models and these algorithms are implemented in different computer software. R software and SAS software are the most preferred programs for analyzing these algorithms. When the independent variables are uncorrelated in the semiparametric regression model, it is quite easy to estimate the semiparametric regression models with many non-parametric variables. In other words, if the independent variables are unrelated, the coefficients of the parametric variables of the model are estimated by applying the least squares method, and the partial regression functions of the non-parametric variables are estimated by non-parametric methods such as spline. However, in semiparametric regression models, the parametric and non-parametric variables of the model may be related to each other. Thus, considering the relationships between the variables, different algorithms are needed. The most preferred among these algorithms are the Newton-Raphson algorithm and the backfitting algorithm (Mammadov, 2005; Liu et al., 2013).

3. Results and Discussion

It is a known fact that in determining the selling prices of agricultural livestock enterprises, the characteristics of the enterprise have an effect on the price. Knowing how these features affect the sales prices of the enterprises, determining the production cost, presenting the products in the supply-demand chain effectively and profitably will give the business owner important information about the sustainability of production and the future of the business. Because the presence of milking unit, quarantine zone, shelter area, ventilation area, number of ventilation, quarantine area, presence of infirmary area, presence of manure pit, presence of birthing unit and distance to the city center are directly or indirectly related to the efficiency of production and additional investment.

In this study, the results of the semiparametric regression model, which includes the multivariate parametric regression model, in which some of the variables affecting the firm price are linear, and the non-parametric regression model, which includes some nonlinear variables, were obtained. The data set used in the study belongs to 60 agricultural livestock enterprises in Kahramanmaraş province and its surroundings. SAS 9.4 package program was used in the analyzes for parametric regression, non-parametric regression and semiparametric regression models.

First of all, assuming that all independent variables have a linear effect on the selling price of the agricultural enterprise, the linear regression model expressed in equation 5 was defined.

$$y = \beta_0 + x_1\beta_1 + x_2\beta_2 + x_3\beta_3 + x_4\beta_4 + x_5\beta_5 + x_6\beta_6 + x_7\beta_7 + x_8\beta_8 + x_9\beta_9 + \varepsilon \qquad (5)$$

Data number (N), arithmetic mean ($\bar{x}$), standard deviation (S) for the price, area, ventilation area, ventilation number, quarantine area, quarantine zone, infirmary area, manure pit, milking unit, distance to the center variables of this model, median and minimum-maximum values are given in Table 1.

Estimated coefficients for the area, ventilation area, ventilation number, quarantine area, quarantine zone, infirmary area, manure pit, milking unit, distance to the center variables of this model, standard error values, t-calculus value, significance levels (P), determination coefficient, corrected coefficient of determination, sum of squares of error and deviation values are given in Table 2.

Table 1. Descriptive statistics values of variables

Variables	N	$\bar{x}$	S	Median	Min-Max
Price (y)	60	1913524	706285	1982230	955230-3150120
Area (x_1)	60	1627.07	616.77	1600	780-2790
Ventilation area(x_2)	60	283.40	1115.51	80	37-8635
Number of ventilation (x_3)	60	13.72	4.77	12.50	5-28
Quarantine zone (x_4)	60	0.58	0.49	1.00	0-1
Quarantine area(x_5)	60	26.82	23.82	40	0-80
Infirmary area (x_6)	60	47.58	24.71	45	0-142
Manure pit (x_7)	60	311.4	97.56	320	100-500
Milking unit (x_8)	60	0.68	0.46	1.00	0-1
Distance from center (x_9)	60	69.85	38.10	56	20-160

