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KAPAK FOTOĞRAFI (COVER PHOTO)
Foliar and fruit symptoms of samples collected from Kumluca and Demre districts
Photo: Nihan GÜNEŞ

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Detection and Characterization of *Tomato spotted wilt virus* and *Cucumber mosaic virus* on Pepper Growing Areas in Antalya

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ABSTRACT

The most efficient method to control the plant virus diseases is breeding resistant cultivars. However, the resistance could be broken down after using resistant cultivars. This study was aimed to determine the prevalence and also serological and molecular characterization of *Tomato spotted wilt virus* (TSWV) and *Cucumber mosaic virus* (CMV) that cause infections, especially, in resistant pepper cultivars. For this reason, samples were collected from pepper growing greenhouses and open fields during vegetation period of 2015 in different parts of Antalya province including Kumluca, Demre, Serik and Aksu districts. Out of 148 pepper samples collected, 53 (35.81%) were infected with TSWV and 11 (7.34%) with CMV as a result of Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) test. Some regions on S RNA (nucleocapsid protein gene), M RNA (glycoprotein gene) and L RNA (RNA-dependent RNA polymerase gene) of TSWV genome; RNA 1 (helicase/methyltransferase gene) and RNA 3 (coat protein gene) of CMV genome of DAS-ELISA positive samples were amplified by RT-PCR with specific primers. Nucleotide similarity rates of nucleocapsid protein gene, glycoprotein gene and RNA-dependent RNA polymerase gene regions of TSWV isolate varied between 92-98% identity with other isolates in GenBank and CMV isolate varied between 89-96%. TSWV isolate showed nucleotide identity varied between 92-97% with *Tsw* resistance breaking isolates from Capsicum plants in Turkey, Italy and Spain. Genetic determinant for overcoming pepper *Tsw* resistance is located in S segment but aminoacid substitutions responsible for TSWV breakdown remain contradictory in several reports.

Keywords: *Cucumber mosaic virus* (CMV); *Tomato spotted wilt virus* (TSWV); DAS-ELISA; RT-PCR; Detection; Characterization

1. Introduction

TSWV (*Tomato spotted wilt virus*), type member of the genus Orthotospovirus within the Bunyaviridae family, first reported on capsicum in Turkey by Yurtmen et al (1999), is rated among the ten most economically devastating plant viruses worldwide (Adkins 2000). TSWV is transmitted persistently through several species of thrips especially *Frankliniella occidentalis* (Mound 2001) and one of the major problem in pepper production areas in Antalya province of Turkey. The genome of TSWV consists of three negative or ambisense single-stranded RNAs designated as L (large), M (medium) and S (small) (Adkins 2000). The most efficient method to control the virus in pepper crops is breeding resistant cultivars harboring the pepper

resistance gene *Tsw* toward TSWV. However, the resistance gene *Tsw* has broken down in several Mediterranean countries after using resistant cultivars (Roggero et al 2002; Margaria et al 2004). Antalya is one the province in Turkey where the most resistant pepper cultivars against TSWV disease are being cultivated. Even though it has been reported that *Tsw* has broken down in Samsun province of Turkey (Deligoz et al 2014), there is no record about *Tsw* resistance in pepper production areas of Antalya province.

One of the most prevalent viral constraint that affecting pepper production is CMV (*Cucumber mosaic virus*), type member of the genus Cucumovirus within the Bromoviridae family (Moury & Verdin 2012), was found on pepper growing areas in Turkey first by Yılmaz & Davis (1985). It was detected in pepper plants at different rates in various provinces in Turkey by DAS-ELISA mostly (Arli-Sokmen et al 2005; Buzkan et al 2006; Uzunoğulları & Gümüş 2015). CMV transmitted by more than 75 aphid species in non-persistent manner especially by *Myzus persicae* and *Aphis gossypii* (Perry et al 1998). The genome of CMV consists of three positive-sense single stranded RNAs designated as RNA 1, RNA 2 and RNA 3 (Kumari et al 2013). It has been reported that CMV resistant hot pepper cultivars which belongs pathotype 0 (P0) showed severe mosaic symptom in Korea and their causal agent was identified as CMV. Pepper isolate of CMV was described as P0 resistance breaking virus. The result suggests that CA-P1-CMV isolate can overcome P0 resistant pepper cultivars (Lee et al 2006).

This study was initiated to determine the prevalence of TSWV and CMV infection especially on resistant pepper cultivars and investigation whether TSWV resistance breaking isolates emerged or not in intensively pepper growing areas of Antalya province of Turkey. Here we report the serological and molecular characterization of TSWV and CMV isolates collected from peppers. The PCR products of nucleocapsid protein gene (S RNA segment), glycoprotein gene (M RNA segment) and RNA-dependent RNA polymerase gene (L RNA segment) regions of TSWV isolate and helicase/methyltransferase gene (RNA 1 segment) and coat protein gene (RNA 3 segment) regions of CMV isolate were sequenced and phylogenetic trees constructed based on nucleotide homology.

2. Material and Methods

2.1. Surveys and collection of virus infected samples

Surveys were conducted in randomly selected pepper growing greenhouses and open fields in Antalya province including Kumluca, Demre, Serik and Aksu districts. The samples was collected from the virus-like symptom showing pepper cultivars during vegetation period in spring and summer of 2015. All samples were stored at -20 °C before testing.

2.2. Enzyme linked immunosorbent assay

DAS-ELISA was performed for detection of TSWV and CMV in the collected plant samples. Specific antibodies for TSWV and CMV were applied according to manufacturer's instruction (Bioreba Switzerland).

2.3. Total RNA extraction, reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted from TSWV or CMV positive samples determined by DAS-ELISA using a previously reported method by Foissac et al (2001) as recommended. RNA extracts were used as template for reverse transcriptase-polymerase chain reaction (RT-PCR). The cDNA synthesis was carried out using 10 µL RNA with 2.0 µL random primers, 0.8 µL of 100 mM dNTP mix, 1.0 µL of RNase inhibitor, 2.0 µL of 10x RT buffer, 1.0 µL of SuperScript™ reverse transcriptase and 3.2 µL Nuclease-free H_2O (Thermo Scientific, USA) in a total reaction mixture of 20 µL. The reaction mixture was incubated at 25 °C for 10 min and 37 °C for 120 min followed by incubation at 85 °C for 5 min. Polymerase chain reaction (PCR) was carried out using 2 µL of cDNA with 25 µL Dream Taq Green PCR Master Mix (Thermo Scientific, USA),

0.5 µL of 10 µmolar forward primer, 0.5 µL of 10 µmolar reverse primer, and 22 µL nuclease free water (Applied Biosystems, USA) in a reaction mix of 50 µL. The PCR amplification was performed in an automated termal-cycler (Gene Amp PCR 9700 systems, Applied Biosystems, USA). Primer sets are mentioned in Table 1. PCR products were electrophoresed in 1% agarose and stained with ethidium bromide.

2.4. Sequencing and sequence analysis

One positive sample was randomly selected for each TSWV and CMV isolate and their amplicons for each segment was sequenced. Nucleotide consensus sequences were assembled and edited using Chromas Pro Version 2.5.1. The sequences were analyzed by NCBI-BLAST analysis. After confirming the identity of the sequences, they were submitted to GenBank. The nucleotide sequences were aligned with 20 other isolates from different countries and hosts. Sequence identity were compared and phylogenetic trees constructed using CLC RNA Workbench Version 6.8.2 (CLC Bio, Denmark) and Vector NTI Software Programme (Invitrogen, USA).

3. Results

3.1. Survey and collection of virus infected samples

To collect virus-like symptoms showing pepper plants 62 samples from Kumluca, 42 samples from Demre, 26 samples from Serik and 18 samples from Aksu districts were collected. The samples were showing stunting, yellowing, leaf deformation and curly top symptoms. Foliar symptoms of samples were chlorosis, ringspots, mosaic, mottling, vein clearing, reduction, curling, chlorotic and necrotic spots. Pepper fruits of samples were showing reduction, ringspot, chlorotic spots and roughness. Symptoms were showed in Figure 1.

3.2. Enzyme linked immunosorbent assay

Out of 148 plants, 11 plants were positive for CMV with overall incidence 7.34% and 53 samples were infected with TSWV with an overall incidence 35.81%. The number of CMV positive samples from Demre was 8 and Serik was 3, the incidence was found 19.04% and 11.53%, respectively. There was no CMV positive samples from Kumluca and Aksu districts. The number of TSWV positive samples was 12 from Kumluca, 10 from Demre, 14 from Serik and 17 from Aksu. The TSWV incidence was found in these districts were 19.35%, 23.80%, 53.84% and 94.44%, respectively. The ELISA results are mentioned in Table 2 and Table 3.

3.3. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Nucleocapsid protein gene, glycoprotein gene and RNA-dependent RNA polymerase gene regions on S RNA, M RNA and L RNA segments of TSWV and helicase/methyltransferase gene and coat

Table 1- Primer sets used for RT-PCR

Primer	*Sequence (5'-3')*	*Region*	*Reference*
TSWV S RNA	F ATGTCTAAGGTTAAGCTCAC R TTAAGCAAGTTCTGC GAGTT	Nucleocapsid protein gene	Nour et al (2013)
TSWV M RNA	F TGCTCACCATCCAACATTTC R CGAGAAGAAGAATCAACCATCC	Glycoprotein gene	Designed by author
TSWV L RNA	F TGTCAAAATCACTGCCGATG R TTCCCCAAAACCCTGCTACT	RNA-dependent RNA polymerase gene	Designed by author
CMV RNA 1	F TCGTTTGACATGCGTTTCTC R TTTAGCCGTAAGCTGGATGG	Helicase/methyltransferase gene	Designed by author
CMV RNA 3	F GTAGACATCTGTGACGCGA R GCGCGAAACAAGCTTCTTATC	Coat protein gene	De Blas et al (1994)

Figure 1- Foliar and fruit symptoms of samples collected from Kumluca and Demre districts. a, Banana pepper, roughness and chlorotic spots; b, Banana pepper, mosaic; c, Sweet bell pepper, chlorotic and necrotic spots; d, Sweet bell pepper, curly top and mottling; e, Sweet bell pepper, chloroticring spots; f, Sweet bell pepper, mottling; g, Long green pepper, stunting and yellowing; h, Long green pepper, chlorotic spots as line shaped; i, Capia pepper, ringspots; j, Capia pepper, chlorotic ringspots

Table 2- The rate of infection with TSWV according to the districts

District	*Number of tested samples*	*Number of infected samples*	*Number of healthy samples*	*Rate of infection (%)*
Kumluca	62	12	50	19.35
Demre	42	10	32	23.80
Serik	26	14	12	53.84
Aksu	18	17	1	94.44
Total	148	53	105	35.81

Table 3- The rate of infection with CMV according to the districts

District	*Number of tested samples*	*Number of infected samples*	*Number of healthy samples*	*Rate of infection (%)*
Kumluca	62	0	62	0.00
Demre	42	8	34	19.04
Serik	26	3	23	11.53
Aksu	18	0	18	0.00
Total	148	11	137	7.34

protein gene regions on RNA 1 and RNA 3 segment of CMV were amplified by RT-PCR with specific primer pairs for molecular characterization (Figure 2 and 3).

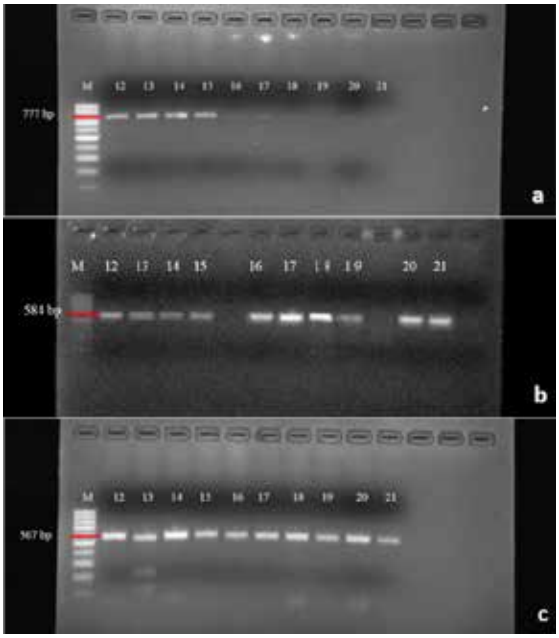

Figure 2- Detection of TSWV by RT-PCR. a, Amplification of T SWV genome with specific primer pair for S RNA segment. DNA marker (M), PCR products: 12–21 (Aksu isolates); b, Amplification of TSWV genome with specific primer pair for M RNA segment. DNA marker (M), PCR products: 12–21 (Aksu isolates); c, Amplification of TSWV genome with specific primer pair for L RNA segment. DNA marker (M), PCR products: 12-21 (Aksu isolates). Isolate number 14 is TSWV Aksu isolate

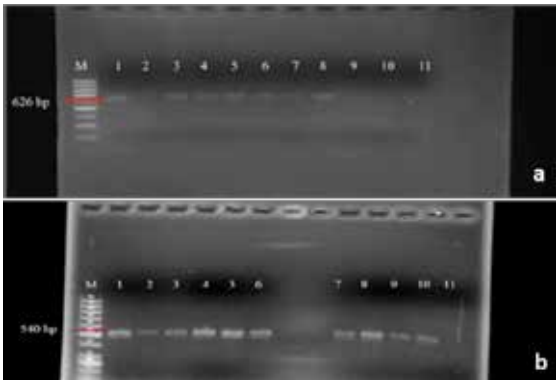

Figure 3- Detection of CMV by RT-PCR. a, Amplification of CMV genome with specific primer pair for RNA 1 segment. DNA marker (M), PCR products: 1-8 (Demre isolates), 9-11 (Serik isolates); b, Amplification of CMV genome with specific primer pair for RNA 3 segment. DNA marker (M), PCR products: 1-8 (Demre isolates), 9-11 (Serik isolates). Isolate number 8 is CMV Demre isolate

3.4. Sequencing and sequence analysis

Molecular characterization was performed for each segment of TSWV and CMV isolates thereafter sequenced. The PCR products for different segments of TSWV Aksu and CMV Demre isolates were sequenced. The sequences of PCR products were submitted to NCBI under accession numbers KY973676 (partial sequence of RNA 3 of CMV isolate), KY973677 (partial sequence of L RNA of TSWV isolate), KY973678 (partial sequence of RNA 1 of CMV isolate), KY973679 (partial sequence of S RNA of TSWV isolate) and KY973680 (partial sequence of M RNA of TSWV

isolate). The sequences obtained by amplification of the region of S RNA, M RNA, L RNA segment of TSWV isolate and RNA 1, RNA 3 segment of CMV isolate was compared with other 20 isolates from all over the world by NCBI-BLAST. The features of isolates from world mentioned in Table 4 and Table 5. Phylogenetic relationships were determined. The Aksu-TSWV isolate S RNA shared 96-98% nucleotide identity and phylogenetic tree was constructed with these isolates (Figure 4). The isolate shared 98% homology with Italy-pepper isolate (GU369722), South Korea-pepper isolate (HQ267713) and New Zealand-chrysanthemum isolate (KC494495). It shared 97% homology with tomato isolates of Samsun (KT192623), New Zealand (KC494501), France (FR693058) and pepper isolates of Antalya (KM407603), France (FR693046), Italy (DQ431238). It also showed 97% homology with *Tsw* resistance breaking (RB) isolates from Capsicum plants from Samsun, Turkey (KM379141) and Italy (DQ431237). It showed 96% homology with Hungary-pepper isolate (KJ649612) and non-resistance breaking pepper isolate (KM379142) from Samsun, Turkey. The Aksu-TSWV isolate M RNA shared 92-97% nucleotide identity and phylogenetic tree was constructed with these isolates (Figure 5). The isolate shared highest homology (97% nt) with USA-dahlia isolate (AY744486). It shared 96% nucleotide identity with South Korea-lettuce isolate (KC261966), Australia-pepper isolate (KT717692), Sw-5 resistance breaking (HM015520) and Sw-5, *Tsw* non-resistance breaking (HM015512) tomato isolates from Spain. It also showed 96% homology with resistance breaking pepper isolate from Italy (HQ830185) while showed 95% homology with Australia-tomato isolate (KM365065). It shared 92% nucleotide identity with *Tsw* resistance breaking pepper isolate from Spain (KP008133) and pepper isolates of Italy (KJ575621), USA (AY744489, KT160281), South Korea (KC261948, KC261957) and China (KM657119). It also showed 92% homology with South Korea-tomato isolate (KC261969), South Korea-chrysanthemum isolate (KC261975), USA-tobacco isolate (AY744490), USA chrysanthemum isolate (AY744483), China-lettuce isolate (JN664253) and China-tomato isolate (JF960236). The Aksu-TSWV isolate L RNA shared 92-98% nucleotide identity and phylogenetic tree was constructed with these isolates (Figure 6). The isolate shared highest homology (98% nt) with New Zealand-tomato isolate (KC494520) and Sw-5 resistance breaking tomato isolate (KP008130) from Spain. It shared 97% nucleotide identity with Italy-pepper isolate (KJ575619), New Zealand-pepper isolate (KC494508), South Korea-lettuce isolate (KC261965) and wilt type tomato isolate (KP008128) from Spain. It showed 96% homology with South Korea-pepper isolates (KC261947, KC261956), Australia-pepper isolate (KT717691), Australia-tomato isolate (KM365064) and South Korea-tomato isolates (HM581934, KC261968). It showed 95% homology with pepper isolate (KM657122) and tomato isolate (JF960237) of China while showed 94% homology with *Tsw*-resistance breaking pepper isolate (KP008132) from Spain, China-tobacco isolate (KM657121) and China-lettuce isolate (JN664254). It showed 93% homology with South Korea-chrysanthemum isolate (KC261974) and South Korea-pepper isolate (HM581937) while showed 92% homology with USA-pepper isolate (KT160280). The Demre-CMV isolate RNA 1 shared 89-96% nucleotide identity and phylogenetic tree was constructed with these isolates (Figure 7). The isolate shared highest homology (96% nt) with Egypt-tomato isolate (KT921314) and 95% homology with Malaysia-cucumber isolate (JN054636). It shared 94% nucleotide identity with India-pepper isolate (KM272277) while 92% with Italy-pepper isolate (HE962478) and India-banana isolate (EU159528). It showed 91% homology with South Korea-potato isolate (KM047509), South Korea-corn isolate (JN180309) and Japan-spinach isolate (LC066420) while showed 90% homology with South Korea-pepper isolates (KC527784, KC527785, KC527787, KC527794), China-tomato isolate (EF216866) and USA-bean isolate (HF572914). It showed 89% homology with South Korea-pepper isolate (KC527789), Japan-cucumber isolate (AB188231), China-chinese cabbage isolate (EF213023) and tomato isolates of France (HE793683), Spain (AM183117) and Japan

(AB368499). The Demre-CMV isolate RNA 3 shared 89-95% nucleotide identity and phylogenetic tree was constructed with these isolates (Figure 8). The isolate shared 95% homology with Malaysia-cucumber isolate (JN054635), India-eggplant isolates (GU906293, HQ343232), India-pepper isolates (KM272275, KM272276) and India-banana isolate (EF178298). It shared 94% nucleotide identity with Italy-pepper isolate (HE962480), India-chrysanthemum isolate (EF153733) and India-bottle gourd isolate (KJ874250). It showed 92% homology with Iran-squash isolate (JX025989), Iran-tomato isolate (JX025999) and Bangladesh-eggplant isolates (KM516898, KM516899) while showed 90% homology with South Korea-pepper isolate (KC527749) and tomato isolates of India (GU111229, JF279606) and Spain (AM183116, AJ829779). It showed 89% homology with tomato isolates of Spain (AJ829778) and China (EF216867).

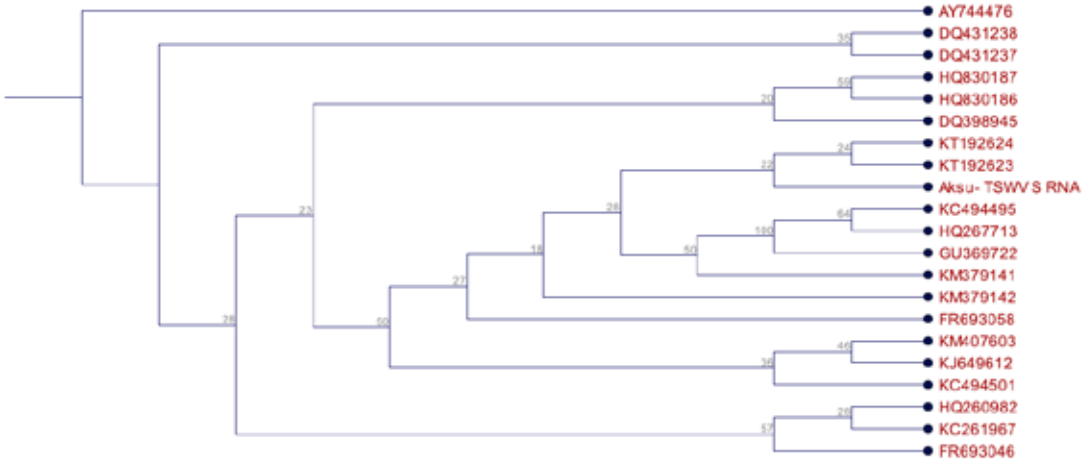

Figure 4- Phylogenetic tree analysis of Aksu isolate based on S RNA segment of TSWV

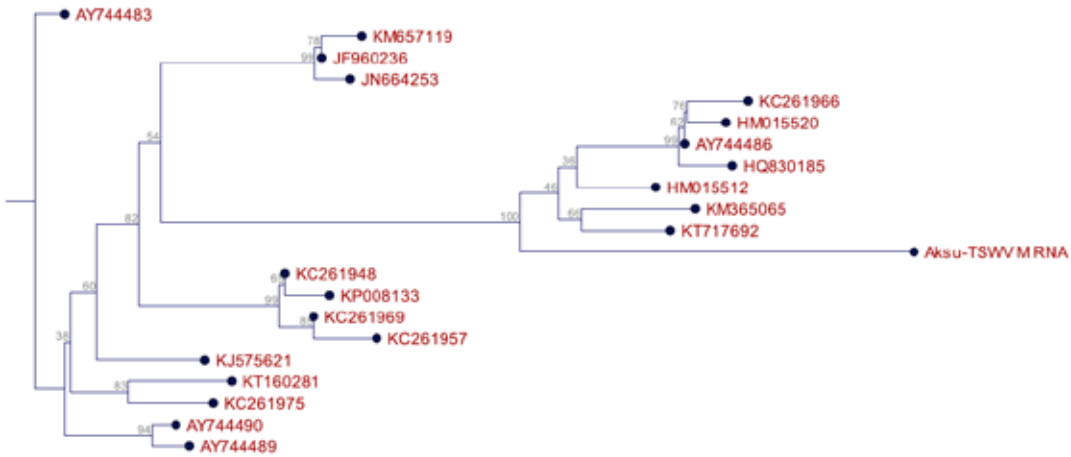

Figure 5- Phylogenetic tree analysis of Aksu isolate based on M RNA segment of TSWV

Table 4- Features of world isolates compared to Demre-CMV isolate

Isolates compared to Demre-CMV RNA 1		
GenBank Accession number	Origin	Host
KT921314	Egypt	Tomato
JN054636	Malaysia	Cucumber
KM272277	India	Pepper
HE962478	Italy	Pepper
KC527794	South Korea	Pepper
LC066420	Japan	Spinach
HF572914	USA	Bean
HE793683	France	Tomato
AM183117	Spain	Tomato
AB368499	Japan	Tomato
KM047509	South Korea	Potato
JN180309	South Korea	Corn
AB188231	Japan	Cucumber
EU159528	India	Banana
KC527785	South Korea	Pepper
KC527784	South Korea	Pepper
EF216866	China	Tomato
KC527787	South Korea	Pepper
EF213023	China	Chinese cabbage
KC527789	South Korea	Pepper
Isolates compared to Demre-CMV RNA 3		
GenBank Accession number	Origin	Host
GU906293	India	Eggplant
JN054635	Malaysia	Cucumber
KM272275	India	Pepper
HE962480	Italy	Pepper
KC527749	South Korea	Pepper
JX025989	Iran	Squash
JX025999	Iran	Tomato
KM516898	Bangladesh	Eggplant
AM183116	Spain	Tomato
EF153733	India	Chrysanthemum
HQ343232	India	Eggplant
EF178298	India	Banana
KJ874250	India	Bottle gourd
AJ829779	Spain	Tomato
EF216867	China	Tomato
JF279606	India	Tomato
KM272276	India	Pepper
KM516899	Bangladesh	Eggplant
AJ829778	Spain	Tomato
GU111229	India	Tomato

Table 5- Features of world isolates compared to Aksu-TSWV isolate

Isolates compared to Aksu-TSWV S RNA		
GenBank Accession number	Origin	Host
KT192623	Turkey, Samsun	Tomato
KM379141	Turkey, Samsun	Pepper
(*Tsw* resistance breaking isolate)		
KM407603	Turkey, Antalya	Pepper
KC494501	New Zealand	Tomato
FR693058	France	Tomato
FR693046	France	Pepper
DQ431237	Italy	Pepper
(*Tsw* resistance breaking isolate)		
DQ431238	Italy	Pepper
KM379142	Turkey, Samsun	Pepper
(Non-resistance breaking isolate)		
KJ649612	Hungary	Pepper
KT192624	Turkey, Samsun	Tomato
AY744476	USA	Dahlia
KC494495	New Zealand	Chrysanthemum
HQ830187	Italy	Pepper
HQ830187	Italy	Pepper
DQ398945	Italy	Pepper
(Resistance breaking isolate)		
KC261967	South Korea	Lettuce
HQ267713	South Korea	Pepper
HQ260982	South Korea	Pepper
GU369722	Italy	Pepper
Isolates compared to Aksu-TSWV M RNA		
GenBank Accession number	Origin	Host
AY744486	USA	Dahlia
HM015520	Spain	Tomato
HM015512	Spain	Tomato
KC261966	South Korea	Lettuce
HQ830185	Italy	Pepper
(Resistance breaking isolate)		
KT717692	Australia	Pepper
KM365065	Australia	Tomato
KP008133	Spain	Pepper
(*Tsw* resistance breaking isolate)		
AY744489	USA	Pepper
KC261957	South Korea	Pepper
KJ575621	Italy	Pepper
KC261969	South Korea	Tomato
JN664253	China	Lettuce
AY744483	USA	Chrysanthemum
KM657119	China	Pepper
KC261975	South Korea	Chrysanthemum
KC261948	South Korea	Pepper
KT160281	USA	Pepper
JF960236	China	Tomato
AY744490	USA	Tobacco
Isolates compared to Aksu-TSWV L RNA		
GenBank Accession number	Origin	Host
KP008130	Spain	Tomato
KC494520	New Zealand	Tomato
KJ575619	Italy	Pepper
KP008128	Spain	Tomato
KC261965	South Korea	Lettuce
KT717691	Australia	Pepper
KM365064	Australia	Tomato
KC261956	South Korea	Pepper
KP008132	Spain	Pepper
(*Tsw* resistance breaking isolate)		
KT160280	USA	Pepper
HM581934	South Korea	Tomato
KM657122	China	Pepper
KM657121	China	Tobacco
JN664254	China	Lettuce
JF960237	China	Tomato
KC261974	South Korea	Chrysanthemum
KC494508	New Zealand	Pepper
KC261947	South Korea	Pepper
KC261968	South Korea	Tomato
HM581937	South Korea	Pepper

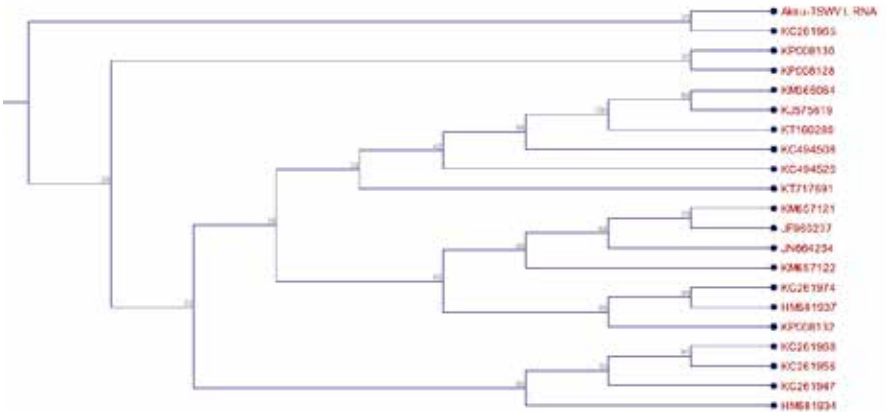

Figure 6- Phylogenetic tree analysis of Aksu isolate based on L RNA segment of TSWV

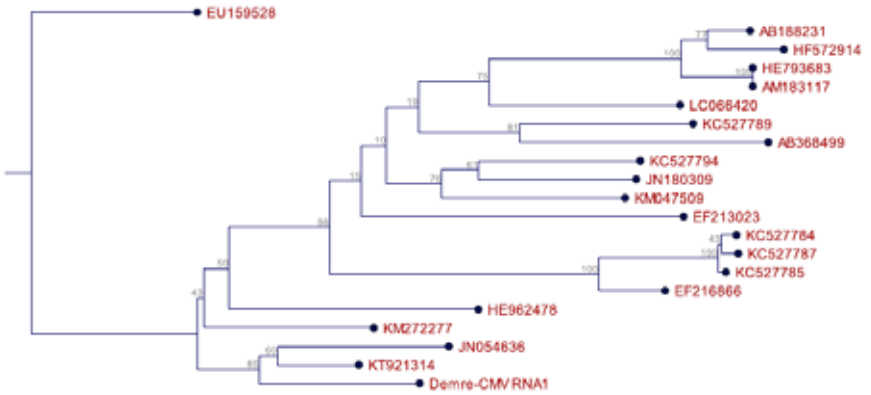

Figure 7- Phylogenetic tree analysis of Demre isolate based on RNA 1 segment of CMV

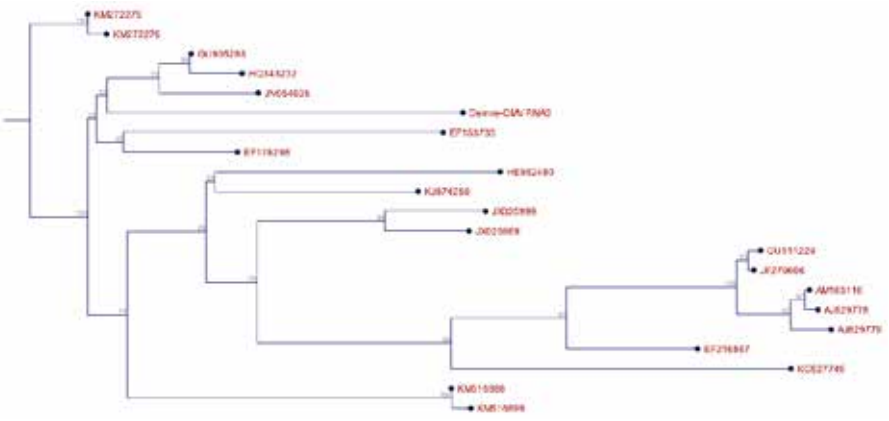

Figure 8- Phylogenetic tree analysis of Demre isolate based on RNA 3 segment of CMV

4. Discussion

The findings of the present study based on serological and molecular detection demonstrated the occurrence of TSWV and CMV causing damage in pepper plants in some districts of Antalya province. In our study, foliar symptoms of infected pepper plants were mosaic, mottling, chlorotic spots, necrotic spots, ring spots, chlorosis, curling and leaf deformation. Fruits were showing necrotic spots, chlorotic ring spots and roughness. Also, infected plants were showing curly top and stunting symptoms. TSWV can cause infection in pepper production areas worldwide and the rate is up to 100%. Infections are generally tested in Turkey and around the world using electron microscopy, ELISA and RT-PCR methods (Mavric & Ravnikar 2001; Sharman & Persley 2006; Ferrand et al 2015). TSWV infection has been detected 67.16% of pepper plants in the Western Mediterranean region of Turkey (Yardımcı & Çulal Kılıç 2009). CMV infection in pepper plants have been identified in many countries using methods such as electron microscopy, biological indexing, DAS-ELISA, RT-PCR and infection rates have been found to vary within the years (Burgmans et al 1986; Vozelj et al 2003; Biswas et al 2013). The studies were conducted in Turkey by using methods such as biological indexing, RT-PCR and RFLP but DAS-ELISA method was mostly used one and the infection in rates were determined in some provinces. In the samples collected from the pepper production areas in Samsun, DAS-ELISA test was used to detect CMV infection. Incidence of CMV was found 7.7% of 222 samples collected in 1998 while 2% of 91 samples were collected in 1999 (Arli-Sokmen et al 2005). In the pepper production areas of Hatay, Şanlıurfa, Kahramanmaraş and Gaziantep provinces 8.3% of CMV infection were detected by DAS-ELISA method (Buzkan et al 2006). In the pepper samples collected from Bursa, Yalova, İstanbul, Bilecik and Sakarya provinces 69% of CMV infection were detected using DAS-ELISA and real-time RT-PCR methods (Uzunoğulları & Gümüş 2015). CMV infected plants were showing mosaic, leaf deformation and stunting in our study. Disease incidence in the surveyed samples from different districts from Antalya, TSWV was found major virus as compared to CMV among the DAS-ELISA tested pepper samples. Further effort was also made to partial molecular characterization of TSWV and CMV from infected plants. The sequence obtained by amplification of the region of the S RNA segment of Aksu isolate showed high homology with *Tsw* resistance breaking pepper isolate from Samsun and Italy. The sequence of the region from the M RNA segment of Aksu isolate showed homology with *Tsw* resistance breaking pepper isolate from Italy and Spain. The sequence of the region of the L RNA segment of Aksu isolate showed homology with *Tsw*-resistance breaking pepper isolate from Spain. There are several reports displaying that TSWV resistance breaking isolates have emerged in Italy (Roggero et al 2002), Spain (Margaria et al 2004) and Australia (Sharman & Persley 2006). Also, TSWV resistance-breaking strains have been reported from Hungary (Gabor et al 2012), Argentina (Ferrand et al 2015) and Turkey (Deligoz et al 2014) in Capsicum species carrying the *Tsw* gene. In several reports it has been identified that the genetic determinant for overcoming pepper *Tsw* resistance is located in S segment but amino acid substitutions responsible for TSWV breakdown remain unidentified (Debreczeni et al 2015). The sequence obtained by amplification of the region of the RNA 1 segment of Demre isolate showed high homology with Egypt-tomato isolate and Malaysia-cucumber isolate. The sequence of the region of the RNA 3 segment of Demre isolate showed high homology with Malaysia-cucumber isolate, India-eggplant isolate and India-pepper isolate. There is a report that CMV resistance breaking isolate has been described in Korea. Sequence homology of RNA 3 segment of resistance breaking CMV isolate revealed high similarity with known CMV strains. The resistance and resistance-breaking mechanisms of CMV in pepper remain to be investigated (Lee et al 2006).

5. Conclusions

Virus resistant pepper cultivars are important management technique for TSWV and CMV control in pepper. These resistances are mostly based on the resistance genes and they have broken down. There is a need to understand the ability of TSWV and CMV isolates to overcome resistance which can be further useful in breeding programs to develop pepper cultivars resistance against TSWV and CMV. Further effort should be taken to identify TSWV and CMV breakdown mechanism of resistance breaking TSWV and CMV isolates.

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Determining of Risk Sources and Risk Management Strategies in Dairy Farms: A Case of Çanakkale Province

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ABSTRACT

This study was aimed to determine farmers' risk sources and risk management strategies in dairy cattle farms in Çanakkale Province. Data were obtained from 302 farms selected by stratified random sampling method from dairy farms in Biga and Çan districts of Çanakkale province in Turkey between May 2017 and September 2017. Descriptive statistics and factor analysis were used to analyze the data. The results of this study show that the most important risk source for farmers was lack of credit availability. This risk was followed by inadequacy of artificial insemination and increase in debt amount. Parasite control was the most important risk management for farmers. This was followed by off-farm work, off-farm investment and on-farm measures. As a solution to the lack of credit availability, it should be provided to ease of repayment in credit use and to inceasing opportunities to loan use with low interest rate of farmers. In terms of parasite control in farms, it is important to the use of regularly parasitic drugs and determining of an effective parasite control program. As a result, it is expected to contribute to farmers and agricultural policy makers of the findings of this research.

Keywords: Cattle; Factor analysis; Off-farm work; Artificial insemination

1. Introduction

Agricultural sector is exposed to various risks from production to marketing depending on problems resulted from nature of agriculture (Akçaöz et al 2009). These risks can have negative effects on crop and livestock, and so farmers don't estimate their own income and yield because of some risks that can't be controlled and predicted (Hall et al 2003; Hazneci & Ceyhan 2011). For this reason, farmers are obliged to take precautions against various risks. Furthermore, livestock is one of the sectors that plays a significant role in socio-economic development of the region and in people's nutrition. Therefore, there is needed improvement of this sector for fulfill the increasing demands in animal food products and to increase farmers' income living in rural area (Bishu et al 2016). Thus, it can be said that practices that will be performed to assessment of farmers' risk behaviours and determining of risks negatively affecting agricultural production are important in terms of production planning. There is an extensive literature that are investigating farmers'

attitudes and behaviors concerning risk sources and its management strategies in farms (Bosch & Johnson 1992; Huirne et al 2000; Meuwissen et al 2001; Flaten et al 2005; Çukur et al 2011; Zhou et al 2012; Bishu et al 2016). However, in Turkey, there exists limited literature on this topic (Özsayın & Çetin 2004; Akçaöz et al 2009; Hazneci & Ceyhan 2011; Hayran & Gül 2015). In Turkey, the number of total bovine animal was about 14 million head in 2016. It is about 205.855 head in Çanakkale province which has an important potential in terms of used technology and milk yield (Turkstat 2016). It constitutes about 1.5% of total bovine animal and 2.9% of total milk production in Turkey. Out of total milk production about 88.0% is provided from dairy cattle. The main reason for selecting of Biga and Çan districts of Çanakkale province as research area is to be milk production activities of the source of income for the great majority of agricultural farms in these districts. Besides, it hasn't reached to any study that was previously conducted to determine farmers' risk sources and risk management strategies in research area. Therefore, it is necessary to identification of risks encountered in farms and to determining of strategies to be taken against these risks in order to reach to expected income levels by making the right decision of farmers in research area. The objective of this study was to evaluate farmers' risks and risk management strategies in Biga and Çan districts of Çanakkale province. This study is expected to contribute to development of dairy farming activities in research area and to other studies in this topic, also.

2. Material and Methods

To estimate risk sources of farmers and management strategies, the data was obtained by survey using face to face interview technique from farms in Biga and Çan districts that have the highest number of dairy cattle in Çanakkale province between May 2017 and September 2017. These districts are constituted 42% of total dairy farms in Çanakkale province. The stratified random sampling method was used in study and the sample size was determined by Neyman Method (Yamane 1967).

$$n = \frac{\left(\sum N_h S_h\right)^2}{N^2 D^2 + \sum N_h S_h^2} \qquad D^2 = \frac{d^2}{z^2} \qquad (1)$$

n is the sample size (302 farms), N is the number of farm in districts (7112 farms), N_h is the number of farm in the h stratum; the standard deviation for the h stratum is S_h, the variance for h stratum is S_h^2, d is desired absolute precision, z is desired confidence level (1.96 for 95%), D is acceptable error limit in mean. Thus, the sampling size was formed by farms selected randomly from these strata by dividing into strata with regards to the number of dairy cattle to farms in these districts. Farms were categorized as 3 to ≤11 cattle (103 farms), 12 to ≤21 cattle (72 farms) and equal 22 and >21 cattle (127 farms). All farms were evaluated together because there weren't important differences between strata of farms. Farmers' socio-economic characteristics was determined by descriptive statistics. In order to rank the importance of each risk and strategies, it was asked questions to farmers by using 5-point likert-type scale with a range from 1 (not important) to 5 (very important) (Çukur et al 2011; Ağır et al 2015). Factor analysis was used in order to determine to farmers' risk sources and risk management strategies in dairy cattle farms. Factor analysis is multivariate statistical technique used to analyze interrelationships among a large number of variables and to explaine variables in terms of common underlying dimensions. The factors are simply a weighted sum of the observed variables that the weights regarding to the variables differ from each other. Factors are interpreted by their factor loadings. It was categorised factor loadings by using another rule of thumb as ±0.30 minimal, ±0.40 important, and ±0.50 rather important (Hair et al 1995; Ağır et al 2015; Hayran & Gül 2015). Therefore, factors were explicated for factor loadings greater than ±0.40 in this study. To measure the internal reliability and consistency of given responses to questions including ranked responses, it was calculated Cronbach's alpha ($\alpha \geq 0.7$). The Kaiser Meyer Olkin (KMO≥0.6) and Barlett's test of Spherincity statistics ($P<0.01$) were calculated in order to test to suitability for factor analysis of the

data (Hair et al 1995). The data were analyzed by using SPSS statistical analysis programme (SPSS 2008).

3. Results and Discussion

3.1. Farmers' general characteristics

The average household size was determined as 3.2 person in farms (Table 1). This size at national level is about 3.5 person (Turkstat 2016). Thus, this value is below the average household size at national level. Farmers' the average age and their dairy farming experience were determined 45.2 years and 20.3 years, respectively. The highest education level was primary school. The majority of farmers (67.8%) had primary education and 16.4% of the farmers had the highest income (≥€ 5432.1).

3.2. Perception of risk sources in dairy cattle farms

The sixteen risk sources were determined for farmers (Table 2), and the most important risk source with the highest mean was lack of credit availability. One possible reason of this result is their considerations about financial incapability of farmers in research area. This was followed by inadequacy of artificial insemination use, increase in debt amount, injury, illness and death of operator, inability to use modern technologies, lack of technical knowledge, lack of harmonisation to hygiene rules, decrease in the number of dairy cattle, and adverse weather conditions, respectively. Hall et al (2003) reported that risk sources were severe drought, cattle price variability and weather, and disease. In another study, these risks were determined as volatility in feed and milk price, production diseases, and misuse

Table 1- Farmers' some socio-economic characteristics

Characteristics	*5-11 cattle*	*12-21 cattle*	*≥ 22 cattle*	*Mean*
Age (year)	44.3	47.1	45.6	45.2
Education level (%)				
- Primary school	70.0	68.7	66.0	67.8
- Secondary school	17.4	18.8	19.3	18.6
- High school	7.9	9.4	12.5	10.3
- University	4.7	3.1	2.2	3.3
Household size (person)	2.8	3.4	3.6	3.2
Farmers' dairy farming experience (year)	22.3	23.9	17.6	20.3
*Household income (€ year^{-1}) (%)				
≤ €2716.1	44.4	28.1	19.3	29.5
€2716.2 - €5431.8	52.4	62.5	52.3	54.1
≥ €5432.1	3.2	9.4	28.4	16.4

*, 1 Euro= 4.05 TRY (Turkish lira) in June 2017

of drugs (Hayran & Gül 2015). The sixteen items were distributed among six factors by factor analysis. KMO, Barlett's test and Cronbach's alpha values were calculated as 0.765, 598.34 and 0.769, respectively. These results supported to use of the factor analysis for risk sources. The six factors from 1 to 6 were labeled as production loss, institutional, disease, financial, technological and price, respectively. These factors explained 64.67% of total variance. Risk factors were determined as change in farming situation, legislation, production, and financial situation by Meuwissen et al (2001). In another study, these factors were defined as technology and cost, political and economics, land value and insurance (Hayran & Gül 2015). Factor 1, production loss, had high loadings decrease in milk yield, injury, illness and death of operator, decrease in the number of dairy cattle and inadequacy of artificial insemination use. There are positive relationship between Factor 1 and their risk sources. The decrease in milk yield is an important criterion for production, and this risk has negative effects on

production. This result was supported by findings of study conducted by Schaper et al (2009). Thus, it is expected to decrease of milk yield in farms due to problems negatively affecting milk yield such as death, disease of animals. Hence, farmers face with production losses. Another risk for farmers is operator's injury, illness, and death. It is important to keep healthy of farmer for continuity of production activities in farms. Because, various production losses come to exist in case such as injury and death of operator. These results were supported by findings of study conducted by Akçaöz et al (2009). The decrease in the number of animal is an important risk in milk production. The number of animal in farms decreases in the event of obligatory slaughtering, death, and disease, and so the quantity of milk production may change. Therefore, production losses can occur in farms. Similar results were reported in study conducted by Akçaöz et al (2009). Artificial insemination has important effects on accelerating animal breeding (Ferraz et al 2012). Hence, the inadequacy of artificial insemination use can affect adversely milk production. That is, it can cause to important losses in milk production when artificial insemination isn't successful in farms. Therefore, it is important to increasing of farmers' success in implementing artificial insemination and to the number of animal applied artificial insemination in order to decrease production loss. These results were supported by findings of study conducted by Akçaöz et al (2009). Factor 2, institutional, had high loadings increase in interest rates, inadequacy of agriculture and livestock supports, increase in input costs. There are positive relationship between Factor 2 and their risk sources. The increase in interest rates is important risk for farmer. Similar result was reported in study conducted by Akçaöz et al (2009). Thus, feed prices may increase depending on the increase in interest rates. The changes in feed prices cause a considerable increase in milk production cost of farms that don't make enough forage crops production. Hence, the profitability of farms decrease depending on the fall in milk prices. Therefore, the institutional regulations need in decreasing of risks related to interest rates. Livestock supports are more important for sustainable dairy farming. However, these supports haven't direct effect on farm income. They have positive effects on farmers' costs in preproduction and on their income in post-production. Therefore, there is a need to increasing of livestock supports in order to decrease to income and production losses of their arising from various risks of farmers. Feed costs account for about 60-70% of the all expenses in farms (Turan & Altuner 2014). Thus, it is expected to increasing milk production costs of the increases in input prices forming production costs of farms. Factor 3, disease, had high loadings lack of harmonisation to hygiene rules, livestock diseases, increase in veterinary and drug prices. There are positive relationship between Factor 3 and their risk sources. Lack of harmonisation to hygiene rules is one of important risk for farms (Akçaöz et al 2009). Because, the animals in farms face with various diseases due to emergent microbial contamination depending on lack of hygiene and deficiency of other health protection precautions (Noordhuizen & Cannas 2014). As a result of this, production of milk and its quality may decrease. Therefore, farmers about hygiene rules by institutions given animal health protection service in research area should inform, and the increasing of their harmonisation to hygiene rules in their own farms should provide, also. Furthermore, the increases in drug and veterinary prices cause to disregarding to the health of own animals of low-income farmers and not sufficiently benefiting from the advantages of animal health protection services. Factor 4, financial, had high loadings lack of credit availability and increase in debt amount. There are positive relationship between Factor 4 and their risk sources. This result are similar with the findings of Flaten et al (2005), which indicated that there was positive relationship between credit availability risk and credit factor. Dairy farming as in other business are also desired to be high of the working capital amount. Because, there is a need to adequate working capital to reach more rational working conditions and to reach targeted income level by increasing labor productivity. Therefore, farmers tend towards borrow from their external resources in order to meet own financial needs due to financial inadequacy and indebtness situation in farms. Hence, financing and

debt situation of farms have great importance in terms of continuity of production activities and improvement. Therefore, it should provide to ease of repayment in credit use and to inceasing the use of loan with low interest rate of farmers. Furthermore, it can be expected to decreasing of the debt amount in farms by diversification of product and increasing of quantity of off-farm income. Factor 5, technological, had high loadings lack of technical knowledge and inability to use modern technology. There are positive relationship between Factor 5 and their risk sources. In farm, development of technical knowledge and modern technology need in terms of the productivity and sustainability. However, it is not easy to use of modern technologies and to adoption of their agricultural innovations by farmers due to inadequate financial power of farmers (Thornton 2010). Hence, they prefer to conventional livestock techniques due to problems such as lack of technical and up-to-date information and their difficulties in adaptation to modern technology. Due to these problems, it can be said that the development of dairy farming is affected negatively. Factor 6, price, had high loadings decrease in milk price and adverse weather conditions. There are positive relationship between Factor 6 and their risk sources. The high input costs and low milk prices have an important impact on farm. Because, the decrease in milk prices in spite of increase in input costs cause to decreasing of profit margin between product and input prices. Also, adverse weather conditions have important effects on the livestock sector and animal products (Akçaöz et al 2009; Thornton 2010). These risks affect negatively to total annual profit of farms. Hence, it is rather important to protection of dairy animals as far as possible from adverse weather conditions and their consequences. These results concerning risk sources show that all factors have negative effects on sustainability of dairy farming activity in research area. Therefore, it is necessary to take necessary precautions to reduce or eliminate to these risks.