Table 2. Estimation results of the variables of the linear regression model

Variables	Coefficients	$S_{\bar{x}}$	t	P
Fixed	-30858.28	22117.62	-1.40	0.890
Area (x_1)	827.109	134.654	6.142	0.000**
Ventilation area (x_2)	-52.074	67.963	-0.766	0.447
Number of ventilation (x_3)	23792.965	12433.654	1.914	0.062
Quarantine zone (x_4)	12812.820	11259.863	1.138	0.261
Quarantine area (x_5)	-694084.866	510136.495	-1.361	0.180
Infirmary area (x_6)	3513.317	2719.605	1.292	0.202
Manure pit (x_7)	516.844	626.670	0.825	0.414
Milking unit (x_8)	-235931.494	122664.109	-1.923	0.060
Distance from center (x_9)	1171.255	1388.689	0.843	0.403
$R^2 = 0.820$ $\bar{R}^2 = 0.783$ F= 22.274 Error Sum of Squares=530700 S= 63944.903				

**the parameters are statistically very significant at the 0.01 significance level.

As seen in Table 2., while the effects of ventilation area, ventilation number, quarantine area, quarantine zone, infirmary area, manure pit, milking unit and distance to the center on the sales price of agricultural holdings were found to be statistically insignificant (P>0.05), the effect on the area variable was found to be insignificant. Effect was found to be very significant (P<0.01).

When multiple linear regression is applied, it is seen that the variables of ventilation area, quarantine area and milking unit have a negative effect on the sales price, while other variables have a positive effect. In addition, the sum of squares of the error and the deviation value were quite high. Thus, it has been seen that the linear parametric model is not sufficient to determine the variable effects on the selling prices of the agricultural enterprise. For this purpose, a semiparametric regression model was created for the variables.

Since the discrete variables included in the study do not affect the curvature of the function, in other words, they are included in the model parametrically since they do not need correction. On the other hand, other variables whose type of relationship with the dependent variable is not known precisely were included in the model as non-parametric part. In order to determine the appropriate semiparametric regression model, the semiparametric regression model in which both parametric and non-parametric variables are included and the relationship between the sales prices of agricultural enterprises and the characteristics of the agricultural enterprise is examined in order to see how the predictions of the model are interpreted, is defined with the equation 6.

$$y = \beta_0 + x_4\,\beta_1 + x_8\beta_2 + s(x_1) + s(x_2) + s(x_3) + s(x_5) + s(x_6) + s(x_7) + s(x_9) + \varepsilon \quad (6)$$

Parameter estimates, standard error values, Chi-square calculation value and significance levels (P) for the quarantine zone and milking unit variables of this model are given in Table3.

Table 3. Estimation results of parametric variables in semiparametric regression model

Variables	Coefficients	$S_{\bar{x}}$	χ^2	P
Fixed	13.569	0.048	77645.992	<0.001**
Milking unit (x_8)	-0.016	0.005	9.854	<0.001**
Quarantine zone (x_4)	-0.812	0.113	51.02	<0.001**

**= the parameters are statistically very significant at the 0.01 significance level.

When the parametric variables in the application are examined according to Table 3, it is seen that all the parametric variables in the model are statistically very significant (P<0.01). Among these variables, the milking unit and the quarantine zone negatively affect the price variable. Among the parametric variables, the variable that most negatively affects prices is the quarantine zone variable.

Table 4. Estimation results of nonparametric variables in semiparametric regression model

Component	EDF	F	P
Area (x_1)	6.8554.04	188.17	<.001**
Ventilation area (x_2)	6.05	68.22	<.001**
Number of ventilation (x_3)	5.01	110.35	<.001**
Quarantine area (x_5)	7.07	1162.24	<.001**
Infirmary area (x_6)	7.48	144.04	<.001**
Manure pit (x_7)	6.61	260.36	<.001**
Distance from center (x_9)	1.00	25.96	<.0002**

**the parameters are statistically very significant at the 0.01 significance level. EDF= effective degrees of freedom

The additive representation of the parametric and non-parametric regression models, the results of which are shown in Table 3 and Table 4, are shown in equation 7.

$$y = 13.569 + x_4(-0.812) + x_8(-0.016) + s(x_1) + s(x_2) + s(x_3) + s(x_5) + s(x_6) + s(x_7) + s(x_9) + \varepsilon \quad (7)$$

Equation 7 consists of two parts as parametric and non-parametric regression. Coefficient interpretations and inferences for these two sections are analyzed with separate methods. The interpretations and inferences for the parametric regression part of the semiparametric regression model are similar to the linear regression models. While the comments for the non-parametric regression part are analyzed with the help of graphics, the inferences are examined with the help of the F test.
Since the non-parametric part obtained contains many coefficients, in other words, it is obtained as a vector, it is not possible to express it parametrically, and thus non-parametric components can only be displayed with graphics (Turanlı and Bağdatlı, 2012). Therefore, the relationship between the price and the variables included in the model in non-parametric form is given in Figure 1 to Figure 7.

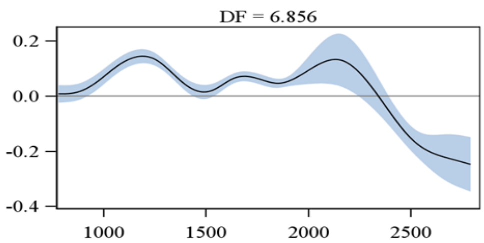

Figure 1. Area and S(A) graph.

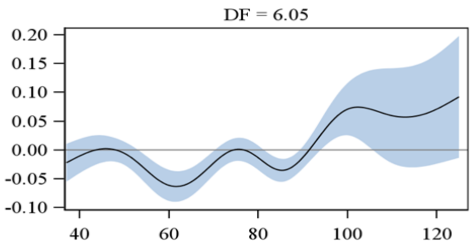

Figure 2. Ventilation area and S(VA) graph.

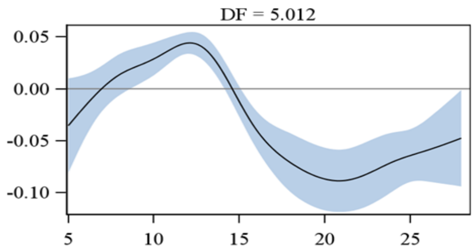

Figure 3. Number of vents and S(NV) graph.

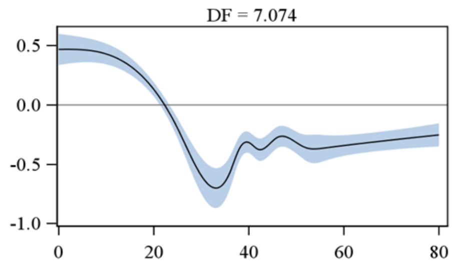

Figure 4. Quarantine area and S(QA) graph.

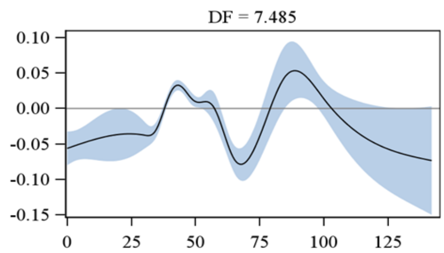

Figure 5. Infirmary area and S(IA) graph.

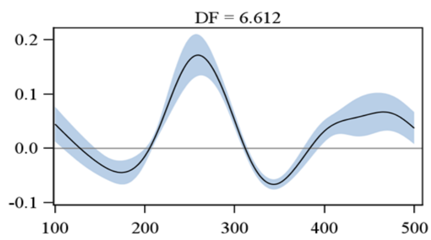

Figure 6. Quarantine area and S(QA) graph.

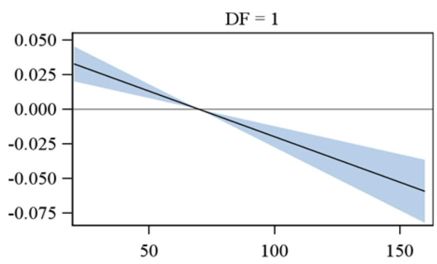

Figure 7. Distance to the center and S(DC).