Table 2- Mean and factor analysis results for risk sources

Risk sources	*Mean[a] (n= 302)*	*SD[b]*	*Important factors[c]*					
			F_1	F_2	F_3	F_4	F_5	F_6
Lack of credit availability	4.65	0.63	0.186	-0.004	0.037	0.783	-0.147	0.160
Inadequacy of artificial insemination use	4.51	0.59	0.492	0.165	0.146	0.116	0.253	0.327
Increase in debt amount	4.48	0.75	-0.040	0.261	0.097	0.754	0.261	-0.011
Injury, illness and death of operator	4.38	0.55	0.703	-0.077	0.112	0.316	0.282	-0.094
Inability to use modern technologies	4.27	0.69	0.305	-0.072	0.274	0.136	0.698	-0.219
Lack of technical knowledge	4.26	0.64	0.036	0.291	-0.091	-0.053	0.704	0.252
Lack of harmonisation to hygiene rules	4.09	0.76	0.050	-0.259	0.774	0.073	0.074	0.160
Decrease in the number of dairy cattle	4.08	0.65	0.627	0.118	0.164	0.026	0.229	0.173
Adverse weather conditions	4.05	0.86	-0.146	0.062	0.150	0.324	-0.016	0.618
Livestock diseases (epidemic/non-epidemic)	3.98	0.74	0.205	0.304	0.716	0.093	-0.098	-0.024
Increase in input costs (e.g., feed)	3.94	0.70	0.138	0.649	0.335	-0.192	-0.011	0.280
Increase in interest rates	3.88	0.69	0.056	0.783	0.108	0.169	0.124	0.131
Decrease in milk price	3.80	0.65	0.335	0.013	0.041	-0.066	0.067	0.713
Decrease in milk yield	3.79	0.75	0.779	0.166	0.021	-0.095	-0.195	-0.006
Inadequacy of agriculture and livestock supports	3.69	0.62	0.156	0.662	-0.104	0.243	0.097	-0.285
Increase in veterinary and drug prices	3.65	0.75	0.098	0.386	0.668	0.017	0.212	0.101
Percent of the variance explained (%)			13.13	25.73	37.43	47.27	55.99	64.67

[a,b], mean and standard deviation (SD) (1, not important; 5, very important); c, factors 1 to 6 are labelled as F_1, production risk; F_2, institutional risk; F_3, disease risk; F_4, financial risk; F_5, technological risk and F_6, price risk; Factor loadings for value greater than 0.4 are in bold

3.3. Risk managemet strategies in dairy cattle farms

Ten risk management strategies were determined for farmers (Table 3). The most important risk management strategy with the highest mean was parasite control. This was followed by off-farm work, off-farm investment and on-farm measures. Hall et al (2003) reported that the most important risk management strategies were being a low-cost producer, off-farm investments and maintaining credit reserves. In another study, they were determined as take precautions for diseases, the lowest possible cost for production, and highly efficient animal breeds (Hayran & Gül 2015). The ten items were distributed among three factors by factor analysis. KMO, Barlett's test and Cronbach's alpha values were determined as 0.783, 611.72 and 0.787, respectively. These results supported to use of the factor analysis for risk strategies. Thus, three factors (from 1 to 3) were labeled as disease control, diversification, and financial management, respectively (Table 3). They explained 66.41% of total variance. Meuwissen et al (2001) explained that the most important risk management strategies were insurance, diversification, and certain income. In another study, they were determined as planning and insurance, off-farm income and diversification in production, and cost reduction (Hayran & Gül 2015). Factor 1, disease control, had high loadings applying hygiene rules, use of veterinary services, parasite control and livestock insurance. There are positive relationship between Factor 1 and their risk management strategies. In farms, disinfection and cleaning applications that is made periodically have great importance on milk production. Thus, risks related to milk production can decrease as a result of obeying regularly to practices such as cleaning of feeding and milking equipments, and the use of veterinary services regularly to minimize risks relation to death and disease of animals. Although the pasture provides many benefits to farms, there are some diseases that affect adversely animals on pasture (Hawkins 1993). Hence, animals that are grazed on pasture are often exposed to high parasite, and so the effects of these diseases of animals are also felt economically. Thus, disease risk of animals can decrease as a result of using of regularly parasitic drugs and determining of effective parasite control programs. Livestock insurance is rather important in reducing the impact of the risks. Because, it can reduce the degree of risks by compensating the economic losses occurred depending on the risks such as accident, death, disease of animal. With livestock insurance, farmers can both protect against to other risks or disease their own animals and prevent to economic losses. Thus, it can be said that disease control factor have positive effects on animal diseases and hygiene practices in farms. Factor 2, financial management, had high loadings liquidity, producing at the lowest possible cost, record keeping in farm and livestock insurance. There are positive relationship between Factor 2 and their risk management strategies. Financial management is important in terms of improvement of productivity, increasing profitability, and fulfilling long-term goals. Thus, it can enhance to the profitability, the liquidity, and the solvency of farmers with financial management by decreasing negative financial consequences of risk sources (Aydın & Günlü 2010). Liquidity have important role in the maintain to continuity of farms, and it affects to efficiency and profitability of farmers. Due to problems such as cash-flows, delays in payment, and falls in income, the maintaining adequate liquidity in farms protects to farmers from financial crisis occurred. Hence, farms need to have adequate liquidity. Also, the significant point in managing of working capital is ensured maintaining liquidity in day-to-day activity to assure its smooth running (Eljelly 2004). According to this result, it can be said that farms that have high liquidity in terms of working capital may have more low risks, and this situation is an important in terms of financial management of farms. Production with the lowest cost is essential to its profitability and sustainability of farms. Because, production cost should be at lowest possible level while obtaining a particular product by using input more than one in dairy cattle farms (Welch & Welch 2016). Record keeping may provide an opportunity to farmers for evaluating both as technical and as economic of farms. Because,

records kept help to farmers in selection of animals, in determination of disease status, and in evaluation of the amount of profit/loss each year. This practice is an important for sustainable livestock. Livestock insurance reduce to the degree of risks in farm by compensating the losses resulting from death, disease, and injury of animals. Therefore, farmers may show tendency to doing insurance in order to minimize to livestock losses. Thus, it can be said that livestock insurances is important in terms of financial management and losses of farms. Factor 3, diversification, had high loadings off-farm investment, off-farm work and on-farm measures. There are positive relationship between Factor 3 and their risk management strategies. Diversification aims combining activities of farms in order to reduce the variability of revenues. It is a self-defense strategy used by farmers to preserve against various risks. However, many factors influence to the diversification in farmers' off-farm activities. Financial reasons is one of the factors affecting the diversification (Bradshaw 2004). As it is know, farmers face with some risk factors such as market and production. Therefore, off-farm investment, off-farm work and on-farm measures are important in decrease of the adverse impact of fluctuations on yield and/or price of these risks. Because, the variability in farm income is problem for farmers, and so it is essential to support risk management of farmers by diversification of assets, income, and activities (Bradshaw 2004). Hence, farmers may select to diversification to reduce variability in income. The aim of activities in relation with off-farm investment, off-farm work, and on-farm measures is closed with income provided from other activity areas of this deficiency when income provided from one activity area decrease. These results concerning risk management strategies show that they have positive influence on risks relation to dairy activity in research area. Also, it is neccessary to increase of the number of these precautions for improvement of farms.

Table 3- Mean and factor analysis results for risk management strategies

Risk management strategies	*Mean[a] (n= 302)*	*SD[b]*	*Important factors[c]*		
			F_1	F_2	F_3
Parasite control	4.48	0.57	0.599	0.040	0.321
Off-farm/non-farm work	4.37	0.74	0.317	-0.052	0.687
Off-farm/non-farm investment	4.27	0.73	-0.171	0.081	0.851
On-farm measures (diversification of product)	4.00	0.76	0.180	0.242	0.610
Use of veterinary services	3.89	0.79	0.854	0.110	0.022
Livestock insurance	3.86	0.76	0.575	0.494	0.189
Producing at the lowest possible cost	3.83	0.76	0.282	0.806	-0.013
Record keeping in farm	3.68	0.76	0.278	0.780	0.115
Applying hygiene rules	3.65	0.87	0.880	0.168	-0.005
Liquidity (keep cash on hand)	3.58	0.85	-0.158	0.866	0.140

[a,b], mean and standard deviation (SD) (1, not important; 5, very important); [c], factors 1 to 3 are labelled as F_1, disease control; F_2, diversification; F_3, financial management; factor loadings for value greater than 0.4 are in bold

4. Conclusions

This study aimed to determine farmers' risks and their strategies in dairy cattle farms in Çanakkale province and its districts (Biga and Çan). The results of this study shows that the most important risk resource of farmers is arise from lack of credit availability. As a solution to the lack of credit availability, it should be provided to ease of repayment in credit use and to inceasing opportunities to loan use with low interest rate of farmers in research area. One of the risk sources is inadequacy of artificial insemination use by farmers. Therefore, it should be provided to increasing of the number of farmers that use artificial

insemination and of their success in implementing artificial insemination in order to decrease production losses in farms. Another risk source is increase in debt amount of farms. The adverse effect of this risk can decrease by diversification of product and increasing of quantity of off-farm income. Parasite control was determined as the most important risk management strategy in farms. Another important risk management strategies are also off-farm work, off-farm investment and on-farm measures. These management strategies can be affective in decreasing of the adverse impacts of fluctuations on yield and/or price in production. All these findings show that farmers engage in cope with various risk sources in order to secure livelihood and continue to farm activities and thus it is important of risk management strategies that will be determined according to farm conditions. Thus, the results of this research may provide useful information for farmers, researchers and policy makers, and to helpful in cope with farmers' risks. Also, these information can also be important for institutions and services regarding livestock.

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Effect of Pregelatinization and Retrogradation on Some Physicochemical Changes of Wheat-Potato Starches

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ABSTRACT

The freezable (FW) and unfreezable water (UFW) contents of wheat and potato starches and their physically modified forms [pregelatinized (PGS) and retrograded (RS)] were analyzed by Differential Scanning Calorimetry (DSC) at various hydration levels (25, 35, 45, 55, 65, 75 and 85%). In all the starch samples, the UFW content increased with increasing hydration level. Potato starch samples (native, pregelatinized and retrograded) had higher UFW contents than wheat starch samples at all hydration levels. Similarly, with the increase of hydration level in all starch samples, onset (T_o), peak (T_p) and endset (T_e) temperatures of the peaks also increased. It was obtained that physical modifications in starches had significant effects ($P<0.05$) on water absorption index (WAI) and water solubility index (WSI) of starch samples. The highest WAI (10.51) and WSI (2.31) values were determined in pregelatinized potato starches. Rapid Visco Analyzer (RVA) profiles revealed that physically modified starches had higher viscosity values than native starches. The results clearly showed that pregelatinization and retrogradation had positive effects on the physicochemical properties studied.

Keywords: Unfreezable water; Wheat-potato starch; Physical modifications; DSC; RVA

1. Introduction

Starch affects texture, viscosity, gel structure, gel formation, adhesion, binding, water holding capacity, film formation and product homogeneity (Kaur et al 2012; Fu et al 2014). Because of low shear resistance, thermal resistances, thermal decompositions and high retrogradation tendency, the use of native starches is limited in some industrial food applications. Thus, there is a need to make some modifications to enhance the functional properties of starch (Gryszkin et al 2016). The modifications in starches can be carried out in three different ways: chemical (derivatization, acid thinning/hydrolytic depolymerization, dextrinization, oxidation, hydrolysis), physical (pregelatinized and granular cold-water-swelling starches) and genetic (waxy starches, high-amylose starches) (Ashogbon & Akintayo 2014; Kaur & Singh 2015). Since starches are not treated with any chemicals in physical modification, these starches are safe for human consumption and, therefore, more preferred (Kaur et al 2012; Huang et al 2016).

Physical modifications of the native starch granules involve different temperature/moisture

combinations, shear, pressure, and irradiation. These modifications also include mechanical abrasion to change the physical size of starch granules (Ashogbon & Akintayo 2014). Physical modifications affect physicochemical properties such as chemical reactivity, solubility, swelling power, pasting properties, thermal stability, gelatinization and retrogradation characteristics. (Huang et al 2016; Wang et al 2016). Pregelatinization process is the one of the most common methods of physical modification of starch and widely used in food industry (Zia-ud-Din et al 2017). Because of pregelatinized starch quickly hydrates and swells in water without heating, it is commonly used as a thickening agent in instant soups, instant desserts and baby foods (Ashogbon & Akintayo 2014).

The physical and structural properties of starch vary depending on the interactions of water and starch molecule. Starch-water interactions can be detected by changes in the physical state of the water, commonly analyzed by DSC. The physical state of water in starch can be described as freezable (free) and unfreezable (bound) water. The UFW is strongly bound to starch molecules and it does not act as a solvent for solutes, such as free water. This water cannot be frozen even at very low sub-zero temperatures, and significantly affects the stability of starch-based products. Determining the content of UFW in dehydrated foods also provides important information in both establishing appropriate process conditions and determining storage conditions (Suzuki & Kitamura 2008; Fu et al 2014). FW and UFW content by DSC can be studied across a wider range of moisture and temperature than most techniques. Although there is a great deal of research on starch gelatinization (Fredriksson et al 1998; Wang et al 2014), the number of studies on starch-water interactions is limited. In that way, the aim of this study was: (1) to determine the FW and UFW contents of wheat and potato starches both native and physically modified forms (pregelatinized and retrograded) at different hydration levels by means of DSC, (2) to define water absorption-water solubility indexes and (3) to determine the RVA properties of all the starches.

2. Material and Methods

2.1. Materials

Native wheat (NWS, moisture content 12.9%) and potato starches (NPS, moisture content 16.6%) used as material were purchased from Soyyigit Food Co., Ltd. (İstanbul, Turkey). Pregelatinized wheat (PGWS), pregelatinized potato (PGPS), retrograded wheat (RWS) and retrograded potato (RPS) starches were obtained from these starches by using the physical modification technique indicated in the method section.

2.2. Preparation of pregelatinized-retrograded starches and determination of pasting properties

The 8% suspensions (w/w) of wheat and potato starch samples were prepared in RVA tubes with distilled water and the samples were loaded into the RVA (RVA 4500, Perten, Sweden). The prepared suspensions were stirred in the RVA at 400 rpm for 10 s and then held for 10 min at 30 °C. Then, they were heated up to 95 °C at 10.83 °C min^{-1} and held for at this temperature for 5 min. The samples were cooled from 95 °C to 50 °C in 4 min and kept at this temperature for 10 min. PGS samples were obtained at viscosity peak (peak viscosity and peak temperature values for wheat starch 104 RVU, 95 °C; for potato starch 787 RVU, 80 °C, respectively) from the RVA profile, while RS samples were obtained after being stored for 120 h at 4 °C of the completed RVA samples (Yglesias & Jackson 2005). After the mentioned periods, the samples were frozen by adding liquid nitrogen, and then freeze drying were carried out in the freeze drier (Operon FDU-8612, Korea). The dried samples were ground by using a 1 mm mesh screened cyclone mill (Retsch ZM 200, Germany) for the analysis. The moisture content of the prepared starch samples was determined by the oven drying method in an oven at 105 °C for 24 h. Starch concentration of the slurry used in pasting analyses was adjusted to 8%, and the pasting properties of starch samples (native pregelatinized and retrograded) were determined by applying the above-mentioned RVA procedure. The RVA parameters (initial, peak, trough, breakdown,

final and setback viscosity) were obtained from the RVA viscogram data according to Ragaee & Abdel-Aal (2006).

2.3. Water absorption and water solubility indexes

The WAI and WSI of the samples (NWS, PGWS, RWS, NPS, PGPS and RPS) were determined based on the method described by Anderson et al (1969). Briefly, 2.5 g sample was weighed into 50 mL tared centrifuge tubes, and 30 mL distilled water was added to form a suspension. The prepared samples were centrifuged (Hanil Combi 514R, Korea) at 3000 g for 10 minutes. Then, the supernatant was carefully transferred to tared drying vessels and dried 105 °C for 12 h in a drying cabinet. The pellet in centrifuge tube was weighed and WAI was calculated according to Equation 1. Water solubility index (WSI) in dried samples was calculated according to Equation 2.

$$WAI = \frac{\text{pellet weight (g)}}{\text{dry weight of original starch (g)}} \quad (1)$$

$$WSI = \frac{\text{weight after drying (g)}}{\text{dry weight of original starch (g)}} \quad (2)$$

2.4. Freezable and unfreezable water contents

The DSC studies were conducted on samples at various hydration levels (25, 35, 45, 55, 65, 75 and 85%). For this purpose, accurately 10 mg samples were weighed into the hermetic DSC pans (product number 03190029, Perkin Elmer, USA), and then distilled water was added with a micro syringe to obtain the desired hydration-level samples. Pans were sealed and weighed (BEL, M214Ai, Italy). The sealed pans were kept for 24 h at room temperature to ensure homogenous distribution of water. The FW and UFW contents of the samples were determined by using the DSC (DSC 6000, Perkin Elmer, USA) with an intercooler system. The temperature and heat flow calibration of the DSC were performed by using indium (melting point: 156.6 °C, ΔH= 28.47 J g^{-1}) and water (melting point: 0 °C, ΔH= 333.20 J g^{-1}). An empty pan was used as reference. The following temperature program was used to determine FW and UFW contents of samples: (1) cooling from 20 °C to -80 °C at 5 °C min^{-1}. cooling rate; (2) holding at -80 °C for 5 min.; (3) heating from -80 °C to 50 °C at 5 °C min^{-1}. heating rate. To determine the FW and UFW contents of the samples at the end of the DSC analyses, the method described by Fu et al (2014) was used. The method contains the ratio of the area corresponding to the melting enthalpy of the ice in the material to the pure water melting enthalpy (333.20 J g^{-1}). UFW content was defined by using equation 3. The exact moisture content of each sample was confirmed after collecting the calorimetric data. The sealed DSC pans were punctured and dried in an oven at 105 °C for 24 h and the reweighed to determine the exact water content in the sample.

$$\text{Unfreezable water content} = \frac{m_w}{m_s} - \frac{\Delta H_s}{\Delta H_w} \quad (3)$$

Unfreezable water content: (g water/g dry starch); m_w, water content in samples (g); m_s, dry starch in samples (g); ΔH_s, the ice melting enthalpies of moisture in starch samples (J g^{-1}); ΔH_w, the ice melting enthalpy pure water (J g^{-1}).

2.5. Statistical analysis

All the tests in the study were carried out in triplicate and the results were reported as mean values ± and standard deviations. The acquired data were subjected to analysis of variance by using SPSS 22.0 (IBM Corp., Armonk, NY, USA) package program, and the average values of meaningful main variance sources were compared using Duncan's multiple comparison tests at 5%.

3. Results and Discussion

3.1. Water absorption and water solubility indexes

The WAI and WSI of the studied starches are given in Table 1. As can be seen the Table 1, the WSI values of physically modified starch samples were significantly higher than those of the NS samples ($P<0.05$). Similarly, the PGPS samples had higher WSI values than those of the RS samples. The highest WAI value were obtained in PGPS. The

highest WAI values of the PGS may be explained by the fact that the water is much more and much better bound to the swollen granules. This may also be explained for potato starch by the repulsion between the phosphate groups weakly bonded in the crystalline structure and thus the further swelling. A similar situation was reported by Wang et al (2014). Also, Ashogbon & Akintayo (2014) emphasized that the PGS had higher water absorption and water solubility properties than native starches.

Table 1- The water absorption and water solubility indexes of starch samples

Starch	*WAI*	*WSI*
Wheat	0.69±0.00^{e}	0.26±0.00^{b}
Pregelatinized wheat	6.08±0.08^{b}	2.07±0.05^{a}
Retrograded wheat	5.68±0.04^{c}	2.07±0.02^{a}
Potato	0.69±0.00^{e}	0.37±0.04^{b}
Pregelatinized potato	10.51±0.26^{a}	2.31±0.64^{a}
Retrograded potato	3.47±0.02^{d}	1.97±0.03^{a}

$^{a, e}$, mean values in the same column with the same superscript letter are not significantly different (P<0.05); mean values (n= 3) ± standard deviation

The WAI and WSI values of the RWS samples were significantly higher than those of the NWS samples (P<0.05). The high WAI and WSI values determined in RWS may due to the appearance of B-type crystalline structure. Wang et al (2015) reported that the RS had a typical B-type XRD pattern, irrespective of whether it was present as A- or B-type polymorphs in its native state. There are four or eight water molecules per unit cell in A-type polymorphs whereas 36 water molecules per unit cell are in B type polymorphs (Bogracheva et al 2002).

3.2. RVA profiles

The RVA results for the starch samples are shown in Table 2. As can be seen in Table 2, the PGPS had the greatest initial viscosity value (the viscosity value at the starting point of gelation) than those of the other starch samples. It was also found that each form of the potato starch (NPS, PGPS, RPS) had higher (P<0.05) peak, trough, breakdown and final viscosity values than the wheat starches. The finding of our study were agreed with the results reported by Jane et al (1999). The researchers reported that the peak, trough and final viscosity values of potato starch were higher than wheat starch, while the setback viscosity value of potato starch was lower than that of wheat starch. Higher viscosity values in potato starches as compared to wheat starches may be attributed to that the potato starch contains more amylopectin and phosphate esters. While potato starch contains about 80% amylopectin (Hovenkamp-Hermelink et al 1988), wheat starch contains about 72% amylopectin (Fredriksson et al 1998). While amylopectin significantly affects the swelling and pasting properties of starch granules, lipids and amylose inhibit swelling (Jane et al 1999). Singh et al (2003) reported that properties of starch pastes in aqueous systems depend on the physical and chemical characteristics of the starch granules, such as granule size distribution, mean granule size, mineral content, and amylose/amylopectin ratio. There are phosphate monoesters covalently bonded to the amylopectin fraction of starch in potato starches and these groups lead to an increase in starch viscosity, while wheat starches have higher phospholipid contents and results in lower-viscosity pastes. Moreover, Gomand et al (2010) reported that chain length distribution had significant effect on the RVA properties in potato and cassava starches and indicated that both peak viscosity and breakdown viscosity increased with higher levels of short chains and lower levels of long chains. The researcher also noted that higher levels of short chains resulted in less regular lamellar structure formation, allowing easier penetration of water into the crystalline zones of the granules, and thus higher peak viscosity. As shown in Table 2, the potato starch samples produced higher (P<0.05) breakdown drop as compared to the wheat starches. Pasting properties of starches vary depending on amylose/amylopectin ratio, size, swelling power, and rigidity of the granule. Viscosity breakdown is considered as a measure of granule degradation and the stability of the paste (Huang et al 2015). During breakdown, the swollen granules are destroyed, and most of the amylose

Table 2- The viscosity values of starch samples

Starch	*Viscosity (RVU)*					
	Initial	*Peak*	*Trough*	*Breakdown*	*Final*	*Setback*
Wheat	2.00±0.00^{e}	104.67±0.58^{e}	75.33±0.57^{e}	30.00±0.00^{d}	167.83±0.29^{d}	92.67±0.58^{c}
Pregelatinized wheat	9.00±0.00^{b}	91.00±1.00^{f}	74.66±0.58^{e}	16.00±0.00^{e}	115.50±0.50^{f}	40.57±0.60^{d}
Retrograded wheat	6.00±0.00^{c}	110.66±0.57^{d}	100.93±0.12^{d}	10.00±0.00^{f}	130.83±0.76^{e}	30.00±0.00^{e}
Potato	1.00±0.00^{f}	787.00±1.00^{b}	200.43±0.75^{a}	587.00±1.00^{b}	208.80±0.75^{c}	8.97±0.06^{f}
Pregelatinized potato	106.97±0.45^{a}	462.83±0.29^{c}	158.00±0.00^{c}	304.97±0.25^{c}	271.73±0.75^{b}	113.57±0.51^{a}
Retrograded potato	3.00±0.00^{d}	1016.67±1.53^{a}	180.07±0.40^{b}	836.66±0.57^{a}	286.43±0.51^{a}	105.93±0.40^{b}

$^{a, f}$, mean values in the same column with the same superscript letter are not significantly different (P<0.05); mean values (n= 3) ± standard deviation

molecules pass into the solution. The height of the peaks at a given concentration reflects the ability to swell freely of the granules. Potato starch has the ability to swell higher than cereal starches and is less resistant to breakdown during cooking. For this reason, a larger decrease in the viscosity is obtained after reaching the maximum viscosity value (Colussi et al 2017).

Setback values of starch samples are shown in Table 2. The setback values of PGWS and RWS were significantly lower than that of the NWS, but higher in NPS (P<0.05). The increase in viscosity during cooling is called setback. This increase may be due to the reduction in system energy in addition to amylose association (Alamri et al 2013). Setback is considered to be a measure of gelling ability or retrogradation tendency of starch (Huang et al 2015). High setback values in starches are positively correlated with amylose content of starch, while gelatinization temperature and peak viscosity are inversely related to the amylose content of the starch.

Final viscosity values of starch samples are shown in Table 2. The values were significantly different from each other (P<0.05), and the highest value was obtained in the RPS. Increase in the final viscosity provides information on the extent of the formation of the starch-lipid complexes (Blazek & Copeland 2009). Final viscosity also indicates the ability of the starch to form a viscous paste or gel after cooking and cooling (Cozzolino 2016).

3.3. Freezable and unfreezable water contents

The T_o, T_p and T_e values of the endotherms (except for NPS with 25% moisture content) obtained from starch samples are shown in Table 3. As can be seen in Table 3, in general, the T_o, T_p and T_e values of all the samples studied were significantly different from each other (P<0.05). With the increase in hydration level, these temperatures were shifted toward higher values. Similar results were reported by Suzuki & Kitamura (2008), Grunina et al (2015) and Tananuwong & Reid (2004). The NS had higher (P<0.05) T_o values than those of the PGS and RS at hydration levels above 35% (Table 3). At the hydration levels lower than 65%, the T_o values of the peak belonging to freezable water in all of the physically modified starches (except for PGPS) were lower than 0 °C. Similar results were reported by Grunina et al (2015), and researchers stressed that the lower T_o values were due to the presence of a higher fraction of small size water cluster in these systems. As can be seen from the Table 3, the T_p and T_e values of PGS' in comparison to their respective of native starches generally shifted to higher temperatures as the moisture content increased. In the case of RS, the T_p values generally were decreased, while the T_e values were increased. Achieving higher T_p and T_e values in pregelatinized starches may be attributed to the establishment of better molecular interactions between water and modified starches.

The UFW contents calculated from the melting enthalpy of ice in the different hydration levels are

Table 3- T_o, T_p and T_e values of the endotherms of starch samples with various hydration levels

	Hydration levels (%)						
	25	*35*	*45*	*55*	*65*	*75*	*85*
T_o (°C)							
Wheat	-11.64±0.14^{cD}	0.74±0.02^{aC}	1.04±0.10^{aBC}	0.99±0.02^{aBC}	1.10±0.01^{aB}	1.19±0.02^{aB}	1.87±0.40^{bA}
Pregelatinized wheat	-8.52±0.60^{aE}	-3.61±0.01^{bD}	-1.99±0.15^{dC}	-0.38±0.06^{bB}	-0.16±0.14^{dB}	0.46±0.13^{dA}	0.83±0.07^{cdA}
Retrograded wheat	-8.06±0.89^{aE}	-3.72±0.06^{bD}	-0.54±0.34^{eC}	-0.76±0.25^{cC}	-0.12±0.03^{dBC}	0.49±0.05^{dAB}	0.72±0.03^{dA}
Potato	nd	-6.02±0.89^{cC}	1.01±0.00^{aB}	1.07±0.04^{aB}	1.14±0.03^{aB}	1.20±0.06^{aB}	3.47±0.24^{aA}
Pregelatinized potato	-10.20±0.30^{bD}	-3.53±0.27^{bC}	0.06±0.04^{bB}	0.94±0.09^{aA}	0.90±0.05^{bA}	0.88±0.00^{bA}	1.04±0.09^{cdA}
Retrograded potato	-14.10±0.43^{dG}	-4.21±0.03^{bF}	-0.69±0.05^{eE}	-0.28±0.21^{bD}	0.22±0.07^{eC}	0.71±0.01^{eB}	1.10±0.07^{eA}
T_p (°C)							
Wheat	-3.15±0.01^{bG}	2.17±0.16^{bF}	3.29±0.14^{bE}	4.07±0.34^{bcD}	6.48±0.29^{bC}	8.34±0.60^{bB}	10.32±0.12^{bA}
Pregelatinized wheat	-2.68±0.24^{aG}	1.10±0.08^{dF}	3.33±0.04^{bE}	4.47±0.04^{bD}	6.53±0.11^{bC}	7.65±0.34^{eB}	9.99±0.13^{bA}
Retrograded wheat	-2.32±0.37^{aG}	1.25±0.10^{cdF}	3.00±0.18^{eE}	3.74±0.58^{cD}	6.39±0.45^{bC}	7.46±0.28^{eB}	10.51±0.46^{bA}
Potato	nd	1.42±0.00^{eF}	3.25±0.03^{bE}	4.31±0.03^{bcD}	6.69±0.06^{bC}	7.64±0.34^{eB}	10.25±0.77^{bA}
Pregelatinized potato	-2.70±0.13^{aG}	2.57±0.23^{aF}	3.97±0.09^{aE}	6.24±0.18^{aD}	8.58±0.24^{aC}	10.00±0.10^{aB}	11.88±0.59^{aA}
Retrograded potato	-4.81±0.13^{eG}	1.37±0.17^{eF}	2.71±0.08^{dE}	4.13±0.33^{bcD}	5.82±0.20^{eC}	8.11±0.06^{bcB}	11.43±0.30^{aA}
T_e (°C)							
Wheat	1.31±0.01^{bG}	3.95±0.14^{eF}	6.17±0.10^{bE}	8.04±0.24^{eD}	9.86±0.48^{eC}	12.95±0.90^{cdB}	17.79±0.21^{bA}
Pregelatinized wheat	0.55±0.19^{eG}	3.72±0.02^{eF}	6.22±0.06^{bE}	7.95±0.42^{eD}	13.28±0.60^{bC}	14.28±0.20^{bcB}	17.85±0.50^{bA}
Retrograded wheat	1.09±0.28^{bF}	5.22±0.70^{bE}	7.71±0.13^{aD}	9.40±1.33^{bC}	15.23±1.03^{aB}	14.56±1.72^{bB}	18.18±1.37^{bA}
Potato	nd	3.48±0.02^{eF}	5.49±0.15^{eE}	7.50±0.69^{eD}	10.05±0.04^{eC}	11.71±0.01^{dB}	18.09±0.20^{bA}
Pregelatinized potato	3.31±0.18^{aG}	6.27±0.18^{aF}	7.84±0.31^{aE}	11.92±0.82^{aD}	15.01±0.06^{aC}	18.47±0.45^{aB}	21.32±0.30^{aA}
Retrograded potato	-0.18±0.08^{dG}	4.85±0.51^{bF}	6.15±0.08^{bE}	7.55±0.10^{eD}	9.68±0.25^{eC}	13.65±0.34^{bcB}	17.60±0.60^{bA}

[a, e], for each DSC temperature and hydration level, mean values in the same column with the same superscript letter are not significantly different (P<0.05); [A-G], for each DSC temperature and starch sample, mean values in a row with the same uppercase superscript letter are not significantly different (P<0.05); mean values (n= 3) ± standard deviation; nd, peak non-detected

given in Table 4. As can be seen in Table 4, the type of starch and hydration level had a significantly effect on the UFW content ($P<0.05$). This may due to the fact that the numbers of available water binding sites of all starches are different. The main binding sites of the starches are hydroxyl groups and inter-glucose oxygen atom. The availability of these regions to interact with water varies depending on the structural and compositional properties of the starches (Fu et al 2014). Hydrogen bonds can be formed between the water molecules and the hydroxyl groups of the starch, and the number of these bonds increases with increasing moisture content up to certain moisture content. The differences between UFW content of starches from different sources may also be due to differences in morphological structure of starch granules. Alcazar-Alay & Meireles (2015) reported that water–binding capacity was significantly related to the morphological characteristics, such as shape and size of the starch granules.

The UFW contents of physically modified starches were higher than those of the NS in hydration levels up to 35%, while the UFW contents were lower than those of the NS samples in hydration levels above 35% (Table 4). Similarly, Fu et al (2014) reported lower UFW content in the gelatinized starch samples. The researchers attributed the decrease in the UFW content to the decrease in water binding sites due to starch-lipid interaction. In hydration levels above 55%, the PGS had higher ($P<0.05$) UFW contents than those of the RS (except for 85% hydration level PGWS). This is an indication that the PGS can establish better starch-water interactions. Fu et al (2014) reported that the gelatinization process destroyed the weak bonds in the amorphous regions of the granules and thus increased the hydration property of the starch molecules. Similarly, Tananuwong & Reid (2004) determined that when the starch was heated, the amount of UFW was increased, and fully gelatinized starch samples contained more UFW content than the partially gelatinized starch samples.

As the hydration level increased, there was an increase in the UFW content of NS. Eliasson & Gudmundsson (2006) indicated that the NS granules swell completely at the hydration level approximately 55%. However, in this research, it was determined that there was significant increase in hydration levels above 55%. Tang et al (2001) reported that increasing the water concentration in starches caused the appearance of inter-granular water, and the thermal properties of this water significantly differed from that of intra-granular water. For the practical consequences these results suggest that controlling the amount of freezable water is more important for moisture contents above 30% considering that only low amounts of freezable water detected in limited water systems.

Table 4- The unfreezable water contents of the starch samples with various hydration levels (g water/g dry starch x100)

Starch	*Hydration levels (%)*						
	25	*35*	*45*	*55*	*65*	*75*	*85*
Wheat	27.63 ± 0.48^{dG}	30.48 ± 0.47^{eF}	37.01 ± 0.07^{dE}	50.77 ± 0.29^{cD}	54.67 ± 0.54^{cC}	60.73 ± 0.16^{dB}	99.92 ± 2.81^{bA}
Pregelatinized wheat	30.49 ± 0.32^{cG}	33.14 ± 0.14^{dF}	35.45 ± 0.08^{eE}	38.25 ± 0.17^{dD}	45.56 ± 0.24^{dC}	54.87 ± 0.07^{eB}	59.79 ± 0.09^{eA}
Retrograded wheat	30.02 ± 0.02^{cG}	33.45 ± 0.38^{dF}	35.56 ± 0.06^{eE}	38.78 ± 0.36^{dD}	43.39 ± 0.32^{eC}	45.47 ± 0.18^{fB}	70.81 ± 0.48^{dA}
Potato	nd	42.26 ± 0.27^{bF}	48.70 ± 0.08^{aE}	58.89 ± 0.57^{aD}	69.94 ± 0.09^{aC}	89.58 ± 1.18^{aB}	123.57 ± 1.01^{aA}
Pregelatinized potato	32.87 ± 0.74^{bF}	35.10 ± 0.35^{cE}	38.79 ± 0.10^{cD}	38.73 ± 0.22^{dD}	59.68 ± 0.89^{bC}	71.06 ± 0.17^{bB}	86.73 ± 0.33^{cA}
Retrograded potato	35.18 ± 0.93^{aG}	43.74 ± 0.09^{aF}	44.67 ± 0.37^{bE}	51.48 ± 0.43^{bD}	55.01 ± 0.19^{cC}	66.05 ± 0.36^{cB}	69.43 ± 0.43^{dA}

[a, e], mean values in the same column with the same superscript letter are not significantly different ($P<0.05$); [A-G], mean values in a row with the same uppercase superscript letter are not significantly different ($P<0.05$); mean values (n= 3) ± standard deviation; nd, peak non-detected

4. Conclusions

The potato starch and its physically modified forms had higher UFW contents than those of the wheat starch and its physically modified forms in all the studied hydration levels. The UFW contents of the starch samples increased with increase in hydration level. The T_o, T_p and T_e values of the peak belonging to FW also increased with increasing hydration level. The lowest and the highest WAI were obtained in the native wheat and the gelatinized potato starches, respectively. A similar situation was also determined for the WSI values. Significant differences were found in all of the properties identified in starches, thus, further researches are needed for different starch combinations of this characteristic properties.

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Influence of Different Cutting Dates on Cornell Net Carbohydrate and Protein System (CNCPS) Parameters and the Fatty Acid Compositions of Caramba Hay *(Lolium multiflorum cv. caramba)*

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ABSTRACT

The aim of the study was to determine the influence of different cutting dates on Cornell Net Carbohydrate and Protein System (CNCPS) parameters and the fatty acid (FAs) compositons of caramba hay (*Lolium multiflorum cv caramba*). The samples were taken from the five randomized plots at the three different cuts (first cut:before blossom, second cut:blossom 50%, and third cut:after blossom). The samples were analyzed including the crude protein (CP), ether extract (EE), CP fractions (A= NPN, B_1= fast, B_2= intermediate, B_3= slow and C= not fermented and available for the animal), degradable intake protein (DIP), undegradable intake protein (UIP) and the FAs compositions (C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3). The CP, EE and A_1 fraction were negatively affected by the different cutting dates ($P<0.05$), while the B_2, B_3 and C fractions were increased by growing stage ($P<0.05$). Different cutting dates affected total major FAs, and decreased the concentration of C18:3 ($P<0.05$) and increased those C16:0 ($P<0.05$) and C18:2 ($P>0.05$). A positive strong linear relation was found between the C18:3 and CP contents in caramba hay ($R^2= 0.769$, $P<0.001$). The study showed that CP, soluble protein ($A+B_1$ fraction) and C18:3 were significantly decreased, the other crude protein fractions (B_2, B_3 and C) and other major FAs (C16:0 and C18:2) were increased by growing stage.

Keywords: CNCPS parameters; Fatty acids; Cutting date; Caramba

1. Introduction

Caramba (*Lolium multiflorum, cv. caramba)* which is a perennial forage grass, is rich especially protein, minerals and water-soluble carbohydrate content. Further its stem does not mature quickly until the time of harvest (Dewhurst et al 2001). In recent years it is stated that caramba is quite well adapted to Turkey climate and soil condition, so caramba has been recognized as potential forage for ruminant animals (Özelçam et al 2015). The livestock breeding in Turkey is largely based on pasture. However, because of agricultural mechanization, total range or pasture areas of Turkey have been drastically reduced (Kusvuran & Tansı 2011).

It is very important to know the crude protein (CP) fractions and total fatty acids (FAs) of the dietary feedstuffs to determine diet composition for target milk or meat composition (Ferlay et al 2006). The CP and FAs of perennial forages are affected by numerous factors such as plant species and variety, climate, day length, rainfall, fertilization, stage of growth and storage methods (Amrane & Michalet-Doreau 1993; Aganga et al 2004; Kalač & Samková 2010). In perennial grasses, the highest level of CP and the C18:3 content of FAs in young plants at the first cut, and then it decreases during summer, particularly around blooming (Dewhurst et al 2001; Boufaïed et al 2003). Also, knowledge of the factors that influence the CP and FAs of forages could help farmers to optimize cultivation and harvest conditions and thereby improve the quality of their forages (Khan et al 2012). The efficiency of nitrogen use and decrease nitrogen excretion to the environment is the important sustainability parameters on ruminant farms (Haugen et al 2006). The organic forages are the most necessary key to prevent the protein deficiency in organic ruminant rations, so forage protein analysis comes on the top of the list in accurately formulating rations (Pacheco et al 2012). Some researchers stated that rumen CP degradability may be reliably predicted from Cornell Net Carbohydrate and Protein System (CNCPS) parameters (Shannak et al 2000; Branco et al 2012). The perennial grass has been used as an animal feed as a fresh grass with grazing or after the processes of silage making or haymaking. The important losses occur in the content of FAs in perennial ryegrass, because of the loss of precursor fatty acids during the processes of silage making or haymaking. Therefore, the major FAs of fresh caramba are higher than silage or hay forms (Aii et al 1988). Although hays contain relatively low level of FAs on lipids, they are cheapest and often the major source of unsaturated FAs in ruminant diets (Kalač & Samková 2010). Also, the data on chemical composition and the FAs of caramba hay have been scarce compared with fresh or silage forms (Glasser et al 2013; Özelçam et al 2015).

The objective of the study was to investigate the influence of three cutting dates on CNCPS parameters and the FAs compositons of caramba hay.

2. Material and Methods

In this study, caramba *(Lolium multiflorum cv. Caramba*) is used as the material from Küçük Menderes basin at the Aegean Region which has the characteristics of Mediterranean climate. The summer season is warm and dry, and winters are temperate and rainy. The caramba was planted the research plots of Ege University, Ödemiş Vocational School Experiment Farm at Izmir (38°13'03" North, 27°57'50" East) from November in 2010 to June in 2011. Caramba samples were taken from the five randomized plots (2 m x 5 m) of the experimental farm at the three cuts of 2011 year. The three cuts were the first cut (before blossom), second cut (50%, blossom) and third cut (after blossom). The dry matter (DM) contents of the fresh caramba were 180.1, 200.1 and 264.7 g kg^{-1} for the first cut, second cut and third cut, respectively.

2.1. The climate, soil, planting and harvesting conditions

The soil and climate conditions of the experimental farm did not show a restrictive effect on caramba planting in irrigated conditions. The average temperature is 15.3 °C and total rainfall precipitation is 510 mm from November in 2010 to June in 2011 (Anonymous 2014). The soil (0-20 cm) where the study conducted had pH 7.28, salt 0.030-0.095%, organic matter 1.13-1.58%, $CaCO_3$ 1.44-21.52%, N 0.11-0.16%, P 20.50-40.52 mg kg^{-1}, K 110-400 mg kg^{-1}. The soil was generally sandy-loam texture. In the experiment, the raw spacing was 20 cm and the amount of the seed per hectare was 25 kg. Before planting, the plots were received 50 kg N (nitrogen) and P_2O_5 (phosphorus) per hectare by taking 15.15.15 compose base fertilizer. In spring, each plot was received 50 kg N per hectare as base application. Harvesting were made at 4-5 cm height at the before blossom, blossom (50, %) and after blossom.

2.2. The chemical compositions and the Cornell net carbohydrate and protein system parameters

The DM, CP and ether extract (EE) values of the caramba hay (air-dried samples) were measured by AOAC-approved methods (AOAC 1995). The standardized method of Licitra et al (1996) was used to determine the CNCPS parameters. The caramba hay samples were grinded using 1 mm sieve. The A fraction (non-protein nitrogen/NPN) is traditionally the nitrogen passing into the filtrate after precipitation with tungstic acid. The B_1 fraction is true soluble protein in borate-phosphate buffer at rumen pH (6.7-6.8). The Whatman#54 filter paper was used for filtration without vacuum to determine the A and B_1 fractions. The A+B_1 fractions generate total soluble protein (SolP). The cell wall components of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by Van Soest (1994). After the *r*esidue paper of NDF and ADF were transferred into a Kjeldhal flask for protein determination of neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP). Degradable intake protein (DIP, g kg^{-1} DM) was calculated by using the following equations and undegradable intake protein (UIP) was calculated by CP-DIP. The equations: A fraction (NPN): RDPA, rumen soluble protein; B_1 fraction (fast soluble protein): $RDPB_1$, $(B_1x(Kd_{B1}/Kd_{B1}+Kp_{1x}))$; B_2 fraction (intermediate degradable protein): $RDPB_2$: $(B_2x(Kd_{B2}/Kd_{B2}+Kp_{1x}))$; B_3 fraction (slow degradable protein): $RDPB_3$, $(B_3x(Kd_{B3}/Kd_{B3}+Kp_{1x}))$; DIP (Degradable intake protein): RDP_{TOTAL}= RDPA+$RDPB_1$+$RDPB_2$+$RDPB_3$. In these calculations the values stated in Sniffen et al (1992) and Fox et al (2003) were used for the coefficients of outflow rate on the different levels of DM intake (Kp) and degradation rate of B fractions (Kd), respectively. (DIP_{1X}= at 1x maintenance level of intake, DIP_{2x}= at 2x maintenance level of intake, and DIP_{3x}= at 3x maintenance level of intake)

2.3. The fatty acids (FAs)

The lipid fraction (approximately 0.5 g) from caramba hay was extracted with chloroform-methanol at a ratio of 2:1; the lipid was then isolated in the chloroform phase after adjustment of the solvent ratio to 2:2:1 (chloroformmethanol-water, v/v). The chloroform phase was removed and evaporated to dry under vacuum heater below 40 °C (Folch, Lees & Sloane-Stanley). The lipid is refluxed with a 1M solution of potassium hydroxide in 95% methanol. Then all lipid samples were analyzed using a gas-liquid chromatograph to determine the FAs (Agilent Technologies 6890 N Network GC System, Anaheim, CA, USA, Thermo Scientific TRACE TR-FAME GC Column; 60 mL, 0.25 mm ID, 0.25 um thick) at the University of Ege, Central Analytical Laboratory. Detector temperature: 250 °C, injection block temperature: 250 °C, Owen temperature: gradually from 2 °C to 240 °C, Split flow 119.9 mL min^{-1}, helium as the carrier gas. The FAs were identified by comparing their retention time and fragmentation pattern with an established standard (SUPELCO 37 Comp. Fame mix 10 mg mL^{-1} in $CH_{12}Cl_2$). The FAs; saturated (myristic C14:0, palmitic C16:0 and stearic C18:0), monounsaturated (oleic C18:1 and linoleic C 18:2) and polyunsaturated (γ-linoleic C18:3) were expressed as the percentages of total lipids.

2.4. Statistical analyses

All data were subjected to one-way ANOVA by using the statistical package of SPSS (15.0®) (SPSS 2006). Significant differences among the means were determined by the Duncan's multiple range tests. The relationship between the values the unsaturated FAs and the CP in caramba hay were determined by stepwise simple linear regressions.

3. Results and Discussion

3.1. The chemical compositions and crude protein fractions

The results of chemical compositions and CP fractions of caramba hay are shown in Table 1. When the growth period is increased by the different cuts (before blossom, 50% blossom and after blossom), the results of the CP and EE were decreased as an expected (Table 1). The differences between these contents were largely due to the different cutting

dates. The first cut had the highest CP content of caramba hay compared to the other cuts (P<0.05). The CP results in this experiment were agreed with Amrane & Michalet-Doreau (1993) and Aganga et al (2004) that CP of caramba hay is decreased depending on different cutting dates. Similar to these references, first cut had the highest CP content. The CP and EE contents of second cut of caramba hay in the present experiment were close to the CNCPS ver. 5 feedbank data (Fox et al 2003) (respectively, 86.0 g kg^{-1} DM and 22.0 g kg^{-1} DM). Also, similar to the this study with third cut, Özelçam et al (2015) stated that the CP was 63.5 g kg^{-1} DM and EE was 18.4 g kg^{-1} DM in the for caramba hay.