When the graphs in the figure (Figure 1, 2, 3, 4, 5, 6 and 7) are examined, it can be observed that there is a non-linear relationship between the price and the variables included in the model in non-parametric form. Regarding the estimation of the semiparametric model, the values of spline values on the vertical axis and non-parametric variables on the horizontal axis were obtained. That is, it shows how the coefficient estimates change in response to the change in the value of each nonparametric variable. The shaded areas in the figure (Figure 1, 2, 3, 4, 5, 6 and 7) indicate that it is in the 95% confidence interval band.

4. Conclusion

In practice, the variables that should be included in the model by smoothing were examined and since there were both parametric and non-parametric variables in the model, it was found appropriate to apply semiparametric regression analysis. The most important feature of the semiparametric regression model is that it can examine the relationship between the dependent variable and the independent variables with statistical tests. In other words, it decides whether to include a variable in the model by smoothing it, linearizing it, or linearizing it by transforming methods. It also shows which model is suitable by comparing the models. In addition to modeling with the semiparametric regression method, determining the structures of the variables using this method also provides the best estimates.

The fact that there are many investment and environmental factors that determine the costs and therefore sales prices of agricultural enterprises clearly reveals how important the correct modeling is. Because, when the variables examined here are taken into the model parametrically, an erroneous result emerges that many variables known to be very effective on cost and selling price have an insignificant effect. In the semiparametric model, on the other hand, inclusion of some of the variables in the parametric and non-parametric form of the variables, which are known to be important in practice, turned out to be statistically very important.

As a result, it can be said that the correct definition of the variables (parametric and non-parametric) and the selection of the appropriate model are extremely important in determining the operating price of agricultural enterprises and accordingly the sales price, and it can be said that semi-parametric models can be easily used in this area.

Author Contributions

E.Y.: initiated the research idea, developed, organized, analyzed and interpreted the data and wrote the manuscript. M.S.: supervised the research, suggested the research methods, structured the paper and edited the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Approval

Ethical approval is not required, because this article does not contain any studies with human or animal subjects. Also the data used in the study were obtained from Agriculture and Rural Development Support Institution and the permission and approval of the institution were obtained (Date: April 01, 2004, Approval number: E70805362-622.03-49453).

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COMPARATIVE STUDIES ON BLOOD SERUM PROFILES OF LIVER ENZYMES, PROTEINS, TOTAL BILIRUBIN AND LIVER ORGAN MORPHOMETRY OF MUSCOVY DUCKS

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Abstract: Twenty adult apparently healthy Muscovy ducks *Cairina moschata* (10 males, 10 females) were used in a study to compare between sexes blood serum alanine aminotransferase (ALT), aspartate aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, and total bilirubin profiles using jugular venous blood. Comparative biometric measurements (weight, length, width and thickness) of their liver organs were also done. Total protein and albumin were significantly (P<0.05) higher in female ducks than in males while ALT, AST ALP and total bilirubin were unaffected (P > 0.05) by sex. The biometric measurements of the liver of Muscovy ducks according to sex were significantly (P < 0.05) higher in males than females. Liver biometric measurements according to lobation were significantly (P < 0.05) higher in the right lobe than in the left lobe, particularly, in terms of liver lobe weight and length of both sexes of ducks while liver lobe width and thickness were statistically similar (P > 0.05) in both sexes. Therefore, sex had a significant influence on serum total protein and albumin, as well as the biometric measurements of the liver of Muscovy ducks studied.

Keywords: Muscovy ducks, Serum biochemistry, Liver organ, Biometry

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1. Introduction

The liver is the largest gland of the body and it is dark red or red brown in colour, bilobated into right and left lobes with the right usually larger than the left (Dyce et al., 2010). The bulk of the liver lies to the right in all species (Dyce et al., 2010). The right and left liver lobes are joined cranially at the midline and the lobes are with specific shapes and sizes (Iqbal et al., 2014). The liver plays a crucial role in numerous physiological processes such as synthesis of blood proteins, production and secretion of bile, detoxification, nutrients absorption, metabolism of several substances, and storage of metabolites (Odokuma and Omokara, 2015).

Abnormal weights of internal organs such as the liver and kidney arise because of the increase in metabolic rate of these organs in an attempt to reduce toxic elements or anti-nutritional factors into non-toxic metabolites (Bone, 1979). Toxins such as mycotoxins, heavy metals and some poisonous plants can have serious effect on the physiological performance of the avian liver, thus leading to elevations in serum liver enzymes and other blood metabolites. Some of the enzymes used for assessing liver functions include alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT). Other metabolites like proteins (total protein and albumin) and total bilirubin are also associated with liver function.

ALT and AST are the most specific markers of hepatic injury (Vernon et al., 2011; Music et al., 2015) but they lack specificity as they are also present in muscle (cardiac and skeletal), kidney and red blood cells (Clementine and Tar Choon, 2010). ALP is found in the liver, bone, kidney, intestine and placenta and is helpful in detecting liver damage due to bile duct obstruction (Clementine and Tar Choon, 2010). Gamma glutamyltransferase (GGT) is present in liver, kidney, pancreas and intestines and considered to be a sensitive indicator of liver damage but it is not specific (Penn and Worthington, 1983). Nearly all proteins are synthesized in the liver, hence, total protein is used to assess the synthesis and maintenance of proteins in circulation, although, it is not a sensitive measure of hepatic failure (Betholf, 2014). Albumin helps maintain osmotic balance and also acts as a transport protein for calcium, unconjugated bilirubin and thyroid hormones (Bertholf, 2014). Bilirubin is the end product derived from liver breakdown of heme in hemoglobin of red blood cells (Fevery, 2008). It is transported in the blood bound to albumin and secreted in bile juices which is stored in the gall bladder

(Clementine and Tar Choon, 2010). Bilirubin serves as a diagnostic marker of liver disorder due to jaundice.

Literature on serum metabolites as well as biometric studies of the internal organs of indigenous ducks in Nigeria, particularly, the Muscovy duck *Cairina moschata* is generally lacking. Therefore, this study sought to contribute to the pool of existing data on ducks through comparative evaluations of some blood serum liver enzymes, proteins, total bilirubin, and gross morphometric measurements of the liver organs between male and female Nigerian Muscovy ducks.

2. Material and Methods

2.1 Experimental Birds and Procedure

Twenty apparently healthy extensively managed adult Muscovy ducks *Cairina moschata* (10 males, 10 females) were sourced from rural household farmers in three (3) villages near Joseph Sarwuan Tarka University, Makurdi and used for the study. Makurdi falls within the southern guinea savanna vegetative zone and is geographically located between latitude 6° 5" N and 8° 5" N and between longitude 7° 47" E and 10° E. The ducks were typically bred under makeshift structures with little or no water bath provided. Common feed resources that formed the bulk of the birds feeding include kitchen wastes, grain supplements, scavenging for insects, worms, grasses etc. and their availability depended on cropping activities and season of year. The sampling of ducks was on the basis of their breeding potential using visual appraisal of body size and caruncle development while determined body weights of at least 1.8 kg (males) and 1.3 kg (females) was ensured. The ducks were sacrificed by neck slaughter while jugular venous blood was collected into a set of test tubes without an anticoagulant and plasma was obtained using standard procedures. The sera were analysed for alanine aminotransferase (ALT), asparte aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, and total bilirubin. After slaughter, the carcasses were scalded, eviscerated, and the liver organs excised. The biometric measurements such as weight, length, width and thickness of liver organs were carried out using methods described by Iqbal et al. (2014).

2.2 Statistical Analysis

Statistical analysis of data was performed using IBM SPSS version 21.0 software. The mean concentrations of serum liver enzymes, proteins, and total bilirubin were compared between male and female using t-test analysis at 95% confidence interval. The means of the biometric measurements of the liver were compared between male and female as well as between liver lobation locations using t-test analysis at 95% confidence interval.