The CNCPS parameters of chemical compositions were compared with the values of the CNCPS ver. 5 feedbank and those determined by Fortina et al (2003). The CNCPS parameters of caramba hay on the CNCPS ver. 5 feedbank were similar to the second cut of the our results (bloosom) except NPN (SolP%). The average NPN (96 of SolP%) on the feedbank was higher than in our results for the all cutting times (Table 1). The differences between the NPN could be attributed to the different reagents (tungistic acid vs tricloratic acid) and filtration methods (Fortina et al 2003). However, we used tungistic acid in our study. On the other hand, when we compared our results with Fortina et al (2003), for caramba hay, although CP content (176 g kg^{-1} DM) is higher than our results, SolP (37.2 CP, %) was very close for the second cut. SolP, NPN (Solp%) and A fraction of the study were decreased by increasing vegetation stage and this decrease was important for Solp and A fraction (P<0.05). Similarly, Villiers & Ryssen (2001) stated that **t**he soluble N fraction, rate of degradation of the potentially degradable fraction and effective N degradability of herbage decreases with advancing stage of growth. Also, the results of B_1 fraction for the all cuts were low as explained in Sniffen et al (1992) that B_1 fraction of forages is lower than other fractions. The crude protein fractions of the Fortina et al (2003) study were close to the results for A, B_1 and B_2 fractions, were higher for B_3 fractions and lower for C fractions. The variations for the B_3 and C fractions could be due to the conventional or filter bag methods to determine NDIP and ADIP (Bovera et al 2003). Like Polat et al (2014), DIP values decreased and UIP values increased in accordance with the increased feeding level of DM intake (1x,

Table 1- Chemical compositions and crude protein fractions of caramba hay

Parameters	*1st cut (Before blossom)*	*2nd cut (Blossom, 50%)*	*3th cut (After blossom)*	*P value*
DM, g kg^{-1}	923.50±1.70 b	932.20±1.50 a	935.50±0.90 a	0.003
CP, g kg^{-1} DM	106.10±4.30 a	92.40±3.10 b	62.70±3.10c	0.000
EE, g kg^{-1} DM	28.40±1.30 a	24.40±2.10 ab	20.30±1.20 b	0.013
SolP, % of CP	50.08±2.01 a	36.73±1.59 b	17.63±1.58 c	0.000
NPN, % of SolP	84.30±3.32	82.80±3.99	78.41±4.58	0.568
NDIP, % of CP	24.93±0.79 c	32.17±1.05 b	36.95±1.64 a	0.000
Crude protein fractions, % of CP				
A= NPN	42.44±2.51 a	29.97±1.16 b	13.56±1.37 c	0.000
B_1	7.64±1.30	6.87±1.74	5.82±0.98	0.650
B_2	18.76±1.64 c	25.96±2.04 b	35.81±1.62 a	0.000
B_3	18.21±1.27 b	23.69±1.62 a	25.35±1.44 a	0.005
C (ADIP)	6.72±0.91 b	8.48±1.15 ab	11.60±1.21 a	0.013

DM, dry matter; CP, crude protein; EE, crude fat; SolP, soluble protein; NPN, nonprotein nitrogen (% of SolP); NDIP, neutral detergent insoluble protein; A fraction (NPN), non-protein nitrogen; B_1, fast true soluble protein; B_2, intermediate degradable protein; B_3, slow degradable protein; C (ADIP), acid detergent insoluble protein; Different letters (a, b, c) in the same row are statistically different (P<0.05)

2x and 3x) (Figure 1). Because of high protein solubility at the first cut, all DIP values are higher than second and third cuts. Similar to the feeding levels, DIP values were decreased by growing period from the first cut to third cut, all UIP values were significantly increased (P<0.05). We found UIP_{1X} values of caramba hay according to the first cut, second cut and third cut respectively, 32.19, 33.39 and 34.26 g kg^{-1} DM. Similarly, Fox et al (2003) reported the amount of the UIP_{1x} values were 30.53 g kg^{-1} DM for caramba hay.

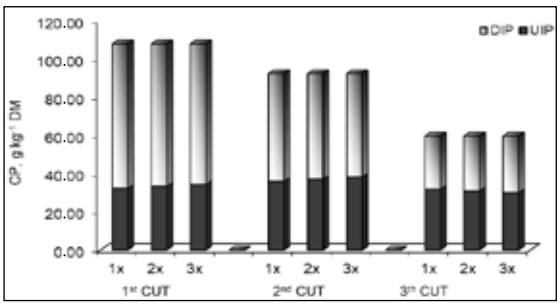

Figure 1- Rumen degradable intake protein (DIP) and Rumen undegradable intake protein (UIP) values of caramba hay according to the DM intakes based on CNCPS. CP, crude protein; DIP, degradable intake protein; UIP, undegradable intake protein (fed at 1x maintenance level, at 2x maintenance level of intake, and at 3x maintenance level of intake)

3.2. The fatty acids and relationship between major fatty acids and crude protein

The FAs of caramba hay with the different cuts are presented in Table 2. The C18:3 was the main FAs present in caramba hay ranging from first cut (43.06%) to third cut (20.71%). The other major FAs were C18:2 and C16:0, which both represented on an average from first cut to third cut 27.93% and 36.73%, respectively (Table 2). The C14:0, C18:0 and C18:1 represented the lower percent of total FAs. This is in agreement with the finding of Boufaïed et al (2003), Elgersma et al (2005) and Ferlay et al (2006). The C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3 content of caramba (fresh) were reported as 1.04, 16.7, 1.73, 2.42, 12.3 and 61.0%, respectively (Aii et al 1988). Ferlay et al (2006) confirmed that C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3 content of caramba hay (contain 133 g kg^{-1} DM of CP) were 0.6, 15.8, 1.8, 2.0, 14.0 and 55.9%, respectively. Ferlay et al (2006) study was close to our study than Glasser et al (2013). Because, as reported in Glasser et al (2013) that haymaking induced a slight decrease in total fat and FAs, among the FA a decrease in C18:3, mainly compensated for by an increase in C16:1. Even this decrease in C18:3 were higher when the drying conditions were not good, because of lipolysis and oxidation of polyunsaturated FAs (Aii et al 1988).

Similar to the study, Dewhurst et al (2001) concluded that the C18:3 of major FAs content (20.4 g kg^{-1} DM) were highest in early season and cutting date were found significantly important for *Lolium multiflorum* (n= 4, P<0.001). This high concentration of C18:3 in first cut (early summer), were explained by Elgersma et al (2005) for perennial ryegrass that the leaf-stem ratio of the herbage probably has effect on FAs content. After first cut, total FAs contained

Table 2- The major fatty acids of caramba hay

The fatty acids (g 100 g^{-1} lipid extract)	*1st cut (Before blossom)*	*2nd cut (Blossom, 50%)*	*3th cut (After blossom)*	*P value*
C14:0	0.71±0.01	0.74±0.07	0.96± 0.03	0.065
C16:0	15.40±0.60 b	17.87±1.13 b	20.61±0.20 a	0.003
C18:0	3.25±0.37 a	2.09±0.14 b	3.20±0.25 a	0.016
C18:1	7.99±1.03 a	4.88±0.61 b	6.29±0.61 ab	0.047
C18:2	12.53±1.03	14.27±1.17	16.12±1.38	0.149
C18:3	43.06±0.96 a	37.96±3.65 a	20.71±1.33 b	0.000
Others	17.10±1.90 b	22.20±3.00 b	31.90±2.90 a	0.006

Different letters (a, b, c) in the same row are statistically different (P<0.05)

lower concentrations of FAs for caramba hay (Table 2) with the consistent with Dewhurst et al (2001). Glasser et al (2013) reported that predominant factor was the vegetation stage and when the forage grew older, CP decreased, along with the EE, total FAs and the content of C18:3. These variations are first due to the decrease in the proportions of leaves that are richer than stems and seeds in membrane lipids. Also, Witkowska et al (2008) stated that because of lower temperature (the lowest daily total radiation), C18:3 content of FAs in early summer was higher than mid-summer in perennial ryegrass. In line with findings of Elgersma et al (2005), Boufaïed et al (2003) and Witkowska et al (2008), the positive linear relation was found between the content of major FAs (C16:0, C18:2 and C18:3) and CP in caramba hay (Figure 2). The simple regression relationship between C18:3 and CP (n= 15, R^2= 0.769, P<0.001) was the highest and significantly important compared to C16:0 and C18:2 (Figure 2). This relationship must be a result of indirect associations. Photosynthetic tissue is formed during growth, which is simulated by nitrogen. Furthermore, with the consistent in our study, the relation between the C18:3 and crude protein (R^2= 0.84, P<0.001) was the highest in Boufaïed et al (2003) study compared to the C16:0 and C18:2. However, in our result was disagreement with Boufaïed et al (2003) that the relationship between the C16:0 and C18:2 with crude protein were insignificant.

In conclusion, the crude protein, the soluble protein ($A+B_1$ fraction) and C18:3 were significantly decreased by growing stage from first cut to third cut. Because of photosynthetic activity, the plant has a higher CP contents at the first cut and this decreases as the crop ages. On the other

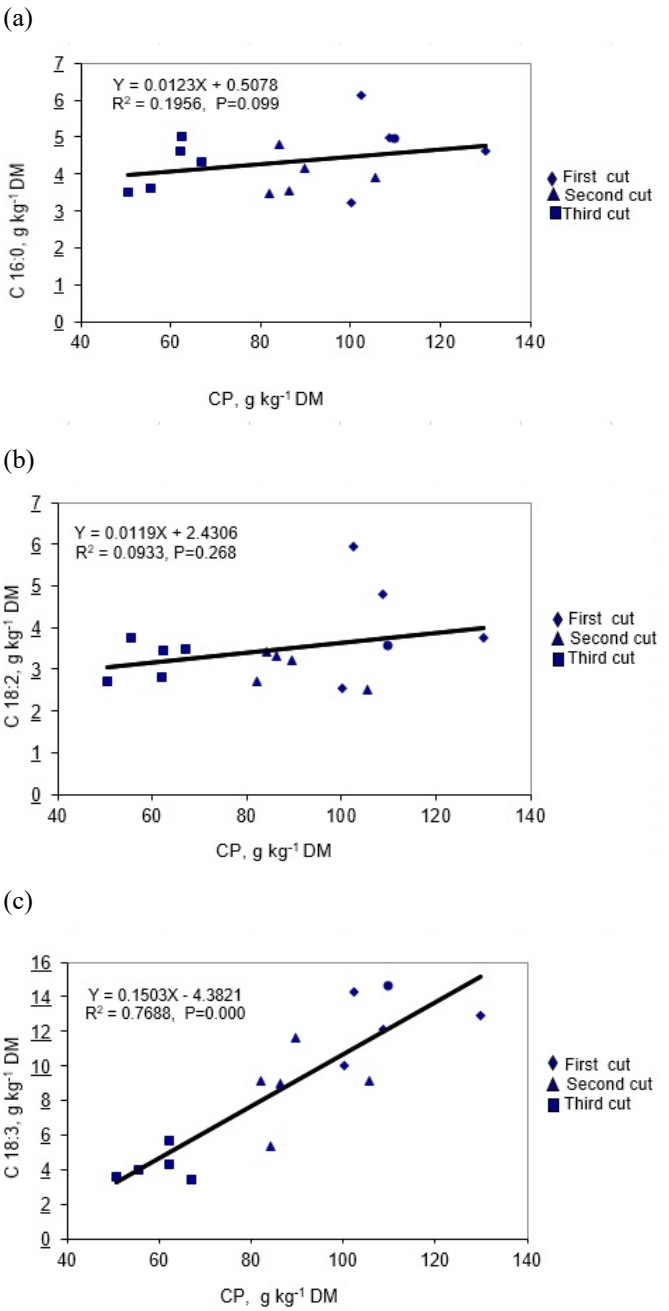

Figure 2- The relationship between the major FAs: C16:0 (a), C18:2 (b), C18:3 (c) and the crude protein in caramba hay harvested at three cutting dates

side, other CP fractions (B_2, B_3 and C) and major FAs (C16:0 and C18:2) values of in caramba hay were increased from first cut to third cut. Further studies are needed to determine the effects of the growth stage and storage methods on relationship between major fatty acids and CP in perennial ryegrass.

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Growing Degree Day and Seed Yield Relationships in Mustard (*Brassica juncea* L.) Under Different Sowing Seasons and Locations of Turkey

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ABSTRACT

Mustard is grown in mild winter regions as late fall and in hard winter regions as late spring crop. Mustard has high degree of adaptability under wide range of climatic conditions in Turkey. Temperature is an important weather parameter affecting the growth and development of the mustard. The sum growing degree day (GDD) for a growing season is related to plant development which is depends on the accumulation of heat. The aim of this study was to determine the adaptation of mustard, under sowing seasons (spring and fall sowing) and locations in terms of crop growth (emergence, 50% flowering, physiological maturity, and sum growing degree days) and seed yield of mustard. Two-year field experiments in a split-plot design with four replications were carried out during 2013-14 and 2014-15 growing seasons at eight different ecological locations. These locations included Ankara, Aydın, Erzurum, Eskişehir, Isparta, Tekirdağ, Tokat and Şanlıurfa provinces of Turkey-as classified by Köppen-Geiger ecological conditions. The *Brassica juncea* L. (mustard seeds) were collected from wild conditions in the Konya province of Turkey. The results showed that, sowing seasons and locations significantly affected seed yield and GDD. The maximum seed yield of 3754.9 kg ha^{-1} was obtained from Tokat (warm humid) during fall sowing with total accumulated GDD of 1512.1 °C for mustard. Sum growing degree-days accumulated in different sowing seasons and locations occurred between 1132.0 °C and 2285.1 °C depending on the related ecological conditions. Fall season crop in Aydın location had the maximum growing degree days. Overall, fall season accumulated more growing degree days due to longer period of sunshine in comparison to spring season with less sunshine days resulting in longer vegetation period.

Keywords: *Brassica juncea* L.; Fall and spring sowing; Growing degree day; Mustard

1. Introduction

Mustard is used for production of vegetable oil or biodiesel production, and for extraction of vegetable in the world (Başbağ et al 2010; Mao et al 2012). Mustard is generally grown in mild winter climates as fall crop, in hard winter climates as spring crop (Wu et al 2011). Two species of mustard *Sinapis alba* L. and *Sinapis arvensis* L. widely found in Turkey belong to Cruciferae family. These grow widely in Turkey as weed and could be commercially exploited for production of biodiesel (Blackshaw et al 2011; Kayaçetin et al 2016). Seed yield and oil quality of mustard depend on genetic, ecological conditions and agronomic factors interactions among them (Johnson et al 2003). Temperature is a major factor that affects and determines crop growth, development and productivity (Qadir et al 2007; Kaleem et al 2009; Singh & Lallu-Singh 2014). Mustard plant behave differently under different sowing seasons and environmental conditions that are based on temperature prevailing during the crop life cycle. Variation in maximum and minimum temperature largely alters the growth pattern of the crop by affecting the duration as well as onset of different phenophases. Quantification of the effect of temperature on crop growth can best be evaluated by GDD (mean ambient temperature minus the threshold temperature required for survival of crop). This quantification helps to quantify the thermal requirement for the start of different phenophases of crops (Dutta et al 2011). There are certain base temperatures for each plant species (Morrison et al 1989). Different sowing seasons and locations might cause different environmental conditions from emergence to maturity. The accumulation of GDD determines the maturity of crop and yield. According to Miller et al (2001) mustard cultivars are available, each with specific GDD requirements, for emergence 110-136, for flowering 680-750, for maturity ranging from 1510 to 1610 growing degree days using a 5 °C base temperature. The best growth of mustard occurs between 12 and 25 °C. The optimum temperature for maximum and minimum growth and development are estimated at just over 20 °C and 5 °C in the same order. GDD has influenced the productivity and profitability of mustard under different weather conditions in locations and sowing seasons (Ghosh & Chatterjee 1988; Wahhab et al 2002).

The target of this study was to determine the adaptation of mustard, in two sowing seasons (spring and fall) and differently selected Köppen-Geiger ecological locations of Turkey in terms of crop growth (emergence, 50% flowering, physiological maturity, and sum growing degree days) and seed yield.

2. Materials and Methods

The study was conducted during the growing seasons of 2013-14 and 2014-15 under the Ankara (warm tempetare climates, dry summer, warm summer-Csb), Aydın (warm tempetare climates, dry summer, hot summer-Csa), Erzurum (snow climates, fully humid, warm summer-Dfb), Eskişehir (snow climates, summer dry, warm summer-Dsb), Isparta (warm tempetare climates, dry summer, hot summer-Csa), Tekirdağ (warm tempetare climates, dry summer, hot summer-Csa), Tokat (snow climates, summer dry, warm summer-Dsb) and Şanlıurfa (arid climates, steppe, cold arid-BSk) Köppen-Geiger ecological conditions of Turkey (Kottek et al 2006; https://en.climate-data.org 2018).

The seeds of mustard used in this study were selected from the plants growing under wild conditions in the Konya province. The identification of the plants was carried out by Department of Biology, Gazi University, Ankara, Turkey. Treatment combinations were arranged in a split-plot design with 4 replication, at all locations during both years. The effect of locations was studied in the main plots and fall-spring sowing in the sub-plots. Plot length was 5 m and consisted of 10 rows (30 cm). The sowing dates were determined for favorable climatic conditions at all locations. Nitrogen, phosphorus and sulphur fertilizers were applied at the rate of 100, 50 and 35 kg ha^{-1} in the form of diammonium phosphate, ammonium nitrate and ammonium sulfate respectively (Pyare et al 2008). The total quantity of phosphorus and

sulphur fertilizer was applied at the time of sowing. Total nitrogen fertilization was applied in two equal doses, at the time of sowing and rosette formation. No irrigation was done to the experimental plots during the two years study period.

The soil samples took from each location at a depth of 0-20 and 21-40 cm during two analysed for the minerals, organic contents texture, the saturation percentage, total salts, pH, lime, phosphorus, potassium and organic contents. The soil samples charachteristics belonging to each experimental areas are shown in Table 1. All soils had low organic contents in range of low inorganic matter (Table 1).

Table 1- Physical and chemical soil charachteristics of the experimental areas sampled at depth of 0-20 and 21-40 cm

Location	*Year*	*Depth (cm)*	*Texture*	*Saturation percentage (%)*	*Total salt (%)*	*pH*	*Lime (%)*	*Phosphorus (P)*	*Potassium (K)*	*Organic Contents (%)*
Ankara	2013-14	0-20	Clay loam	64.0	0.041	7.79	28.12	6.63	162.04	1.31
		21-40	Clay loam	63.0	0.035	7.85	27.40	4.87	149.86	1.31
	2014-15	0-20	Clay loam	63.0	0.028	7.75	31.45	7.35	234.55	0.90
		21-40	Clay loam	63.0	0.037	7.76	24.82	7.81	219.99	1.49
Aydın	2013-14	0-20	Loam	49.0	0.017	8.00	14.23	22.29	52.61	0.53
		21-40	Loam	49.0	0.017	8.06	13.98	17.17	50.13	1.16
	2014-15	0-20	Clay loam	51.0	0.028	7.90	13.29	19.17	77.10	1.30
		21-40	Clay loam	51.0	0.029	7.96	16.41	15.86	63.00	1.45
Erzurum	2013-14	0-20	Loam	50.0	0.018	7.93	5.94	11.23	105.60	0.64
		21-40	Clay loam	51.0	0.018	7.98	6.20	12.77	92.44	0.53
	2014-15	0-20	Clay loam	54.0	0.450	7.80	5.99	9.61	109.02	0.97
		21-40	Clay loam	52.0	0.254	7.84	5.17	9.68	86.15	1.20
Eskişehir	2013-14	0-20	Clay loam	61.0	1.000	8.08	10.99	8.59	132.00	3.45
		21-40	Clay loam	60.0	0.836	7.99	8.06	8.51	136.00	3.87
	2014-15	0-20	Clay loam	58.0	0.043	7.57	22.55	7.96	105.60	1.53
		21-40	Clay loam	58.0	0.039	7.71	20.17	8.25	102.24	1.71
Isparta	2013-14	0-20	Loam	45.0	0.011	7.88	31.19	7.08	40.74	0.26
		21-40	Clay loam	53.0	0.014	7.83	30.44	5.04	89.27	0.14
	2014-15	0-20	Loam	43.0	0.011	7.88	30.55	3.69	145.90	0.67
		21-40	Loam	42.0	0.008	7.93	32.93	4.68	149.86	0.99
Tekirdağ	2013-14	0-20	Clay loam	53.0	0.022	7.88	8.10	5.18	92.44	0.13
		21-40	Clay loam	52.0	0.022	7.83	8.21	4.05	40.74	0.25
	2014-15	0-20	Clay loam	57.0	0.032	7.30	0.74	7.32	57.70	1.61
		21-40	Clay loam	56.0	0.016	7.62	0.74	6.57	52.61	1.37
Tokat	2013-14	0-20	Loam	46.0	0.015	7.74	11.85	7.44	43.01	0.40
		21-40	Loam	46.0	0.018	7.79	11.28	5.16	34.21	0.55
	2014-15	0-20	Clay loam	51.0	0.022	7.64	11.65	8.05	65.72	1.27
		21-40	Loam	49.0	0.023	7.57	15.41	5.39	32.14	1.18
Şanlıurfa	2013-14	0-20	Clay loam	69.0	0.045	7.68	30.00	6.01	160.80	1.74
		21-40	Clay loam	68.0	0.053	7.73	30.00	2.63	72.00	1.49
	2014-15	0-20	Clay loam	54.0	0.023	7.98	32.93	4.05	102.24	0.75
		21-40	Clay loam	55.0	0.026	8.02	32.78	1.85	71.31	0.25

Data were obtained from Soil Fertilizer and Water Resources Institute

Daily maximum and minimum temperature value (%) of the 2013-14 and 2014-15 vegetation periots of mustard are presented in Table 2; Montly rainfall, minimum and maximum temperatures values recorded during mustard development in experimental areas are presented in Figure 1. During

Table 2- Monthly maximum and minimum temperature value (%) of the 2013-14 and 2014-15 vegetation periods of mustard

Location	*September*				*October*				*November*			
	2013-14		*2014-15*		*2013-14*		*2014-15*		*2013-14*		*2014-15*	
	Max.	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*
Ankara	25.14	11.03	25.18	13.48	19.33	4.86	19.21	8.65	15.30	2.980	12.51	3.54
Aydın	32.01	16.99	31.35	18.22	25.64	11.17	26.57	14.53	20.16	9.90	19.34	9.36
Erzurum	21.29	3.93	23.01	5.18	13.49	-2.45	14.92	2.48	8.27	-4.63	6.78	-5.04
Isparta	26.22	10.45	24.89	11.71	19.14	3.76	19.53	7.56	15.64	3.17	13.06	2.12
Tekirdağ	25.55	17.17	24.97	16.78	18.08	11.02	19.12	12.35	15.93	9.90	14.40	8.72
Tokat	25.72	11.34	27.56	14.02	18.78	5.53	20.36	9.49	15.11	4.15	12.13	3.02
Location	*December*				*January*				*February*			
	2013-14		*2014-15*		*2013-14*		*2014-15*		*2013-14*		*2014-15*	
	Max.	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*
Ankara	3.71	-4.51	9.54	3.00	7.33	0.07	5.00	-1.86	11.73	0.52	7.94	0.47
Aydın	13.27	3.60	16.38	8.63	16.00	7.50	12.74	4.75	17.10	6.46	14.33	5.53
Isparta	7.14	-3.35	10.7	2.86	9.41	-0.30	6.27	-1.59	12.12	-0.14	7.81	0.05
Tekirdağ	9.66	3.18	12.31	6.84	11.16	5.32	9.17	2.82	11.64	5.66	10.04	4.28
Tokat	2.56	-4.21	10.81	3.64	9.50	-0.06	6.90	-1.33	13.18	3.06	10.31	1.88
Şanlıurfa	9.66	3.18	12.31	6.84	11.16	5.32	9.17	2.826	11.64	5.66	10.04	4.28
Location	*March*				*April*				*May*			
	2013-14		*2014-15*		*2013-14*		*2014-15*		*2013-14*		*2014-15*	
	Max.	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*
Ankara	14.58	2.76	12.56	3.05	19.77	7.28	14.90	3.41	22.61	10.59	23.28	10.80
Aydın	19.38	7.98	17.75	7.89	23.36	11.64	21.98	9.38	27.83	15.07	29.58	15.50
Erzurum	8.22	-3.40	4.52	-7.12	14.24	0.66	11.10	-0.35	18.45	4.60	17.33	3.09
Isparta	12.95	1.95	11.70	2.26	17.26	5.30	15.33	2.81	20.76	8.71	22.75	8.82
Tekirdağ	14.10	6.36	11.40	6.01	17.18	10.03	15.80	7.74	21.61	13.61	22.85	14.56
Tokat	16.02	4.88	13.72	3.74	22.60	8.64	16.27	5.38	24.47	11.60	24.54	10.83
Şanlıurfa	14.10	6.36	11.40	6.012	17.18	10.03	15.80	7.74	21.61	13.61	22.85	14.56
Location	*June*				*July*				*August*			
	2013-14		*2014-15*		*2013-14*		*2014-15*		*2013-14*		*2014-15*	
	Max.	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*
Ankara	26.52	13.41	24.57	13.31	32.31	18.29	31.19	16.97	32.93	18.41	31.60	17.78
Aydın	32.65	18.84	31.31	18.42	36.08	21.23	37.62	22.05	37.05	22.36	37.58	22.61
Erzurum	23.51	6.31	24.28	6.79	29.25	11.44	29.50	10.05	30.54	11.66	29.67	10.92
Isparta	26.42	12.51	24.82	11.28	31.32	16.23	31.28	15.96	32.35	16.39	31.23	16.17
Tekirdağ	26.16	17.70	25.80	17.32	29.17	20.17	29.51	19.92	30.08	20.89	30.47	21.75
Tokat	27.97	14.00	26.11	14.60	31.95	17.50	29.21	15.59	32.79	18.91	31.31	18.46
Şanlıurfa	26.16	17.70	25.80	17.32	29.17	20.17	29.51	19.92	30.08	20.89	30.47	21.75

Data were obtained from the Directorate of State Meteorological Observatory at Ankara

the vegetation period in 2013-14 and in 2014-15. There was total of 302.2 and 603.0 mm, average temperature of 11.4 °C and 12.3 °C, and an average humidity of 55.5% and 69.3% in Ankara. There was total of 302.2 and 603.0 mm, average temperature of 17.9 °C and 17.4 °C, and an average humidity of 58.6% and 63.9% in Aydın. There was total of 317.7 and 467.9 mm, average temperature of 6.4 °C and 6.4 °C, and an average humidity of 65.3% and 68.4% in Erzurum. There was total of 257.4 and 571.9 mm,

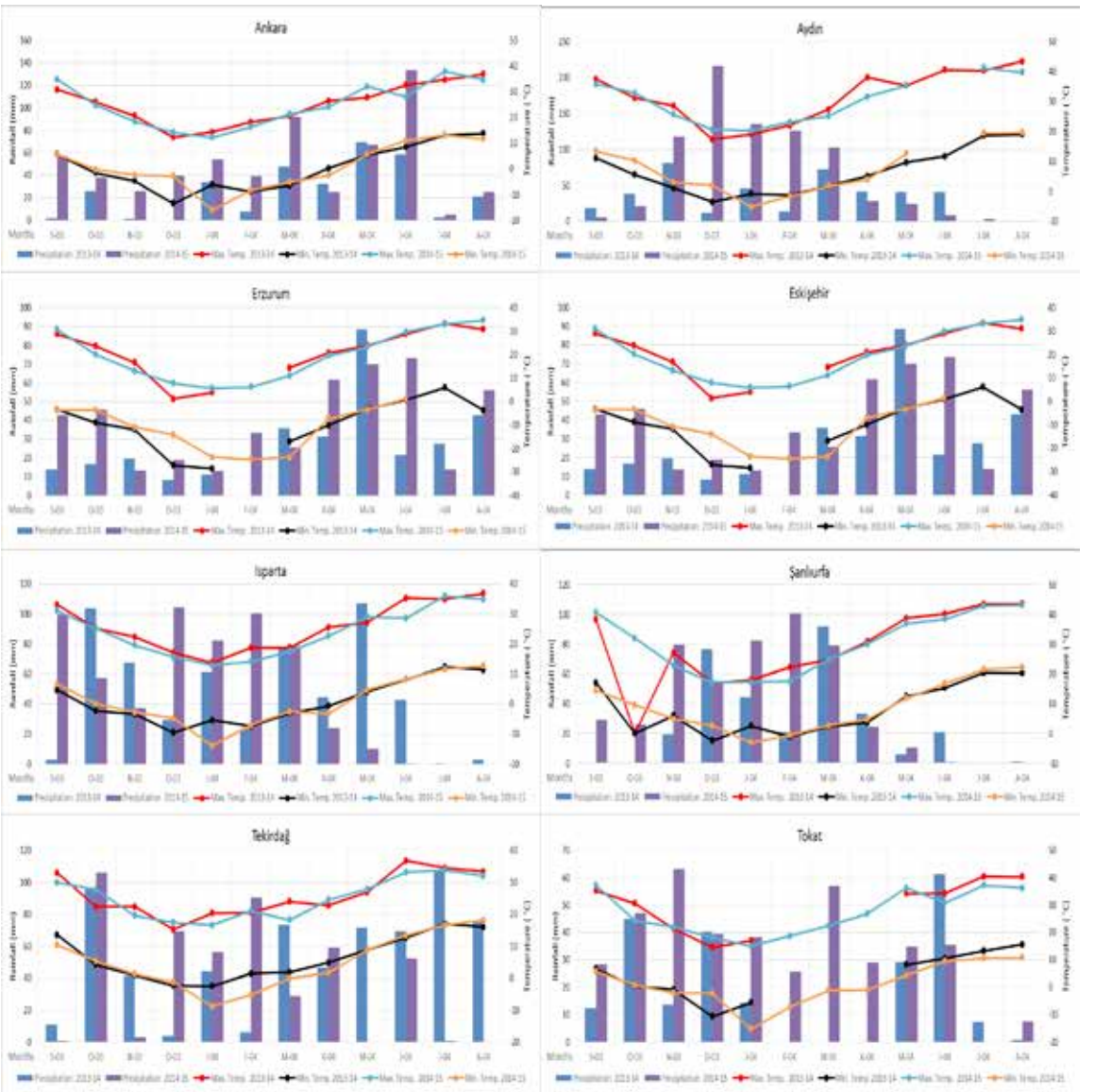

Figure 1- Monthly rainfall, minimum and maximum temperatures values recorded during mustard development in experimental areas (S, September; O, October; N, November; D, December; J, January; F, February; M, March; A, April; M, May; J, June; J, July; A, August)

average temperature of 12.7 °C and 11.1 °C, and an average humidity of 62.2% and 73.3% in Eskişehir. There was total of 565.9 and 596.6 mm, average temperature of 12.6 °C and 11.9 °C, and an average humidity of 57.1% and 63.4% in Isparta. There was total of 647.9 and 468.7 mm, average temperature of 15.4 °C and 14.5 °C, and an average humidity of 77.1% and 78.1% in Tekirdağ. There was total of 224.8 and 406.7 mm, average temperature of 14.4 °C and 13.3 °C, and an average humidity of 52.1% and 62.4% in Tokat. There was total of 313.8 and 487.0 mm, average temperature of 18.6 °C and 18.9 °C, and an average humidity of 41.4% and 50.6% in Şanlıurfa (Figure 1).

Emergence, 50 percent flowering and physiological maturity were identified based on visual observations. Growing degree days requirement for attaining different phenological events were calculated from weather data recorded through out crop life cycle by the following equation (Berti & Johnson 2008).

$$GDD = \Sigma \left[\left[(T_{max} + T_{min}) / 2 \right] - T_{base} \right]$$

T_{max} and T_{min} are daily maximum and minimum air temperatures in degree centigrade, respecticely. T_{base} is the 5 ºC base temperature for mustard development (Stannard et al 2000). Daily maximum and minimum air temperature data from eight locations are used in the study. GDD were accumulated by adding each day's GDD contribution as the season progressed.

Seed yield data were subjected to analysis of variance (ANOVA) using the MSTAT-C computer Statistical software. The significant differences among group means were separated using Duncan's Multiple Range Test.

3. Results and Discussion

Effects of sowing season and locations on growing degree days from emergence to maturity and seed yield of mustard are presented in Table 3. Emergence, flowering, maturity, sum growing degree days and seed yield of mustard varied according to sowing seasons, years and locations. Sum growing degree-days were accumulated between 1473.0 °C and 2098.9 °C during 2013-14 and 1132.0 °C and 2008.2 °C during 2014-15 under conditions of varied ecological conditions.

In fall sowing, no results could be obtained because of cold damage at Ankara, Eskişehir and Isparta locations during 2013-14. No emergence was noted at Erzurum, despite irrigation for both years due to high coldness.

The seed yield (1847.5 kg ha^{-1}) of the second year was higher than the first year (1146.9 kg ha^{-1}) (Table 3). This differences were occured due to higher rainfall during the growing period of plants during second year. Degree days from sowing to emergence in all locations was earlier at Tokat and Şanlıurfa compared to other locations during 2013-14, whereas it was earlier at Ankara compared to other locations during 2014-15. Flowering was earlier at Tokat compared to other locations during 2013-14, at Ankara compared to other locations during 2014-15. Degree days from sowing to maturity at all locations was earlier at Tokat during 2013-14, at Ankara in 2014-15 compared to other locations. Different locations exhibited differences for growing degree days (GDD) accumulation during both years. Sowing season is important for higher seed yield. Soil moisture is the major constraint for seed germination as well as for plant establishment and plays a key role under rainfed conditions, besides temperature. Statistically significant differences were found between the two consecutive years in terms of seed yield of mustard. The highest seed yield (1962.5 and 3754.9 kg ha^{-1}) was obtained at Aydın and Tokat, and the total GDD of mustard accumulated between 1473.0 and 2098.9 °C during 2013-14 and 1132.0 and 2008.2 °C during 2014-15, respectively. Total GDD of mustard increased at Aydın during both years, but its seed yield did not increase during both years. Aydın location had higher rainfall during the growing period of plants in the second year (791.0 mm) compared to the first year (407.8 mm). However, the seed yield in the second year was lower compared to first year due to diseases and long drought period before flowering (İptaş & Kolsarıcı 1988). At Tokat location, rainfall during the growing period

Table 3- Effects of sowing season and locations from emergence to maturity on growing degree days and seed yield of mustard

Year	*Sowing Season*	*Location*	*Sowing date*	*Emergence date*	*Flowering date*	*Harvest date*	*Emergence (°C)*	*Flowering (°C)*	*Maturity (°C)*	*Sum growing degree-days (°C)*	*Seed yield (kg ha^{-1})*
2013-14	Fall sowing	Aydın	25-Oct-2013	15-Nov-2013	06-Mar-2014	10-Jun-2014	237.5	573.7	1287.7	2098.9	1962.5a
		Şanlıurfa	31-Oct-2013	12-Nov-2013	25-Mar-2014	22-May-2014	137.5	688.0	837.3	1662.8	1235.0b
		Tekirdağ	02-Oct-2013	24-Oct-2013	21-Mar-2014	17-Jun-2014	206.1	692.0	969.7	1867.8	876.7bc
		Tokat	08-Oct-2013	25-Oct-2013	06-Apr-2014	09-Jun-2014	137.4	511.6	824.0	1473.0	513.3c
						Mean	179.6	616.3	979.7	1775.6	1146.9
						Max.	237.5	692.0	1287.7	2098.9	1962.5
						Min.	137.4	511.6	824.0	1473.0	513.3
										$Fvalue_{location}$	47.9007**
										CV (%)	26.31
	Spring sowing	Ankara	16-Apr-2014	29-Apr-2014	14-Jun-2014	4-Aug-2014	108.4	453.3	872.6	1434.3	1831.9a
		Aydın	20-Mar-2014	28-Mar-2014	20-Apr-2014	15-Jul-2014	83.8	251.0	1633.4	1968.2	330.8de
		Erzurum	24-Apr-2014	18-May-2014	11-Jul-2014	18-Aug-2014	220.7	653.7	697.0	1571.4	126.2e
		Eskişehir	08-Apr-2014	24-Apr-2014	02-Jun-2014	22-Jul-2014	120.7	445.6	835.8	1402.1	1587.0b
		Isparta	21-Mar-2014	10-Apr-2014	03-Jun-2014	19-Jul-2014	90.7	466.5	745.1	1302.3	768.9c
		Şanlıurfa	26-Feb-2014	07-Mar-2014	26-Apr-2014	16-Jun-2014	70.5	545.7	950.1	1566.3	104.8e
		Tekirdağ	04-Apr-2014	19-Apr-2014	22-May-2014	26-Jul-2014	114.8	356.8	1143.5	1615.1	474.2d
		Tokat	05-Mar-2014	21-Mar-2014	07-May-2014	7-Jul-2014	79.5	461.2	940.5	1481.2	152.5e
						Mean	111.1	454.2	977.3	1542.6	672.0
						Max.	220.7	653.7	1633.4	1968.2	1831.9
						Min.	70.5	251.0	697.0	1302.3	104.8
										$Fvalue_{location}$	11.7733**
										CV (%)	31.43
2014-15	Fall sowing	Ankara	14-Oct-2014	02-Nov-2014	26-May-2015	15-Jul-2015	71.3	440.5	620.2	1132.0	3225.5b
		Aydın	03-Nov-2014	22-Nov-2014	18-Feb-2015	26-Jun-2015	204.7	483.0	1597.4	2285.1	922.8e
		Eskişehir	14-Oct-2014	27-Oct-2014	08-May-2015	30-Jun-2015	108.9	557.0	709.3	1375.2	1267.5d
		Isparta	23-Oct-2014	05-Nov-2014	07-May-2015	13-Jul-2015	73.9	442.4	879.6	1395.9	1328.0cd
		Şanlıurfa	27-Oct-2014	11-Nov-2014	10-Apr-2015	1-Jun-2015	136.4	763.8	778.8	1679.0	1516.8c
		Tekirdağ	16-Oct-2014	02-Nov-2014	20-Apr-2015	8-Jul-2015	152.6	734.2	1121.4	2008.2	916.7e
		Tokat	14-Oct-2014	25-Oct-2014	04-May-2015	29-Jun-2015	96.3	607.1	808.7	1512.1	3754.9a
						Mean	120.6	575.4	930.8	1626.8	1847.5
						Max.	204.7	763.8	1597.4	2285.1	3754.9
						Min.	71.3	440.5	620.2	1132.0	916.7
										$Fvalue_{location}$	65.3092**
										CV (%)	15.44
	Spring sowing	Ankara	01-May-2015	12-May-2015	22-Jun-2015	10-Aug-2015	84.6	452.5	795.5	1332.6	585.4b
		Aydın	17-Apr-2015	24-Apr-2015	7-Jun-2015	09-Jul-2015	70.4	731.3	669.1	1470.8	146.0d
		Erzurum	15-May-2015	30-May-2015	30-Jun-2015	01-Sep-2015	141.2	407.4	879.8	1428.4	624.2b
		Eskişehir	04-Mar-2015	06-Apr-2015	11-Jun-2015	10-Jul-2015	100.1	650.1	423.5	1173.7	295.4c
		Isparta	17-Mar-2015	10-Apr-2015	1-Jun-2015	17-Jul-2015	56.5	434.9	673.1	1164.5	574.0b
		Şanlıurfa	27-Feb-2015	12-Mar-2015	3-May-2015	09-Jun-2015	88.2	473.8	688.5	1250.5	311.5c
		Tekirdağ	16-Apr-2015	30-Apr-2015	10-May-2015	15-Jul-2015	110.1	121.0	1068.6	1299.7	82.8d
		Tokat	28-Feb-2015	17-Mar-2015	30-May-2015	06-Jul-2015	76.5	592.9	573.4	1242.8	2862.3a
						Mean	91.0	483.0	721.4	1295.4	685.2
						Max.	141.2	731.3	1068.6	1470.8	2862.3
						Min.	56.5	121.0	423.5	1164.5	82.8
										$Fvalue_{location}$	192.1848**
										CV (%)	19.03

**, P<0.01 significantly different according to the Duncan. Data was the means of 4 replications

of plants during the second year (406.7 mm) was higher compared to the first year (224.8 mm). Low temperatures at flowering and maturity during 2014-2015 prolonged vegetation period and provided suitable environmental conditions for good growth (Schuster & Taghizadeh 1981; Kondra et al 1983). Due to higher regular and sufficient rainfall (90.75 mm) during the flowering and maturity (March-April-May-June) of second year growing season compared to the first year (56.5 mm) at Tokat locations. The second year seed yield was higher compared to the first year. The results of the previous studies support that the differences in yield could be derived from various years and locations which have different ecological conditions including air temperature, precipitation and agronomic practices (Saran & Giri 1987; Shafii et al 1992; Walton & Bowden 1999).

In spring sowing, the seed yield (685.2 kg ha^{-1}) of the second year was higher compared to the yield of the first year (672.0 kg ha^{-1}) (Table 3). This differences was a result of raising rainfall and air temperature during the growing period of plants.

Comparing GDD from sowing to emergence among all locations; it varied. It was earlier at Şanlıurfa and Isparta during 2013-14 and during 2014-15 compared to other locations in the same order. Flowering was shorter at Aydın compared to other locations during 2014. Flowering was shorter at Tekirdağ compared to other locations in 2015. Degree days from sowing to maturity within all locations was obtained earlier at Erzurum and Eskişehir during 2014 and 2015 compared to other locations.

Statistically significant differences were found between the two consecutive years in terms of seed yield of mustard. The highest seed yield (1831.9 and 2862.3 kg ha^{-1}) was obtained at Ankara and Tokat locations. The total GDD of mustard at Ankara and Tokat was obtained between 1302.3 and 1968.2 °C during 2014 and 1164.5 and 1470.8 °C during 2015, respectively. Sum GDD of mustard was observed the highest at Aydın during both years, but without increase in seed yield. The germination, growth, flowering stages, and ripening periods of the plants were determined as by temperatures and genetic factors. Environmental factors, especially temperature during the growing period of the plants is important. Especially, during flowering and ripening, as high temperatures and water stress caused decreases in seed yield (Hocking et al 1997; Kaleem et al 2010). The threshold temperature during flowering, resulting in seed yield losses, was 29.5 °C and high mean maximum temperature during vegetative development caused a reduction in induction of number of flowers for all of the tested Brassica species (Malcolm et al 2002). The precipitation of 69.2 and 58.6 mm during flowering, began and continued through May and June until the maturity of capsules after fertilization that affected positively during 2014. Erzurum had the highest altitude among locations; where the plants were not able to complete theire vegetative growth and entered generative phase at an earlier stage of growth that resulted in non development of their morphological features before generative maturity, therefore this affected completation of grain formation and yield. Thus, the grains were quite weak and feeble (Amirnia et al 2012). Varying results in yields among locations clearly demonstrated that, these differences among locations could be due to varying air temperatures, precipitation (Sra 1978; Christensen et al 1985; Walton & Bowden 1999).

Variation in maximum and minimum temperature largely alters the growth pattern of the crop by affecting the duration as well as onset of different phenophases. Quantification of the effects of temperature on crop growth can best be evaluated by GDD (mean ambient temperature minus the threshold temperature required for survival of crop). This quantification helps to know the thermal requirement for the start of different phenophases of crops (Dutta et al 2011). According to Miller et al (2001) mustard cultivars are available, each with specific GDD requirements, for emergence 110-136, for flowering 680-750, for maturity ranging from 1510 to 1610 growing degree days using a 5 °C base temperature. It is a cool loving crop with thermo and photo-sensitivity (Ghosh & Chatterjee 1988).

The best growth of mustard occurs between 12 and 25 °C. The optimum temperature for maximum and minimum growth and development are estimated at just over 20 °C and 5 °C in the same order. GDD has influenced the productivity and profitability of mustard under different weather conditions in locations and sowing seasons (Wahhab et al 2002).

In fall sowing, GDD were accumulated between 1473.0 and 2098.9 °C during 2013-14 and between 1132.0 and 2285.1 °C during 2014-2015. Seed yield was obtained from 513.3 to 1962.5 kg ha^{-1} during 2013-14 and from 916.7 to 3754.9 kg ha^{-1} during 2014-2015. In spring sowing, GDD were accumulated between 1302.3 and 1968.2 °C during 2014 and 1164.5 and between 1628.4 °C during 2015 depending on sowing dates under locations and ecological conditions. Seed yield in spring sowing was between 104.8 and 1831.9 kg ha^{-1} during 2013-14 and between 82.8 and 2862.3 kg ha^{-1} during 2014-2015. The results of this research showed that the locations and sowing seasons of mustard affected growing degree days for emergence, 50% flowering and physiological maturity during both years. Sum GDD of mustard increased at Aydın during both years compared to other locations, but its seed yield remained unchanged. This confirms that GDD accumulation plays a important role for higher seed yields. However, not only rainfall but also low and high temperatures are usually the most limiting factors for crop growth (Loss & Siddique 1994). Vegetative growth rate is also restricted by low temperatures (0-7 °C) in mid-winter and seed yield is adversely affected by high temperatures (25-40 °C) at the end of the season in spring and early summer (Turner et al 2001).

4. Conclusions

Different sowing seasons and locations might cause different environmental conditions from emergence to maturity. The accumulation of GDD determines the maturity of crop and yield. The target of this study was to determine the adaptation of mustard, in two sowing seasons (spring and fall) and differently selected Köppen-Geiger ecological locations of Turkey in terms of crop growth (emergence, 50% flowering, physiological maturity, and sum growing degree days) and seed yield. The Turkey is facing acute shortage of underground water levels and there is need to grow high vegetable oil producing crops suitable for biodiesel production with minimum input. This study reports the feasibility of mustard cultivation in different climatic zones of Turkey based on GDD or heat unit availability during the growing season and evaluates two years data. This evaluation report suggests minimum GDD for identifying useful sowing dates for harvesting profitable oil yield. In addition, the experimental locations lying in the North and West of Turkey were more useful, compared to the Erzurum location lying in the cold location with insufficient GDD suggesting non suitability of the location for commercial cultivation of mustard. These results clearly show that mustard is a suitable alternative crop for warm climatic regions of Turkey, irrespective of the location. It is assumed that there is a high potential of mustard for cultivation under arid climatic conditions of Turkey irrespective of the type of soil and environmental relative humidity status, if the farmers could be convinced to grow mustard in provinces that could accumulate GDD of at least 1132.0 °C. Differences among various locations might be due to the different climatic conditions that are based on prevailing temperatures during the growing period of mustard at these locations. The plants from fall sowing showed increased seed yield compared to the plants obtained from spring sowing; because mustard has tendency to mature (completes its life cycle) earlier (short duration) by accumulating less heat units.

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Effect of the Application of Foliar Selenium on Canola Cultivars as Influenced by Different Irrigation Regimes

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ABSTRACT

Selenium (*Se*) is an essential micro-nutrient element for animals and human, which also has some beneficial roles in many plant species. This study aimed to evaluate the application of foliar *Se* on canola cultivars under different irrigation regimes. The study was carried out in two consecutive years, in the form of a factorial split plot experiment, based on an RCB design with three replications. Sodium selenate solution was sprayed on the leaves of 6 winter canola exposed to 3 different irrigation regimes. The results revealed that most of the studied traits were affected by foliar selenium, especially seed yield, seed oil yield, leaf proline content and leaf chlorophyll a content. Under drought stress conditions, foliar selenium caused a significant increase in seed yield, seed oil yield, and the relative water content of leaves. According to partial regression analysis, foliar selenium changed the nature of relationships governing the traits, especially under drought stress conditions. The results showed that, selenium reduced the effects of drought stress through improving the relative water content of the leaves. Therefore, foliar selenium can be a useful strategy to achieve sustainable agriculture, especially under water deficit conditions.

Keywords: Irrigation regime; Partial regression; Rapeseed; Seed yield; Sodium selenate

1. Introduction

Iran, with an average annual rainfall of 240 mm, is located in the arid and semi-arid belt regions of the world. More than 60% of Iranian lands is located in the arid and semi-arid regions in which, water scarcity is the most important limiting factor for agricultural activities (Modarres & de Paulo Rodrigues da Silva 2007). Studies have shown that under water deficit condition, oxidative stress reduces plant growth, respiration, and photosynthesis through damaging cellular compounds, such as carbohydrates, lipids, nucleic acids, and proteins (Ahmad et al 2016).

Canola (*Brassica napus* L.) is one of the world's leading edible oil crops which favors human consumption due to its high oil and low saturated fat content (Turinek et al 2016). Studies have shown water scarcity has an undesirable effect on most of the morphological and agronomic characteristics of this plant (Ullah et al 2012; Badrooj et al 2016; Jaberi et al 2016; Pavlista et al 2016; Said-Al Ahl et al 2016). Most crop plants are sensitive to drought

stress, especially at flowering, pollination, and seed filling stages (Thomas et al 2004). Masoud Sinaki et al (2007) reported that the highest decrease in canola performance was observed during water deficit at the pod development stage.

Selenium (*Se*) is an essential micro-nutrient element for animals and human (Woo & Lim 2017). Although its necessity for higher plants is still unproven, however it is still of interest to biologists due to its beneficial role in many plant species (El-Ramady et al 2015). Studies have shown that *Se* plays a beneficial role in plants through enhancing growth, reducing damage caused by oxidative stress, enhancing chlorophyll content under light stress, stimulating senesce to produce antioxidants, and improving plant tolerance to drought stress by regulating water status (Ahmad et al 2016). As reported by Hasanuzzaman & Fujita (2011), selenium conferred enhanced tolerance to drought stress in rapeseed seedlings. As a secondary *Se* accumulator, canola takes up *Se* in proportion to the amount of *Se* available in the soil (White et al 2004). Commercial selenium-enriched fertilizers are in the forms of selenate and selenite. Recently, Deng et al (2017) showed that a greater accumulation of *Se* was obtained when selenate-base fertilizers was used. Also, foliar spray of *Se* was found to be a more economical and effective method than when incorporated with soil (Wang et al 2017).