3. Results and Discussion

There is paucity of literature on serum biochemical characteristics partitioned according to sex for indigenous ducks, especially, Muscovy ducks in Nigeria. The blood components of indigenous and exotic duck breeds found in different regions of the world have been steadily reported upon (Chen et al., 2014; Gerzilov and Petrov, 2015; Rath et al., 2019), although, to a large extent not in the context of sex. The serum profiles of liver enzymes, proteins and total bilirubin of Muscovy ducks is presented in Table 1. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin did not show significant (P>0.05) variation between male and female ducks. ALT and AST are the most specific markers of hepatic injury (Music et al., 2015) while ALP is also useful in detecting biliary related liver damage (Clementine and Tar Choon, 2010). The findings on serum ALT, AST and ALP in this study were in line with Mulley (1979) for black ducks. Jerabek et al. (2018) also found similar result for serum ALT and ALP of fattened Mallard ducks but observed a significant effect of sex on AST which contrasted from the present investigation. In contrast, Rath et al. (2019) also observed significant ALT and AST concentrations in different breeds of female ducks. These observed significant differences from previous studies cited in comparison to the results of this study may have been due to differences in reproductive stages. The influence of sex and different reproductive stages on serum liver enzymes of ducks have been reported (Fairbrother et al., 1990).

The concentrations of serum ALT, AST and ALP obtained in this study were lower than the findings of Fairbrother et al. (1990) for adult Mallard ducks and this could be attributed to differences in age, breed and reproductive stages.

Table 1. Serum profiles of liver enzymes, proteins and total bilirubin of Muscovy ducks (Mean ±SEM)

Organ measurement	Sex		P-value
	Male	Female	
ALT (IU/L)	16.14±2.36	13.91±1.37	0.427^{ns}
AST (IU/L)	51.85±5.23	53.60±5.29	0.817^{ns}
ALP (IU/L)	291.10±37.69	282.30±60.56	0.903^{ns}
Total protein (g/dL)	2.82±0.13^{b}	3.48±0.16^{a}	0.005
Albumin (g/dL)	1.08±0.04^{b}	1.27±0.05^{a}	0.006
Total bilirubin (mg/dL)	0.078±0.003	0.074±0.004	0.372^{ns}

^{a,b}Means in the same row with different superscripts differ significantly (P<0.05), SEM= standard error mean, ns= not significant (P>0.05).

In addition, age-related decreases have been observed in ALP levels of ducks (Fairbrother et al., 1990), turkeys (Franchini et al., 1990a; Franchini et al., 1990b) and broiler chickens (Meluzzi et al., 1992). This has been attributed to decreased osteoblastic activity (Protais et al., 1982; Franchini et al., 1988a; Franchini et al., 1988b).

Total bilirubin concentrations from the present investigation recorded non-significant (P>0.05) differences between the male and female ducks which were also corroborated by Mulley (1979). This study recorded lower total bilirubin concentrations compared to the findings of Mulley (1979) and Fairbrother (1990) for black and Mallard ducks respectively. This may have been due to reductions in haemolytic activity of the liver, thus, suggestive of normal liver function. Female ducks recorded significantly (P<0.05) higher serum total protein and albumin values than the male ducks. Serum total proteins are considered important blood parameters because these estimates are indicative of immune status of the species due to antibody fractions contained in them (Rath et al., 2019). The significantly higher total protein concentration observed in female ducks from this study agreed with the findings of Ologbose and Dick (2021) and Okeudo et al. (2003) for Muscovy ducks. Orji et al. (1986) and Verma et al. (1975) also corroborated this observation in adult guinea fowls and domestic chickens respectively. This phenomenon according to Swathi and Sudhamayee (2005) was the result of increased levels of estrogens during the laying period thereby inducing protein synthesis. Total protein concentrations from this study were lower than the findings of the previous works of Okeudo et al. (2003) and Mulley (1979) for ducks. This is probably due to variations in the plane of nutrition as influenced by the management system of the birds. The influence of age, sex, diet and sampling time on serum total protein has been reported (Gattani et al., 2016).

Albumin is the most abundant circulating protein found in the plasma (Moman and Varacallo, 2018) and it is useful in assessing liver function or its ability to synthesize proteins and factors vital to overall homeostasis (Chang and Holcomb, 2016). According to Rath et al. (2019) and Santos et al. (2019), female ducks recorded significantly higher albumin values than the males which may be attributed to reproductive physiological processes. This is in conformity with the results of this study, however, Ologbose and Dick (2021) did not observe any significant sex effect on albumin concentration of Muscovy ducks. These authors posited that differences in age, blood collection procedures, animal housing and nutrition may account for differences in blood albumin values.

Literature on comprehensive morphometric studies of duck organs, especially the liver, irrespective of partitioning according to sex is very limited. However, available reports on morphometric studies of the chicken liver are few (Iqbal et al., 2014; Ripa et al., 2020; Ishi et al., 2000). Table 2 shows the biometric measurements of the liver of Muscovy ducks. The male ducks recorded significantly (P<0.05) higher values than the females in all the biometric measurements considered. The paired liver weight, right lobe weight and left lobe weight were significantly higher in the male ducks. Ishi et al. (2000), Iqbal et al. (2014) and Rani et al. (2020) observed significant differences in intact liver weights of broiler chickens at different ages. However, Etuk et al. (2006) observed non-significant (P>0.05) differences in intact liver weights of Muscovy ducks reared under different management systems. This may have been due to differences in the managements systems the birds were exposed to which impacted their plane of nutrition.

The mean liver weight of Muscovy ducks from the present study was comparable to the report of Ishi et al. (2000) but however contrasted with the higher values observed by Iqbal et al. (2014) for intact liver weights of broiler chickens. The male ducks recorded significantly (P<0.05) higher biometric measurements (i.e. paired, right lobe and left lobe) for liver length, liver width and liver thickness than the female ducks.

Table 2. Biometric measurements of liver organ of Muscovy ducks (Mean ± SEM)

Organ measurement	Sex		Mean±SE	P-value
	Male	Female		
Paired liver weight (g)	50.38±4.11^a	27.66±0.95^b	39.02±3.32	0.000
Right lobe weight (g)	33.14±2.81^a	17.92±0.65^b	25.53±2.24	0.000
Left lobe weight (g)	17.25±1.38^a	9.74±0.42^b	13.49±1.11	0.000
Paired liver length (cm)	17.10±0.48^a	13.84±0.42^b	15.47±0.46	0.000
Right lobe length (cm)	10.33±0.32^a	8.29±0.24^b	9.31±0.30	0.000
Left lobe length (cm)	6.77±0.23^a	5.55±0.08^b	6.16±0.18	0.000
Paired liver width (cm)	7.63±0.34^a	6.38±0.23^b	7.01±0.25	0.008
Right lobe width (cm)	3.80±0.15^a	3.26±0.14^b	3.53±0.12	0.019
Left lobe width (cm)	3.83±0.22^a	3.12±0.12^b	3.48±0.15	0.013
Paired liver thickness (cm)	3.06±0.09^a	2.65±0.09^b	2.86±0.08	0.004
Right lobe thickness (cm)	1.51±0.06^a	1.33±0.04^b	1.42±0.04	0.021
Left lobe thickness (cm)	1.55±0.05^a	1.32±0.07^b	1.44±0.05	0.017

^{a,b}Means in the same row with different superscripts differ significantly (P<0.05), SEM= standard error mean, SE= standard error.