The present experiment aims to determine the effect of foliar sodium selenate on some agro-physiological traits of canola under drought stress conditions. The experiment is based on the assumption that foliar selenium can possibly alleviate limiting effects due to drought stress.

2. Materials and Methods

2.1. Experimental design

The experiment was carried out at the research farm of Seed and Plant Improvement Institute, Karaj, Iran (latitude 35° 59' N, longitude 50° 75' E, altitude 1313 m above sea level) during two consecutive years (2014-15 and 2015-16). The soil texture was clay loam with 0.64 of organic matter (Table 1). Studied factors included i) irrigation regimes and ii) foliar application of sodium selenate and six winter canola genotypes i.e. Ahmadi, SW102, Okapi, GKH2624, GK-Gabriella, and Elvis. The factors were arranged as a factorial split plot based on a randomized complete block statistical design with three replications. The levels of irrigation regimes and foliar application of sodium selenate were randomly assigned to main plots, while canola genotypes were randomly distributed among sub plots.

Table 1- Physico-chemical parameters of the experimental field soil

Depth (cm)	*Potassium (mg kg^{-1} of soil)*	*Phosphorus (mg kg^{-1} of soil)*	*N%*	*Clay%*	*Silt%*	*Sand%*	*Ec (Ds m^{-1})*	*PH*
0-30	276	154	0/06	42	29.33	29	1.72	7.75

On October 2nd of each crop year, the seeds were sown at a depth of 1 to 1.5 cm, with a density of 100 seeds per square meter. Each plot, with an area of 10.8 m^2, consisted of 6 rows, which were 6 m long and 30 cm apart. The seeds were planted on rows and were spaced 4 cm apart. The distance between the plots and blocks was 2.4 m and 7 m, respectively. Two lateral lines were considered as margins and only the 4 middle lines were used for sampling and measuring of traits. Nitrogen, phosphorous and potassium chemical fertilizers were added to the soil at the rate of 150: 60: 50 kg ha^{-1}, respectively. Phosphorous and potassium fertilizers together with one-third of nitrogen were added to the soil concurrent with seed sowing, while the remaining two-third of nitrogen was distributed equally at the beginning of stem elongation and flowering stages. Weed control was done mechanically and

chemically. Plots were irrigated by furrow method until the reproductive stage.

2.2. Selenium and drought treatments

On April 27th after the planting year, sodium selenate solution (Na_2SeO_4) was sprayed on the leaves of the crop in two concentrations of Se1: 0 and Se2: 30 g L^{-1}. Three irrigation regimes were considered as drought stress treatment levels including I1: normal irrigation I2: irrigation cut off from flowering stage (A week after selenium spray, concurrent with the emergence of 50% of flowers) and I3: irrigation cut off from pod development stage (three weeks after selenium spray, concurrent with the emergence of 50% of pods).

2.3. Studied traits

Studied traits included plant height (*PH*), number of pods $plant^{-1}$ (*NPP*), number of seeds pod^{-1} of main stem (*NSPMS*), number of seeds pod^{-1} of lateral branches (*NSPLB*) thousand-seed weight (*TSW*), seed yield (*SY*), see oil yield (*SOY*), the relative water content of leaves (*RWC*), leaf proline content (*LPC*), leaf chlorophyll a content (*Chla*), and leaf chlorophyll b content (*Chlb*). To measure physiological traits such as chlorophyll and proline content of the leaves, 10 leaves were randomly selected from the middle lines of each plot. Also, to measure the agronomic traits, 10 plants were randomly selected from the middle lines of each plot. Stress intensity (SI) was calculated using the Equation proposed by Fischer & Maurer (1978) as:

$$SI = 1 - \left(\frac{\overline{Y_s}}{\overline{Y_p}} \right) \quad (1)$$

Where; $\overline{Y_s}$ and $\overline{Y_p}$ are mean yield under stress and non-stress conditions, respectively. The leaf relative water content was determined as follows:

$$RWC\% = \frac{FW - DW}{FW} \text{x100} \quad \text{(Ritchie et al 1990)} \quad (2)$$

Where; *FW* and *DW* are defined as the fresh weight and the dry weight of leaf. The proline content of the leaves was estimated as described by Bates et al (1973). Leaf chlorophyll content was measured according to Arnon (1949).

All the data were subjected to combined analysis of variance (ANOVA) using General Liner Model procedure in SAS software version 9.1.3, 2003, SAS Institute Inc., Cary, NC, USA. Means were compared at a 0.05 probability level. Partial regression coefficients were calculated in Microsoft Excel software based on the method described by Akintunde (2012).

3. Results and Discussion

Combined analysis of variance (ANOVA*)* revealed that different irrigation regimes had significant effects on all studied traits except for *TSW* and *Chlb*. Also, selenium spray significantly affected *PH*, *NSPMS*, *NSPLB*, *SY*, *SOY*, *LPC*, and *Chla*. Selenium by irrigation interaction effect was statistically significant for *SY*, *SOY*, and *RWC*. Furthermore, variation due to genotype was significant for all traits (data not shown).

3.1. Effects due to irrigation regime

Drought is a form of environmental stress that affects the physiological and biochemical processes in plants (Ahmad et al 2016). In general, mean trait values were found to be higher in the second year of the experiment except for *LPC* and *RWC* (Table 2).

Estimation of stress intensity revealed that, in both years, irrigation cut off from flowering caused more stress compared to irrigation cut off from pod development stage. In most references, accumulation of reactive oxygen species (*ROS*) has been known to be the main cause of devaluation of traits under drought stress (Feng et al 2013). Therefore, it can be concluded that irrigation cut off from flowering stage caused more accumulation of *ROS* which resulted in a further decrease in mean trait values. Increase in stress intensity affected most of the studied traits. For example, irrigation cut off from flowering caused a remarkable decrease in *SY* up to 46.09% compared to the control plants (Table 2). This result was in contrast with Masoud Sinaki et al (2007) who

stated that most of the reduction in canola yield was achieved when drought stress occurred during pod development stage. Water deficit significantly affects crop evapotranspiration and yield. According to Léllis et al (2017), when water supply does not meet crop water requirements, actual evapotranspiration will fall below the maximum evapotranspiration. In such circumstances, the response of crop yield to water deficit is a function of the proportion of relative yield decrease to relative evapotranspiration deficit, which is variable depending on the growth stage when water deficit occurs (Shirani Rad et al 2013). In the case of water shortages, dry matter reduction can be due to decreased cellular turgor pressure and biochemical restrictions, resulting in lower leaf chlorophyll content and photosynthesis (Shirani Rad & Abbasian 2011).

Irrigation cut off from flowering reduced *SOY* up to 49.32% compared to control plants (Table 2). Seed oil yield is a function of seed oil percentage and seed yield. Furthermore, changes in the content of seed oil in modified cultivars is low, thus the *SY* has the highest effect on *SOY*. Therefore, selecting the cultivars for higher *SY* will lead to increase in *SOY*.

Plant height also declined up to 32.11% when the irrigation was cut off from flowering (Table 2). Reduction of *PH* is a consequence of reduced chlorophyll content and photosynthesis area, increased energy consumed by the plant to absorb water, increased protoplasm density, change in respiration pathways, the activation of the pentose phosphate route and/or the reduction of root volume, etc (Moaveni et al 2010).

The cut off of irrigation from the flowering stage caused a decrease in the number of pods per plant by up to 56%. It seems that under the stress of water shortage, reduction of the number of pods in the higher-order branches plays a role in decreasing the number of pods per plant (Shirani Rad et al 2013). Research has shown that the flow of water to the leaf depends on the presence of water potential gradients between the xylem and the leaves, so that reduction in the water potential of xylem reduces the water potential gradient between the xylem and the leaf. Therefore, the number of seeds per plant under water deficit stress is decreased (Afkari Bajeh Baj 2012).

The results showed that water deficit reduced *TSW* significantly. Under drought stress conditions, photosynthesis rate decreases due to chlorophyll drop and consequently, reducing the storage and accumulation of photosynthetic material in the seeds causes a reduction in *TSW*. Also, reduction in seed weight is a consequence of the declines in the number of seeds plant^{-1} as well as the number of seeds pod^{-1}.

Statistically, drought stress led to a non-significant decrease in *Chlb*, showing a relative resistance to the drought stress applied in this study (Table 2). Compared to other studied traits, the highest reduction due to drought was observed for *NPP* and *Chla* (54.4% and 50.88%, respectively, Table 2). Previous studies indicated that drought stress causes a significant decline in leaf chlorophyll content. Under drought stress conditions, decrease of photosynthetic pigments content is a common symptom which mainly results from damage to chloroplasts caused by *ROS* (Mafakheri et al 2010). Moreover, the observed decline in *Chla* was almost two that of *Chlb*. This result was in agreement with Ghorbanli et al (2013).

Drought stress reduced the relative water content of the leaves. An increase in drought caused a further decline in *RWC*. As an indicator of water status in plants, *RWC* reflects the balance between water supply to the leaf tissue and transpiration rate. Therefore, it seems that there would be a significant correlation between *RWC* and relative yield loss. In our experiment, this correlation was high, negative, and significant at 0.01 probability level (-0.929**). Soltys-Kalina et al (2016) also confirmed the existence of such a significant and negative correlation. The *LPC* increased from 11.80 at I1 to 21.02 μM g^{-1} at I3 level, showing an increase of up to 78.13% (Table 2). As one of the most important osmolytes, proline accumulation protects the chloroplasts against destruction, consequently

Table 2- Mean ± standard error of mean of some agro-physiological traits of six winter canola as influenced by different irrigation regimes and foliar sodium selenate during 2015 and 2016

Factor	*Level*	*PH (cm)*	*NPP*	*NSPMS*	*NSLP*	*TSW (g)*	*SY (kg h^{-1})*	*SOY (kg h^{-1})*	*RWC (%)*	*LPC (µM g^{-1} FW)*	*Chla (mg g^{-1} FW)*	*Chlb (mg g^{-1} FW)*
Year	2015	134.05±2.32	149.88±4.77	21.89±0.37	14.72±0.26	3.16±0.06	3964.10±111.44	1725.09±52.65	86.46±0.31	18.69±0.44	0.95±0.03	0.35±0.01
	2016	145.79±2.28	162.18±5.27	24.93±0.45	17.34±0.30	4.28±0.10	4720.76±122.43	2071.03±58.28	86.12±0.28	13.98±0.36	1.04±0.03	0.41±0.00
LSD (P<0.05)		1.76	7.29	0.93	0.51	0.20	496.20	228.52	1.05	1.48	0.05	0.02
Irrigation regime	I1	167.39±1.33	217.01±2.94	28.07±0.33	18.94±0.27	4.63±0.11	5626.88±85.03	2519.76±40.26	89.28±0.23	11.80±0.24	1.34±0.02	0.41±0.01
	I2	138.72±1.33	152.11±2.33	23.31±0.32	16.17±0.21	3.73±0.08	4367.00±87.14	1897.41±39.84	85.95±0.24	16.18±0.39	0.99±0.01	0.38±0.00
	I3	113.64±1.19	98.95±1.83	18.85±0.24	12.98±0.23	2.80±0.06	3033.42±76.19	1277.02±33.54	83.65±0.24	21.02±0.38	0.66±0.01	0.34±0.01
LSD (P<0.05)		1.88	14.29	2.81	0.81	1.63	285.61	153.73	2.00	3.96	0.18	0.07
Selenium spray	S1	136.10±2.29	148.27±4.89	22.85±0.43	15.65±0.30	3.60±0.10	4178.72±121.39	1818.78±57.00	85.92±0.28	15.77±0.45	0.95±0.03	0.37±0.01
	S2	143.73±2.38	163.79±5.12	23.97±0.44	16.41±0.31	3.84±0.10	4506.15±121.85	1977.35±57.98	86.67±0.30	16.9±0.46	1.04±0.03	0.38±0.01
LSD (P<0.05)		6.34	3.51	0.60	0.34	0.80	52.82	57.18	0.89	0.88	0.07	0.06
Genotype	Ahmadi	142.52±3.21	160.86±6.18	24.02±0.62	16.41±0.41	3.83±0.15	4505.89±159.07	1970.13±74.83	86.38±0.34	15.62±0.66	1.04±0.04	0.39±0.01
	SW102	146.56±4.32	171.32±9.52	24.57±0.80	16.89±0.56	3.96±0.18	4674.54±217.77	2060.68±104.85	87.08±0.56	15.22±0.78	1.08±0.05	0.39±0.01
	Okapi	135.24±3.83	145.40±8.02	22.57±0.70	15.44±0.5	3.56±0.17	4113.03±201.94	1786.41±94.66	85.79±0.46	17.16±0.81	0.93±0.05	0.37±0.01
	GKH2624	135.66±4.28	146.65±9.22	22.59±0.78	15.47±0.55	3.57±0.18	4114.41±227.51	1789.51±106.61	85.86±0.53	17.18±0.84	0.93±0.05	0.37±0.01
	GK-Gabriella	133.6±4.19	141.75±8.94	22.25±0.78	15.23±0.55	3.49±0.18	4009.55±224.85	1740.42±105.34	85.65±0.53	17.51±0.85	0.91±0.05	0.36±0.01
	Elvis	145.93±4.28	170.17±9.35	24.47±0.79	16.73±0.54	3.91±0.18	4637.19±218.03	2041.23±103.92	86.99±0.58	15.31±0.78	1.07±0.05	0.39±0.01
LSD (P<0.05)		2.43	3.01	0.35	0.12	0.14	18.44	14.83	0.21	0.37	0.01	0.02

PH, plant height; *NPP,* number of pods plant^{-1}; *NSPMS*, number of seeds pod^{-1} of main stem; *NSPLB*, number of seeds pod^{-1} of lateral branches; *TSW*, thousand-seed weight; *SY*, seed yield; *SOY,* seed oil yield; *RWC,* relative water content; *LPC,* leaf proline content; *Chla*, chlorophyll a content; *Chlb,* chlorophyll b content

preventing chlorophyll depletion under stress conditions. Therefore, proline accumulation is directly related to the degree of resistance to drought stress (Mwenye et al 2016).

3.2. Effects due to foliar selenium application

Physiological and antioxidant properties of *Se* drew the attention of scientists and led to various researches being conducted in this area. In this study, most of the studied traits were affected by foliar selenium. Selenium spray remarkably increased *NPP*, *SOY*, *Chla*, and *SY* by up to 10.47, 8.72, 8.70, and 7.84%, respectively. Various literatures have noted the increase of leaf chlorophyll content under the influence of selenium. In accordance with the results of this study, Mozafariyan et al (2017) reported an increase in chlorophyll content of tomato leaves when the plants were fed with 7 and 10 µM of selenium. Feng et al (2013) believed that the addition of *Se* to the growth substrates can reduce the excess ROS generation, especially of O_2- and/or H_2O_2, in plants subjected to stress. In chloroplasts, Fe-S clusters have a vital role for the operation of cytochrome B/F complex (Raven et al 1999). On the other hand, formation of Fe-Se clusters may occur under *Se* supplementation which plays an important role in the electron transfer chain, the emergence and quenching of *ROS* and the responses of antioxidants in stressed plants (Feng et al 2013). Although, the addition of appropriate levels of *Se* can increase the chlorophyll content, however, more selenium levels have had stressful effects on *Chlorella vulgaris* leading to loss of chlorophyll content (Chen et al 2005).

Also, *LPC* significantly increased up to 7.21% (Table 2). Increase in the proline content of leaves under the influence of foliar selenium has been earlier reported (Djanaguiraman et al 2005). According to El-Ramady et al (2015), *Se* acts as an antioxidant and as a result inhibits lipid peroxidation via increased levels of thiols and GSH. They also suggested that, *Se* activates plant protective mechanisms, thereby alleviating oxidative stress, and improving heavy metals or trace elements uptake in higher plants. Nevertheless, despite the numerical increase, no significant improvement was observed for *TSW*, *RWC*, and *Chlb* (Table 2).

As shown in Figure 1, under drought stress conditions, foliar selenium alleviated the adverse effects of drought stress on some important traits. At the second level of the irrigation regime (i.e. when the irrigation was cut off from flowering stage), the *SY* measured for selenium-sprayed plants showed a significant increase of up to 12.48%. Likewise, at the third level of the irrigation regime (i.e. irrigation

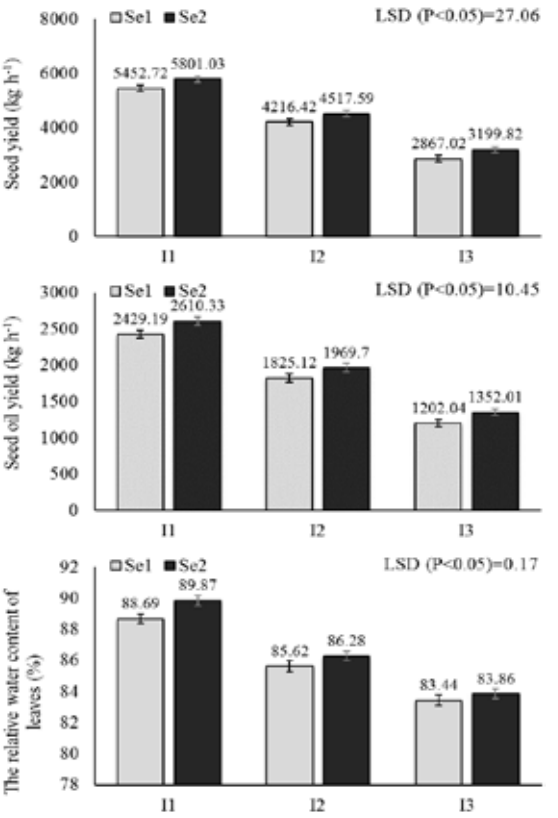

Figure 1- Mean comparison of seed yield (A), oil yield (B), and the relative water content of leaves (C) of six winter canola as influenced by different irrigation regimes and foliar sodium selenate (Se1, no selenium (control); Se2, selenum solution (30 g L^{-1}) spryaed on leaves; I1, normal irrigation; I2, irrigation cut off from pod development stage; I3, irrigation cut off from flowering stage)

cut off from pod development stage), foliar selenium led to an increase of up to 7.92% for *SY*. A similar trend was observed for *SOY* and *RWC* (Figure 1). Increase in *RWC* for selenium-fed plants could have resulted from the improvement of plant water management or a significant interaction between the effects of water deficit and *Se* on respiratory potential (Hasanuzzaman & Fujita 2011). Similar findings have been reported in wheat (Nawaz et al 2015) and rice (Xu & Hu 2004).

3.3. Partial regression coefficients

Partial regression coefficients were estimated to determine the relative importance of traits affecting *SY* (Table 3). Considering that the data were standardized before the regression analysis, therefore, the regression coefficients were comparable with each other and hence, the higher coefficient represents the greater weight of the corresponding traits.

I1, normal irrigation; I2, irrigation cut off from pod development stage; I3, irrigation cut off from flowering stage; Se1, no selenium (control); Se2, exposed to foliar sodium selenate solution (30 g L^{-1}); *PH*, plant height; *NPP*, number of pods $plant^{-1}$; *NSPMS*, number of seeds pod^{-1} of main stem; *NSPLB,* number of seeds pod^{-1} of lateral branches; *TSW*, thousand-seed weight; *RWC*, relative water content; *LPC,* leaf proline content; *Chla,* chlorophyll a content; *Chlb*, chlorophyll b content

According to the results, the direct effects of traits on *SY* varied when the plants were exposed to foliar selenium. At I2-Se1 level, the highest positive direct effects on *SY* belonged to *Chla* and *NSPMS* while, at I2-Se2 level, *PH*, *Chla*, *RWC* and *NSPLB* had the highest positive direct effects (Table 3). Although *PH* is a vegetative trait, it should be noted that increased *PH* causes an increase in *NPP* which leads to increased *SY*. According to these results, foliar selenium seems to increase the *RWC* which leads to decrease in the intensity of drought stress. Moreover, at I3-Se1 level, *Chla*, together with *TSW* had the highest positive direct effects on *SY* while, at I3-Se2 level, *Chla*, *RWC*, *PH* and *NSPLB* had the highest positive direct effects on *SY* (Table 3). As reported by Sabaghnia et al (2010), *TSW* was one of the most important traits related to *SY* under both normal and water-stressed conditions.

According to the results, under the influence of drought stress, selenium spray application caused *RWC*, *Chla*, *NSPLB*, and *PH* to have a direct and important effect on *SY*. Consequently, the selection of these traits can be useful in plant breeding programs.

These results imply that under drought stress, selenium spray varied the nature of the causal relationship between *SY* and other studied traits.

Table 3- Partial regression coefficients of seed yield over some agro-physiological traits in six winter canola as influenced by different irrigation regimes and foliar sodium selenate

Model	*I1*		*I2*		*I3*	
	Se1	*Se2*	*Se1*	*Se2*	*Se1*	*Se2*
Intercept	0.000	0.000	0.000	0.000	0.000	0.000
PH	0.285	0.381	0.057	0.305	-0.278	0.218
NPP	-0.268	0.406	0.083	-0.551	-0.903	-0.050
NSPMS	0.014	1.014	0.441	0.164	0.013	-0.442
NSPLB	-0.182	-0.306	-0.749	0.279	0.133	0.210
TSW	0.250	-0.025	0.198	-0.226	0.956	0.133
RWC	0.095	-0.127	-0.198	0.277	0.254	0.190
LPC	-0.388	0.001	-0.294	-0.427	0.041	-0.374
Chla	0.474	-0.545	0.464	0.323	0.937	0.448
Chlb	-0.084	0.150	0.168	-0.113	0.138	-0.092

It seems that the relationships between traits were affected by the strategies and mechanisms adopted by the plant to deal with drought. Therefore, regression coefficients were variable among different levels of the drought stress.

3.4. Cluster analysis of genotypes

Cluster analysis categorized the genotypes into two groups each with three members (Figure 2). Cluster I consisted of 3 genotypes including Ahmadi, Elvis, SW102, and cluster II consisted of genotypes GK-Gabriella, GKH2624, and Okapi. Except for *LPC*, trait mean values for cluster I were found to be higher compared to cluster II (Table 4). Based on this result, cluster I genotypes are recommended for cultivation compared to genotypes clustered in group II.

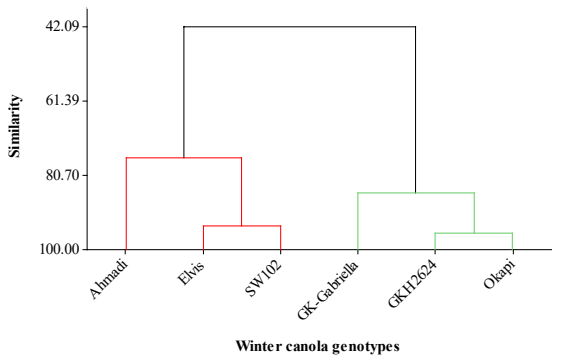

Figure 2- Dendrogram obtained from cluster analysis of six winter canola subjected to different irrigation regimes and foliar sodium selenate during 2015 and 2016

4. Conclusions

The results of this study showed that the use of foliar selenium can be considered as a useful strategy to cope with the adverse effects of drought stress in order to achieve sustainable agriculture. Also, genotypes Ahmadi, Elvis, SW102, are recommended for cultivation in semi-arid regions.

Abbreviations and Symbols	
ANOVA	Analysis of variance
Chla	Leaf Chlorophyll a Content mg g^{-1} of leaf fresh weight
Chlb.	And Leaf Chlorophyll b Content mg g^{-1} of leaf fresh weight
LPC	Leaf Proline Content µM g^{-1} of leaf fresh weight
NPP	Number of Pods $Plant^{-1}$
NSPLB	Number of Seeds Pod^{-1} Lateral Branches
NSPMS	Number of Seeds Pod^{-1} of Main Stem
PH	Plant Height (cm)
ROS	Reactive Oxygen Species
RWC	The Relative Water Content of Leaves (%)
Se	Selenium
SI	Stress Intensity
SOY	See Oil Yield (kg h^{-1})
SY	Seed Yield (kg h^{-1})
TSW	Thousand-Seed Weight (g)

Table 4- Mean ± standard error of mean for groups derived from cluster analysis of six winter canola as influenced by different irrigation regimes and foliar sodium selenate during 2015 and 2016

Cluster membership	*PH*	*NPP*	*NSPMS*	*NSPLB*	*TSW*	*SY*	*SOY*	*RWC*	*LPC*	*Chla*	*Chlb*
Ahmadi, Elvis, SW102	139.41±1.80	160.96±2.66	22.75±0.21	15.34±0.14	3.31±0.04	4224.42±48.54	1848.40±25.10	87.04±0.21	17.73±0.19	1.02±0.01	0.36±0.001
GK-Gabriella, GKH2624, Okapi	128.68±0.60	138.79±1.49	21.03±0.10	14.10±0.07	3.01±0.02	3703.79±34.67	1601.78±15.50	85.88±0.06	19.64±0.07	0.88±0.01	0.34±0.003
LSD (P<0.05)	5.26	8.47	0.64	0.43	0.12	165.62	81.91	0.62	0.56	0.04	0.01

PH, plant height; *NPP*, number of pods $plant^{-1}$; *NSPMS,* number of seeds pod^{-1} of main stem; *NSPLB*, number of seeds pod^{-1} of lateral branch; *TSW*, thousand-seed weight; *SY,* seed yield; *SOY*, oil yield; *RWC,* relative water content; *LPC,* leaf proline content; *Chla,* chlorophyll a; *Chlb,* chlorophyll b

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Genomic Variability and Recombination Analysis of Grapevine *leafroll-associated virus-1* Isolates from Turkey

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ABSTRACT

Grapevine leafroll-associated virus-1 (GLRaV-1), one of the causal agents of Grapevine leafroll disease (GLRD), is one of the most important viral diseases of grapevine worldwide. In this study, the prevalence of GLRaV-1, genetic variation and recombination events among GLRaV-1 isolates in Turkey were investigated. Initially, 197 grapevine samples from different provinces of the country were serologically tested. Of the total samples, 109 (55.32%) were identified as GLRaV-1 infected. Subsequently, 9 samples representing different geographic distribution were selected for further sequence analysis of the heat-shock protein 70 homolog (HSP70h), open reading frame 9 (p24), coat protein (CP) and coat protein duplicate 2 (CPd2). Among the four gene regions, CPd2 was found the most divergent region while HSP70h gene exhibited the lowest genetic diversity. The phylogenetic analysis of four genomic regions including GenBank records clustered all variants in two major groups and grouped Turkish isolates mostly together. However, the isolate clusters were not correlated to their geographic origin. Furthermore, several putative recombination events were detected with trace to moderate evidence support of algorithms implemented in Recombination Detection Program (RDP). Taken together, the results provide a better understanding on genetic variation of Turkish GLRaV-1 isolates in the country and worldwide and can help to improve sanitation of propagated material programs for the grape growers.

Keywords: *Vitis vinifera* L.; CP; CPd2; p24; HSP70h; Genetic variation

1. Introduction

Grapevine (*Vitis* sp.) is one of the most widely grown woody crops worldwide. Turkey produces 4.175.356 tons of grapes and ranks the 6th in production after China, USA, Italy, Spain and France (FAOSTAT 2014). Many viruses can cause damages to grapevine in the form of yield losses, short survival life and quality decreases. Among the grapevine virus diseases, the most widespread and economically destructive one is grapevine leafroll disease (GLRD), which was first described in the mid-19th century. The main symptoms of GLRD include, leaf rolling, interveinal reddening of the leaves and reduced pigmentation in red-fruited cultivars, leaf chlorosis in white-fruited cultivars and reduced yields, lower Brix and uneven ripening. GLRD effects on fruits are poor maturation of berries, lower Brix content and yield, and reduced wine pigmentation. The phloem limited filamentous-

shape viruses associated with that disease are named as Grapevine leafroll associated viruses (GLRaVs). They are represented by the species GLRaV-1, -2, -3 and -4 and recently, GLRaV-5, -6, -9, GLRaV-Pr, GLRaV-De, and GLRaV-Car were recognized as strains of GLRaV-4 (Martelli et al 2012). All these viruses belong to the family *Closteroviridae*, with GLRaV-2 belonging to the genus *Closterovirus*, GLRaV-7 belongs to a newly proposed genus *Velarivirus* and the other GLRaVs to the genus *Ampelovirus* (Martelli 2014).

The GLRaV-1 genome has a positive (+) sense single-stranded (ss) RNA genome 19.5 kb in size organized into ten major open reading frames (ORFs) and those frames encoding proteins have different functions. A putative RNA helicase is encoded by ORF-1a and an RNA-dependent RNA polymerase is encoded by ORF-1b. Other ORFs encodes one hydrophobic protein, capsid protein (CP), a HSP70 family of heat shock proteins-homologue (HSP70h), a HSP90-like protein, two diverged CP gene copies (CPd1 and CPd2) and two more proteins. Moreover, GLRaV-1 is the only one among the GLRaVs which includes two diverged CP gene copies (Fazeli & Rezaian 2000).

Understanding the genome of the species, variants of the virus and genetic diversity of viral populations are of significant importance for epidemiological studies and certification testing to prevent its spread and improve efficient control strategies. RNA viruses, such as GLRaVs, have high ratio of genetic diversity due to an error-prone replication with high mutation rates; therefore, the evolutionary processes which allow them to spread throughout the species need to be clarified (Fazeli & Rezaian 2000). Although GLRaV-1 is a common virus infecting grapevine plants worldwide, limited information is available in literature about the genomic variability and molecular evolution of GLRaV-1 (Little et al 2001; Kominek et al 2005; Alabi et al 2011; Predajna et al 2013; Cseh et al 2013; Fan et al 2015).

GLRaV-1 has been detected in Turkey since 1997 (Çağlayan 1997; Köklü et al 1998; Çığsar et al 2002; Akbaş et al 2007; 2009; Değer et al 2015; Önder 2016) however the nucleotide sequence variation between isolates was unknown. Therefore, genomic variability and recombination events of *Grapevine leafroll-associated virus-1* isolates in Turkey were evaluated through the sequence analysis of four different genomic regions of the virus. For this purpose, the HSP70h, CP, ORF9 (p24) and CPd2 genes of GLRaV-1 were amplified, sequenced and possible recombination events were investigated.

2. Materials and Methods

Grapevine leaves showing suspicious GLRD symptoms were collected from Hatay, Gaziantep and Tekirdağ provinces of Turkey during autumn 2015. In total, 197 grapevine samples, cvs. Antep karası, Pafu, Kalecik which are the local varieties and cv. Syrah, were collected and the leaves were stored at 20 ºC until use.

The collected samples were analyzed by a double antibody sandwich, enzyme-linked immune sorbent assay (DAS-ELISA) using the Bioreba's commercial kit (Bioreba, Switzerland) based on Clark & Adams (1977) method. BIOTEK-EL800 spectrophotometer (BioTek, USA) was used for measurement of absorbance values at 405 nm. Assays were done following the manufacturers' instructions.

The RNAs were isolated and purified from leaf tissues by a commercial RNA isolation kit following the manufacturer's instructions (RNeasy Plant Mini Kit, Qiagen Sci., Germany) and their yield and quality were estimated by a NanoDrop spectrophotometer (NanoDrop2000c, Thermo Sci., USA).

The cDNA was synthesized based on the two-step protocol and used as a template for PCR analysis. For reverse transcription of total RNAs, random hexamer primers and the cDNA synthesis kit were used (SuperScript™ Choice System for cDNA Synthesis, Thermo Sci., USA). The PCR was conducted with 2 µL of template, 0.5 µL of 10 mM dNTPs, 1.5 µL of 25 mM $MgCl_2$, 2.5 µL of 5x

green reaction buffer and 0.5 µL of 10 µM of each specific primers for HSP70h, p24, CP, CPd2 genes of GLRaV-1 (Alabi et al 2011) adding with 0.2 µL of 5 units μL^{-1} Taq DNA polymerase (GoTaq® DNA Polymerase, Promega Corp., USA). PCR amplifications were performed as: denaturation at 94 °C for 5 min; 40 cycles of annealing at 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 1 min; extension at 72 °C for 10 min.

The PCR amplicons were sequenced in both directions (forward and reverse) by an Automated Genetic Analyzer (ABI3730, MedSanTek Company, Turkey). The obtained nucleotide sequences were analyzed by the program Molecular Evolutionary Genetics Analysis (software MEGA 6.06, Tamura et al 2013). BLASTn and BLASTx modules were used to determine nucleotide and amino acid identities of GLRaV-1 Turkish isolates to reference isolate (Nucleotide ID: Acc. No. NC016509, Protein ID: Acc. No. YP004940644 (Hsp70), YP004940646 (CP), YP004940648 (CPd2), YP004940650 (p24)) at BLAST on NCBI.

Phylogenetic analysis was conducted on the 4 different partial gene regions of GLRaV-1. The GenBank (NCBI) nucleotides of different GLRaV-1 isolates were included in the analysis. Multiple nucleotide and amino acid alignments were performed with CLUSTAL W (Larkin et al 2007) and the phylogenetic tree was drawn based on the neighbor-joining method implemented in the program MEGA 6.06 (Tamura et al 2013). The Bootstrap analysis was performed with 1000 replications. *Little cherry virus-2* (LCV-2, Acc.No. NC005065) was used as out-group.

The aligned nucleotides of the isolates were analyzed for the recombination events using seven recombination detection algorithms in Recombination Detection Program (RDP) version 4.16 (Martin et al 2015). Sequences were masked to be sure for optimal recombination detection before algorithm analysis. ‘‘Sequences are linear’’ option and Bonferroni-corrected P value cut-off of 0.05 were selected.

3. Results and Discussion

According to DAS-ELISA results, 109 out of 197 collected from Hatay (61 out of 107 samples), Gaziantep (23 out of 46 samples) and Tekirdağ (25 out of 44 samples) grapevine samples were found to be infected by GLRaV-1. Hatay and Gaziantep are Southeast regions of the country and rather far from the third region, Tekirdağ. Although they distantly located, they exhibited approximately same level of the virus prevalence. To date, there are several studies on GLRaV-1 incidence in grapevine growing areas of Turkey. Özaslan & Yılmaz (1985) reported GLRaV-1 as a common virus in some provinces and Çağlayan (1997) found GLRaV-1 always as mixed infection with GVA in Hatay province of Turkey. Moreover, GLRaV-1 was found to be the most common virus in Central Anatolia with the infection rate of 8.36% by DAS-ELISA (Akbaş et al 2007). Recently another survey was conducted in Eastern Mediterranean Region of Turkey and the most common virus was found to be GLRaV-1 with the infection rate of 55.56% followed by GLRaV-4 (43.14%), GLRaV-2 (15.69%) and GLRaV-3 (12.42%) by RT-PCR analysis (Değer et al 2015). Önder (2016) studied on prevalence of GLRaVs (GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5, GLRaV-6, GLRaV-7, GLRaV-9, GLRaV-Pr and GLRaV-De) at Manisa, Denizli, İzmir, Aydın and Uşak provinces and reported that 133 out of 424 samples were infected at least by one GLRaVs and regardless the mixed infections, GLRaV-Pr was the most widespread one with 12% infection rate which followed by GLRaV-De (12%), GLRaV-3 (8.5%), GLRaV-2 (2.8%), GLRaV-4 (2.4%), GLRaV-9 (0.9%), GLRaV-1 (0.5%), GLRaV-5 (0.2%) and GLRaV-7 (0.2%), respectively. Our results are in accordance with previously reported RT-PCR analysis results on grapevine plants infected with GLRD. The high incidence of this virus and poor sanitation conditions for grapevines were indicated in Turkey. The reason of such a high incidence of this virus can be due to vector transmissions, since most samples were infested by mealybugs, a vector responsible for transmission of this virus (Sforza et

al 2003). Another reason could be the usage of uncertified materials (scions, rootstocks etc.).

Among DAS-ELISA positive samples for GLRaV-1, nine representative isolates were chosen according to their geographical region and cultivar for genetic analysis. All these isolates were successfully amplified for four genomic regions of GLRaV-1 based on RT-PCR assays using with specific primers for CP, CPd2 and p24, HSP70h genes with amplification product sizes 734 bp, 398 bp, 634 bp, of 540 bp, respectively.

All obtained PCR amplicons were purified and sequence analysis was done. After nucleotide assembly, the sequences of each RNA segment were deposited in GenBank with accession numbers of KU362237-KU362263 and KU362270-KU362278. Based on the BLAST analysis, the nucleotide and amino acid sequence identities of the nine Turkish GLRaV-1 isolates to reference isolate ranged from 79 to 94% and from 75 to 96%, respectively. The CP sequences obtained in this study showed the nucleotide identities ranged from 85 to 87%; whereas amino acid identities ranged from 93 to 96%. For CPd2 sequences, it ranged from 78 to 80% and from 75 to 79%, respectively. The level of nucleotide and amino acid sequence identities for p24 gene ranged between 79-82% and 80-84%, respectively. Only the isolate 141 showed highest identity among them (94% nt identity and 93% a.a. identity). Based on HSP70h gene sequence analysis nucleotide identities ranged from 82 to 84% and amino acid identities ranged between 90-91% (Table 1). The overall mean distance values of CP, CPd2, p24 and Hsp70h regions' nucleotides were 0.044, 0.065, 0.110 and 0.049, respectively. Moreover, the overall mean value of nucleotide diversity for 4 gene regions were 0.067, 0.110, 0.081 and 0.142. The observed most divergent region was CPd2 while the lowest region was HSP70h. These findings confirm two previous studies reported that GLRaV-1 genes have a high genetic variation and the CPd2 gene is the most variable gene (Little et al 2001; Alabi et al 2011).

Table 1- Nucleotide and amino acid identities of four different genes of *Grapevine leafroll-associated virus*-1 Turkish isolates to reference isolate (Nucleotide ID: Acc. No. NC016509, Protein ID: Acc. No. YP004940644 (Hsp70), YP004940646 (CP), YP004940648 (CPd2), YP004940650 (p24)) based on BLAST (blastn and blastx) analyses

Isolate name	*Gene*	*Accession number*	*Nucleotide identity (%)*	*Amino acid identity (%)*
81	Hsp70h	KU362237	83	91
86	Hsp70h	KU362238	83	91
89	Hsp70h	KU362239	82	90
102	Hsp70h	KU362240	82	91
126	Hsp70h	KU362241	82	90
129	Hsp70h	KU362242	83	91
130	Hsp70h	KU362243	83	91
141	Hsp70h	KU362244	84	91
147	Hsp70h	KU362245	83	91
81	p24	KU362246	82	83
86	p24	KU362247	81	81
89	p24	KU362248	81	84
102	p24	KU362249	81	83
126	p24	KU362250	80	83
129	p24	KU362251	80	83
130	p24	KU362252	79	80
141	p24	KU362253	94	93
147	p24	KU362254	80	80
81	CPd2	KU362255	79	75
86	CPd2	KU362256	80	77
89	CPd2	KU362257	79	75
102	CPd2	KU362258	78	75
126	CPd2	KU362259	80	79
129	CPd2	KU362260	80	78
130	CPd2	KU362261	80	78
141	CPd2	KU362262	80	77
147	CPd2	KU362263	80	79
81	CP	KU362270	87	96
86	CP	KU362271	87	93
89	CP	KU362272	86	94
102	CP	KU362273	87	96
126	CP	KU362274	88	96
129	CP	KU362275	86	96
130	CP	KU362276	85	95
141	CP	KU362277	88	95
147	CP	KU362278	85	95

81, 86, 89, cv. Antep Karası-Hatay; 102, 126, cv. Pafu-Hatay; 129, 130, cv. Antep Karası-Gaziantep, 141, cv. Kalecik-Tekirdağ; 147, cv. Syrah-Tekirdağ

Phylogenetic relationships among Turkish GLRaV-1 isolates were determined for four gene regions and compared with the other isolates deposited in GenBank from different countries such as California, Washington and New York-USA, Portugal, Slovenia, Czech Republic, Iran, Hungary, China, Poland, India, Poland, Chile, Canada, Italy. There are a few studies on genetic diversity of GLRaV-1 from Europe. Kominek et al (2005) reported that GLRaV-1 can be grouped into two sequence variants based on sequences derived from the HSP70h gene of eight isolates from Slovakia and the Czech Republic. Also, from partial nucleotide sequences of this gene, the authors reported that GLRaV-1 isolates consisted of two variant groups, tentatively designated as groups A (North America and Australia) and E (Europe). However, more recent data from the USA supported the grouping of a wider range of GLRaV-1 isolates into three main variant groups based on the p24 and HSP70h gene sequences (Alabi et al 2011).

In this study, the 54 partial CP gene sequences (15 from China, 13 from USA, 13 from Portugal, 9 from Turkey, 1 from Iran, 1 from Poland, 1 from Canada and 1 as reference isolate (RefSeq) were analyzed and segregated into two major groups. Group 1 includes most of the isolates from Portugal, China, USA, Canada, Poland, and Iran additional to Turkish isolates. Turkish GLRaV1 CP isolates were closely clustered in the same subgroup of Group 1. Group 2 includes only some of the isolates from China and USA (Figure 1a). Based on the dendrogram, there is no distinct separation within and between the isolates collected from the same geographical conditions. Here, it can be concluded that there is no high degree of variation at the CP gene of analyzed Turkish GLRaV1 isolates. In accordance with other studies (Alabi et al 2011; Esteves et al 2013) the results of phylogenetic analysis did not show a clear correlation between phylogeny and geographical origin. The most common GLRaV-1 variants obtained from CP gene sequences belonged to Group I (Esteves et al 2013) whereas the majority of Chinese GLRaV-1 variants belong to Group II (Fan et al 2015). They reported that natural selection rather than a random process has led to the evolution of CP gene sequence variants in Group II. For the CPd2 analysis; 23 isolates from USA, 9 from Turkey, 1 from Poland, 1 from Iran, 1 from Chile, and 1 RefSeq were used. Two main groups were obtained and the Turkish isolates were clustered into Group 2. Some of the Californian isolates (CA3, CA6, CA10, CA11, CA16, CA18, CA20) were found highly similar to Turkish isolates with high bootstrap values. The GenBank sequences obtained from Poland, Iran and most of the American isolates were clustered into Group 1 with reference sequence (NC016509) while none of the Turkish isolates were clustered there (Figure 1b).

Phylogenetic analyses of 45 sequences of p24 (USA: 32, India: 2, Iran: 1, Turkey: 9, RefSeq: 1) resulted into two main groups with three subgroups. The Turkish isolates were mostly clustered together into group 1 and showed highest similarity with three Californian (CA21, CA2, CA6) and one Iranian (IR-S7) isolates. The isolate 141p24 which was extracted from a local cultivar, Kalecik, was found highly similar to RefSeq (NC016509) isolate. The out-group control LCV-2 was distinctly separated from all the isolates as expected (Figure 1c). Global HSP70h-specific sequences of GLRaV-1 (Total 51; USA: 19, Slovenia: 11, Portugal: 6, Czech Republic: 2, Hungary: 1, Iran: 1, Italy: 1, Turkey: 9, reference isolate (RefSeq):1) segregated into two major phylogroups with two subgroups. Group 1 consists of American, Portugal, Slovenian, Czech, Iranian and Turkish isolates while Group 2 consists of Slovenian, American and Hungarian isolates. Turkish isolates were clearly separated and clustered together in the same group (Figure 1d). According to Kominek et al (2005) this cluster can be separated into two groups that were designated as groups A and E however Alabi et al (2011) reported three distinct variant groups and they could not found any evidence for precisely defined geographical structuring of GLRaV-1 isolates among the three groups. Our phylogenetic analysis of GLRaV-1 HSP70h gene is corresponded to the results of Kominek et al (2005) and Cseh et al (2013) regarding the number of the phylogroups however no correlation was found for

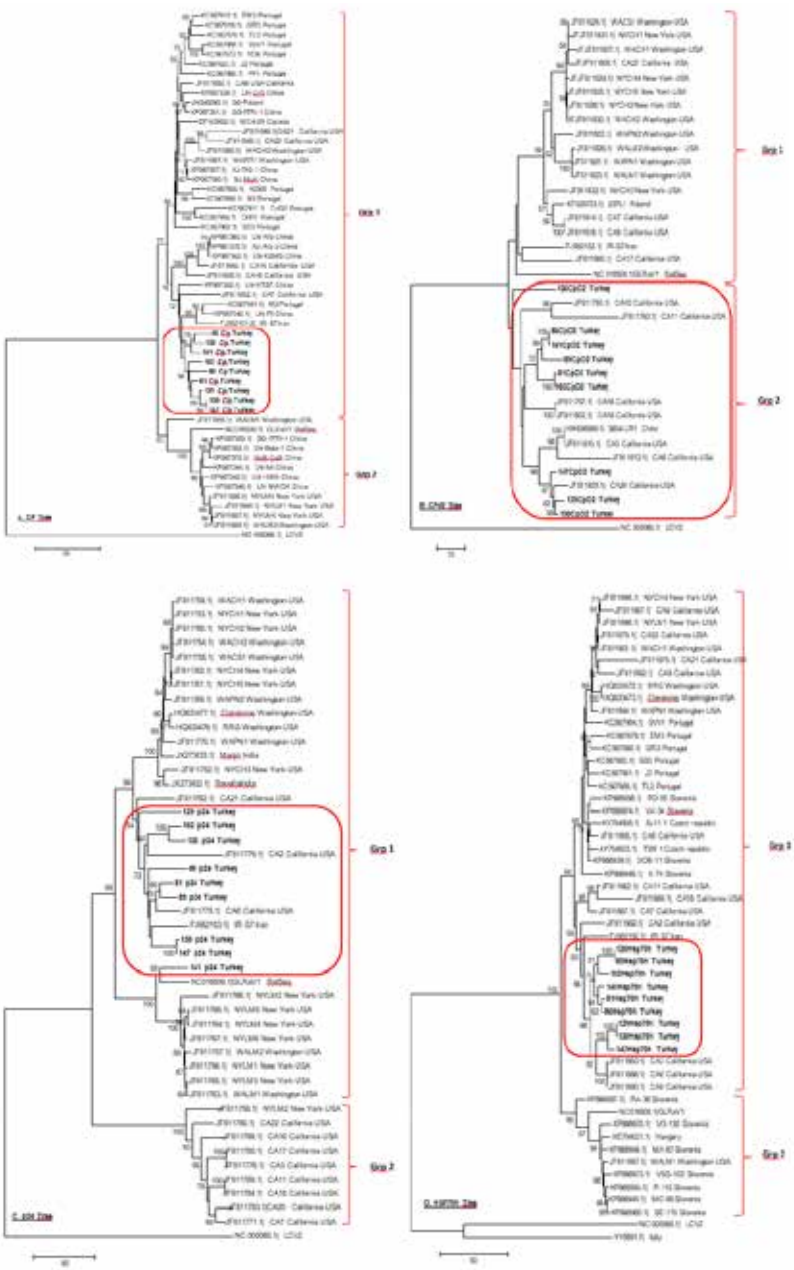

Figure 1- Phylogenetic analysis of *Grapevine leafroll-associated virus* 1 isolates by neighbor-joining method based on nucleotide diversity of the a, coat protein (CP); b, coat protein duplicate 2 (CPd2); c, movement protein (p24); d, heat shock protein 70 homologue (HSP70h). The sequences obtained in this study are marked in bold and their clusters are shown in red circle. *Little cherry virus-2* (LCV-2) was used as out-group control. Bootstrap analysis was done with 1000 replications and the values more than 50 are given at the branch nodes

geographical structure same as Alabi et al (2011). The cell to cell movement protein, which is HSP70 gene product, has highly conserved sequence among *Closteroviridae* family members (Dolja et al 1994). Based on the phylogenetic analysis in this study, the lowest divergent genomic region was also detected as HSP70h region of GLRaV1 among the analyzed gene sequences. GLRaV-1 sequences of CPd2-derived phylogenetic trees constructed with American isolates confirmed recombination event possibilities in this gene. Therefore, it is suggested to use both p24 and HSP70h genes for the significant analysis of the phylogeny of GLRaV-1 variants (Alabi et al 2011).

Recombination analysis of the four gene sequences (the HSP70h, CP, CPd2, and p24) has been performed and several putative recombination events with moderate support were detected in the CP, CPd2, and p24 regions (Table 2). Recombination events in RNA viruses are reported as a powerful inducement for generating new variants (Simon-Loriere & Holmes 2011). No significant recombination events in the HSP70 gene (9 from this study and 22 from GenBank) were detected by any of software implemented in RDP program. Recombination analysis in the p24 region (9 from this study and 22 from GenBank) have resulted in detection of 3 putative recombination events and the event 2 and 3 were included also in Turkish isolates, 141 (GenBank accession no: KU362253), 102 (KU362249) and 129 (KU362251). The putative recombination event 2 involved 102 (KU362249) as potential parent and the GenBank isolate CA3 (JF811776) was used to infer the unknown parent. This recombination event was detected in 8 isolates 141, NJ016509, JF811768, JF811767, JF811766, JF811765, and JF811764. Trace evidence of the same recombination event was also detected in the GenBank records, NJ016509, JF811763, and JF811756. Recombination position of the event 2 was depicted at Figure 2. It is noticeable that based on recombinant sequence of p24, the Turkish isolate 141 placed close to sequence of NYR isolates rather than grouping with the remaining Turkish isolates. Recombination event 3 were found in 8 isolates including a Turkish isolate 129 as major parent but this recombination event was supported with only one program (SiSican). One putative recombination event was detected in CP sequences generating three putative recombinant sequences (JF811846-CA2, JF811848-CA2, and JF811860-WAC). The event involved KC567911-CdG as minor parental sequences and no major parents were identified among the sequences examined. With respect to CPd2 gene, 3 recombination events detected and none of them involved Turkish isolates. The recombination is an important evolutionary trait of *Closteroviridae* family members (Karasev 2000). Thereby, RNA recombinations play a significant role in this virus evolution and variation. Putative recombination events were also previously found in

Table 2- Recombination events detected in p24 gene, CP gene and CPd2 gene of Grapevine *leafroll-associated virus-1* by RDP program

Gene name	*Event number*	*Found in*	*Recombination*	*Major parent*	*Minor parent*	*Detection methods*						
						R	*G*	*B*	*M*	*C*	*S*	*T*
p24	1	1	JF811766.1|NYL	JF8117	Unknown	+	+	-	+	+	-	+
	2	10	JF811757.1|WAL	102_p2	Unknown	+	-	-	-	-	+	-
	3	8	FJ952153.1|IR-	129_p2	Unknown	-	-	-	+	-	-	-
CP	1	3	JF811846.1|CA2	Unknown	KC567911.1|CdG	-	-	-	+	+	+	+
CPd2	1	2	JF811802.1|CA1	JF8118	JF811812.1|CA6	-	-	-	+	-	+	-
	2	1	JF811800.1|CA1	JF811832.1|NYC	89CpD2	-	-	-	+	-	-	-
	3	1	JF811812.1|CA6	86CpD2	JF811832.1|NYC	-	-	-	+	+	+	-

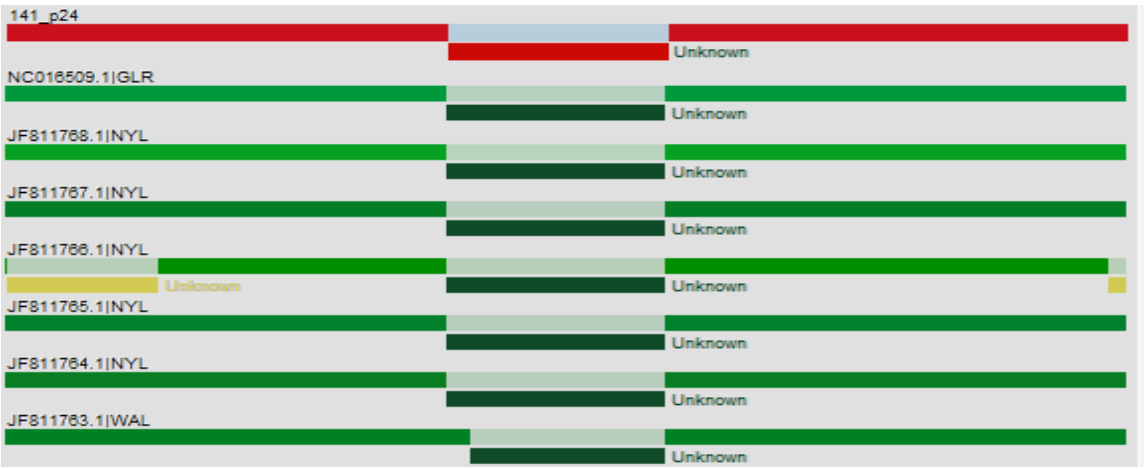

Figure 2- Recombination events of p24 gene of *Grapevine leafroll-associated virus*-1 detected by RDP program. Two events were displayed by RDP program. Event 1 is shown in yellow. Event 2 displayed with dark green and red for Turkish isolate 141

HSP70h gene, CP gene and p24 sequences but not in the CPd2 gene (Alabi et al 2011). More recently 3 new putative recombination events were detected in CP gene of Chinese GLRaV-1 isolates (Fan et al 2015).

4. Conclusions

In conclusion, a high frequency of GLRaV-1 in grapevines was detected. Taken together the nucleotide comparisons, phylogenetic and recombination analysis, the results indicate there is no distinct grouping according to geographic source although samples were taken from distantly located regions. This finding indicates dissemination of the virus occurs via propagation materials transfer and emphasizes use of virus-free plant material in preventing the dissemination of this virus.

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Estimates of Genetic Parameters for Body Weight in Turkish Holstein Bulls using Random Regression Model

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ABSTRACT

The objective of this study was to estimate genetic parameters for the body weights of Turkish Holstein bulls using the random regression model. The data set consists of 1475 body weight records from 395 Holstein bulls raised in the same herd. Body weight records of bulls aged between 32 and 725 days old were collected at approximately two-month intervals from December 2013 to October 2014. In the study body weight measurements made on the same day were accepted as a group and the bulls were grouped into 10 different groups according to their age. The additive genetic and permanent environmental effects were estimated using DFREML algorithm by third order Legendre polynomials. The additive genetic variance estimates ranged from 10.73 to 4867.07, the phenotypic variance estimates ranged from 382.84 to 5514.86 and permanent environmental variance estimates ranged from 0.33 to 63.27. The heritability values were estimated between 0.03 to 0.90. The phenotypic and additive genetic correlations between body weights were positively estimated between 0.085 to 0.89 and 0.53 to 0.94, respectively. It was concluded that use of body weight at an earlier age will give advantage in breeding studies for body weight at slaughter.

Keywords: Holstein; Heritability; Meat; Random regression model

1. Introduction

Approximately 21% of world meat production is obtained from cattle while this rate exceeds 32% for red meat (Anonymous 2016). Even though the breeds and systems used for cattle meat production differs from each other, the basic logic is to use breeds with high growth capacity and/or rate and to grow animals at as low cost as possible. Even if the animals are from the same breed, due to diversity in climate and herd management, growth and efficiency may differ according to the regions (Ferraz & Eler 2010).

There are various researches on the weights and growth rates of cattlefrom different ages and regions of the world. In these researches, the data from the meat-oriented breeds, like Nellore, Angus or Hereford, are used as material (Albuquerque & Meyer 2001; Arango et al 2004; Bohmanova et al 2005; Menéndez-Buxadera et al 2008; Baldi et al 2012; Martinez et al 2012). In Turkey, there are not many animals from breeds listed above. Almost half

of the cattle presence of Turkey consists of Holstein or its crosses. Cattle meat is generally obtained through fattening of male animals at dairy farms that have breeds like Holstein, Simmental and Brown Swiss that are considered to be efficient in both milk and meat purposes. Additionally, in terms of average carcass weight, Turkey is far behind the EU and USA. Recently, the average carcass weight has increased to 250 kg depending on imports. In some countries such as USA, UK, Germany, France, this amount reaches 300-350 kg (Anonymous 2016). In addition, cattle fattening in Turkey is mostly done in a closed farming style and the range lands-based farming is limited and therefore the costs are high. Furthermore, imports are frequently discussed to both meet the domestic market demand and reduce the meat prices. However, the projections of the Ministry of Food, Agriculture and Livestock have shown that in order to meet the increasing demand, Turkey's cattle meat production, which was 882 thousand tonnes in 2014, should be increased by almost 50% in 10 years and one of the solution ideas was the development of policies towards the breeding of multi-purpose breeds (Anonymous 2015).

On the other hand, genetic breeding studies for the improvement of efficiency of the animals used in Turkey for meat production are highly inadequate. Whereas around the world; weights and weight gains at specific ages or during specific periods are commonly applied as selection criteria in most beef cattle breeding programs, since these traits show moderate to high genetic correlations with carcass weight, are easy to measure, and respond to selection (Razook et al 2001; Baldi et al 2012). Parkkonen et al (2000) reported that, the heritability estimates for slaughter weight were estimated between 0.07 and 0.10. Genetic correlations between slaughter weight and fleshiness were estimated to be 0.38 for males and 0.65 for females. In another study, heritabilities for body weights, carcass weights and carcass fleshiness were estimated 0.17 to 0.22, while genetic correlations between them ranged from 0.54 to 0.78 (Liinamo & Van Arendonk 1999).

Growth can be defined as a measurement sequence that changes gradually until reaching a plateau. The measurements at any points on this line have a correlation with each other because of collecting them from same individual. The similarities among measurements increase as interval of time goes closer, but decrease as interval of time increases. When the relationship between the measurements during the growth period of cattle are taken into consideration, genetic parameter and breeding value estimations will become much more reliable and as a result, the precision level will increase in selection. Random regression models are the test day models that consider the change in variance components throughout the growth period (Jaffrezic et al 2002; Schenkel et al 2002; Nephawe et al 2006; Menéndez-Buxadera et al 2008; Silva et al 2013).

As mentioned above, there is a gap in the genetic breeding studies of Turkey for the utilization of the live weight of the animals used for meat production and for the increase of efficiency. This research estimates the (co) variance components, genetic parameters for the live weight of male Holstein, using a random regression model. This way, the aim is to both draw attention to the subject and provide leadership for more extensive studies that will be conducted in this field.

2. Material and Methods

A total of 1475 live weight records taken between December 2013 and October 2014 from 395 male Holstein raised at the same farm, located in the south of Turkey (latitude 37°8′ north and longitude 30°39′ east), were used for the research. This region is characterized by a warm and temperate climate, with average annual temperature and rainfall of 19 °C and 1009 mm, respectively. The numbers of animals, records and subgroups included in the analysis are given in Table 1. Live weight records were taken in approximately two-month intervals from male cattle at the age of 32 to 725 days. The records used in the research were categorized in 10 different stages according to age groups and the descriptive statistics of the terms are given in Table 2.

Table 1- Numerical information of the material

Number of bull used	395
with 2 records	74
with 3 records	105
with 4 records	95
with 5 records	94
with 6 records	27
Number of sire	61
Number of dam	375
Number of group according to the weighing date	17
Number of record	1475

Table 2- Descriptive statistics of live weight measurements taken at different stages

Stage	*Age (month)*	*N*	*Mean*	*SE*	*Min*	*Max*
1	1-2	57	72.56	0.70	58	84
2	3-4	46	150.61	3.87	100	208
3	5-6	192	194.27	2.46	109	278
4	7-8	254	254.50	2.39	137	361
5	9-10	234	312.07	3.00	173	455
6	11-12	215	375.23	3.49	210	487
7	13-14	198	436.36	4.33	288	615
8	15-16	183	475.71	4.53	331	656
9	17-18	73	519.26	7.18	353	739
10	19+	23	550.30	20.70	372	832
	Total	1475	332.69	3.33	58	832

The live weight measurements conducted on the same day at the farm were considered a group in the research while the data shown below have been analyzed through the random regression model. (Co) variances of random regression coefficients and heritability values were estimated by REML using the DXMRR subroutine of the DFREML software package, version 3.0 β (Meyer 1998). Estimates were obtained by using AI-REML algorithm, thereby avoiding problems with "derivative-free" possible local max estimates.Third order Legendre polynomials were used to define the (co)variance structure between the observations of the same individual. The general model can be presented as follows:

$$y_{ijk} = TG_i + \sum_{m=0}^{\theta_a - 1} a_{jm} z_{km} \sum_{m=0}^{\theta_p - 1} p_{jm} z_{km} + e_{ijk}$$

Where; the y_{ijk} is the body weight (kg), TG_i is the effect of i[th] weighting group, a and p is the random regression coefficients for additive genetic and permanent environmental effects, θ_a and θ_p are the orders of fit additive genetic and permanent environmental effects, z_{km} is the m[th] legendre polynomial for k[th] term and e_{ijk} is the error term (assumed to be homogeneous for 10 stages).

3. Results and Discussion

The additive genetic, permanent environmental and phenotypic variances and the heritability estimations are given in Figure 1. It has been determined that additive genetic variance increases rapidly with advanced age and even though it was 10.73 in the first stage, it has reached 4867.07 in the 10[th] stage. Phenotypic variance estimations have also shown the same tendency as the additive genetic variances; while it was 382.84 in the first stage, it was estimated to be 5514.86 in the last stage. Course of the additive genetic variances have shown similarities with the studies of Albuquerque & Meyer (2001), Nobre et al (2003), Lopes et al (2012) and Silva et al (2013) on the Nellore cattle. In Silva et al (2013), phenotypic variances have shown a faster increase than additive genetic variances. In

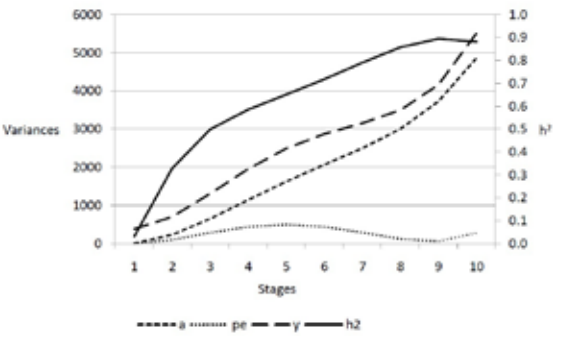

Figure 1- Estimates for additive genetic (a), permanent environmental (pe) and phenotypic (y) variance and heritability (h^2)

Arango et al (2004) research on the live weight of Angus and Hereford cattle, it has been determined that additive genetic variance slowly increases with advancing age. In the aforementioned research, there aren't any reports of an increase in phenotypic variances for the first two years.

On the other hand, in this research, the permanent environmental variances for the first stage and 9th stage live weight were estimated to be respectively 0.33 and 63.27. Permanent environmental variance for the 5th stage live weight was estimated to be the highest with 496.82. Even though permanent environmental variance estimations have a tendency to decrease with advancing age, it has been determined that permanent environmental variances have a much more constant structure than additive genetic and phenotypic variance (Figure 3). Silva et al (2013) has reported that there is some increase in permanent environmental variances especially after one year of age, but it has been identified that this increase is not as much as the increase in other variances. Valente et al (2008) and Lopes et al (2012) have reached the conclusion that the permanent environmental variance estimations increase with the age.

In our research, major differences have been identified between the heritability estimations obtained for the live weight from different age terms. Heritability was estimated to be 0.03 for the 1st stage live weight that consists of the first two months after birth, while it ranged between 0.33 (2nd stage) and 0.90 (9th stage) for other stages. These changes in heritability are thought to be the result of the increase in additive genetic and phenotypic variance estimations for live weight with advancing age, the fact that the change in permanent environmental variance is almost none existent and that the model has the assumption of the existence of a constant error variance. Similar with this research, the previous research on the topic report that the heritability estimations increase with age (Albuquerque & Meyer 2001; Nobre et al 2003; Silva et al 2013). In addition to these researches, there is also researchwhich report that heritability estimations have tendency to decrease or fluctuate with increased age (Arango et al 2004; Valente et al 2008; Baldi et al 2012; Lopes et al 2012).

The additive genetic covariance estimations between the stages have increased with increased age. Additive genetic variance-covariance matrix is presented in Figure 2 as three-dimensional graph. Estimations of additive genetic correlations can be seen in Figure 3. General status of the phenotypic variance-covariance estimations is similar with those of the additive genetics. As seen in Figure 4, there are no sharp transitions between the variances and covariance values on the diagonal. This case is the result of the fact that environmental factors from the term don't play an important role in the

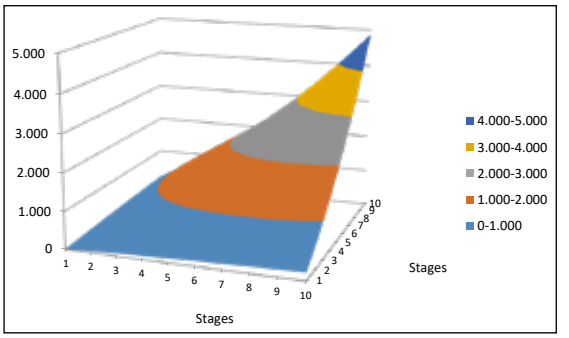

Figure 2- Additive genetic covariance estimations between stages

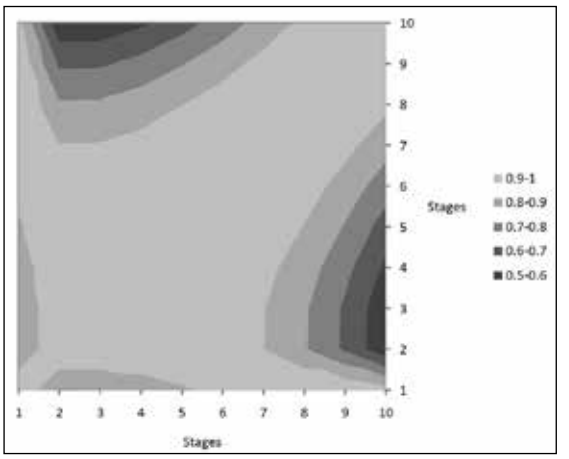

Figure 3- Additive genetic correlation estimations between stages

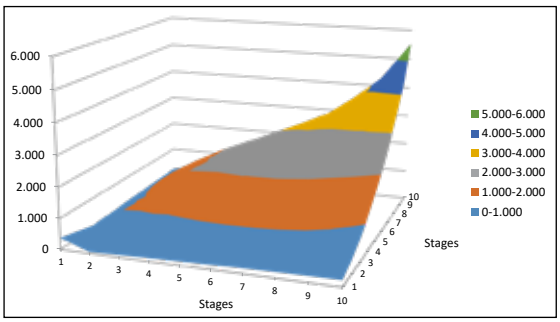

Figure 4- Phenotypic covariance estimations between stages

variation. In phenotypic correlation estimations, the lowest value has been obtained as 0.09 (between 1st and 2nd stages) while the highest value was obtained as 0.890 (between 9th and 10th stages). Compared to this research, Martinez et al (2012) has reported higher phenotypic correlation estimates for the live weight at advanced ages.

At the end of the analysis, it can be seen that the additive genetic correlations between live weights are higher than phenotypic correlations (Figure 3 and Figure 5). The additive genetic correlations have shown a tendency to be high as expected when close to each other while they decreased with the increase in the distance between the stages. The lowest estimation for additive genetic correlations has been observed as 0.53 (between 2nd and 10th stages). While the 7th stage has become the age group with the highest correlation amongst the other stages, with 0.94; the age group with the lowest has become the 10th stage with 0.75.

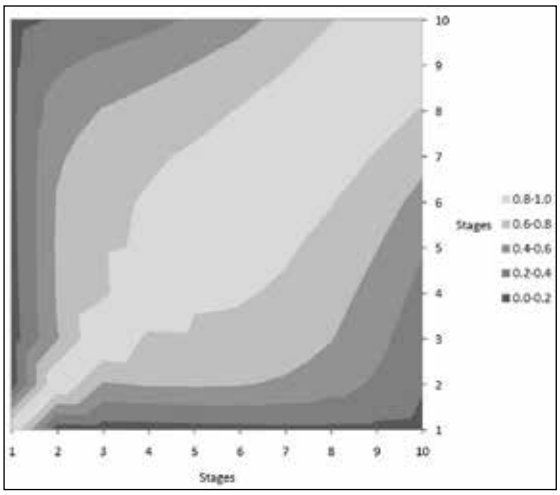

Figure 5- Phenotypic correlation estimations between stages

High and positive genetic correlation estimates indicate the fact that most of the genes that cause higher live weight in those ages are the same. High level of additive genetic correlations between different age groups can also be interpreted as the fact that early period weight can be used for studies of live weight at slaughter age (Boligon et al 2010). The values reported in the research based on the simulation of Bohmanova et al (2005) and the research conducted by Martinez et al (2012) using different breeds, have also similarly varied between 0.50-1.00. While the additive genetic correlations between the birth weight and live weight in advanced ages were low in Silva et al (2013) research, the values between other age groups have been observed to be high. Nobre et al (2003) and Valente et al (2008) have indicated that the correlations between 1 year of age and older are high.

4. Conclusions

This research has estimated parameters for live weight of stock materials raised in Turkey, using a random regression model. In conclusion, it has been observed that the change of variances of live weight at the age of 1-24 months can be adequately defined by using random regression model that contains Legendre polynomials, and as a result, it is possible to take advantages of the random regression models. However, low number of records per animal may cause low level of effectiveness for the random regression models. More research on the use of random regression models about growth is required in order to make sure that numerical problems and/or wrong parameters do not reduce the effectiveness of breeding studies. It has been concluded that younger live weight can be used for the breeding studies of live weight at slaughter age.

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Effect of Microwave, Infrared and Freeze Drying Methods on Drying Kinetics, Effective Moisture Diffusivity and Color Properties of Turmeric

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ABSTRACT

In the present research, effect of methods that use the microwave (90, 160 and 350 W), infrared (60, 70 and 80 °C), and freeze drying for turmeric samples on the drying kinetics, effective moisture diffusivity and color were analyzed. Also ten distinct thin layer models of drying were used to predict their kinetics. Depending on the evaluation of the statistical tests, models of Midilli et al and Wang & Singh models were found the optimum ones for explaining drying characteristics of turmeric. Among the used methods, the fastest and slowest drying time was 65 min with microwave drying (350 W) and 600 min with freeze drying, respectively. The calculations demonstrate that the maximum effective moisture diffusivity value is obtained in microwave drying (350 W). Our study shows that although the freeze-drying increases the drying time, it showed closest color results against to fresh samples. In conclusion, microwave, infrared and freeze drying methods applied to turmeric should improve with the combined drying applications.

Keywords: Turmeric; Drying kinetics; Effective moisture diffusivity; Color

1. Introduction

Turmeric is a member of the Zingiberaceae family and genus Curcuma (Singh et al 2010; Gupta et al 2015). It is originated into South Asia and exported to the United States of America, the United Kingdom, the Netherlands, South Africa, Singapore, Saudi Arabia, United Arab Emirates, Japan, and Iran (Mishra et al 2015).

Turmeric comprises three compounds namely bis-dimethoxy curcumin, dimethoxy curcumin, curcumin which is biologically active (Riaz et al 2015). It has various beneficial effects on cardioprotective, hypolipidemic, antibacterial, anti-HIV, anti-tumor, anti-carcinogenic and anti-arthritic activities (Prathapan et al 2009). Commercially, it is used as a spice for foodstuff with fresh or as dried. However, dried turmeric price for selling worldwide is influenced by many quality factors (moisture content, color, and phenolic contents) (Hirun et al 2014).

Turmeric rhizomes are dried to avoid deterioration after harvesting (Apintanapong & Maisuthisakul 2011). Therefore, drying is defining moisture removal process and resolves the

following problems; improves food stability, lowers shipping weights, minimizes chemical and physical changes in due course of storage, and reduces microbiological activity due to the decrease of the water activity (Laosanguanek et al 2009). To dry distinct food products, various drying methods have been applied. Each one comprises its own advantages and disadvantages. However, some products are heat sensitive. If they remain in high temperature for a significant time, they lose some aroma and flavor.

In the present study, the thin layer fresh cubic turmeric rhizomes were dried with microwave, infrared and freeze methods to specify the impact of distinct methods on the drying characteristics, to identify the most optimal drying model, to figure out effective moisture diffusivity values, and to evaluate the differences color.

2. Materials and Methods

2.1. Drying experiments

Fresh turmeric were bought from a fruiterer in Bursa province of Turkey. During all experiments of this research, mature and healthy turmeric were chosen. The products were kept at 4±0.5 °C temperature levels. Content of moisture on a dry basis at first was confirmed to be 3.99 (g water g dry matter^{-1}) with oven drying method (ED115 Binder, Tuttlingen, Germany) at 105 °C for 24 hours (Aral & Beşe 2016). The samples were cut into cubes of 5x5x5±0.04 mm by means of a slicer (Nicer Dicer, China). In the course of drying experiments, microwave, infrared, and freeze drying methods were utilized. All experiments were repeated three times.

2.2. Microwave drying

For the drying experiment, a microwave oven with 90, 160 and 350 W output levels (AMW 545, Whirlpool, Italy) was used. Turmeric samples of 25 g were disposed in a thin layer on revolving circular glass plate with 245 mm diameter. Loss of moisture in the samples was checked with a 0.01 g precision digital balance (Radwag, Radom, Poland) in every 2 minutes.

2.3. Infrared drying

An infrared dryer (Moc63, Shimadzu, Japan) that radiates electromagnetic radiation ranging from medium to shortwave infrared radiation that has a wavelength between 2 mm and 3.5 mm. By using the device, parameters about moisture content and temperature were defined directly and they are measured on the display of it. Drying procedure was conducted with 10 g samples at three levels of radiation power which was regulated to attain final temperatures of 60, 70 and 80 °C.

2.4. Freeze drying

A freeze dryer (Alpha 1-2 LD Plus, Osterode am Harz, Germany) at -50 °C process temperature with 52 Pa constant pressure was used. The moisture loss of 25 g turmeric sample was gauged in every 2 hours with a ± 0.01 g precision digital balance (Radwag, Radom, Poland) in the course of the drying procedure.

2.5. Mathematical modelling of drying data

The data on moisture ratio (MR) was coupled to ten thin layer models which are characteristically utilized for modeling of drying curves (Table 1). Values of the moisture ratio were figured out by applying Equation 1 and Equation 2.

$$MR = \frac{M_t - M_e}{M_o - M_e} \quad (1)$$

Above M_t stands for the moisture content (g water g dry matter^{-1}) at a given time, M_o stands for the initial moisture content (g water g dry matter^{-1}), M_e stands for the equilibrium moisture content (g water g dry matter^{-1}). In comparison to M_t or M_o, M_e values are relatively small. As a result, several researchers have vulgarized the moisture ratio as follows (Midilli et al 2002):

$$MR = \frac{M_t}{M_o} \quad (2)$$

Table 1- Thin layer drying models used for the turmeric drying kinetics

No	*Model name*	*Model*	*References*
1	Henderson & Pabis	$MR = a\exp(-kt)$	(Westerman et al 1976)
2	Newton	$MR = \exp(-kt)$	(Ayensu 1997)
3	Page	$MR = \exp(-kt^n)$	(Agrawal & Singh 1977)
4	Logarithmic	$MR = a\exp(-kt) + c$	(Yagcioglu et al 1999)
5	Two Term	$MR = a\exp(-k_0 t) + b\exp(-k_1 t)$	(Madamba et al 1996)
6	Two Term Exponential	$MR = a\exp(-kt) + (1-a)\exp(-kat)$	(Sharaf-Eldeen et al 1980)
7	Wang & Singh	$MR = 1 + at + bt^2$	(Wang & Singh 1978)
8	Diffusion Approach	$MR = a\exp(-kt) + (1-a)\exp(-kbt)$	(Kassem 1998)
9	Verma et al	$MR = a\exp(-kt) + (1-a)\exp(-gt)$	(Verma et al 1985)
10	Midilli et al	$MR = a\exp(-kt^n) + bt$	(Midilli et al 2002)

2.6. Determination of effective moisture diffusivity

According to the 2nd law of Fick on the diffusion Equation, drying of agricultural products with a declining rate during a time frame is symbolized by using a mass-diffusion equation as Equation (3):

$$\frac{\partial M}{\partial t} = \nabla M\left[\mathrm{D_{eff}}(\nabla \mathrm{M})\right] \quad (3)$$

The Equation (3) that explains the 2nd law of Fick on unsteady state diffusion can be utilized to figure out the moisture ratio calculated in Equation (4). For an infinite slab, the formula of diffusion equation was set forth (Crank 1975), and uniform initial moisture distribution, steady diffusivity, immaterial shrinkage, and negligible external resistance were expected:

$$MR = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-\frac{(2n+1)^2\pi^2 D_{eff}t}{4L^2}\right) \quad (4)$$

Where; D_{eff} ($m^2\ s^{-1}$) stands for effective moisture diffusivity; t (s) stands for time; L (m) stands for sample's half thickness; n stands for a positive integer.

Regarding for extend drying periods, only the first term in Equation (4) is significant and consequently, the Equation is simplified as The Equation (5) as logarithmically:

$$MR = \frac{8}{\pi^2}\exp\left(-\frac{\pi^2 D_{eff}t}{4L^2}\right) \quad (5)$$

Plotting experimental drying data from the point of ln (MR) versus drying period enables to figure out effective moisture diffusivity values in Equation (6). The slope of the straight line which is generated by the plot is calculated as follows (Doymaz et al 2015):

$$K = \frac{\pi^2 D_{eff}}{4L^2} \quad (6)$$

2.7. Color measurement

With the use of a colorimeter (MSEZ-4500L, HunterLab, USA), L*, a*, and b* values of dried and fresh turmeric samples were classified in ten readings that are realized at random positions on the surfaces of samples. The color parameters, L_0*, a_0* and b_0* of the fresh turmeric samples. Throughout these experiments, before every color determination, white-black plates were used for calibration of the colorimeter. First of all, a glass cell that contains a sample was disposed above the light source that is near the nose cone of the colorimeter and then the

values of the parameters L_0*, a_0*, b_0*, L*, a*, and b* were saved. Moreover, the Chroma C, hue angle α, and the overall color difference ΔE was calculated in Equation (7), Equation (8) and Equation (9), respectively (Delgado et al 2016).

$$C = \sqrt{(a^2 + b^2)} \tag{7}$$

$$\alpha = \tan^{-1}(\frac{b}{a}) \tag{8}$$

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \tag{9}$$

2.8. Statistical analysis

The research was carried out with the help of randomized plots factorial design. During the measurement process of the inspected products, three replicates were utilized. For analyzing the obtained results, JMP (Version 7.0, SAS Institute Inc., Cary, NC, USA) and MATLAB (MathWorks Inc., Natick, MA) technologies were utilized. For significance, testing of mean differences was performed and the least significant difference test (LSD) yielded level of 5% significance. The optimum model that describes drying characteristics of turmeric sample in a thin layer is verified as the one that has the maximum coefficient of determination (R^2) and the lowest reduced chi-squared (χ^2) and the lowest root mean square error (*RMSE*) values (Arumuganathan et al 2009). The mentioned statistical values are described as below:

$$\chi^2 = \frac{\sum_{i=1}^{N}(MR_{\exp,i} - MR_{pre,i})^2}{N - z} \tag{10}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(MR_{pre,i} - MR_{\exp,i})}{N}} \tag{11}$$

Where; $MR_{exp,i}$, stands for the experimental moisture ratio for test number I; $MR_{pre,i}$, stands for the estimated moisture ratio for test number i; N stands for the number of observation and z stands for the count of constants in the drying model (Doymaz & Ismail 2011).

3. Results and Discussion

3.1. Drying kinetics of turmeric

The drying curves of turmeric samples that were dried via different drying methods are depicted in Figure 1. It is clear that the drying method significantly influenced to achieve the final moisture content in terms of total drying time. Among the used drying methods in this study, longest period was realized with freeze drying (600 min) and microwave drying at 350 W (65 min) application took shortest period. These results indicated that with respect to the freeze-drying method when the turmeric samples were dried at 350 W microwave power, drying period declined by 89.17%. Additionally, a remarkable decline took place in the drying period when the microwave level has risen. Accordingly, the drying periods were 255, 125 and 65 min for the samples that were dried at 90, 120 and 350 W, respectively. Similarly, the decline in drying periods along with the rise in the microwave power level has also been confirmed for okra (Dadalı et al 2007), pumpkin (Wang et al 2007), white mulberry (Evin 2011) and onion slices (Arslan & Özcan 2010). As expected, the shortest time in infrared drying (120 min) was obtained at 80 °C in comparison with 60 and 70 °C, which required times of 250 and 170 min, respectively. Thus, an important decrease in the drying period has been realized as drying temperature rises. Identical results were recorded for various samples under infrared dryings, such as apple (Toğrul 2005), wet olive husk (Celma et al 2008), and tomato (Sadin et al 2014).

3.2. Fitting of drying curves

Tables 2-3 denote the statistical analysis values obtained from the nonlinear regression of the all thin layer drying models including the comparison criteria and the drying model coefficients that

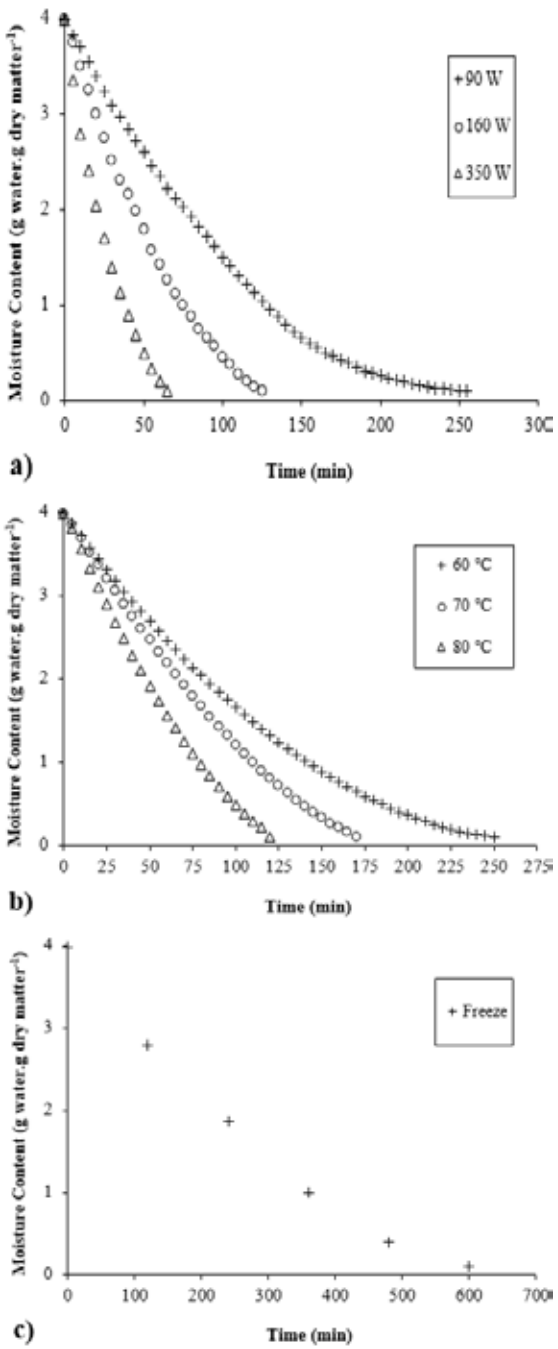

Figure 1- Drying curves of turmeric samples; microwave powers (a), infrared temperatures (b) and freeze (c)

are benefited to assess the suitability quality, R^2, *RMSE,* and χ^2. In all cases, The R^2 values ranged from 0.9606 to 0.9999, *RMSE* values ranged from 0.0027 to 0.0597 and χ^2 values ranged from $0.0807x10^{-4}$ to $35.9287x10^{-4}$, that are pointing out good fit results. The Midilli et al model put forward more suitable statistical values as against the other models for 70 and 80 °C the infrared temperatures and for 160 and 350 W microwave power levels. Furthermore, the Wang & Singh model demonstrated greater R^2 value and smaller *RMSE* and χ^2 values as against other thin-layer drying models at 60 °C infrared temperature, 90 W microwave power level, and freeze condition. In the Midilli et al and the Wang & Singh models, values of the R^2, *RMSE* and χ^2 varied between 0.9985 and 0.9999, 0.0027 and 0.0146, $0.0807x10^{-4}$ and $3.4160x10^{-4}$; and also 0.9963 and 0.9999, 0.0031 and 0.0189, $0.0864x10^{-4}$ and $3.9596x10^{-4}$, in return. Based on these outcomes, the Midilli et al and Wang & Singh models might be accepted as demonstrating the thin-layer drying behavior of the turmeric samples.

Figure 2 demonstrates the variance between the most appropriate predicted models and experimental moisture ratio at selected drying conditions for dried turmeric. Obviously, the results obtained from the models of Midilli et al and Wang & Singh are quite close to the experimental values. So it may be deduced that Midilli et al and Wang & Singh models may identify the drying curves of turmeric samples properly. The outcomes of this study are in line with earlier ones found in the drying of rough rice (Cihan et al 2007), olive pomace (Smail Meziane 2011) and mushroom (Motevali et al 2011) for the Midilli et al model and bamboo shoot (Bal et al 2010), banana (Kadam & Dhingra 2011) and paddy (Manikantan et al 2014) for Wang & Singh model.

3.3. Determination of effective moisture diffusivity

The determined effective moisture diffusivity values for cubic turmeric rhizomes are demonstrated in Table 4 and were ranged between 1.01×10^{-9} and 9.12×10^{-9} $m^2 s^{-1}$. It may be observed that D_{eff} values

Table 2- Estimated coefficients values and statistical analyses resulted from selected thin layer drying models for drying of turmeric at different microwave powers

No	*90 W*				*160 W*				*350 W*			
	Model coefficients	R^2	*RMSE*	χ^2 (10^{-4})	*Model coefficients*	R^2	*RMSE*	χ^2 (10^{-4})	*Model coefficients*	R^2	*RMSE*	χ^2 (10^{-4})
1	a= 1.079 k= 0.01145	0.9838	0.0383	14.8881	a= 1.072 k= 0.01946	0.9809	0.0421	16.8393	a= 1.028 k= 0.03858	0.9873	0.0348	11.3908
2	k= 0.01062	0.9772	0.0455	20.6204	k= 0.01811	0.9746	0.0486	22.1172	k= 0.0375	0.9871	0.0351	11.4009
3	k= 0.002676 n= 1.294	0.9971	0.0162	2.7091	k= 0.005284 n= 1.3	0.9956	0.0201	3.8818	k= 0.02135 n= 1.165	0.9935	0.0248	6.0526
4	a= 1.275 k= 0.008108 c= -0.1616	0.9965	0.0179	3.3063	a= 1.326 k= 0.01136 c= -0.3116	0.9994	0.0077	0.5324	a= 1.188 k= 0.0261 c= -0.1983	0.9994	0.0074	0.5900
5	a= -24.6 k_o= 0.00989 b= 25.69 k_1= 0.009958	0.9845	0.0375	14.3004	a= 14.67 k_o= 0.03093 b= -13.64 k_1= 0.03238	0.9934	0.0248	5.8194	a= -10.12 k_o= 0.05838 b= 11.14 k_1= 0.05587	0.9920	0.0277	7.4034
6	a= 0.00009008 k= 117.9	0.9767	0.0460	21.0478	a= 0.00007655 k= 236.5	0.9735	0.0496	23.0526	a= 0.0005352 k= 70.02	0.9860	0.0366	12.3939
7	a= -0.007882 b= 0.00001597	0.9995	0.0064	0.3583	a= -0.01336 b= 0.0000444	0.9995	0.0063	0.3475	a= -0.028 b= 0.0002038	0.9963	0.0189	3.9596
8	a= -7.706 k= 0.01932 b= 0.9211	0.9965	0.0177	3.0939	a= -9.96 k= 0.03425 b= 0.9324	0.9951	0.0212	4.0292	a= -5.409 k= 0.06271 b= 0.9125	0.9938	0.0243	5.2447
9	a= -11.63 k= 0.01977 g= 0.01861	0.9969	0.0168	2.8688	a= -45.08 k= 0.02878 g= 0.02844	0.9906	0.0295	7.8306	a=-7.652 k=0.05976 g=0.05621	0.9936	0.0247	5.7837
10	a= 0.9732 k= 0.002521 n= 1.288 b= -0.0001231	0.9986	0.0112	1.2095	a= 0.9982 k= 0.008952 n= 1.121 b= -0.0009541	0.9996	0.0059	0.3010	a=0.9984 k=0.0347 n=0.9501 b=-0.00226	0.9996	0.0063	0.3882

Table 3- Estimated coefficients values and statistical analyses resulted from selected thin layer drying models for drying of turmeric at various infrared temperatures and freeze drying condition

No	60 °C				70 °C				80 °C				Freeze			
	Model coefficients	R^2	*RMSE*	χ^2 (10^{-4})	*Model coefficients*	R^2	*RMSE*	χ^2 (10^{-4})	*Model coefficients*	R^2	*RMSE*	χ^2 (10^{-4})	*Model coefficients*	R^2	*RMSE*	χ^2 (10^{-4})
1	a= 1.076 k= 0.01042	0.9837	0.0379	14.6868	a= 1.072 k= 0.0131	0.9718	0.0506	25.4483	a= 1.082 k= 0.01827	0.9738	0.0492	24.4453	a= 1.037 k= 0.003916	0.9705	0.0646	34.6438
2	k= 0.009674	0.9770	0.0451	20.3821	k= 0.01198	0.9618	0.0588	34.8544	k= 0.01679	0.9650	0.0568	32.7320	k= 0.003788	0.9741	0.0606	33.3259
3	k= 0.00255 n= 1.281	0.9964	0.0177	3.2174	k= 0.002143 n= 1.385	0.9941	0.0232	5.0366	k= 0.003697 n= 1.367	0.9944	0.0227	4.9280	k= 0.0004158 n= 1.387	0.9949	0.0268	5.8328
4	a= 1.257 k= 0.006613 c= -0.2395	0.9992	0.0084	0.6673	a= 1.598 k= 0.005827 c= -0.5846	0.9994	0.0074	0.5641	a= 1.57 k= 0.00838 c= -0.5565	0.9997	0.0053	0.3576	a= 1.379 k= 0.002154 c= -0.3737	0.9980	0.0170	3.9332
5	a= -11.4 k_o= 0.01764 b= 12.42 k_1= 0.01667	0.9954	0.0201	4.1449	a= -17 k_o= 0.02218 b= 18.08 k_1= 0.02138	0.9835	0.0386	14.4233	a= 7.299 k_o= 0.01818 b= -6.238 k_1= 0.01823	0.9706	0.0521	27.4593	a= 10.91 k_o= 0.0009416 b= -9.981 k_1= 0.0007938	0.9718	0.0622	32.5007
6	a= 0.00008325 k= 116.2	0.9765	0.0455	20.8117	a= 0.00007223 k= 165.8	0.9606	0.0597	35.9287	a= 0.0000909 k= 184.5	0.9634	0.0581	34.1764	a= 0.0003984 k= 9.5	0.9676	0.0668	35.1904
7	a= -0.007182 b= 0.00001312	0.9999	0.0031	0.0864	a= -0.008585 b= 0.00001645	0.9997	0.0051	0.2791	a= -0.01209 b= 0.0000329	0.9998	0.0044	0.1925	a= -0.002719 b= 0.00000179	0.9997	0.0070	0.9706
8	a= -9.801 k= 0.01792 b= 0.9336	0.9963	0.0182	3.2884	a= -7.233 k= 0.02243 b= 0.9151	0.9901	0.0299	8.5593	a= -17.69 k= 0.03308 b= 0.9568	0.9931	0.0252	5.9042	a= 3.202 k= 0.001601 b= 0.6202	0.9980	0.0166	2.7348
9	a= -11.72 k= 0.01791 g= 0.01689	0.9963	0.0181	3.3519	a= -27.13 k= 0.02351 g= 0.02283	0.9926	0.0259	6.4390	a= -14.14 k= 0.03348 g= 0.03167	0.9931	0.0252	6.1719	a= -0.2749 k= 0.4587 g= 0.004729	0.9827	0.0495	24.8674
10	a= 0.9911 k= 0.003939 n= 1.155 b= -0.0003502	0.9996	0.0057	0.2648	a= 0.9914 k= 0.004069 n= 1.174 b= -0.0009741	0.9998	0.0045	0.1544	a= 0.9994 k= 0.007318 n= 1.126 b= -0.001484	0.9999	0.0027	0.0807	a= 0.9984 k= 0.001107 n= 1.179 b= -0.0001847	0.9985	0.0146	3.4160

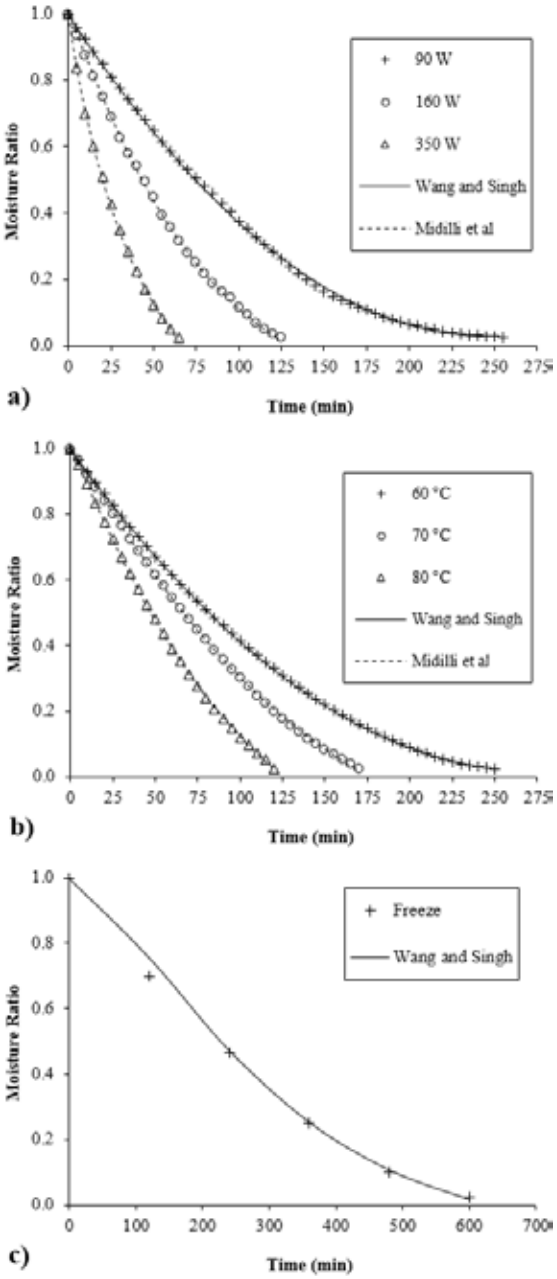

Figure 2- A comparison of the appropriate models to experimental moisture ratios at specific drying times under microwave (a), infrared (b) and freeze (c) drying conditions

have risen significantly with rising infrared radiation and microwave power. During drying, the effective moisture diffusivity value is at its maximum level at 350 W power levels and its lowest level is yielded at freeze-drying. This can be explained by the rapid of vapor pressure. These diffusivity values were good agreement with reported for turmerics such as drying of sliced and solid turmeric with solar conduction dryer which was found $1.852x10^{-10}$ and $1.456x10^{-10}$ m^2 s^{-1}, respectively (Borah et al 2015), and $8.43x10^{-11}$ to $2.51x10^{-10}$ m^2 s^{-1} for drying at 50 °C hot air temperature in a tray drier (Parveen et al 2013). Also, the effective moisture diffusivities at 60, 80 and 100 ºC of blanched rhizomes and unblanched rhizomes for cylinder were $3.23x10^{-10}$, $6.10x10^{-10}$, $10.90x10^{-10}$ m^2 s^{-1} and $1.77x10^{-10}$, $3.73x10^{-10}$, $7.80x10^{-10}$ m^2 s^{-1}, respectively. Similar values for slab were found $11.90x10^{-10}$, $19.60x10^{-10}$, $35.10x10^{-10}$ m^2 s^{-1} and $6.87x10^{-10}$, $14.05x10^{-10}$, $28.00x10^{-10}$ m^2 s^{-1}, respectively (Blasco et al 2005). These values mentioned above are in concordance with the estimated D_{eff} values that are provided for dried turmeric with infrared, microwave and freeze dryers.