The significant differences in these measurements observed in this study were in conformity with the report of Ishi et al. (2000) and Iqbal et al. (2014) for different age groups of broiler chickens. Similarly, Rani et al. (2020) observed highly significant (P<0.01) differences between the right lobe and left lobe measurements for liver length and liver width of broiler chickens. However, the non-significant (P>0.05) findings for liver thickness measurements (right lobe and left lobe) observed by these authors were at variance with the result of this study. This can be attributed to the young age groups (2 weeks and 4 weeks) of birds used by these authors that could not meaningfully influence this part of the liver biometry. The mean values for liver length, width and thickness measurements recorded in this present investigation were similar to the findings of Rani et al. (2020) and comparable to the previous studies of Iqbal et al. (2014). The distinctively higher liver biometrical measurements (weight, length, width and thickness) observed in male Muscovy ducks suggest a dominant effect of sex which was corroborated by Nwachukwu (1998). In addition, Siregar et al. (1982) and Duong (1994) suggested that there may be nutritional related influences on internal organs due to increased consumption of fibrous substances associated with extensively reared poultry.

The biometric measurements of the liver of Muscovy ducks according to liver lobation is presented in Table 3. The right liver lobe produced significantly (P<0.05) higher liver weights and lengths than the left liver lobe in both male and female ducks. However, lobation did not significantly (P>0.05) alter the liver width and thickness measurements in both male and female ducks. It is worthy of note that the values of these measurements according to liver lobation (right and left liver lobes) recorded in this study have not been previously reported upon in literature for any poultry species and therefore limiting comparisons that would have validated this result.

Table 3. Biometric measurements of liver organ of Muscovy ducks according to lobation (Mean ± SEM)

Organ measurement	Lobation		P-value
	Right	Left	
Male:			
liver lobe weight (g)	33.14±2.81[a]	17.25±1.38[b]	0.000
liver lobe length (cm)	10.33±0.32[a]	6.77±0.23[b]	0.000
liver lobe width (cm)	3.80±0.15	3.83±0.22	0.913[ns]
liver lobe thickness (cm)	1.51±0.06	1.55±0.05	0.616[ns]
Female:			
liver lobe weight (g)	17.92±0.65[a]	9.74±0.42[b]	0.000[ns]
liver lobe length (cm)	8.29±0.24[a]	5.55±0.08[b]	0.000[ns]
liver lobe width (cm)	3.26±0.14	3.12±0.12	0.458[ns]
liver lobe thickness (cm)	1.33±0.04	1.32±0.07	0.902[ns]

[a,b]Means in the same row with different superscripts differ significantly (P<0.05), SEM= standard error mean, ns= not significant (P>0.05).

4. Conclusion

Among the blood serum biochemical characteristics investigated, total protein and albumin concentrations were significantly (P<0.05) higher in female ducks. However, ALT, AST, ALP and total bilirubin concentrations did not vary significantly (P>0.05) between male and female ducks. The male ducks recorded significantly (P<0.05) higher biometric measurements for liver weights, lengths, widths and thickness. The right liver lobe was observed to be significantly (P<0.05) higher for liver weights and lengths in both male and female ducks. The liver width and thickness measurements in both male and female ducks were not significantly (P>0.05) affected by lobation. In conclusion, sex had a significant influence on specific serum metabolites as well as liver biometric measurements of Muscovy ducks. Liver lobation effect was significantly pronounced on right liver lobe with emphasis on liver weight and length measurements. Therefore, the liver organ of Muscovy ducks reared extensively in the given local tropical environment, are well adapted and capable of normal functions.

Author Contributions

S.S.C: conceptualized the research idea, developed, supervised the research, structured the paper and wrote the manuscript. J.M.A.: co-supervised the research, organized the data, analyzed and interpreted the data statistics. M.E.A.: co-supervised the research, suggested the research methods and revised the manuscript. S.K.U.: collected the data and proofread the manuscript.

Conflict of Interest

All authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Ethical approval was obtained from the Animal Ethical Review Committee of Joseph Sarwuan Tarka University (2021-2; 05-02-2021).

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