Table 4- Effective moisture diffusivities of dried turmeric samples

Drying method	D_{eff} $(m^2 s^{-1})$
Microwave drying	
90 W	$2.03x10^{-9}$
160 W	$4.05x10^{-9}$
350 W	$9.12x10^{-9}$
Infrared drying	
60 °C	$2.03x10^{-9}$
70 °C	$3.04x10^{-9}$
80 °C	$4.05x10^{-9}$
Freeze drying	$1.01x10^{-9}$

3.4. Color analysis

Color reflects the quality of the dried product samples also it is a determinant of the consumer acceptance. The results concerning the color changes of the fresh turmeric samples and the dried ones throughout distinct drying methods are detailed in Table 5. Drying methods significantly

(P<0.05) affected color values of turmeric. Regarding lightness, freeze-dried turmeric promoted an increase in L^* values (L^*= 46.19 for the fresh sample). In other respects, the fresh turmeric sample (38.42) possessed significantly higher a^* values (P<0.05) in comparison to every other drying methods. The b^* value was significantly at its maximum level (P<0.05) for the freeze-dried sample (66.04) and the lowest for an infrared dried sample at 60 and 70 °C. Further, the maximum C value was obtained with 72.53 in freeze-dried and significantly more vivid (P<0.05) with regard to color as against all other fresh samples and dried ones. In contrast with the sample of fresh, a significant increase (P<0.05) was seen in α value of in all dried samples. Infrared-dried turmeric showed a significantly different ΔE value (P<0.05) at 35.56 and 36.13 (60 and 70 °C, respectively) than freeze-dried turmeric at 12.65, perhaps due to presence of polyphenol oxidase (PPO) and/or peroxidase (POD) compounds that reacted with phenolic to form browning mechanism (Hirun et al 2014) were observed that during infrared drying. Color changes of turmeric in the various drying methods have been reported that color quality of turmeric is more dependent quality attributes than every other and an active ingredient of turmeric (curcumin) is photosensitive and highly responsible for its color (Borah et al 2017). The study of Hirun et al (2014), found that products might remain brighter in color when increasing microwave-vacuum power up to 4000 W. Similarly, hot air drying method culminated in less red color (low a^* value) and a darker color (lower L^* value) as against the combined microwave vacuum drying. However, this drying method yielded in higher yellow color value (Apintanapong & Maisuthisakul 2011).

Table 5- Color values of dried and fresh turmeric samples

Drying method	*Color parameters*					
	L^*	a^*	b^*	C	α°	ΔE
Fresh	46.62±2.43^{b}	38.42±1.21^{a}	58.38±1.82^{b}	69.89±2.18^{b}	56.68±0.06^{a}	-
Microwave drying						
90 W	32.10±1.04^{c}	22.60±0.71^{d}	36.23±1.03^{d}	42.70±1.24^{d}	58.07±0.18^{d}	30.85±1.58^{c}
160 W	32.66±0.34^{c}	24.90±0.30^{c}	40.00±0.21^{c}	47.12±0.33^{c}	58.14±0.20^{d}	26.75±0.42^{b}
350 W	32.29±0.13^{c}	25.14±0.40^{c}	36.65±0.33^{c}	46.95±0.30^{c}	57.56±0.52^{c}	27.07±0.29^{b}
Infrared drying						
60 °C	29.52±0.58^{d}	20.72±0.60^{f}	32.71±0.78^{f}	38.72±0.98^{f}	57.67±0.19^{c}	35.57±1.12^{e}
70 °C	29.85±0.57^{d}	20.07±0.26^{g}	32.15±0.48^{f}	37.90±0.52^{f}	58.04±0.23^{d}	36.13±0.71^{e}
80 °C	29.62±0.78^{d}	22.00±0.49^{e}	34.00±0.50^{e}	40.50±0.68^{e}	57.13±0.25^{b}	33.96±0.96^{d}
Freeze drying	51.99±1.15^{a}	30.00±0.78^{b}	66.04±0.94^{a}	72.54±1.06^{a}	65.61±0.49^{e}	12.65±0.94^{a}

$^{a-g}$, in a column, means within the different letters are significantly different (P<0.05)

4. Conclusions

In conclusion, various methods could be used as a drying opportunity of turmeric. When the drying methods utilized in this research are compared, microwave drying reduced the drying period significantly as against the infrared and freeze methods. However, the best and worst color results are achieved with freeze and infrared methods, respectively. Among the applied drying models, it is found that the Midilli et al and the Wang & Singh models are the most appropriate ones to clarify the drying kinetics of turmeric samples. Further understanding of turmeric drying will be important for the dried food industry to gain a new perspective.

Abbreviations and Symbols	
M_0	Initial moisture content, g water g dry matter^{-1}
M_t	The moisture content at a particular time, g water g dry matter^{-1}
M_e	Equilibrium moisture content, g water g dry matter^{-1}
$MR_{exp,i}$	Experimental moisture ratio at the test number i,
$MR_{pre,i}$	Estimated moisture ratio at the test number i,
N	Observation number
z	Total count of constant
$RMSE$	Root mean square error
R^2	Coefficient of determination
χ^2	Reduced chi-square
$a,b,c,g,n,\ k_o,k_1$	Model constants
D_{eff}	Effective moisture diffusivity
t	Stands for time
L^*	Whiteness/Darkness
a^*	Redness/Greenness
b^*	Yellowness/Blueness
C	Chroma
α	Hue angle
ΔE	Total color differences
L_0^*	Whiteness/Darkness of fresh sample
a_0^*	Fresh sample of fresh sample
b_0^*	Fresh sample of fresh sample

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Investigation of Genotoxic, Antimicrobial and Antioxidant Activities of Leaf and Flower Extracts of *Cynara syriaca* Boiss

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ABSTRACT

The mutagenicity and antimutagenicity of leaves and flower extract of *Cynara syriaca* Boiss were studied with Ames assay in *Salmonella typhimurium* TA98 and TA100 strains. While leaves extract did not show any mutagenic effects against all the tester strains with or without metabolic activation, the flower extract showed mutagenic effect against TA98 strain without metabolic activation. On the other hand, it has been observed that the extracts have antimutagenic activity against mutations induced by sodium azide and daunomycin. The antimicrobial activity of extracts was determined by disc diffusion and MIC value. Both of the extracts possess weak antimicrobial activity. Cupric reducing antioxidant capacity (CUPRAC), DPPH free radical scavenging activity, and ABTS radical cation decolorization methods were carried out to determine the antioxidant activity. Among the tested antioxidant methods, the highest antioxidant capacity was determined in ABTS radical cation decolorization assay in which both of the extracts exhibited the best effect. Flower extract exhibited higher activity also in DPPH free radical scavenging.

Keywords: Mutagenicity; Antimutagenicity; Antioxidant activity; Antimicrobial activity; *Cynara syriaca*

1. Introduction

Plants have significant biological content and in recent years plant polyphenols are increasingly attracted to the role of antioxidants, mutagenic, antimutagenic and anticancerogenic properties as well as the prevention of several diseases such as cancer and cardiovascular diseases (Chulasiri 1998; Caderni et al 2000; Lin et al 2009; Sun et al 2011).

One of the secondary metabolites commonly found in fruits and vegetables is flavonoids. These polyphenol compounds are responsible for the color of many vegetables and fruits and at the same time provide important functions in reproduction and breeding plants. They act as defensive mechanisms against pathogens, parasites, and ruptures. (Báidez et al 2007). Natural phenolic compounds which were included flavonoids, phenolic acids, stilbenes, curcuminoids, tannins, lignans, quinones, coumarins and others have been reported to possess potent antioxidant activity and anticarcinogenic, antimutagenic, antiatherosclerotic, antibacterial,

antiviral anti-inflammatory activities (Owen et al 2000; Veeriah et al 2006; Báidez et al 2007; Han et al 2007). Phenolic compounds have contributed to the induction of apoptosis by arresting the cell cycle. Moreover, they inhibit DNA binding, regulating carcinogen metabolism, cell adhesion, migration, proliferation or differentiation and blocking signaling pathways (Huang et al 2009).

Researches on traditionally used plants have been increasing day by day and very important results have obtained from them. *Cynara syriaca* has been grown wild in a very narrow area in Southern Turkey (Kupicha 1975). There has been no chemical investigation on *C. syriaca* except for the lipid content of the seeds (Heidari et al 2000).

The aims of this study were to investigate the mutagenic, antimutagenic, antioxidant and antimicrobial activity of leaf and flower extracts of *C. syriaca*.

2. Materials and Methods

2.1. Plant extraction

The leaf and flower of *C. syriaca* was picked up from Diyarbakır, Turkey in June 2010 and identified by Prof. Dr. A. Selçuk ERTEKIN (Department of Biology, Faculty Science, Dicle University). The dried and powdered leaf and flowers of *C. syriaca* macerated with methanol at room temperature for 24 h. The solvent was evaporated after filtration and kept at +4 °C in a glass bottle.

2.2. Determination of minimum inhibitory concentration (MIC) and antimicrobial activity

Gram-positive bacteria (*Streptococcus pyogenes* ATCC19615 and *Staphylococcus aureus* ATCC 25923), gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922) and yeast (*Candida albicans* ATCC10231) which were purchased from Refik Saydam Sanitation Center (Turkey) were used for detecting the antimicrobial activity of the samples. The disc diffusion method was employed for this purpose ampicillin and fluconazole were used as positive controls for bacteria and yeast, respectively. The minimum inhibitory concentration of the extracts was determined by broth dilution methods **(**NCCLS 2009).

2.3. Determination of total phenolic and flavonoid contents

The total phenolic (Slinkard & Singleton 1977) and flavonoid contents (Moreno et al 2000) of extracts were expressed as gallic acid and quercetin equivalents, respectively, and calculated according to the following equations.

Absorbance= 0.1741 Gallic acid (µg) - 0.0224 (R^2= 0.9925)

Absorbance= 0.2784 Quercetin (µg) - 0.2872 (R^2= 0.9911)

2.4. Antioxidant activity assays

DPPH free radical scavenging activity (Blois 1958), cupric reducing antioxidant capacity (CUPRAC) (Apak et al 2004) and ABTS radical cation decolorization (Re et al 1999) methods were carried out to determine the antioxidant activity. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as positive controls. The percentage of inhibition was calculated by using the following equation except in the CUPRAC method in which increasing absorbance refers to increasing activity.

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

2.5. Mutagenic and antimutagenic activity

The bacterial mutagenicity and antimutagenicity assays were carried out according to Maron & Ames (1983). The plate incorporation procedure was done to detect reverse mutations from histidine dependence to histidine independence via *S. typhimurium* test strains TA98 and TA100 absence and presence S9 mammalian liver homogenate fraction. The NaN_3 (into distilled water–1.5 µg mL^{-1}) for *S. typhimurium* TA100, 2-aminofluorene requiring metabolic activation (in DMSO-0.1 mg mL^{-1}) and daunomycin (in distilled water- 6 µg mL^{-1}) for *S. typhimurium* TA98 were used as positive controls and 10% DMSO as the negative control.

While the mutagenicity assessment was conducted by a dose-response and a two-fold increase in the number of revertants, the antimutagenicity was assessed by the inhibition percentage of mutagenicity (IP) calculated by the following Equation:

$$IP(\%)= (1 - (A-B) / (C-B)) \times 100$$

Where; *A*, number of revertants on test plates incubated with mutagen and extract; *B*, spontaneous revertants (test strains incubated in the absence of both extract and mutagen); *C*, number of revertants on control plates incubated with the mutagen alone.

Forty percent or more inhibition was considered as strong antimutagenicity; twenty-five to forty percent inhibition was defined as moderate antimutagenicity and twenty-five or less inhibition as no antimutagenicity (Ikken et al 1999).

2.6. Statistical analysis

The results of the antimicrobial and antioxidant activity assays are expressed as mean ± SD of three experiments. The statistical significance was estimated using analysis of variance (ANOVA), Followed by a Dunnett's test to compare the treated groups to the control group. *p* values less than or equal to 0.05 were considered to indicate statistically significance.

3. Results and Discussion

3.1. Antimicrobial activity

The antimicrobial activities of *C. syriaca* leaf and flower extracts against different microorganisms were assessed according to the inhibition zone diameter. Results are given in Table 1. According to the results, both of the extracts possess weak antimicrobial activity (inhibition zone < 12 mm). The highest activity was observed in the leaf extract against *C. albicans* with 12.5±0.7 mm inhibition zone diameter and 250 µg mL^{-1} MIC value.

3.2. Total phenolic and flavonoid contents

Total phenolic and flavonoid contents of the extracts were determined as gallic acid (GAEs) and quercetin (QEs) equivalents, respectively. As depicted in Table 2, the flavonoid contents of the extracts higher than their phenolic contents. The phenolic content of flower extract found to be higher than leaf extract.

3.3. Antioxidant activity

Free radicals play a significant role in a variety of pathological markers and involved in many syndromes such as aging, atherosclerosis, and diabetes in humans. Antioxidants neutralize the free radicals by donating electron and protect from several progressive diseases (Nimse & Pal 2015).

Table 1- Zones of growth inhibition (mm) and MIC values of leaf and flower extracts of *C. syriaca* compared to positive controls

Samples		*Microorganisms*				
		Gram positive		*Gram negative*		*Yeast*
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*C. albicans*
Leaf	[a]DD	9.6±0.8	8.5±0.7	11.5±0.7	10.5±0.7	12.5±0.7
	MIC	600.0±0.1	800.0±0.4	250.0±0.2	500.0±0.2	250.0±0.5
Flower	[a]DD	8.5±0.7	8.6±0.2	9.5±0.1	9±0.0	10.0±0.0
	MIC	800.0±0.5	800.0±0.5	600.0±0.1	600.0±0.7	600.0±0.1
Positive controls	[b]DD	35.0±0.2	19.0±0.2	20.0±0.1	-	30±0.3
	MIC	1.95±0.3	7.8±0.1	7.8±0.4	-	3.1±0.2

-, not active; [a]DD, inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 30 mg mL^{-1} of plant extracts; [b]DD, inhibition zone in diameter (mm) of positive controls that are ampicillin for bacteria and fluconazole for yeast. Minimum inhibitory concentration (MIC) values are given as µg mL^{-1}

Table 2- Total phenolic-flavonoid contents and antioxidant activity[a] of the extracts and positive controls

Samples	*Phenolic content (µg GAEs mg extract^{-1})*[c]	*Flavonoid content (µg QEs mg extract^{-1})*[d]	*IC_{50} (µg mL^{-1})*	
			DPPH Free Radical	*ABTS Cation Radical*
Leaf	4.59±0.17	52.95±0.51	6.13±0.70	2.64±0.50
Flower	7.29±0.28	52.47±0.17	2.34±0.49	1.91±0.20
BHT[b]	-	-	58.86±0.50	13.25±0.27
BHA[b]	-	-	7.88±0.20	17.59±0.10

[a], values expressed are means ± SEM of three parallel measurements (P<0.05); [b], positive controls; [c], *GAEs*, gallic acid equivalents (y= 0.1741 x + 0.0224 R^2= 0.9925); [d], *QEs* quercetin equivalents (y= 0.2784 x - 0.2872, R^2= 0.9911)

The results of DPPH and ABTS assays are expressed as IC_{50} value in Table 2. Lower IC_{50} value indicates the higher activity. The flower extract exhibited excellent antioxidant activity in both DPPH and ABTS assays with 2.34±0.49 and 1.91±0.2 µg mL^{-1} IC_{50} value, respectively. The leaf extract also showed high activity in DPPH and ABTS assays. Both of the extracts possess higher activity than BHT and BHA. On the other hand, positive controls exhibited higher activity than extracts in CUPRAC assay (Figure 1).

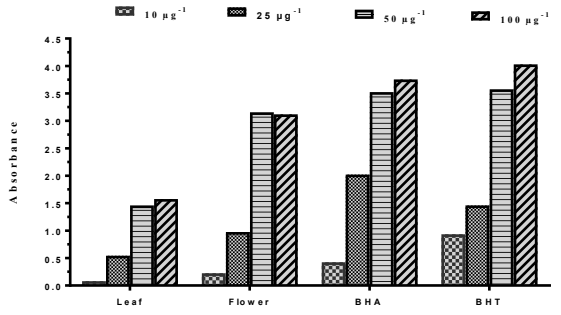

Figure 1- Cupric reducing antioxidant capacity of leaf and flower extracts of *C. syriaca* and positive controls

3.4. Mutagenic and antimutagenic activity

The methanol extracts of *C. syriaca* leaf and flower were evaluated for its mutagenic potentials towards *S. typhimurium* TA98 and TA100, both in the presence and absence of S9 mix. The different amounts of leaf extract (0.1, 0.5, 1, 5, 10, 25, 50, 100, 250, 500 µg plate^{-1}) were tested on the TA98 and TA100 strains neither any mutagenic effects were observed nor mutation frequencies significantly change when compared with spontaneous mutation frequencies (Table 3.). Flower extract increased the number of colonies at 10, 25, 50, 100, 250, 500 µg plate^{-1} concentrations in the absence of S9 mix on TA98 strain. The extract has no toxic effect at the same concentrations in the presence of S9 mix (Table 4.).

The antimutagenic experiments established in the absence of S9 mix. Results indicated that leaf extract has significant (inhibition level > 40%) antimutagenicity at 250 and 500 µg plate^{-1} concentrations (42.63% and 53.98%) on TA98 strain. On the other hand, leaf extract has moderate (inhibition level < 40-25%) antimutagenicity at 5 and 500 µg plate^{-1} concentrations (35.73% and 29.69%) on TA98 and TA100 strains respectively (Table 3.). Moreover, the flower extract showed strong antimutagenicity at 0.1 µg plate^{-1} concentration (44.17%) on TA98 strain. The flower extract has no antimutagenicity on TA100 strain (Table 4.).

According to the analysis of the leaves of *C. syriaca* which reported that it included six flavonoids (apigenin, chrysoeriol, luteolin, apigenin 7-O-glucoside, chrysoeriol 7-O-glucoside, luteolin 7-O-glucoside), four phenolic acids (caffeic acid, chlorogenic acid, 1,5-dicaffeoylquinic acid, cynarin), and three sesquiterpene lactones [11,13-dihydroxy-8-desoxygrosheimin (1) 11,13-dihydrodeacylcynaropicrin (2) solstitialin (3)] (Meriçli & Seyhan 2006).

Table 3- Mutagenic and antimutagenic activities of leaf extract of *C. syriaca*

Doses (µg plate^{-1})	*TA98*			*TA100*		
	Number of Revertants S9(-)	*Number of revertants S9(+)*	*Inhibition of Mutagenesis (%)*	*Number of Revertants S9(-)*	*Number of revertants S9(+)*	*Inhibition of mutagenesis (%)*
0	52±8	31±4	0.00	169±12	189±2	0.00
0.1	43±8	22±5	-23.61	177±15	219±4	5.63
0.5	34±5	25±3	20.55	126±13	195±19	21.48
1	50±8	20±2	-8.89	154±31	179±13	-7.99
5	46±8	23±1	35.73	126±18	183±9	-23.71
10	39±7	26±4	6.13	133±10	195±12	-20.23
50	51±2	27±5	-18.25	94±4	186±12	-21.62
100	56±3	23±3	0.46	171±12	184±10	-17.45
250	46±3	20±1	42.63	145±4	190±9	-11.89
500	60±11	23±3	53.98	122±13	177±13	29.69
DMSO	42±7	25±2	7.82	121±12	205±19	1.11
(+) Control	704±24	368±21	-	1615±45	960±30	-

(-S9) without and (+S9) with metabolic activation. Mean and ± S.D. of three plates. DMSO solvent control; 2-AF, NaN_3 and daunomycin positive control for +S9 and -S9

Table 4- Mutagenic and antimutagenic activities of flower extract of *C. syriaca*

Doses (µg plate^{-1})	*TA98*			*TA100*		
	Number of revertants S9(-)	*Number of revertants S9(+)*	*Inhibition of mutagenesis (%)*	*Number of revertants S9(-)*	*Number of revertants S9(+)*	*Inhibition of mutagenesis (%)*
0	52±8	31±4	0.00	169±12	189±2	0.00
0.1	30±5*	22±3	44.17	168±9	214±18	-0.06
0.5	33±3	24±1	0.92	161±9	175±8	3.12
1	49±5	27±2	17.63	138±13	165±7	13.00
5	343±21	29±3	-11.96	114±8	178±5	-15.50
10	nt	32±6	15.18	125±10	197±15	-1.73
50	nt	25±2	-	112±9	183±10	4.79
100	nt	28±4*	26.84	120±10	165±9	-42.55
250	nt	26±3*	32.20	126±11	176±5	-17.52
500	nt	29±2	8.43	122±8	168±9	-43.04
DMSO	42±7	25±2	7.82	121±12	205±19	1.11
(+) Control	704±24	368±21	-	1615±45	960±30	-

(-S9) without and (+S9) with metabolic activation. Mean and ± S.D. of three plates. DMSO solvent control; 2-AF, NaN_3 and daunomycin positive control for +S9 and -S9; nt, not tested; *, significantly different from the corresponding solvent control value (Dennett's test, $P<0.05$)

According to our results, it could be suggested that the antioxidant activity of the *C. syriaca* extract relates in part to its constituent both flavonoids and phenolic acids such as apigenin, aglycone, luteolin, dicaffeoylquinic acids, caffeic caffeic acid, and chrysoeriol. These constituents act as hydrogen donors, metal ion chelators, and their dividing between aqueous and lipophilic phases further influences the effectiveness.

Studies of these components, which also include *C. syriaca* extracts, have shown antioxidant and antimutagenic activities. Researches on some of these components are: Simsek & Uysal (2013) have reported the inhibitory effects of *C. cardunculus* and *C syriaca* extracts on the proliferation of human colorectal cancer DLD1 cells and inducing apoptotic pathway on DLD1 cells.

Anti-mutagenic, anti-proliferative, antioxidant, anti-inflammatory and anti-cancer activities are some activities of Apigenin. It is a flavonoid compound and found in a variety of fruits and vegetables (Madunić et al 2018). Apigenin Apigenin has shown shown potent antioxidant (Nielsen et al 1999) and strongly inhibited the bacterial mutagenesis induced by nitropyrenes (Kuo et al 1992). Luteolin 7-O-glucoside, luteolin 7-O-rutinoside and luteolin 7-O-glucuronide have shown antimutagenic effects on TA1537 and TA1535 strains (Orhan et al 2012). A number of biological activities of Apigenin 7-O-glucosid such as anticonvulsant, anti-inflammatory, antioxidant, and anticancer have been reported recently (Guzelmeric et al 2015). The pure aglycone, luteolin, have demonstrated an efficacy similar to artichoke extract in inhibiting lipid peroxidation; luteolin-7-O-glucoside, have demonstrated a dose-dependent reduction of LDL oxidation; copper chelating properties of luteolin-7-O-glucoside and luteolin have suggest a potential role for chelation in the antioxidative effects of artichoke extract (Brown & Rice-Evans 1998). Chrysoeriol have shown the ability to inhibit lipid peroxidation in low density lipoprotein induced by Cu^{2+}/O_2^- (Rice-Evans et al 1996), and antimutagenic activity in S. typhimurium TA98 (Kukić et al 2008). Chrysoeriol have found to exhibit antimutagenic activity in Salmonella typhimurium TA98 and Chrysoeriol isolated from Morinda morindoides leaves has been found to be ineffective towards superoxide radicals generated from xanthine and xanthine oxidase (Re et al 1999). Chlorogenic acid and caffeic acid have vicinal hydroxyl groups on an aromatic residue, and they exhibit antimutagenic, carcinogenic and antioxidant activities in vitro, which is to scavenge reactive oxygen species (Rice-Evans et al 1996); caffeic acid completely eliminated the mutagenicity induced by activating Glu-P-2 (2-aminodipyrido [1.2-a: 3'. 2'-d] imidazole). Both caffeic acid and chlorogenic acid effectively decreased the mutagenicity of Trp-P-I (3-amino-1,4-dimethyl-5H-pyrido-(4.3-b)indole) and Glu-P-2 (Yamada & Tomita 1996). Caffeic acid and linoleic acid has reported been that it showed inhibition on lipid peroxidation emulsion and also caffeic acid is an effective $ABTS^+$, DPPH, superoxide anion radical scavenging, total reducing power and metal chelating on ferrous ions activities (Yamada & Tomita 1996). The antioxidant activity of dicaffeoylquinic acids has been found stronger than that of ascorbic acid (Slanina et al 1999). Cynarin is a dicaffeoylquinic acid derivative of artichoke. Although cynarin is found in low amounts in the artichoke, it enhances the effect of artichoke. Choleretic and cholesterol lowering, hepatoprotective, anti-atherosclerotic, antiHIV, antioxidative, anti-diabetic, anti-carcinogenic effects are the potential healt effects of cynarin (Gezer 2017).

4. Conclusions

This study is one of the first studies in the literature investigating the antimutagenic and antioxidant activity of *C. syriaca* extract. Our findings indicate that *C. syriaca* extracts have antioxidant and antimutagenic effect. These activities of the *C. syriaca* extract may be associated, in part, with both flavonoids and phenolic acids or their synergistic effect.

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Measurement and Prediction of Total Friction Losses in Drip Irrigation Laterals with Cylindrical Integrated in-line Drip Emitters using CFD Analysis Method

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ABSTRACT

The objective of this study was to predict total friction losses in drip irrigation laterals with cylindrical integrated in-line emitters at different spacing using Computational Fluid Dynamics (CFD) simulation method. Two types of drip irrigation laterals with different technical specifications were used in the study. In the laboratory, the total friction losses were measured in the laterals for different velocities. In CFD analysis, standard *k-ε*, RNG *k-ε*, realizable *k-ε*, Reynolds Stress (RSM) with Linear Pressure-Strain (LPS) turbulence models and standard wall function, non-equilibrium wall function, enhanced wall treatment were considered. CFD simulation results were compared with experimental total friction losses in laterals. The highest prediction was obtained by RSM turbulence model with LPS using standard wall function with the lowest values of MAPE (2.96%) and RMSE (369 Pa).

Keywords: Drip irrigation; Pipe; Turbulence models; Computational fluid dynamics

1. Introduction

Uniform water distribution along the drip irrigation lateral lines is effected by many factors. Several of them are related to the external influences that are emitter clogging, emitter manufacturing variations, water temperature differences etc. The other important factor is friction losses along the lateral, which is related to construction and spacing of the drip emitter and pipe roughness. The pressure distribution along the lateral line changes with the friction loss and the lateral slope. These pressure changes along the lateral directly affect the flow rates of drip emitter in the lateral line. As a consequence, the water distribution uniformity in the field is negatively affected. This is especially important for full-turbulence flow and non-compensated drip emitters.

Drip irrigation laterals have multiple outlets depending on the emitter type and spacing. In general, the drip emitter flow rate and hydraulic pressure relation is characterized by following Equation (Von Bernuth & Solomon 1986).

$$q = kH^x \quad (1)$$

In Equation; q, flow rate of drip emitter (L h^{-1}); H, inlet pressure of drip emitter (m); k, flow coefficient

(L h^{-1} m^{-x}); x, exponent of inlet pressure. The flow coefficient (k) depends on the physical dimensions of the water passage paths in the drip emitter.

The pressure at anywhere along the lateral line, in other words the inlet pressure of the drip emitter at that point can be calculated by the following equation.

$$H_i = H_{i-1} - \Delta H_K \pm \Delta H_g \quad (2)$$

Where; H_i, i th drip emitter inlet pressure at anywhere along lateral line (m); H_{i-1}, previous drip emitter inlet pressure (m); ΔH_K, friction loss between two emitters (m); ΔH_g, pressure loss or gain due to the incline between two emitters (m).

Therefore, all friction and local losses based on the protrusion of drip emitters in laterals have to be considered for accurate evaluation the total friction losses along the laterals.

$$\Delta H_K = \Delta H_f + \Delta H_k \quad (3)$$

Several studies have been carried out on total friction and local losses for different type emitters. Inner diameter of the integrated cylindrical drip emitter is usually smaller than the pipe. As a consequence, this structure causes contraction of the flow paths at the up-stream of the drip emitter and the expansion of the flow paths downstream from the drip emitters. So, all frictional losses have to be considered in lateral design (Bagarello et al 1997; Juana et al 2002; Provenzano & Pumo 2004).

Provenzano et al (2005a) measured the total head losses for co-extruded laterals, and a new Equation was developed by considering the total local loss on account of the emitter connections. Besides these mathematical based studies, there are different approaches on determining of the head losses and required uniformity of water application. For instance, Anyoji & Wu (1987) used statistical methods, while Kang & Nishiyama (1996) applied finite element method in their studies. Demir et al (2007) developed mathematical model in drip laterals for in-line and on-line emitters using dimensional analysis to prediction total friction losses. Provenzano et al (2014) presented an empirical local loss prediction model for lay-flat drip laterals.

Computational Fluid Dynamics (CFD) is a method which is commonly used for the determination of the performance of product in the design, improvement of the product performance on computer and manufacturing of final product in optimal performance. Some of the advantages of using CFD are providing of minimum number of prototype production for test, decreasing of investment and time necessities. The studies conducted using CFD method in drip irrigation systems can be grouped into two main categories which are the dripper design and the lateral hydraulic. CFD was also applied in the studies related to drip irrigation system for only determining of flow characteristics on streamline and revealing of dripper design parameters (Wei et al 2006; Wang et al 2006; Zhang et al 2007).

The limited numbers of studies conducted using CFD about friction losses in drip irrigation laterals included local losses are summarized below:

Provenzano et al (2005b) and Provenzano et al (2007) evaluated the friction and local losses in laterals with pressure compensating in-line co-extruded emitters by using CFD method. They used standard k-ε turbulence model for CFD analysis obtained using at different Reynolds numbers and found that the differences of total friction losses obtained by experimental and CFD analysis varied between -4.7% and 10.9%. They stated that, the total friction losses in polyethylene laterals can be simulated by CFD method for low turbulence regimes very closely.

Palau-Salvador et al (2006), revealed the behavior of the flow around the protrusion of the on-line type dripper emitters in the lateral by means of CFD analysis that used the Reynolds Stress Model (RSM) and SIMPLE algorithm. They compared experimental and CFD analysis results of local loss data, and found the better simulation for the larger protrusion area and the turbulence.

In the literature, there were numerous analytical and experimental studies carried out to determine the frictional losses in the laterals, accurately and easily. In recent years, researchers continue to work on this subject. In addition, limited numbers of CFD simulation based studies have been carried out on determination of friction losses. However, there is not any study on the comparison of the simulation models.

The main objective of this study was to predict the total friction losses in drip irrigation laterals with cylindrical integrated emitters using CFD analysis method. The experimental data and CFD analysis results obtained by using different turbulence models and wall functions were compared, and it was tried to define the CFD simulation method and wall function which was in harmonious with the experimental data.

2. Materials and Methods

2.1. Experimental studies

In the study, two different drip irrigation laterals (A and B type) with cylindrical type drip emitters were used. The general properties of the drip irrigation laterals are given in Figure 1 and Table 1. Inner diameter of the pipe was determined by using volumetric method (Bagerello et al 1997), and a digital caliper (accuracy of ±0.01 mm) was used in order to measure other dimensions of the drip emitters (Bagarello et al 1997).

A schematic diagram of the test apparatus is illustrated in Figure 2. The total friction losses were measured with piezometric tubes at various flow rates in 6 m section in the middle of 10 m length laterals. The total discharge at the end of the lateral was measured by using volumetric method. For this aim drip emitter outlets were sealed, and discharge was regulated by valves. Water temperature was measured between 18 and 22 °C during the experiments. The flow velocities in lateral were calculated by measured flow rates. The relationship between the flow velocities and the total friction losses in laterals was revealed. The properties of the drip emitter and pipe flow characteristics are given in Table 2.

2.2. CFD analysis studies

The total friction losses of A and B type drip irrigation laterals at different inlet velocities of

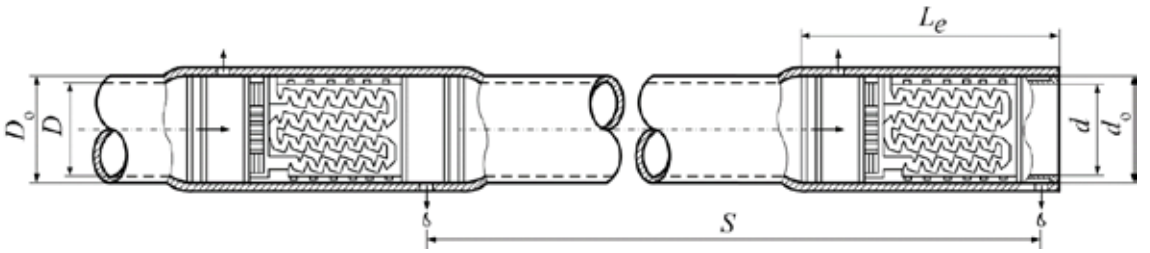

Figure 1- General properties of the drip irrigation laterals

Table 1- General dimensions of drip irrigation pipes and drip emitters

Type of drip irrigation pipe	*Pipe*		*Drip emitter*			*Drip emitter spacing*
	Outer diameter	*Inner diameter*	*Outer diameter*	*Inner diameter*	*Length*	
	D_o *(mm)*	*D (mm)*	d_o *(mm)*	*d (mm)*	L_e *(mm)*	*S (m)*
A	15.57	13.63	15.92	11.58	39.78	0.33, 0.50, 0.75
B	15.67	13.59	15.65	11.62	31.58	

Table 2- Properties of the drip emitter and pipe flow characteristics

Type of drip emitter	*Properties of drip emitter*			*Pipe flow characteristics***		
	Drip emitter flow rate q (L h^{-1})*	*k*	*x*	*Range of measured flow rate* Q (L h^{-1})	*Range of Reynolds number* R_e	*Number of exp.*
A	4.22	1.388	0.483	236.4-873.4	6079-22457	42
B	3.04	0.868	0.545	183.5-855.5	4728-22050	46

*, average drip emitter pressure: 10 m; **, the pipe flow characteristics obtained during friction losses measurements for all drip emitter spacing given in Table 1

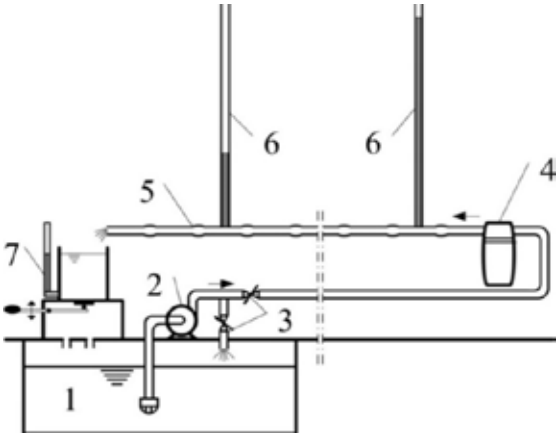

Figure 2- Experimental setup (1, reservoir; 2, pump; 3, valves; 4, disc filter; 5, experimental drip lateral; 6, piezometric tubes; 7, flow rate measurement unit)

water were calculated by using CFD software ANSYS Fluent 16.2 (ANSYS, Inc. Products USA Release 16.2).

2.2.1. Geometrical model and mesh generation

The geometrical models were created for 6 m length of A and B drip irrigation laterals for different drip emitter spacing (0.33, 0.50 and 0.75 m) by using ANSYS Design Modeller software. After created geometrical models, the mesh structures were formed by using ANSYS Meshing software (Figure 3). The number of nodes and elements in this mesh structures had more than 1.3×10^5 and 6.1×10^5, respectively. Elements are used to describe the area to be modeled. Elements are formed by joining nodes. The meshing quality parameters that are minimum orthogonal quality, maximum skewness and maximum aspect ratio occurred at the values of 0.23-0.26, 0.80-0.90 and 9.09-10.05, respectively.

2.2.2. Mathematical model

The flow can be described by the mass and momentum conservation equations. In the Newtonian, incompressible and steady-state flow condition, density of fluid is the constant, and the

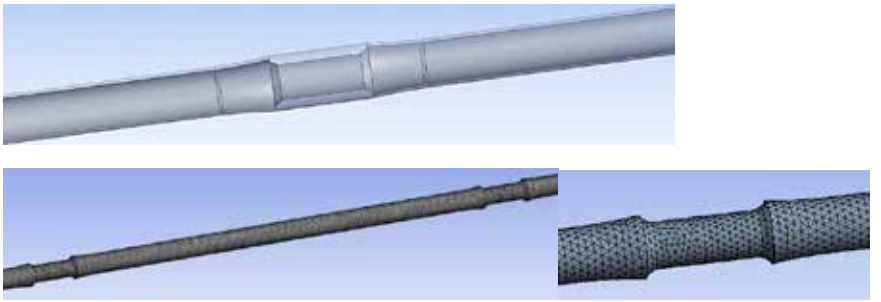

Figure 3- General view of the geometrical model and mesh structure

conservation of mass, or continuity equation is defined as:

$$\nabla \cdot \mathrm{v} = 0 \tag{4}$$

Similarly, an incompressible Newtonian fluid with constant viscosity, in vector notation of the Navier-Stokes Equations is defined as:

$$\rho\left(\frac{\partial \mathrm{v}}{\partial t} + (\mathrm{v}\cdot\nabla)\mathrm{v}\right) = -\nabla p + \rho \mathrm{g} + \mu\nabla^2 \mathrm{v} \tag{5}$$

In Equations; ∇ is the vector operator ($\nabla = \partial/\partial x + \partial/\partial y + \partial/\partial z$); v, mean velocity vector (m s^{-1}); ρ, density of fluid (kg m^{-3}); p, static pressure (Pa); g, acceleration of gravity vector (m s^{-2}); μ, viscosity of fluid (Pa s) (White 2001; ANSYS 2016).

For the numerical analysis of Navier-Stokes Equations in turbulence flow, the approach is called as Reynolds Averaged Navier Stokes (RANS) Equations for the variation of fluctuating velocity, pressure and other scalar quantities considering take the time-average. Various turbulence models are used in the RANS approach to analyze the Reynolds stress tensor term $\left(-\rho\overline{u_i' u_j'}\right)$ appropriately, taking into account the effects of turbulence.

Several researchers have reported that the commonly used turbulence models are *k-ε* turbulence model, *k-ω* turbulence model and Reynolds Stress Model (RSM) for vortex flow in drip irrigation laterals. The studies revealed that the turbulence models of *k-ε* and *k-ω* have given the similar results (Provenzano et al 2005b; Palau-Salvador et al 2006; Provenzano et al 2007; Vijiapurapu & Cui 2010).

In CFD analysis, standard *k-ε*, RNG *k-ε*, realizable *k-ε* turbulence models and Reynolds Stress Model (RSM) with Linear Pressure-Strain (LPS) were used for the friction loss calculations. For all turbulence models; standard wall function, non-equilibrium wall function and enhanced wall treatment were chosen as the Near-Wall Treatment.

2.2.3. Boundary conditions and solution methods

In ANSYS Fluent analysis; the fluid was chosen as water, it was assumed to be steady, incompressible, viscous, and non-gravity effect. The boundary conditions were selected as velocity-inlet and pressure-outlet of the drip irrigation lateral. All inlet flow velocity and outlet pressure values measured at various flow rates in the experiments were defined as the multiple parameters in CFD analysis. Hydraulic diameter values were taken into account. Surface roughness height of the internal pipe wall was accepted as 0.005 mm for PE pipes (White 2001). SIMPLEC (0) algorithms and Second Order Discretization Schemes were used in all solutions. In the study, a limit value of 250 iterations was accepted for the stability of the solution. The solution convergence accuracy was accepted to be 1×10^{-5}.

2.3. Statistical analysis

The mean absolute percentage error (MAPE) and the root mean square error (RMSE) were used to compare the differences between the experimental friction loss data and the predicted data using CFD models (Willmott & Matsuura 2005; Willmott et al 2012). The lowest values of MAPE and RMSE represent the highest model prediction. MAPE and RMSE error parameters were calculated by the following equations.

$$\mathrm{MAPE} = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{\Delta H_{Ki,Exp} - \Delta H_{Ki,CFD}}{\Delta H_{Ki,Exp}}\right| \tag{6}$$

$$\mathrm{RMSE} = \left[\frac{1}{n}\sum_{i=1}^{n}(\Delta H_{Ki,CFD} - \Delta H_{Ki,Exp})^2\right]^{1/2} \tag{7}$$

Where; $\Delta H_{Ki,Exp}$ is experimental and $\Delta H_{Ki,CFD}$ is the simulation values, n is the number of data.

3. Results and Discussion

In CFD analysis, standard *k-ε*, RNG *k-ε*, realizable *k-ε* and RSM with LPS turbulence models, and standard wall function, non-equilibrium wall function and enhanced wall treatment were considered.

The prediction of the total friction losses depending on the lateral type and drip emitter spacing was investigated for different near wall treatments. For this purpose, the CFD analysis results for standard wall function, non-equilibrium wall function and enhanced wall treatment by using standard *k-ε* turbulence model were compared with the experimental results. The comparison results for A and B type drip irrigation laterals are given in Figure 4.

Based on the Figures 4, it was found that the total friction losses were predicted very close to

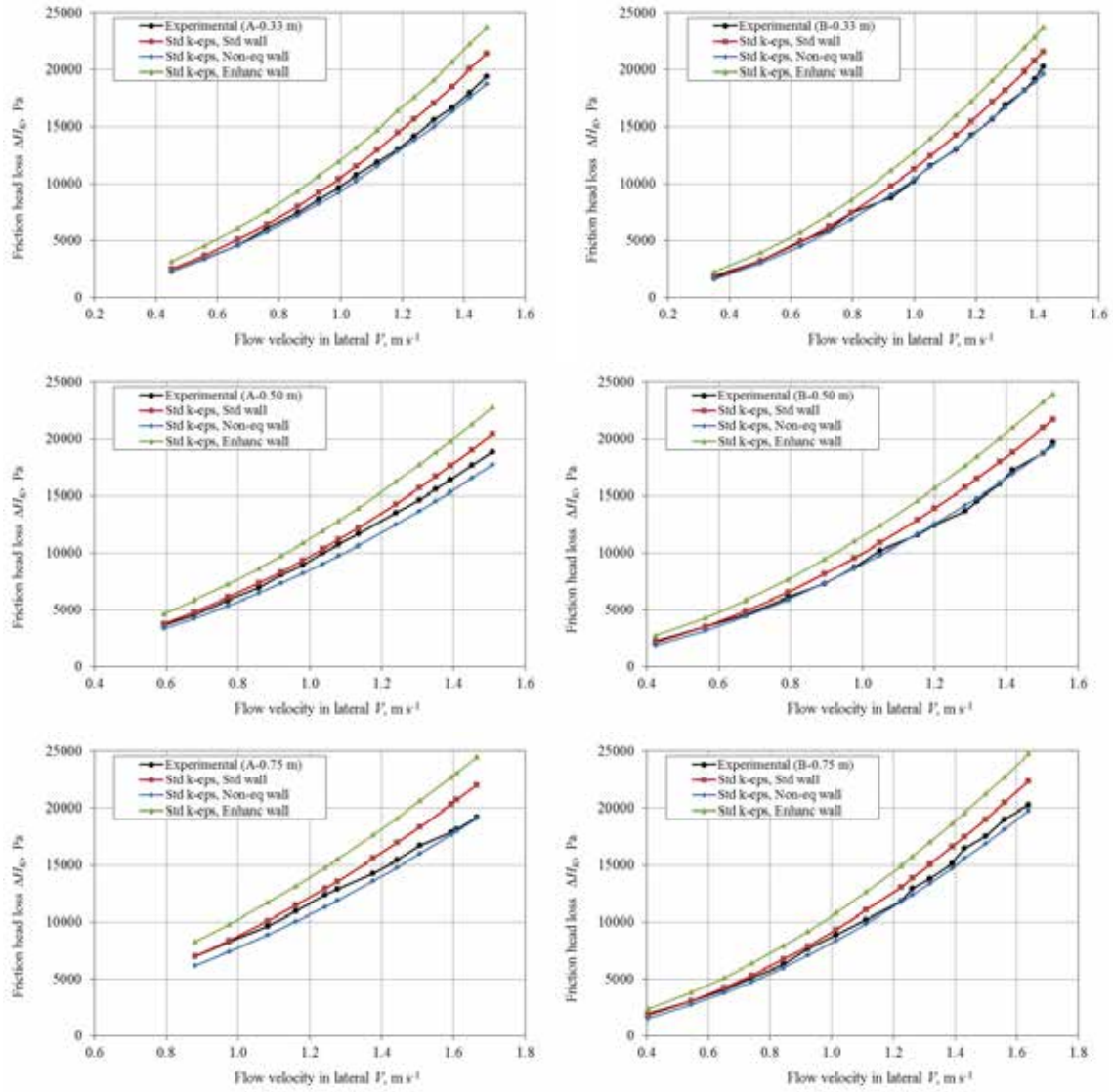

Figure 4- Comparison of experimental and standard *k-ε* CFD analysis results of total friction losses for A and B type drip irrigation lateral for different near wall treatments

experimental data by using non-equilibrium wall function approach in CFD analysis conducted in different near wall treatments of standard *k-ε* turbulence model. Using standard *k-ε* turbulence model with standard wall function approach the experimental data were predicted nearly similar at lower flow velocity. However, it was found that the prediction was negatively affected based on the increasing of velocity. The worst prediction was occurred using enhanced wall treatment.

The mean absolute percentage error (MAPE) and the root mean square error (RMSE) were calculated to compare for the performances of CFD models (Table 3).

As seen in Table 3, among those CFD simulation models, the Reynolds Stress Model (RSM) with Linear Pressure-Strain turbulence model using standard wall function had the minimum MAPE and RMSE values of 2.96 and 369 Pa, respectively. As seen in the table, the MAPE values in three turbulence models (realizable *k-ε* and RNG *k-ε* with standard wall function and standard *k-ε* with non-equilibrium wall function) were found approximately 5% while the RMSE values were found as 855, 738 and 550 Pa, respectively. Within these three turbulence models, the next closest prediction model was standard *k-ε* with non-equilibrium wall function turbulence model with the lowest value of 550 Pa of RMSE.

In addition to the error parameters, to show the harmony between the experimental and predicted friction loss values for four CFD turbulence models having lowest MAPE values are given Figure 5.

As shown in the Figure, realizable *k-ε* and RNG *k-ε* with standard wall function seem to be very similar to each other. These results also overlap with the error parameters results (Table 3). On the other hand, a good agreement between experimental results and the predicted values by CFD simulation models exist for standard *k-ε* with non-equilibrium wall function and especially RSM-LinPressStrain model with standard wall function (Figure 5).

Prediction values obtained with all turbulence models under the same near wall treatments were compared to the experimental total friction losses. Comparisons are given in Figures 6 and 7 for A and B drip irrigation laterals, respectively. The enhanced wall treatment was not considered in the comparison since it has the highest deviation (Table 3 and Figure 4).

It is clear from Figures 6 and 7 that different turbulence models may differently predict the experimental data based on the standard and non-equilibrium wall functions.

Figures 6 and 7 show that the highest predictions were obtained using RSM-LPS with standard wall function and standard *k-ε* with non-equilibrium wall function turbulence models for A and B type drip irrigation laterals in different drip emitter spacings.

These results were in harmonious with the other studies using the similar models (Provenzano et al 2005b; Palau-Salvador et al 2006; Provenzano et

Table 3- The MAPE and RMSE results for all simulation models

CFD models	*Mean absolute percentage error MAPE (%)*			*Root mean square error RMSE (Pa)*		
	Standard wall function	*Non-equilibrium wall function*	*Enhanced wall treatment*	*Standard wall function*	*Non-equilibrium wall function*	*Enhanced wall treatment*
Standard *k-ε*	7.51	4.87	23.29	1184	550	2856
Realizable *k-ε*	5.15	7.60	22.15	855	858	2673
RNG *k-ε*	4.61	8.65	21.29	738	917	2559
RSM-LinPressStrain	2.96	11.85	20.35	369	1276	2453

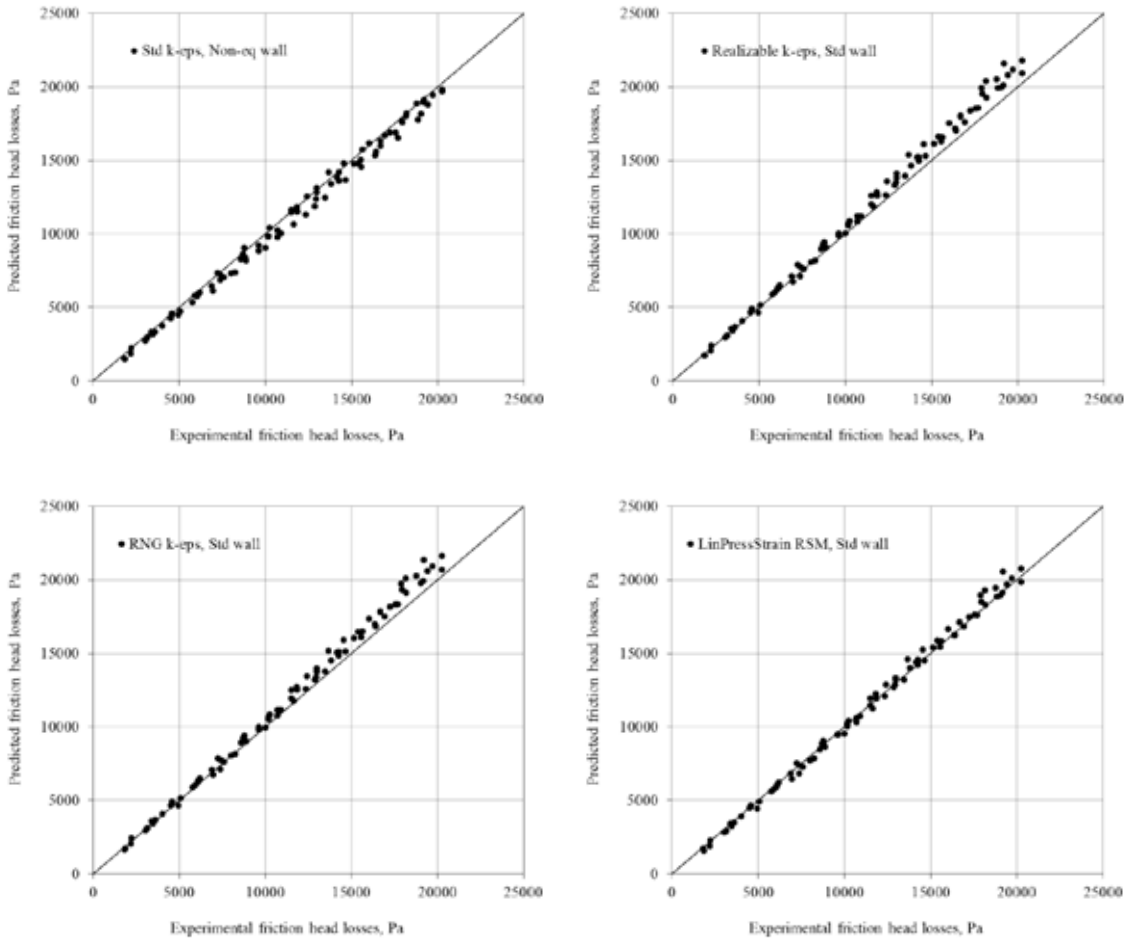

Figure 5- Comparison of the experimental and predicted friction head losses for considered closest prediction turbulence models

al 2007). Vijiapurapu & Cui (2010) used *k-ε*, *k-ω,* RSM and LES (Large Eddy Simulation) turbulence models at constant Reynolds number (*Re*= 100000) to determine the analyzing time using only straight lateral. They found that the head losses results, and *k-ε* and RSM turbulence models results were similar in their study.

The comparison of experimental and CFD analysis results that provide the highest prediction for turbulence models with near wall treatments for A and B type drip irrigation laterals in different drip emitter spacing are given in Table 4. Total friction loses were measured and calculated by CFD at 0.5, 1.0 and 1.5 m s^{-1} water flow velocity in lateral, and deviation between measured and calculated total friction losses for all data were determined as a percentage.

As seen from Table 4, the average percentage differences were found between the -7.66% and 12.43% for considered flow velocity. Also, the average percentage differences were found between the -3.42% and 4.54% for all measured data.

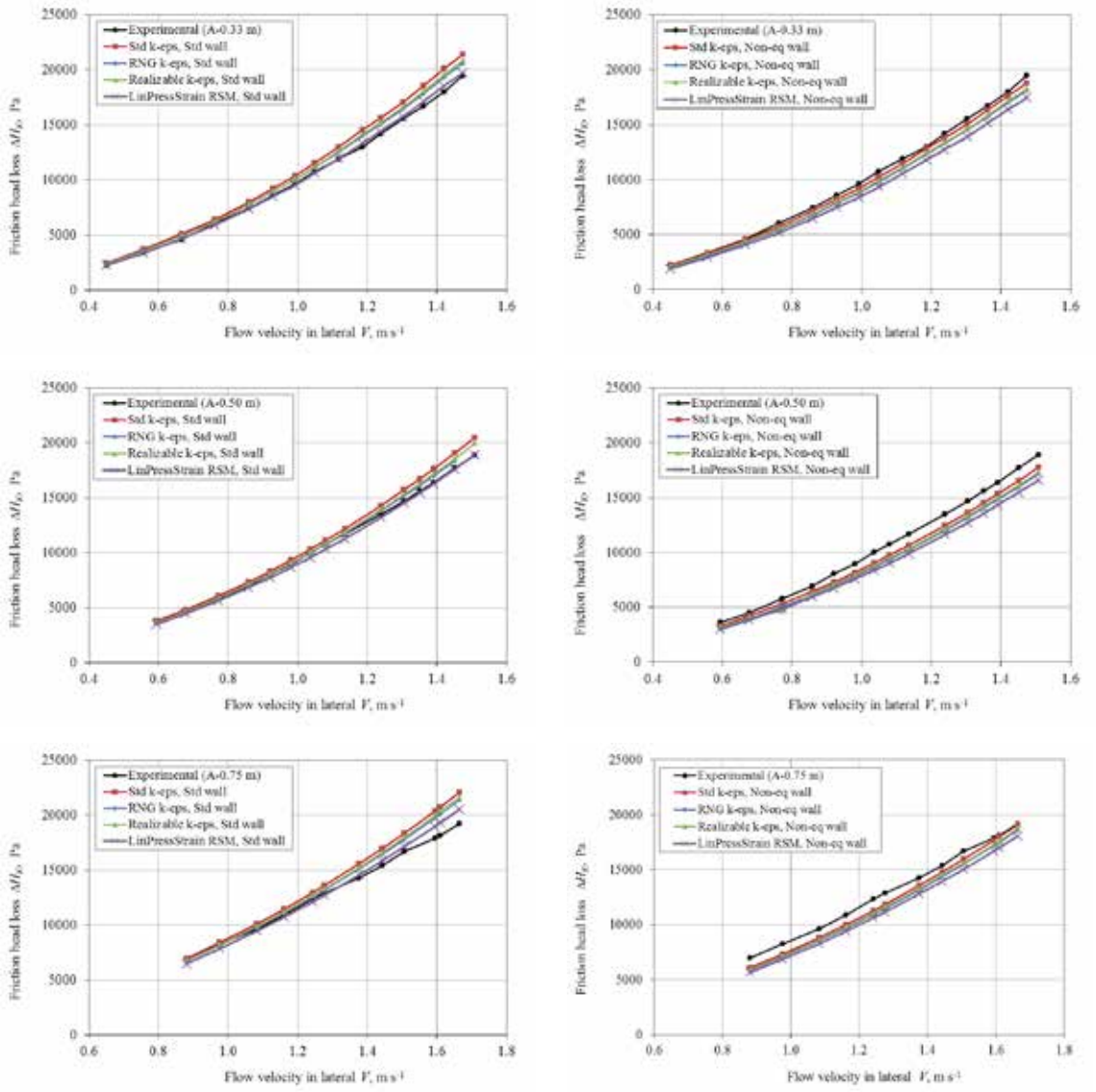

Figure 6- Comparison of experimental and CFD analysis results (*k-ε* and RSM turbulence models with standard and non-equilibrium wall functions) of total friction losses for A type drip irrigation lateral

These results had a similarity with Provenzano et al (2005b) and Provenzano et al (2007). They calculated the deviations between -4.7% and 10.9% using CFD analysis method.

The lowest average percentage difference of all data was found in RSM with LPS turbulence model using standard wall function with 0.92%. Similarly, the same model had the lowest error parameters as MAPE of 2.96% and RMSE of 369 Pa (Table 3). According to these results, it could be said that the RSM with LPS turbulence model using standard wall function was the closest prediction model for the total friction losses.

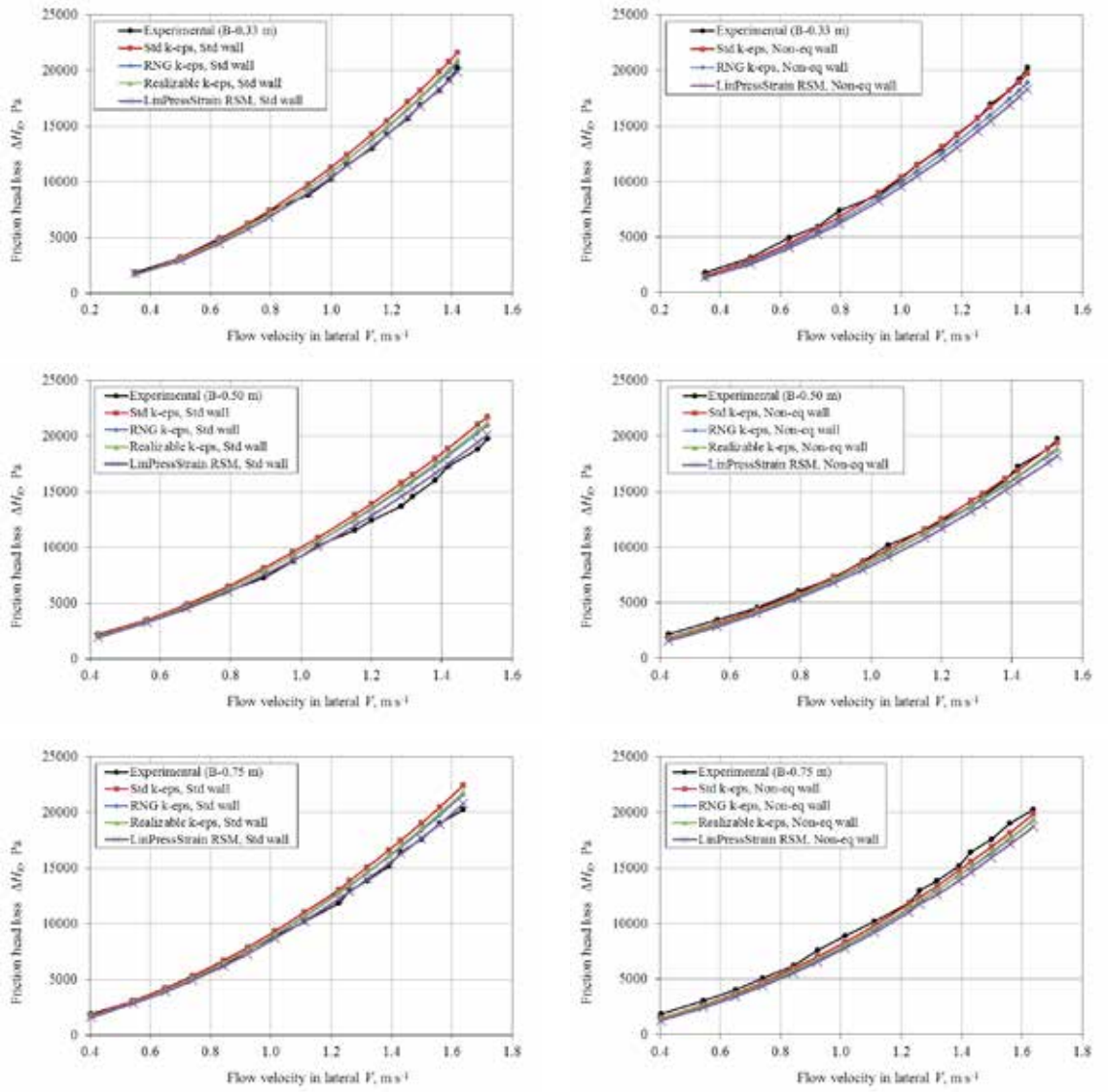

Figure 7- Comparison of experimental and CFD analysis results (*k-ε* and RSM turbulence models with standard and non-equilibrium wall functions) of total friction losses for B type drip irrigation lateral

An example of the pressure distribution along the lateral line due to friction loss according to CFD analysis was shown in Figure 8. The example includes the CFD analysis results for RSM-LPS turbulence model with standard wall function model applied for B type emitter (0.33 m emitter spacing and 1 m s^{-1} inlet velocity).

As can be seen in Figure 8, a considerable amount of friction loss was occurred in the lateral section that was between the sequence emitter spacings. Except this friction loss, the friction losses due to pressure changes resulting from sudden contraction and expansion based on the emitter are also clearly seen

Table 4- Prediction performance of selected CFD analysis models

Type of drip irrigation lateral	*Emitter spacing*	*Average flow velocity of water*	*Reynolds number*	*Exp. total friction loss*	*Predicted total friction loss by CFD analysis method using different turbulence models & near wall treatments*							
					Standard k-ε model & non-equilibrium wall	*Percent diff.*	*Realizable k-ε model & standard wall*	*Percent diff.*	*RNG k-ε model & standard wall*	*Percent diff.*	*RSM model LPS & standard wall*	*Percent diff.*
	S (m)	*V* (m s^{-1})*	*Re**	*$\Delta H_{K,Exp}$ (Pa)*	*$\Delta H_{K,CFD}$ (Pa)*	*%*	*$\Delta H_{K,CFD}$ (Pa)*	*%*	*$\Delta H_{K,CFD}$ (Pa)*	*%*	*$\Delta H_{K,CFD}$ (Pa)*	*%*
A	0.33	0.56	7517	3383	3318	1.92	3540	-4.64	3563	-5.32	3379	0.12
		0.99	13389	9630	9211	4.35	10025	-4.10	9994	-3.78	9509	1.26
		1.47	19895	19447	18761	3.52	20815	-7.04	20570	-5.78	19698	-1.30
	0.50	0.59	8015	3628	3325	8.36	3649	-0.58	3087	3.15	3472	4.32
		0.98	13224	8924	8175	8.40	9048	-1.38	10823	-5.61	8631	3.29
		1.45	19571	17701	16539	6.57	18568	-4.90	20704	-2.24	17583	0.67
	0.75	0.88	11871	6963	6097	12.43	6759	2.93	3658	-0.81	6441	7.49
		0.98	13162	8247	7348	10.90	8160	1.06	8996	-0.80	7843	4.90
		1.51	20318	16691	15972	4.31	17970	-7.66	18336	-3.59	17128	-2.62
B	0.33	0.50	6751	3187	2951	7.41	3064	3.86	4718	-3.46	2912	8.64
		1.00	13448	10248	10380	-1.29	10879	-6.15	9241	-5.88	10373	-1.22
		1.42	19092	20251	19678	2.83	20918	-3.29	18163	-5.23	19852	1.97
	0.50	0.68	9097	4560	4398	3.56	4709	-3.26	6750	3.06	4514	1.01
		0.98	13161	8728	8618	1.26	9276	-6.28	8128	1.44	8826	-1.12
		1.42	19065	17260	16911	2.02	18383	-6.51	17795	-6.61	17452	-1.11
	0.75	0.65	8770	4021	3741	6.95	4054	-0.82	4060	-0.97	3892	3.21
		1.01	13645	8875	8339	6.04	9033	-1.78	9022	-1.66	8650	2.54
		1.50	20176	17554	16882	3.83	18534	-5.58	18342	-4.49	17629	-0.43
Average percentage differences for all data, %						4.54		-4.06		-3.42		0.92

*, the average flow velocity and the Reynolds number for the average pipe diameter

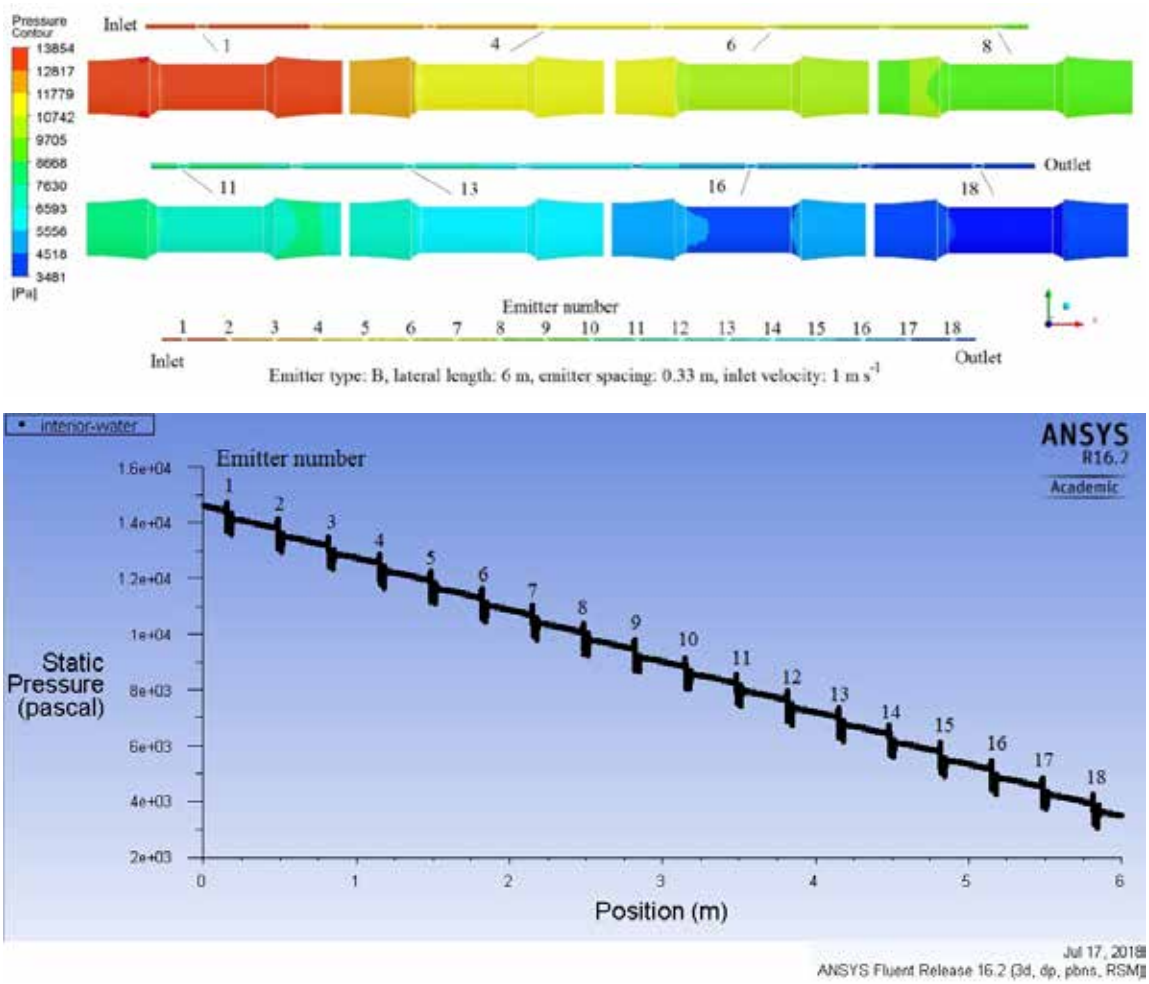

Figure 8- Pressure distribution along the lateral line with CFD analysis for RSM-LPS turbulence model with standard wall function

in Figure 8. Many researchers have expressed that the local friction losses need to be in consideration in the studies (Bagarello et al 1997; Juana et al 2002; Provenzano & Pumo 2004). The analysis results clearly showed the same necessity, too.

4. Conclusions

It can be concluded that the considered turbulence models in CFD analysis can be used in prediction of the total friction losses of drip irrigation laterals with high accuracy if the near wall treatments were considered in the analyzing.

The closest prediction of total friction losses to experimental results was obtained by RSM with LPS turbulence model using standard wall function (MAPE= 2.96%, RMSE= 369 Pa). The next closest prediction was achieved using standard k-ε turbulence model with non-equilibrium wall function with the lowest RMSE value of 550 Pa. It is thought that the study results would be beneficial

for researchers and manufacturers working on this subject.

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Barley Leaf Stripe Disease in Algeria: Evaluation of Virulent *Pyrenophora graminea* Isolates and Identification of Resistant Algerian Barley Genotypes

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ABSTRACT

Nine isolates of *Pyrenophora graminea*, barley leaf stripe disease causal agent, collected from several regions in Algeria were evaluated under greenhouse conditions for their virulence to a collection of barley cultivars including three most cultivated Algerian varieties. Virulence levels were observed among the set of isolates and a mean disease rate ranging from 3.33% to 75.83% was found. Pathogenic variability of *P. graminea* and resistant gene effects in barley cultivars were revealed. Isolate OS was the most virulent among *P. graminea* isolates making it a suitable virulent isolate in future breeding programs. A set of 8 barley genotypes composed of common Algerian cultivars and local developed lines were tested for their reaction to *P. graminea* and yield response. Barley cultivar Minnesota 23 and line 18/17/7L2 were the most resistant of the collection with high grain number/ear and thousand grains weight even when diseased. These genotypes could be useful to integrate as candidate genitor plants into barley breeding programs to develop resistant cultivars to leaf stripe disease.

Keywords: Barley stripe disease; Aggressiveness; Barley; Resistance; Breeding; Algeria

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the largest sown crops in the world. In Algeria, barley is a strategic crop and occupies an important place after durum and bread wheat. Thirty five to 40% of crop areas were reserved for cereals in Algeria (Benmohammed 2004; Rahal-Bouziane 2015; Zairi et al 2016) and a production around 1400 to 2000 kg ha^{-1} occurs (ONFAA 2015). Barley is heavily devastated by barley leaf stripe disease, a widely disseminated seed-born disease caused by the fungal pathogen *Pyrenophora graminea* (Ito & Kuribayashi) (anamorph *Drechslera graminea* ((Rabenh ex. Schlech) Shoemaker). This disease decreases barley yield and quality and induces important economic losses in many countries (Porta-Puglia et al 1986; Arabi et al 2004; Karakaya

et al 2016). It is the most important barley disease in Algeria. Benbelkacem et al (2000a) evaluated the mean incidence of barley leaf stripe averaging 27.94%, which corresponds to one_third of the Algerian potential barley production and estimated an overall yield reduction of 29 kg per hectare for each 1% of the disease incidence. Fungicides are effective for reducing the severity of this disease, but the most practical and environmentally sound means of control is through the use of resistant cultivars (Arabi & Jawhar 2005). Developing resistant cultivars is the best method environmentally and effective one for disease control. However, to breed for resistance to disease, a solid knowledge of the virulence variation information related to pathogen is necessary (Arabi & Jawhar 2012).

The objective of the present study was to investigate the most aggressive isolate of the *P. graminea* among a collection from different regions of Algeria and to evaluate the reaction and yield response of most cultivated Algerian varieties and local selected lines, under greenhouse conditions to *P. graminea*. The study was also carried out in attempt to develop a breeding program for improving barley local cultivars to leaf stripe disease.

2. Materials and Methods

2.1. Fungal isolates

Barley leaves showing *P. graminea* stripes, collected from different regions of Algeria, were used to obtain the fungus isolates (Table 1). Infected leaves were cut into pieces and sterilized using 5% sodium hypochlorite solution (NaOCl) for 5 min and then washed three times with sterile distilled water. Under sterile conditions and using a binocular loupe, a single spore was removed with a handle and put into Potato Dextrose Agar (PDA) medium. The Petri dishes were left for 7 days of incubation at 21±1 °C in the dark. All isolates were derived from single spores cultures.

2.2. Aggressiveness test

Nine isolates of *P. graminea* were assessed for their aggressivity on four barley varieties, known for their reaction to *P. graminea* which ranged from susceptible to resistant (Table 1): Rihane 03 (ICARDA), Saïda and Tichedrett (Algeria) and Minnesota 23 (USA). Rihane 03, Saïda and Tichedrett are frequently cultivated in Algeria whereas Minnesota 23 is experimentally used. The inoculum was prepared according to Hammouda (1986) modified sandwich method. Under aseptic conditions, barley seeds were disinfected, following leaf surface sterilization protocol previously described, and put onto a half part of an 8 days old mycelia culture plates, and the other half of the fungal colony was flipped over seeds. Each prepared plate was sealed. For control treatment, seeds were placed between two half of PDA medium without *P. graminea*. The seeds were incubated at 6 °C for 14 days in the dark. Inoculated and uninoculated seeds were carefully planted into 25 cm diameter plastic

Table 1- *P. graminea* isolates and barley genotypes used in this study

Fungal isolates	*Region*	*Algerian geographic area*	*Barley genotypes*	*Origin*
SST	Sidi taleb/Setif	East	Minnesota 23	USA
BBN	Biskra	South-East	Rihane 03	ICARDA
IP48	Algiers	North	Saïda	Algeria
STF	Setif	East	Tichedrett	Algeria
OS	Algiers	North	P11L4 and	Line developed from Algerian
MSK	Mascara	North-West	P48/L2	Populations 48 and 11 (*)
SFOU	Fouara/Setif	East	18/17/7L2	Doubled haploid line (*)
AD	Aind Defla	South-West	18/3/2BL2	Genealogical line (*)
MBS	Medea	South-West		

(*), developed by Professor Mekliche L.

pots, at rate of 10 seeds per pots and placed under greenhouse conditions in a randomized complete block design, with three replicates. Infected and uninfected plants were scored at heading stage (GS 50) (Zadoks et al 1974). To estimate infection level, percentage of infected plants was calculated, according to Delogu et al (1989) scale, described as follows: highly resistant (*HR*) (0-5% of infected plants), resistant (*R*) (6-11%), moderately resistant (*MR*) (12-26%), susceptible (*S*) (27-78%) and highly susceptible (*HS*) (79-100% of infected plants).

2.3. Assessment of barley cultivars' reaction to leaf stripe and grain production components

Eight barley genotypes were used to evaluate their reaction to the most virulent isolate of the nine *P. graminea* isolates collection. These eight genotypes are composed of the four varieties described above and four lines (Table 1) developed by Professor Mekliche L. at our Plant Production Department. Inoculated and control seeds were treated as previously described and then carefully removed from Petri dishes and planted, in greenhouse, directly to cement vats previously filled with soil. In this experiment, randomized complete block design with three replicates was used. Infected and healthy plants were counted at heading stage and infection level was assessed according to Delogu et al (1989) scale as described above. At maturity, plants of each plot were harvested to evaluate grain number per ear (NG/E) and thousand grain weight (TGW).

2.4. Computation and data analysis

Statistical ANOVA and homogeneous groups (Newman-Keuls tests) analyzes were performed to evaluate differences between experienced different factor values.

3. Results and Discussion

3.1. Evaluation of isolates' aggressiveness

Highly significant virulence levels were observed among five separated homogenous groups (Tables 2 and 3). The mean disease rating was ranged from 3.33% to 75.83%. Çetin et al (1995) observed infection ranging from 15.4% to 96.3%. Isolate OS was the most virulent on four cultivars. Therefore, this isolate may be considered as a suitable virulent isolate in the future plant-breeding programs. MBS isolate was found as the least virulent (Table 3). Reaction of cultivars to *P. graminea* isolates showed highly significant differences (Tables 2 and 3). The cultivar Minnesota 23 was shown to be resistant to all pathogen isolates with <5% of disease reaction. Rihane 03 was resistant to 7 isolates. However, the varieties Saïda and Tichedrett were the most susceptible ones and were resistant to only three isolates. The cultivars Minnesota 23, Rihane 03, Saïda and Tichedrett were suitable for defining virulence because they all provided clear response to the pathogen isolates investigated, whether in aggressiveness test or in barley reactions. They revealed a high level of variability in their reactions. Similar reactions were found by other researchers. Bayraktar & Akan (2012) also found resistant cultivars with <20% of disease reaction. In this study, Minnesota 23 genotype was the most resistant, Rihane 03 was intermediate, Tichedrett and Saïda were susceptible. These results are in agreement with those obtained by Benbelkacem et al (2000b). Analysis of variance showed also highly significant interaction of isolates and cultivars (Table 2) suggesting that cultivars reacted differently to the isolates. Based on those differential reactions between different cultivars and several isolates collected from diverse regions, specific resistance gene effects may exist in the host barley cultivars. Consequently, this indicates the presence of pathogenic variability of *P. graminea* isolates. Variation in pathogenicity of *P. graminea* isolates was mentioned by many researchers (Zriba & Harrabi 1995; Delogu et al 1995; Benbelkacem et al 2000b; Aminnejad et al 2009; Bayraktar & Akan 2012; Çelik et al 2016; Karakaya et al 2017). However, groups clustering showed that isolates OS, IP48, MSK and MBS constituted by themselves as statistically different individual groups (Table 3), suggesting that those isolates can be different distinct physiologic races. On the other hand, Algerian East and

South-West isolates BBN, SFOU, STF, SST, AD (Table1) were gathered into one group (Table 3), with no differential virulence, suggesting that they might be related to the same pathotype. This can be explained by the fact that *P. graminea* is exclusively seed-borne and that Eastern Algerian farmers often supply their needs of barley seeds from the south-western regions and vice versa. The same case happens to South-Western Algerian farmers. Such results were also reported by Benbelkacem et al (2000b).

3.2. Evaluation of barley cultivars' reaction

Differences were observed in the reaction of barley cultivars (Table 2) indicating a high level of variability among barley collection for leaf stripe severity. The line 18/3/2BL2 was highly susceptible barley genotype with disease incidence of 90% followed by Tichedrett (80%). The genotypes P11L4, P48/L2 and the varieties Saïda and Rihane 03 were susceptible with disease incidence ranging from 50 to 77%. The line 18/17/7L2 was moderately resistant (23%). The cultivar Minnesota 23 was highly resistant with 3% disease incidence. Cultivar Minnesota 23 proved to be the most resistant one all through the aggressiveness and the reaction variability tests.

Differences among the reactions of the barley cultivars and lines to the isolates of the barley leaf stripe fungus were also reported by different authors (Ulus & Karakaya 2007; Bayraktar & Akan 2012; Çelik et al 2016; Karakaya et al 2017; Çelik Oğuz et al 2017).

Table 2- Mean squares (MS), degrees of freedom (df) and coefficients of variation (CV) from analysis of variance for the studied traits

Studied traits	*Disease rating aggressivity (%)*		*Incidence (OS isolate inoculation) (%)*		*Number of grain/ear*		*1000-grain weight (g)*	
Sources	*df*	*MS*	*df*	*MS*	*df*	*MS*	*df*	*MS*
Cultivars	3	2.538***	7	0.235***	7	140.952***	7	103.356***
Isolates	9	1.386***	1	8.626***	1	833.333***	1	1631.934***
Varieties isolates	27	0.173***	7	0.235***	7	27.048***	7	166.009***
Error	54	0.006	14	0.023	14	2.610	14	0.698
CV(%)		1.639		5.494		8.073		1.741

***, significant at P<0.001

Table 3- Mean disease aggressiveness rating of *P. graminea* isolates

Isolates	*Mean disease rating (%)*	*Mean disease rating on barley cultivars (%)*			
		Minnesota23	*Rihane03*	*Saida*	*Tichedrett*
OS	75.83 a[y]	10.00 R	96.67 HS	96.67 HS	100.00 HS
IP48	49.17 b	0.00 HR	56.67 S	83.33 HS	56.67 S
MSK	30.83 c	0.00 HR	23.33 MR	66.67 S	33.33 S
BBN	16.67 d	3.33 HR	0.00 HR	23.33 MR	40.00 S
SFOU	14.17 d	0.00 HR	0.00 HR	26.67 S	30.00 S
SST	14.17 d	0.00 HR	0.00 HR	36.67 S	20.00 MR
STF	14.17 d	0.00 HR	0.00 HR	26.67 S	30.00 S
AD	10.83 d	0.00 HR	0.00 HR	23.33 MR	20.00 MR
MBS	3.33 e	0.00 HR	0.00 HR	13.33 MR	0.00 HR

3.3. Evaluation of grain number per ear and thousand grains weight components

Number per ear and thousand grains weight of inoculated barley cultivars was significantly reduced compared to their associated controls and this decrease varied largely between barley genotypes (Table 4). The disease reduced the NG/E of the resistant cultivars Minnesota 23 and 18/17/7L2 by 29% and 18%. For susceptible cultivars, reduction of NG/E ranged between 19% to 87%. On the other hand, the disease did not affect TGW of the resistant cultivar Minnesota 23 and line 18/17/7L2 while for susceptible genotypes Rihane 03, P11/L4, Saïda, Tichedrett, P48/L2 and 18/3/2BL2, disease impact reduced TGW of 9%, 12%, 54%, 56%, 80% and up to 100% respectively. This study showed that inoculation with *P. graminea* affected grain number per ear and thousand grain weights of diseased cultivars considerably. Arabi et al (2004) reported that the thousand grain weight was affected negatively when barley plants were inoculated with *P. graminea.* However, in our study, the resistant cultivar Minnesota 23 and line 18/17/7L2 showed high number/ear and thousand grain weight while the susceptible ones scored reduced values of these traits. Arabi et al (2001) showed that *P. graminea* had a direct impact on element storage (as proteins) of susceptible cultivars, whereas no effects were detected in the resistant ones. In our study, the resistance demonstrated in Minnesota 23 and 18/17/7L2 approached closely to those reported in Arabi et al (2001) study. Therefore, it is suggested that cultivar Minnesota 23 and line 18/17/7L2 should be integrated in breeding studies for introducing leaf stripe disease resistance into most cultivated and high yielding varieties.

4. Conclusions

The present study showed pathogenic variation among Algerian *P. graminea* isolates and provided insights about reactions of Algerian barley cultivars and resistance sources to barley leaf stripe disease. The agressiveness of the *P. graminea* isolate OS could make it suitable as the virulent isolate in future plant breeding programs. The barley cultivar Minnesota 23 and the line 18/17/7L2 were good sources of resistance and may be useful in developing resistant cutivars. Although our sample size was limited, these prelimenary results indicated presence of good resistance gene effects in Algerian barley cultivars and varieties and diversity in the *P. graminea* pathogen populations of Algeria. For more solid protocols to assist breeding programs, a larger set of isolates collected from different localities should be used and more barley cultivars should be investigated and screened under field conditions.

Table 4- Incidence of *P. graminea* (inoculated with OS isolate) in 8 barley genotypes and its effect on the grain number per ear and 1000-grain weight (g)

Cultivars	*Barley genotypes reactions*		*NGE*			*1000-grain weight*		
	Incidence (%)	*Reaction type*	*Mean*		*Reduction rate (%)*	*Mean*		*Reduction rate (%)*
			Uni.	*Ino.*		*Uni.*	*Ino.*	
18/3/2BL2	90 a[y]	HS	39.33 b	21.00 e	87	52.67 a	23.00 h	129
Tichedrett	80 a	HS	29.67 e	23.00 ge	29	44.67 ef	28.67 f	56
P48/L2	77 a	S	31.67 de	26.33 efg	20	45.67 de	25.33 g	80
Saïda	63 ab	S	32.67 de	25.00 fg	31	47.67 bc	31.04 e	54
Rihane 03	53 ab	S	37.67 bc	31.67 bcd	19	44.67 ef	41.00 c	9
P11L4	50 ab	S	34.67 cd	28.33 def	22	39.67 g	35.33 d	12
18/17/7L2	23 b	MR	43.66 a	37.00 a	18	48.67 b	46.67 a	4
Minnesota23	3 c	HR	42.67 a	33.00 b	29	43.67 f	43.00b	2

[y], different letter, means significantly different at P<0.05 (Newman-Keuls test)

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The Effects of Different Growing Media on Growth, Flowering and Quality of *Petunia grandiflora*

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ABSTRACT

A study was carried out to investigate the effects of growing media on physiology, flowering behaviour and longevity of potted petunia plants (*Petunia grandiflora* Juss.) under green house condition. A total of 30 potted petunia plants were used in this experiment and five different growing media namely top soil (control), vermicompost, biochar, cocopeat and peatmoss were tested. Leaf area, lateral branching, stomatal conductance and net photosynthetic rate significantly increased when the plant was grown in peatmoss and cocopeat. The highest length of lateral branch was recorded in the control treatment. In addition, peatmoss and cocopeat medium increased the number of flower, flower diameter, weight of individual flower and petal thickness significantly. Furthermore, chlorophyll *a* & *b* contents in leaves, carotenoids and anthocyanin content in flowers were also increased significantly with peatmoss and cocopeat applications. Ethylene production was reduced significantly and flower longevity of potted petunia plants increased in different growing media. There was a positive correlation between petal thickness and flower senescence, and between anthocyanin content with flower senescence in potted petunia plants. It can be concluded that peatmoss and cocopeat growing media improved the growth, quality and longevity of potted petunia flowers under green house condition.

Keywords: Growth; Development; Flower; Floriculture; Medium; Quality

1. Introduction

Petunia plants are characterized by a short life cycle, color diversity, compact plant size, and relatively easy growth. Petunias are perennials in warm climates and used in building decoration, private garden and landscape, pharmaceutical and cosmetic industries, and phytoremediation. It has been reported that flowering capacity of petunia plants can be influenced by breeding and cultivation technologies (Nishijima et al 2006). Growth regulators and substrates influence the plant growth, flowering potential and longevity of potted flowering plant (Khandaker et al 2010). Nowadays, a very important aspect in the production of potted flowering plants is the controlled and induced vegetative and floral growth by horticultural practices.

Growth medium are known to have been effective in value adding of potted ornamental plant, and should have a best characteristics such as proper aeration, water holding capacity and adequate nutrient supply (Khobragade et al 1997). Besides that, growth medium plays an important role in physiological parameters such as plant height, number of leaves, number and diameter of florets per spike, and yield. It has been reported that the optimum amount of nutrient and environmental factors affect the plant growth, development and flowering of petunia (Zhanga et al 2012)

Petunia seedlings are commonly produced in the greenhouse in mixtures of commercial soilless bedding plant growth media, and sometimes the growth and flower production of petunia plants is low due to unsuitable growing media. In this study, several types of growing media were used as growing components of petunia to select the best adequate growing medium for managing ornamental potted plants. Currently, there is little information available on the effect of growing media on growth, flowering and longevity of petunia flowers. Besides that, the petunia flower has short longevity and floral longevity has received very little attention from ornamental plant breeders. Moreover, no information is available in the literature about the longevity of the petunia flowers. The findings of this study will help to increase floral longevity of petunia as well as other potted flowering plants which will create a significant value to further develop the floriculture industry. It is proposed that different growing media can enhance the growth, development and quality of petunia flowers under green house condition.

2. Materials and Methods

2.1. Plant materials and treatment setting

The study was conducted at the greenhouse and laboratories of the Faculty of Bioresources and Food Industry, University Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia from June 2015 to May 2016. The plant material used in this experiment was *Petunia grandiflora* cultivar pink. All the collected petunia seedlings are produced from sexual reproduction. The seedlings of the cultivar approximately 15 cm height were selected from the Beauty Garden, a commercial nursery located at Sungai Buloh, Selangor, Malaysia. Five different growing media; cocopeat (pH= 4), biochar (pH>9), vermicompost (pH= 6.8), peatmoss (pH= 4.5), and clay loam top soils (pH= 6.5) as control were used. The seedlings were transferred in to 8 cm diameter and 12 cm height size polybags that contain different growing media. After transplanting in the growing media, pinching back of terminal bud of all the seedlings were carried out to encourage branching to produce a bushy growth. The growing temperature in the greenhouse was 24±2 °C with maximum PAR of 500-1000 $\mu Em^{-2} s^{-1}$ and relative humidity of 60% to 90%. A thoroughly watering regime by a handheld sprayer was applied every day. Thirteen gram (13 g) NPK fertilizers (13:13:21) (Baja Yara mila) per polybag were applied once in every two week interval. Two months after transplanting all the petunia seedlings started flowering.

2.2. Preparation of media and transplanting

The growing media was placed into 8 cm-diameter x 12 cm-height polybags, and proper care was taken for uniform filling of all pots by soft tapping to maintain equal compaction levels. The seedlings of petunia with uniform size and age was transplanted (one seedling per polybag) when they have approximately ten (10) leaves each.

2.3. Measurements of physiological and flowering parameters

A young fully expanded leaf from each petunia plant was selected and leaf area was measured by using the Leaf Area Meter (CI-202, CID Bio-science, USA). The average value was recorded and leaf area was expressed in cm^2. The number of bud, number of flowers, length of lateral branch, number of lateral branch, weight of individual flowers, petal thickness and flower longevity, were determined after one (1) month of transplanting of the seedling into polybags. Five flowers from the five potted

petunia plants per treatment were selected for the measurement of petal thickness. Petal thickness of petunia flowers was measured using a Mitutoyo Vernier Scale. Five buds per pot were selected for blooming and longevity measurements. At blooming phase of petunia, eighteen leaves from six plants per treatment were selected for the measurement of plant physiological characteristics. All the measurements were taken from the center of the three (3) youngest fully expanded leaves of each plant. Relative leaf chlorophyll content was measured by using a handheld Chlorophyll Meter (SPAD-502; Minolta Japan). Three data for leaf chlorophyll content and stomatal conductance were recorded per plant and the average was calculated. Stomatal conductance of a leaf was measured by using a portable Porometer (Leaf Porometer, Model SC-1, USA). Photosynthetical characteristics of potted petunia plants were recorded from 11 am to 2 pm every two weeks. Net photosynthetic rate of potted petunia plants was determined, in terms of μmol CO_2 fixation m^{-2} s^{-1}, to measure the activity level of the photosynthetic Carbon metabolism. Data for net photosynthetic rate was measured using a handheld Photosynthesis System (C1-340; CID Bio-Science, USA). Three replications of readings were recorded per plant from each treatment and the data was recorded once during the experimental period.

2.4. Determination of pigments content and ethylene production

The chlorophyll (chl. *a* & chl. *b*) contents of the control and treated petunia leaves were determined according to the methods described in Khandaker et al (2013). Carotene content was measured by using Spectrophotometer (Genesys 20, Thermo Scientific, USA). All the pigments contents were determined at the full blooming stage of petunia flower (5 days after petal opening). The concentration of carotenoids was measured by using a formula of Arnon (1949). The formula to calculate the pigments concentration is as shown below:

Carotenoid ($\mu g\ g^{-1}$)= [A480+(0.114×A663)-(0.638×A645)]/112.5

The total anthocyanin content of the hydrophilic extracts of petunia petals were measured by the pH-differential method using cyanidin-3-glucoside as a standard, as described by Rodriguez-Saona et al (1999). At 5^{th} days of flower opening, petal carotenoid and anthocyanin contents were determined once during the study period. Flower ethylene production of potted petunia plants were carried out according to the method described in Khandaker et al (2013). Six flowers from six plants for each treatment were collected once at fully bloom stage and air samples were analyzed for ethylene using a Shimadzu GC-14A Gas Chromatograph. The ethylene production rates were calculated and expressed as nL $flower^{-1}$ h^{-1}.

2.5. Statistical analysis

A Completely Randomized Design (CRD) with six replications was used for the treatments in the experiment. Statistical analysis was performed by using SPSS 20 software (SPPS Inc). All data were analyzed according to one way repeated ANOVA. The means were separated using Fisher's protected least significant difference procedure when the F test indicated significance at $P \leq 0.05$.

3. Results

3.1. Leaf area, number and length of lateral branch

Our results showed that, leaf area significantly affected by growing media (Table 1). The highest leaf area was recorded in peatmoss treatment, followed by cocopeat and vermicompost treatment. While, the smallest leaf was found in control treatment (Figure 1). The number and length of lateral branch differed significantly among treatments (Table 1). The highest value (6.4) was observed in cocopeat treatment followed by BioChar (6.0), while the lowest value (5.4) was obtained with vermicompost treatment. The highest length of lateral branch was obtained with control treatment (49 cm) followed by cocopeat (46 cm). The lowest length was recorded in peatmoss treatment at 27.86 cm.

Table 1- Effects of different growing media on growth and physiological characteristics of potted *Petunia grandiflora* plants

Treatment	*Leaf area (cm^2)*	*No of lateral/ branch*	*Length of branch (cm)*	*Stomatal cond ($mol\ m^{-2}s^{-1}$)*	*Photosynthesis ($\mu mol\ CO^2\ m^{-2}\ s^{-1}$)*
Control	1.40±0.20 c	5.80±0.90 b	49.00±0.80 a	0.35±0.20 c	6.00±0.42 c
Vermicompost	1.50±0.20 c	5.40±0.24 c	35.00±0.60 c	0.48±0.20 b	7.80±0.72 a
Biochar	1.40±0.20 c	6.00±0.30 a	39.00±0.50 c	0.40±0.20 b	6.50±0.42 b
Cocopeat	1.60±0.20 b	6.40±0.20 a	46.00±0.30 b	0.61±0.20 a	8.00±0.91 a
Peatmoss	1.80±0.20 a	5.80±0.30 b	28.00±1.20 d	0.72±0.20 a	8.50±0.72 a

Data was present in means (± SE). Different means in same column followed by same letter are not different according to LSD test at P= 0.05 of probability

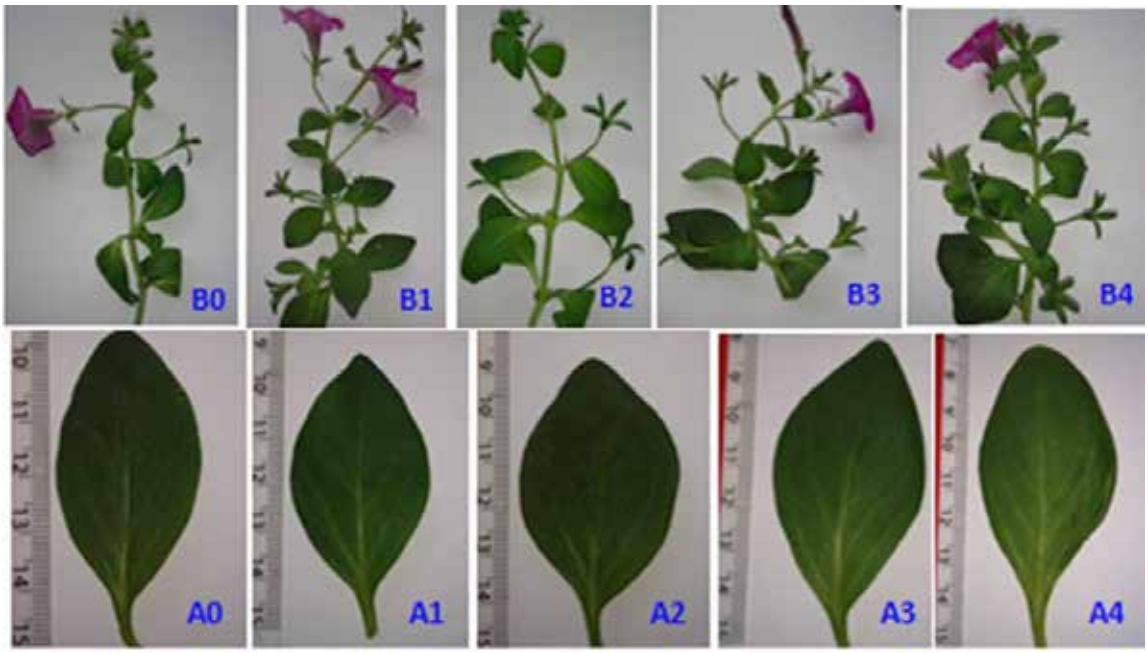

Figure 1- The effect of different growing media on number of flower bud (B) and leaf area (A) of potted petunia plants. 0, Control; 1, Vermicompost; 2, Biochar; 3, Cocopeat and 4, Peatmoss

3.2. Stomatal conductance and photosynthetic rate

Different growing media significantly affect the leaf stomatal activity of potted petunia plants. Stomatal conductance was the highest in peatmoss, followed by cocopeat and vermicompost treatment with a value of 0.72, 0.61 and 0.48 mol $m^{-2}\ s^{-1}$, respectively. While, the lowest stomatal conductance was recorded in the control plant at 0.35 mol $m^{-2}\ s^{-1}$ (Table 1). In this study, different growing media produced significant effect on photosynthesis of petunia plants. The results showed that net photosynthetic rate of petunia plant was the highest in peatmoss treatment, followed by cocopeat and vermicompost treatment, whereas, the control plant showed the lowest photosynthetic rate (Table 1).

3.3. Number of flower bud and flower

The number of buds produced were significantly different among treatments at P≤0.05 (Table 2). As can be seen from Figure 1, the highest bud production was obtained with BioChar (21.8) followed by peatmoss (20.13) while, the lowest number of bud was recorded in control (3.2). The maximum flower production highlights their adaptability to suitable environment. Based on the results of Figure 2 and Table 2, the mean number of flowers were significantly different between the treatments and control at P≤0.05. The number of flowers per plant was the highest with cocopeat treatment (32) followed by peatmoss (29) while, the lowest number of flower was obtained in vermicompost with the mean value of 21.

3.4. Diameter of flower, weight of individual flower and petal thickness

Based on Table 2 and Figure 2, the results showed that diameter of flower differed significantly among the growing media. The peatmoss treatment had the highest value (52 mm) followed by vermicompost (47 mm) whereas, the lowest flower diameter was recorded with BioChar treatment (41 mm). The different growing media thus produced significant

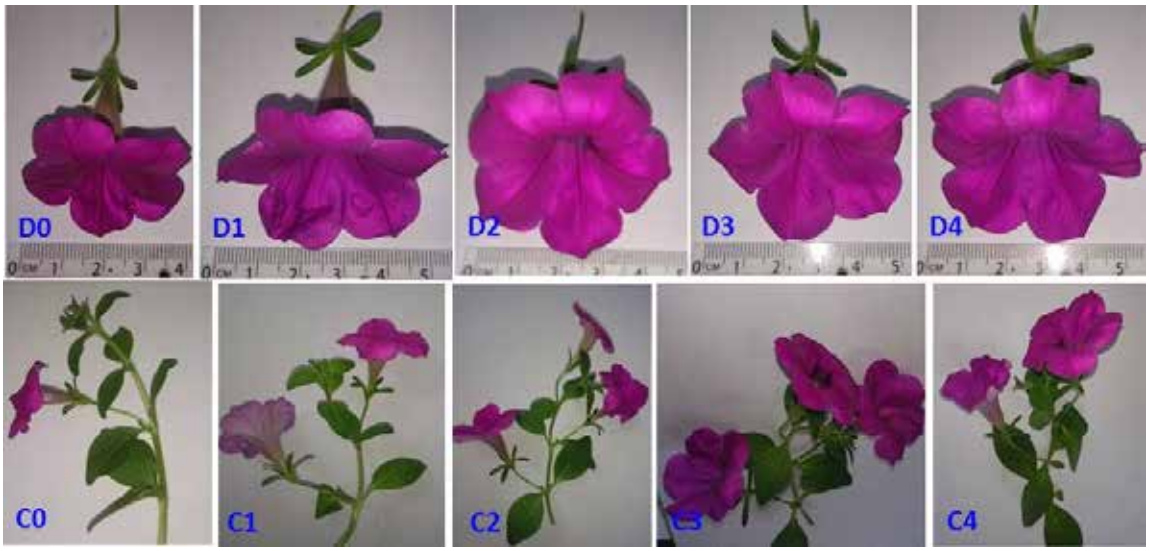

Figure 2- The effect of different growing media on flowers number (C) and flower diameter (D) of potted petunia plants. 0, Control; 1, Vermicompost; 2, Biochar; 3, Cocopeat and 4, Peatmoss

Table 2- Effects of different growing media on flowering behaviour and quality of potted *Petunia grandiflora*

Treatment	*Number of Bud/ plant*	*Number of flower/plant*	*Flower diam (mm)*	*Wt of ind flower (g)*	*Petal thickness (mm)*
Control	3.20±0.60 b	3.00±0.80 c	33.50±0.90 c	0.12±0.02 c	0.08±0.00 c
Vermicompost	18.40±2.00 a	21.70±2.00 b	47.10±0.60 a	0.18±0.02 b	0.09±0.00 c
Biochar	21.80±9.00 a	28.50±2.30 a	41.90±3.00 b	0.21±0.02 b	0.08±0.00 c
Cocopeat	18.00±5.00 a	32.40±2.00 a	46.80±2.00 a	0.22±0.01 b	0.10±0.00 b
Peatmass	20.10±1.50 a	29.80±1.20 a	52.50±3.40 a	0.29±0.03 a	0.12±0.00 a

Data was present in means (± SE). Different means in same column followed by same letter are not different according to LSD test at P= 0.05 probability

effect on weight of individual flower. Based on Figure 2 and Table 2, the highest value was recorded in peatmoss at 0.29 g followed by cocopeat at 0.22 g. The lowest value was observed in the control treatment at 0.12 g. Based on Table 2, the highest petal thickness was recorded in peatmoss treatment at 0.12 mm, followed by the cocopeat and vermicompost with a value of 0.10 and 0.09 mm, respectively. Control and BioChar treatments produced the thinner petal with a petal thickness of 0.08 mm.

3.5. Chlorophyll content (SPAD) and Chlorophyll a & b

The results showed that chlorophyll content of potted petunia plant was similar among the growing media except for the control. Results showed that growing media used in this study enhanced relative chlorophyll content as compared to control (Table 3). Based on Table 3, the SPAD chlorophyll value was the highest in peatmoss treatment with a mean value of 24, followed by cocopeat and vermicompost treatments. For Chlorophyll *a*, peatmoss growing media produced the highest amount of leaf chlorophyll *a*, followed by cocopeat, vermicompost and biochar. While, the lowest amount of chlorophyll *a* was recorded in control treatment (Table 3). Similar trend was also obtained for chorophyll *b* content, which was 1.75 times higher in peatmost treatment as compared to the control (Table 3).

3.6. Carotene and anthocyanin contents

Based on Table 3, there were significant differences for both caratone and anthocyanin contents among the treatments. The highest carotenoid content was obtained with peatmoss treatment at 1.00 µg g^{-1}, followed by cocopeat with a value of 0.70 µg g^{-1}. The lowest reading was recorded in the control plant with carotenoid content of 0.30 µg g^{-1}. Anthocyanin content in petunia flowers petal was also significantly affected by growing medium under potted condition (Table 3). The results showed that petunia grown in peatmoss medium produced 2.3 times more anthocyanin content as compared to the control treatment. It was also seen that all the treatments produced darker petal colour compared to control (Figure 3).

3.7. Flower longevity, ethylene production and correlation between petal thickness and flower longevity

All the treatments increased the longevity of potted petunia flowers compared to control and the best growing media were peatmoss and vermicompost (Figure 3). The highest flower longevity (days) was recorded in peatmoss treatment at 15 days while the less flower longevity was recorded in the control treatment (9 days) (Figure 4a). Results showed that the rate of ethylene production of potted petunia flowers was significantly affected from growing media (Figure 4b). Petunia plants grown in top soil (control) produced the highest amount of ethylene than the peatmoss, biochar, cocopeat and vermicompost. The results showed that the petal thickness and anthocyanin content have had positive correlation with the flower longevity of petunia flower (Figures 5a and b). Flower longevity was enhanced with the increase in petal thickness and anthocyanin content of petunia petal (Figures 5a and b).

Table 3- Effects of different growing media on accumulations of pigments in potted petunia flowers

Treatment	*Chlo cont (SPAD)*	*Chlo a*	*Chlo b*	*Carotene cont (µg g^{-1})*	*Anthocyanin content (mgg^{-1} FW)*
Control	13.40±0.80 b	6.50±0.80 c	2.00±0.10 b	0.30±0.00 d	0.10±0.02 c
Vermicomposte	22.40±0.40 a	7.80±0.80 b	3.10±0.30 a	0.40±0.10 c	0.20±0.01 a
Biochar	18.60±0.40 a	7.70±0.90 b	2.90±0.20 a	0.55±0.00 c	0.15±0.03 b
Cocopeat	22.80±0.60 a	8.50±0.50 b	3.20±0.40 a	0.70±0.20 b	0.18±0.02 a
Peatmoss	24.30±0.50 a	9.50±0.70 a	3.50±0.40 a	1.00±0.10 a	0.23±0.02 a

Data was present in means (± S E). Different means in same column followed by same letter are not different according to LSD test at P= 0.05 of probability

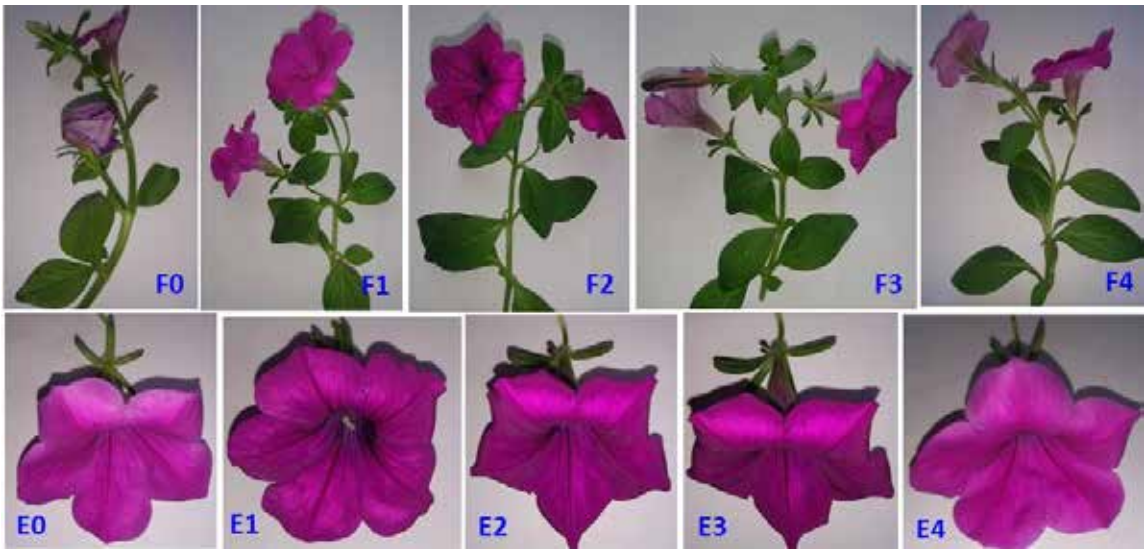

Figure 3- The effect of different growing media on petal colour (e) and flower logevity (f) of potted petunia plants. 0, Control; 1, Vermicompost; 2, Biochar; 3, Cocopeat and 4, Peatmoss

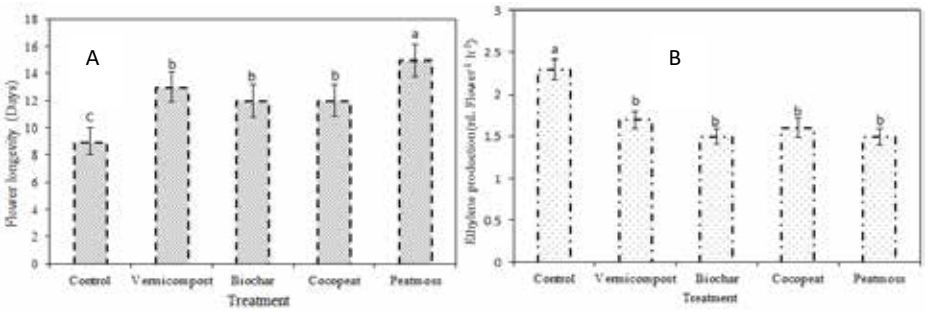

Figure 4- Effects of different growing media on flower longevity (a) and ethylene production rate (b) of potted petunia flowers

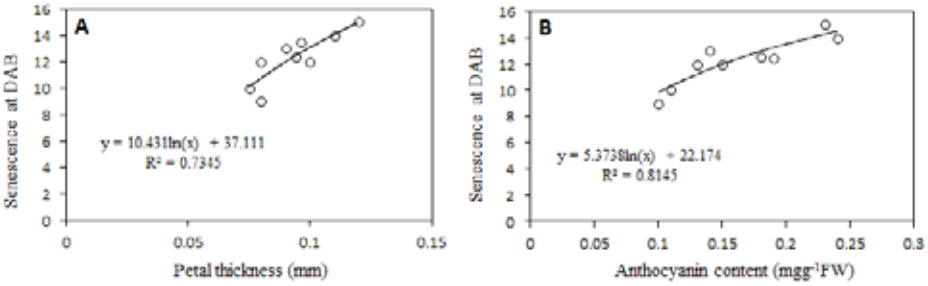

Figure 5- Correlation between petal thickness (a) and anthocyanin (b) with flower longevity of potted petunia plants. DAB, Days After Bloom

4. Discussion

Plant productivity is largely dependent on leaf area, which is an important determinant of light interception, net photosynthetic rate, transpiration and other metabolic acitivities. In the present study, growing medias; peatmoss and cocopeat significantly increased the leaf area of potted petunia plants. Mehmood et al (2013) also stated that peatmoss-containing growing substrate greatly affects the size of leaves of *Antirrhinum majus* L. (cv. Floral Shower). This might be due to the high availability of nutrients in growing substrate during the vegetative and reproductive growth periods. Our results also showed that growing media produced significant effect on number and length of lateral branch of potted petunia plants. Cocopeat increased the number of lateral branches as compared to the other treatments. The results from current study are in accordance with the findings of Riaz et al (2008), who stated that the coconut compost increases the number of lateral branches. In our study, the vegetative growth; length of lateral branches was the highest in control treatment. The top soil pH was around 5.5 to 6.5 and it has higher percentage of clay and humus. The higher amount of organic matter and nitrogen present in the clay loam top soil might have stimulating impact on vegetative growth of petunia plant. Moreover, moisture holding capacity of the top soil is also very high thus it may stimulate more vegetative growth rather than reproductive growth.

Stomata occupy a central position in the pathway for the transport of water vapour for CO_2 and O_2 (Jones 1998). Different growing media produced a significant effect on stomatal aperture of potted petunia plants. Peatmoss and cocopeat increased the stomatal aperture of petunia plants. Peatmoss is considered as an optimal organic amendment to improve the physical properties of soil's pH, moderate temperature, fine texture and good water retention ability. These improved physical conditions of growing media may increase the production of cytokinins, a hormone that is known to favor the opening of stomata (Stoynova et al 1996).

Results from this study indicated that growing media increases net photosynthetic rate of potted petunia plant. Among the growing media tested, peatmoss and cocopeat produced significant positive effect on photosynthesis of petunia plant. Similar results was reported by Nazari et al (2011), who stated that cocopeat singinificantly increased net photosynthetic rate, efficiency of mesophyll cells, fresh weight of flowering stem, and length of floret of potted hyacinth plant (*Hyacinthus orientalis* L.). It has been stated earlier that peatmoss and cocopeat stimulate the growth regulators activities and these growth regulators has a stimulatory effect on photosynthesis. Khandaker et al (2013) reported that application of Triacontanol to the potted Bougainvillea plant (*Bougainvillea glabra*) had increased the net photosynthetic rate and enhanced accumulation of photosynthates, thus enhance the flowering of potted plants.

In this current study, cocopeat and peatmoss significantly increased the number of flower bud and flower diameter in potted petunia plants. Enhanced floral quality by cocopeat and peat moss may be attributed to the stimulating effect of them on the activity of growth regulators which play a significant role in flower and fruit developments (Moneruzzaman et al 2013). Similar to our findings in petunia plants. Talukdar & Barooah (1987) reported that cocopeat resulted with 'superior flowering' in *Dendrobium densiflorum*. Plants with the largest flowers are normally regarded as high quality and preferred by consumers for most purposes including bedded plants, cut flowers or potted plants. In this current research, potted petunia plants had better performance with the acidic growing media of peatmoss in which pH is around 4 because it is as acid loving ornamental plant (Argo & Fisher 2008). Our results are in agreement with the findings of Hasan et al (2014), who reported that peatmoss and sheep manure increased the flowers number, floral diameter and petals number per flower of marigold (*Calendula officinalis* L.).

Peatmoss and cocopeat media produced positive effect on weight of individual flower. This might be due to the slightly acidic growing media with

pH of around 4.0. The increment in the flower weight of petunia treated with peatmoss may be due to the peatmoss growing medium enhancing the biosynthesis of the plant growth hormones and protein, which subsequently affects the cell division and cell expansion in the reproductive organ of the petunia plant. May be this growing medium suppresses the vegetative growth and induces the reproductive growth of petunia. Petal thickness was increased significantly with peatmoss and cocopeat treatments. These results were in line with Hasan et al (2014), who reported that peatmoss significantly increased the number and size of petal of marigold. Applying peatmoss and cocopeat significantly increased the number of flowers of potted petunia plants and this may be due to the impact of nutrients in these organic extracts.

In this study, we measured the greenness of leaf by using the SPAD meter and determined the Chlorophyll *a* and *b* contents of potted petunia plant. All of the growing media significantly increased the chlorophyll content of leaves of petunia as compared to control. Our results showed an aggrement with the results of Hasan et al (2014), who reported that peatmoss and sheep manure increased the leaf chlorophyll content of marigold plant. Carotenoids are important in plant system due to light harvesting complex and in the photoprotection of the photosystem. Peatmoss significantly increased the carotenoids content of petunia flower. Flower quality was enhanced with the use of peatmoss as a growing media. These results were supported by the findings of Sardoei & Rahbarian (2014), who reported that peatmoss growing media increased photosynthetic pigments and carotenoids content in ornamental plants.

The red or pink colour that appears on the flowers or fruits arises from the accumulation of anthocyanins (Khandaker et al 2012). The results from this study showed that a growing medium produced a significant effect on anthocyanin content of petunia petal, and the highest anthocyanin content was recorded in acidic growing media of peatmoss and cocopeat-treated petunia plant. Our results were also supported by the findings of Schmitzer & Stampar (2010), who reported that the rose plants planted in growth medium with a lower pH would had more anthocyanins in rose petal. They also stated that acidic growing media may increase the availability of Zn in root zone and this in turn increase the accumulation of plant anthocyanin content. It has also been also reported that the mircronutrients iron, manganese, and zinc become more available as pH decreases in the plant growing medium (Hannan 2016).

The results showed that peatmoss increased the longevity of potted petunia flower around 6 days as compared to control plant. Asghari (2014) also reported that growing medium significantly affects the flower character as well as longevity of carnation (*Dianthus caryophyllus* L.) flowers. This might be due to increases of available moisture supply, nutrient content and cation exchange capacity (CEC) of potted plant. Moreover, it has been reported earlier that peatmoss is an acidic growing media with pH of around 4, and this low pH value may also reduce the pH of plant cell (pH 6) and decrease the rate of ethylene production after flowering. Flower longevity and abscission of bract is also correlated with ethylene production (Moneruzzaman et al 2010). The findings of this current study indicated that petunia plants grown in soilless media decreased the ethylene production. This might be due to increased availability of water and nutrients, and production of gibberellin and cytokinin hormones in plants grown in the peatmoss and other growing media, which would extend the reproductive growth of petunia plants. This improved growth might have retarded the ethylene production and increased flower longevity. Aharoni (1989) reported that exogenous treatments of GA_3 and kinetin retards the senescence, as well as reduced the biosynthesis of ethylene in plant parts.

In our study, we have found positive correlation among the petal thickness and longevity of petunia flower on the plant. Similary, Breadmore & Kirk (1998) reported that the amount of petal demages strongly correlated with petal thickness of herbaceous plant, which may be due to higher carbohydrate reserve and enables to maintain the

dry matter in the petals which help in extending the keeping quality. Khandaker et al (2011) also reported that girdling increase the carbohydrates availability in the above parts of girdle branch and reduced the floral bud drop. It was observed that anthocyanin concentration in petal affect the vase life of petunia flower and both parameters were positively corrected. Flowers from petunia plants treated with peatmoss and cocopeat have high anthocyanin content as well as flower longevity. Our results were suported by the findings of Emami et al (2011), who reported that anthocyanin content had the highest direct effect on flower longevity of lily and flower longevity has a positive correlation with initial fresh weight of flower, and anthocyanin and carotenoid contents.

5. Conclusions

Based on results, it can be concluded that the peatmoss and cocopeat enhanced the growth, development and improved the floral quality of *Petunia grandiflora*. Cocopeat showed better performances in number of flower, number of lateral branch and length of lateral branch. On the other hand, peatmoss showed a better performance in diameter and weight of flower, petal thickness, chlorophyll content, stomatal conductance, carotene content and flower longevity. In addition, peatmoss and cocopeat siginificantly increased the net photosynthetic rate of potted petunia plants, chlorophyll *a* and *b*, and carotenoids and anthocyanin contents in flowers petal. Moreover, a decreased ethylene production but an increased flower longevity were of advantages of peatmoss and cocopeat. Hence, for potted *Petunia grandiflora*, the use of peatmoss and cocopeat as a growing media is strongly recommended to improve production.

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Influence of Harvesting on Quality of Alfalfa Forage used for Haylage and Hay

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ABSTRACT

The paper presents the three years efficiency results (2011-2013) of the work and ways of harvesting (three types of mowers, with or without spreading the forage) influencing the quality of the forage (the content of crude protein and crude fiber) during the three days drying process. These results indicate that the harvesting method can strongly affect the work efficiency, energy consumption, the forage drying intensity and the quality of forage used for hay and haylage preparation. The best quality of the forage was achieved when a drum rotating mower PÖTTINGER CAT 185 was used. Negative correlation was found between content of crude protein and the crude fibre content, depending on the type of mower, varying in the range between r= -0.978 and r= -0.882 (PÖTTINGER CAT 185 r= -0.882 P≤0.05 to JF STOLL SB 200 r= -0.978 P≤0.001).

Keywords: Mowers; Cutting; Drying; Crude protein; Crude fiber

1. Introduction

In many regions of the world, alfalfa (*Medicago sativa*) is an important fodder plant for the provision of high-quality animal feed for ruminant (Khadda et al 2015; Ahmad et al 2016). It is very adaptable to the environmental conditions, which enables a wide area of growing (in the northern hemisphere it is grown up to 69° N - in Scandinavian countries, and in the south up to 45° S (New Zealand), as well as on 55° S in Argentina and Chile (Ivanov 1988). Its preference lies in the fact that it is used in different ways: fresh, hay, silage, haylage and/or for pasturage. It has high nutritive values. All of this gives it the title of Queen of forage crops. High variability of yield and quality of alfalfa forage is defined in the world, be it influenced by genetics, cultivation technology, harvesting manner (Brummer 2004; Ahmad et al 2016; Karayilanli & Ayhan 2016), and seed yield (Stanisavljević et al 2012; Zhang et al 2017). Bagg (2004) recommends manipulation of the mowed alfalfa mass of humidity up to 50% using a rotary

spreader. Subsequent treatment of the mass with a lower percentage of humidity results in a large loss of leaves. In the southeastern and southern Europe, alfalfa is mainly used for drying and haymaking, but in smaller areas it is used for storing haylage. Given that the process of hay storing and/or alfalfa haylage is done on the fields, it is largely dependent on climatic factors. Based on rainfall simulation, Coblentz & Muck (2012) reported consistent reductions in concentrations of WSC and starch. However, changes in WSC were relatively modest, and postwetting concentrations of WSC could be buoyed by hydrolysis of starch. In addition, they found much less desirable indicators of ensilability, when forages were subjected to natural rainfall events followed by prolonged exposure under field conditions. Therefore, the selection of the appropriate mowing apparatus and timely alfalfa mowing is essential for protein and fibre content, being the most important parameter of the forage quality. Mowers are agricultural machines that consume a lot of energy, and the special attention has to be paid to optimal energy consumption and quality of work (Hosseini & Shamsi 2012).

The aim of this study was to determine the impact on yield, quality of forage and energy consumption by examining the ways of alfalfa harvesting.

2. Material and Methods

Field experiment was established in Central Serbia, (43^0 33' 33''N; 21^0 12' 53''E) on alfalfa Cultivar Kruševacka-28 in the third to fifth year of use when in the phase ½ of flowering. Random block system with three repetitons over the plots of 30 m x 10 m= 300 m^2 was used. Cultivar is characterized by high genetic potential for yield of forage (over 80 t ha^{-1}) and dry matter (over 20 t ha^{-1}). It is suitable for intensive production, achieves 6 cuts per year with applied irrigation. This cultivar is tolerant to lodging, frequent cutting and possesses a high resistance to low temperatures and drought, providing excellent forage quality: crude protein content is 20-22 g kg^{-1} and crude fiber (CF) about 32 g kg^{-1} (Institute for Forage Crops 2011).

Alfalfa seeding have been performed following classical soil tillage system, after deep autumn plowing and secondary soil tillage, characterised with a seed norm of 20 kg h^{-1} during each experimental year, the mineral fertilizer consisting of 8% (N): 16% (P_2O_5): 24% (K_2O) 150 kg h^{-1} was deposited over the plot.

At the first alfalfa harvest during 2011 (A_1), 2012 (A_2), 2013 (A_3) - factor A, three mowers were studied: IMT 627 667 (B_1), whose cutting apparatus consists of fixed and mobile part. Fixed part consists of steel beam with fingers. Each finger has a counter knife attached it by clinch. The movable part is a rod with moving blades having a straight line and return movement, Pottinger CAT 185 (B_2), rotary mower with two drums that work in pairs. Each drum has three movable blades which cut the stem cut off with free and high velocity of the blades (60 to 80 m s^{-1}), and JF-STOLL SB 200 (B_3), rotary mower with five disks with two knives each - factor B. After cutting, forage was spread on one half of the plot during the first two days (C_2), whereas on the other half it was dried without spreading (C_1) (factor C). Samples of forage were analyzed immediately after cutting (D_0), on the cutting day after 8^h (18^h) (D_1), on the second day at 18^h (D_2) and on the third day at 18^h (D_3), factor D time after cutting. Speed is determined by the chronometer method based on distance travelled per unit of time. The quality of work is determined through the working width, the stems cutting height and losses while mowing, assuming that the optimum cutting height for alfalfa is 6 cm. Stem cutting height is determined by the on-site determination of loss, by measuring the height of stubble for each probe (probe within three replicates) on a suitable surface. The mean values are determined for each trial on the basis of obtained parameters. During mowing, losses were measured on the surface of one length meter of swath in working width, of tested mowers, on the same place where the height of cut was determined. Total losses (L_Σ [%]) represent the sum of the losses incurred due to the cut height (L_{Hcut} [%]) and losses incurred due to chopping (L_{Chop} [%]). After cutting, humidity of the

mowed mass was measured on the first, second and third day at 18 h (after drying in a drier at 105 ^{0}C), and the forage quality parameters in the dry matter were measured: Crude protein (CP, g kg^{-1}), using the Kjeldahl method (AOAC 1990) and crude fibre (CF, g kg^{-1}), using the Weende method (AOAC 1990).

Statistical analysis of forage quality: analysis of variance (ANOVA F-test) was applied to determine the influence of the factors, whereas Tukey's Multiple Range test was used for assessing the influence of the middle of the treatment. The relationship between traits was established by the Pearson's Correlation Test (r). The program Minitab 16.1.0 was used for data processing.

3. Results and Discussion

Under the agroecological conditions in South East Europe, alfalfa is generally mowed four times, wherein the first cutting contributes to an overall forage yield with about 50% (Strbanović et al 2015). Therefore, this work depicted the results of the first cut. During experiment (2011-2013), there was no rainfall in the alfalfa drying period (D_1-D_3), and the temperature had not varied for more than 2%. Also, the relative humidity did not vary for more than 2% (Figure 1).

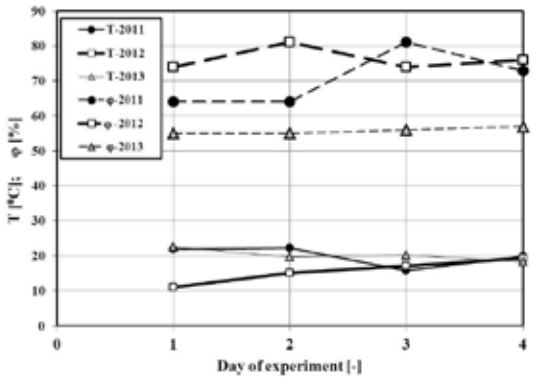

Figure 1- Atmospheric conditions during experimental mowing: the mean temperature T [^{0}C] and relative humidity ϕ [%]

3.1. Work efficiency of the mowers

In our tests, the maximum value of the utilization coefficient of working swath of 0.97 (1.55 m), was found at classical mower in the first year of research. The lowest value of 0.91 (1.81 m) was recorded at the rotational disc mower in the third year of research. The cutting height depends on the mower movement speed. The maximum value of the cutting height from 7.25 cm was recorded at the rotational disc mower in the third year of research, at speed of 12.4 km h^{-1}. The minimum value of the cutting height of 5.52 cm in the first year of research was recorded at rotary mower with drums at speed of 9.1 km h^{-1} (Figure 2). The obtained results are consistent with the results of other researchers (Wiersma & Wiederholt 2001).

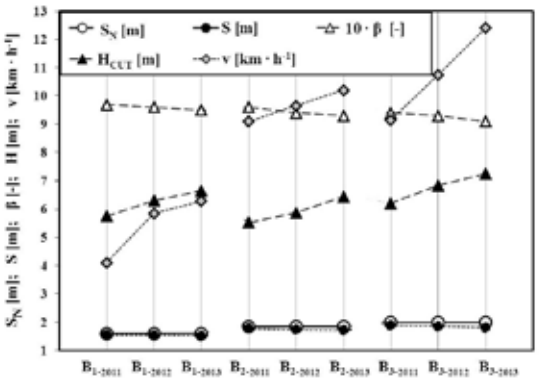

Figure 2- Operational parameters of the harvesters: nominal working swath S_N [m]; achieved working swath S [m]; coefficient of achieved working swath β [-]; cutting height H_{CUT} [m]; working speed v [km · h^{-1}]

Figure 3 shows the losses of the tested mowers. Minimum losses of 1.03% were recorded in conventional mowers in the first year, whereas maximum of 3.03% were recorded at rotary mowers with drums in the third year of research. The evidenced losses correspond with the results of other researchers (Bagg 2004; Barać et al 2012).

Using variance analysis (F test) we found that years (factor A) and interactions AxB, AxC, AxD did not seem significant for the moisture content,

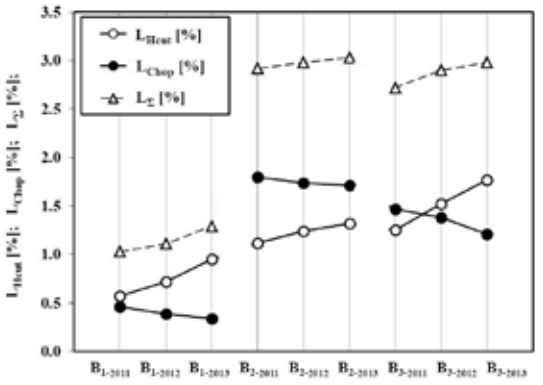

Figure 3- Harvesting losses due to: cutting height L_{HCut} [%], chopping L_{Chop} [%] and total L_{Σ}[%]

crude protein and crude fiber in alfalfa forage (hereinafter the average values for 2011-2013 are given). Influence of different mower (factor B), hay spreading (C) and drying time (D) had significant impact (P≤0.05 to P≤0.001), as well as their interaction on the moisture content, crude protein and crude fiber in the alfalfa forage (Table 1).

3.2. Forage moisture

Forage moisture should be up to 20% (Undersander et al 2004) for drying process and hay preparation without additives that can be stored. Optimum moisture content is 55%-60% for making haylage without additives (Dinić & Djordjević 2005). Due to possible rainfall, it is usefull to make green fodder into stored hay or silage. As expected, moisture ranged from 76% (B_1) to 71% (B_2 and B_3) immediately after mowing, so that due to high moisture, forage could have not been used even for silage. After eight hours, moisture from the treatments B_1 C_1 and C_2 (56.8% and 55.5%) B_2 C_1 (55.1%) was ideally suited for the preparation of haylage. Forage referred to in the treatment B_3 C_2 and B_2 C_2 (47.7% and 49.0%) had a significantly lower moisture (P≤0.05) than forage from B_1 C_1 (56.8%). As expected, in all B_1-B_3 treatments of the forage spreading (C_2), moisture was lower compared to the forage not spread (C_1) (Table 2).

On the second day of forage drying, moisture between spread and non-spread feed was as follows: 7.6% for the treatment B_1; 6.5% for the treatment B_2 and 4.7% for B_3. However, after two days of drying, the moisture of the applied treatments was high for hay making, but low for haylage, so the different mowers (B_1-B_3) and forage spreading could not influence enough on forage moisture to make it sufficient for hay storing. After the third day (D_3) the forage treatment: B_3 C_1 and C_2 (moisture content 18.9% and 17.3%); B_2 C_2 (moisture content 18.6%), and B_1 C_2 (moisture content 18.7%) met conditions

Table 1- Results of analysis of variance (ANOVA) for forage moisture, crude proteins, crude fiber. Sources of variation: (A), year; (B), type of mower; (C), hay spreader; (D), time after cutting

Source of variation	*Forage moisture (%)*	*Crude proteins (CP, g kg^{-1})*	*Crude fibre (CP, g kg^{-1})*
Year (A)	ns	ns	ns
Type of mower (B)	*	*	*
Hay spreader (C)	**	*	*
Time after cutting (D)	***	***	***
AxB	ns	ns	ns
AxC	ns	ns	ns
AxD	ns	ns	ns
BxC	**	*	*
BxD	**	*	*
CxD	*	*	*

***, significant F tests at the P≤0.001 level of significance; **, significant F tests at the P≤0.01 level of significance; *, significant F tests at the P≤0.05 level of significance; ns, not significant F tests at the P≥0.05 level of significance

Table 2- Influence of way of alfalfa harvesting to forage moisture during three days of drying

Type of mowers-B treatmens B_1-B_3	*Forage moisture (%)*					
	Before cutting	*Immediately after cutting (10 h) D_0*	*C*	*Cutting day 18 h D_1*	*Second day 18 h D_2*	*Third day 18 h D_3*
B_1	78.0 ^{a}	76.0 ^{a}	C_1	56.8 $^{a\,A}$	35.0 $^{a\,B}$	25.4 $^{a\,C}$
			C_2	55.5 $^{ab\,A}$	27.4 $^{ab\,B}$	18.7 $^{ab\,C}$
B_2	76.0 ^{a}	71.5 ^{b}	C_1	55.1 $^{ab\,A}$	33.4 $^{a\,B}$	22.0 $^{a\,C}$
			C_2	49.0 $^{b\,A}$	26.9 $^{b\,B}$	18.6 $^{b\,C}$
B_3	75.0 ^{a}	71.5 ^{b}	C_1	50.6 $^{ab\,A}$	29.6 $^{ab\,B}$	18.9 $^{ab\,C}$
			C_2	47.7 $^{b\,A}$	24.9 $^{b\,B}$	17.3 $^{b\,C}$

a, b, (different small letters) significant effect (P≤0.05; Tukey's Multiple Range test) for the column; A, B, C (different capital letters) significant effect (P≤0.05; Tukey's Multiple Range test) for the row

for hay making, which can be stored for long time (Undersander et al 2004).

3.3. The content of crude protein and crude fiber

Strbanović et al (2017) found the maximum crude protein content of 212 g kg^{-1} dry matter for the researched sorts at the beginning of flowering process, whereas the lowest recorded content 174 g kg^{-1} of dry matter. Thus, depending on a sort, the content of CP in the forage varied for 41 g kg^{-1} dry matter. Our research has shown lower levels of crude protein, which can be explained by the fact that mowing was done at a stage when a ½ plant was in flowering process. In general, the quality of alfalfa forage got worse as the days of drying passed (the protein content in the alfalfa forage decreased, a crude fibre content increased), which was statistically significant between the first and third day (P≤0.05) (Table 3 and 4).

Following Fonnesbeck et al (1986) yield loss from soluble nutrients was 9.7% (losses of 18.8% available carbohydrate, 10.2% of crude protein, 19.8% of lipids and 14.0% of soluble minerals). Influence of rain damage on hay quality reducing was more expressed than the influence of advancement in maturity.

Table 3- Influence of alfalfa harvesting on crude protein content in dry matter alfalfa forage during the three days of drying

Type of mowers-B treatmens B_1-B_3	*C*	*CP, g kg^{-1}*		
		Cutting day 18 h D_1	*Second day 18 h D_2*	*Third day 18 h D_3*
B_1	C_1	163.0 $^{a\,A}$	138.4 $^{ab\,B}$	125.8 $^{ab\,B}$
	C_2	160.9 $^{ab\,A}$	124.4 $^{b\,B}$	112.2 $^{b\,B}$
B_2	C_1	164.8 $^{a\,A}$	147.9 $^{a\,AB}$	137.1 $^{a\,B}$
	C_2	160.5 $^{ab\,A}$	139.6 $^{a\,B}$	128.6 $^{a\,B}$
B_3	C_1	157.0 $^{b\,A}$	125.6 $^{b\,B}$	125.6 $^{ab\,B}$
	C_2	158.8 $^{b\,A}$	133.7 $^{ab\,AB}$	119.8 $^{b\,B}$

a, b, (different small letters) significant effect (P≤0.05; Tukey's Multiple Range test) for the column; A, B, (different capital letters) significant effect (P≤0.05; Tukey's Multiple Range test) for the row

Table 4- Influence of alfalfa harvesting on crude fibre content in dry matter of alfalfa forage during the three days of drying

Type of mowers-B treatmens B_1-B_3	*C*	*CF, g kg^{-1}*		
		Cutting day 18 h D_1	*Second day 18 h D_2*	*Third day 18 h D_3*
B_1	C_1	34.96 $_{a\,B}$	38.35 $_{a\,AB}$	39.85 $_{a\,A}$
	C_2	34.57 $_{a\,B}$	37.57 $_{a\,AB}$	40.41 $_{a\,A}$
B_2	C_1	35.24 $_{a\,B}$	36.99 $_{a\,AB}$	38.45 $_{a\,A}$
	C_2	34.59 $_{a\,B}$	36.02 $_{a\,AB}$	39.26 $_{a\,A}$
B_3	C_1	35.28 $_{a\,B}$	38.53 $_{a\,AB}$	39.55 $_{a\,A}$
	C_2	34.81 $_{a\,B}$	37.58 $_{a\,AB}$	40.39 $_{a\,A}$

a, b, (different small letters) significant effect (P≤0.05; Tukey's Multiple Range test) for the column; A, B, (different capital letters) significant effect (P≤0.05; Tukey's Multiple Range test) for the row

The highest contents of crude proteins was recorded 8^h after mowing (D_1), on the second (D_2), and on the third day (D_3) from the treatment of B_2 C_1. The crude protein content was lower from treatments with the spread forage, as a consequence of leaves loss (having the highest protein content) during the mass spreading. (Table 3).

Influence of mower had no statistically significant effect after any time (D_1-D_3) on the crude fibre content (Table 4). According to Strbanović et al (2017) the average value of the crude fibre content for fifteen alfalfa sorts is 275 g kg^{-1} with differences of 99 g kg^{-1} of dry matter, caused by impact of a sort, and the total variability expressed by the coefficient of variation 10.48%. It also indicates a high impact on the exploitation phase of the crude fiber content.

After application of various mowers for the collection of alfalfa forage, a negative correlation between the content of crude protein and crude fiber was found, but of different intensity (P≤0.01 B_1; B_2 P≤0.05; B_3 P≤0.001) (Table 5). The results of the negative interdependent correlation (P≤0.001) between content of protein and cellulose are consistent with the results of Heuze et al (2013), Strbanović et al (2017).

Table 5- Coefficient of simple correlation (r) between the content of crude protein and crude fiber content in the forage cut by different mowers (n= 6)

Type of mowers-B tretmani B_1-B_3	*r*
B_1	-0.929 **
B_2	-0.882 *
B_3	-0.978 ***

Statistical significance level; *, P≤0.05; **, P≤0.01; ***, P≤0.001

4. Conclusions

If the alfalfa forage is used to store haylage under the given conditions and in similar regions throughout the world, ten hours are sufficient if the collection is performed by a rotary mower with a drum PÖTTINGER CAT 185 (B_2) or less, using a rotary device with discs JF-STOLL SB 200 (B_3) with spreading. For hay storing (humidity under 20%), the application of a rotary device with discs STOLL JF-SB 200 (B_3) provides moisture, be the forage spread or not. That is also provided by the other two mowers (B_1 and B_2), with hay spreading (C_2). The best forage quality was achieved by mower PÖTTINGER CAT 185 (B_2), which is reflected in the highest crude protein content and the lowest dependence correlation with the crude fibre content (r= -0,882; P≤0.05).

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Abbreviations and Symbols	
r	Index of correlation
P	Signifficance level
L_{Σ}	Total losses, (%)
L_{Hcut}	Cut-height losses, (%)
L_{Chop}	Chopping losses, (%)
CP	Crude protein content, (g kg^{-1})
CF	Crude fibre content, (g kg^{-1})

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Grain Yield and Some Physiological Traits Associated with Heat Tolerance in Bread Wheat (*Triticum aestivum* L.) Genotypes

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ABSTRACT

This research was carried out in the experimental fields of Department of Field Crops, Faculty of Agriculture, the University of Namık Kemal in 2014-2015. In the study, totally 30 bread wheat (*Triticum aestivum* L.) genotypes (15 cultivars; early, medium-early and late-maturing; 10 lines are tolerant to the heat-temperature stress which were provided by CIMMYT-International Maize and Wheat Improvement Center), 5 lines (were taken from the same university's wheat breeding program which was collaborated by the CIMMYT) were used as an experimental material. The experiment was adjusted in a split-plot design with 3 replicates. Sowing dates (Normal (NS ≈ November 09, 2014) and Late sowing (LS ≈ January 09, 2015)) were constituted the main plots, and the genotypes constituted the sub-plots. These physiological traits ((membrane thermostability (MT), canopy temperature (CT), leaf chlorophyll content (LCC) and stomatal conductance (SC)) were measured at the LS stage due to giving much more correct, logical and meaningful results, but grain yield (GY) was fixed for all the sowing dates. Obtained findings are: The GY was varied between (4.35-6.34 t ha^{-1}) for genotypes; the MT was changed between (10.58-66.25%); the CT was realized between (17.67-22.00 °C); the LCC was varied between (38.30-53.30 SPAD) and the SC was changed between (25.20-166.80 mmol m^{-2} s^{-1}). It was observed that most of the CIMMYT originated genotypes are tolerant to high-temperature stress and most of the wheats that are grown in Thrace Region are negatively affected by the high-temperature stress.

Keywords: Heat tolerance; Canopy temperature; Chlorophyll content; Membrane thermostability; Stomatal conductance

1. Introduction

Bread wheat (*Triticum aestivum* L.) is one of the oldest and most important staple foods of the world agriculture because of its high adaptation ability, stress-tolerant genotypes, easy transportation and storage. It has expanded in quite different ecologies, undergoes many restrictive abiotic stress factors. Abiotic stress factors negatively influence the growing and grain yield by causing morphological, physiological, biochemical and molecular changes (Wang et al 2001). The stress factors such as high temperatures, salinity, and drought which occurred as a result of global climate change that has been highly felt in recent years influence plant yield quite a lot (Mathur & Jajoo 2014).

The abiotic stress conditions such as high-temperature stress are being threatened to agriculture and agricultural fields in many regions in the world

(Wang et al 2003). In addition, cultivated many plants grow well between (15-45 °C) temperature limits, and in outside of these limits their growth, development, metabolism, quality and quantity, etc. are highly (negatively) affected depending on regions. However, under high-temperature conditions, heading or flowering of plants may be negatively influenced, abnormalities such as pollen viability reduce, flower shedding happens, respiration, photosynthesis, fertilization evens decreases, and finally all of them stop (Balla et al 2011). Besides, Kirby et al (1985) and Longnecker et al (1993) stated that plants react differently to high-temperature stress in different phenological periods, and their development and survival durations depend on genotype, growing duration, cultivation technique applications and especially the temperature of the region in which they grow.

The researches revealed that the yield decrease which is caused by high-temperature stress in the development process of wheat is related to the decrease in the number and weight of grains per spike (Hays et al 2007). Reynolds et al (1994) explained that high-temperature stress in temperate environments is an important restrictive factor in heading and grain filling periods. Balla et al (2011) identified that high temperature is the most effective factor at the early embryo development stage in bread wheat. The reductive effects of high temperature on grain yield were revealed by Bluementhal et al (1995) and Wardlaw et al (2002). Kosina et al (2007) stated that high temperatures at heading and grain filling stages caused significant decreases in grain size and weight. Mentioned researchers' studies of wheat's tolerance to high-temperature stress, to know the physiological traits of genotypes which they use to resistance high-temperature stress will increase the effectiveness of breeding programs in the improvement of new high-temperature tolerant genotypes. Reynolds et al (2001) manifested that the LCC of bread wheat and high photosynthesis rate, stay-green duration, CT, MT and SC are physiological traits that are related to wheat's tolerance to the high-temperature stress.

The aim of this study is to identify the effects of high-temperature stress on the grain yield and selected physiological traits and to detect and use them as selection criteria for the high-temperature tolerant wheat breeding programs as genitor.

2. Material and Methods

2.1. Experimental site and growing conditions

This research was conducted out at the University of Tekirdağ Namık Kemal, Faculty of Agriculture, Department of Field Crops, Tekirdağ, Turkey in the 2014-2015. Geographically, Tekirdağ district locates at latitude 40° 36'-40° 31' and longitude 26° 43'-28° 08'and asl is 10 m. The mean temperature, total rainfall and relative humidity in the 2014-2015 with long-term means are presented in Table 1. As seen in Table 1, the mean temperature in November 2014 and July 2015 is 13.1 °C and the long-term annual mean value since this period is 12.7 °C. While the total temperature is 915.4 °C during a heading-maturation stage in the NS; in the LS, total temperature becomes 1084.8 °C during the heading-maturation stage. The difference between the total temperature in a heading-maturation stage in the NS and in a heading-maturation stage in the LS is 169.4 °C. Likewise, while the total rainfall is 62.1 mm during a heading-maturation stage in the NS, in the LS total rainfall becomes 88.6 mm during the heading-maturation stage. The difference between the total rainfall in a heading-maturation stage in the NS and in a heading-maturation stage in the LS is 26.5 mm.

According to soil analysis results, experimental area's soil was clay-loam, slightly acidic (pH 6.5), limeless, and poor (1.08%) in the organic matter.

2.2. Experimental materials and design

Thirty bread wheat (*Triticum aestivum* L.) genotypes were used as experimental material in this study. They are (15) registered cultivars (Namely, Nota, Kate A1, Basribey, Gelibolu, Esperia, Saraybosna, Syrena, Flamura 85, Krasunia, Dropia, Tina, Golia, Tekirdağ, Pehlivan and Yubileynaya 100) with different phenological and agronomical traits (such as early maturing, medium-early maturing and late

Table 1- Some meteorological parameters during the 2014-2015

Months	*Mean temp. (°C)*	*Rainfall (mm)*	*Humidity (%)*	*Long-Term*		
				Mean temp. (°C)	*Rainfall (mm)*	*Humidity (%)*
November-2014	11.2	35.2	85.2	11.3	62.5	84.0
December-2014	9.3	80.3	89.1	7.2	82.5	83.6
January-2015	5.8	61.5	81.9	5.2	62.1	84.0
February-2015	6.5	90.3	86.0	5.7	64.9	81.4
March-2015	8.5	29.4	81.9	8.0	57.4	80.7
April-2015	11.4	60.1	74.3	12.2	41.5	78.2
May-2015	18.6	1.4	76.3	17.6	33.8	75.1
June-2015	21.3	58.4	73.3	22.2	35.0	72.6
July-2015	24.9	0.5	70.6	25.0	26.7	69.6
Total	-	417.1	-	-	466.4	-
Mean	13.1	-	79.8	12.7	-	78.8
Total temperature is 915.4 °C and total rainfall is 62.1 mm during a heading-maturity stage under the NS conditions.						
Total temperature is 1084.8 °C and total rainfall is 88.6 mm during a heading-maturity stage under the LS conditions.						

Source, Tekirdağ meteorology station

maturing); (10) lines obtained from the Heat Tolerance Nursery (HTN) of CIMMYT (International Maize and Wheat Improvement Center-Mexico) collection which are known as tolerant to the heat-temperature stress (CIMMYT-HTN 2014/15-1, CIMMYT-HTN 2014/15-2, CIMMYT-HTN 2014/15-3, CIMMYT-HTN 2014/15-4, CIMMYT-HTN 2014/15-5, CIMMYT-HTN 2014/15-6, CIMMYT-HTN 2014/15-7, CIMMYT-HTN 2014/15-8, CIMMYT-HTN 2014/15-9 and CIMMYT-HTN 2014/15-10) and (5) lines are (CIMMYT-HTN 2013/14-4464, CIMMYT-HTN 2013/14-4488, CIMMYT-HTN 2013/14-4489, CIMMYT-HTN 2013/14-4490 and CIMMYT-HTN 2013/14-4492). Except them, Basribey bread wheat was used as a standard "tolerant" cultivar to the high-temperature stress in this study.

On the other hand, to be able to synchronize of growth stages, the experiment was arranged in a split-plot design with 3 replicates at two sowing dates (Normal sowing date ≈ 09 November 2014 and Late sowing time date ≈ 09 January 2015). Sowing dates were adjusted as main plots and genotypes were allotted as subplots. Sowing procedure was done in 1.2 m x 5 m plots, consisted of 6 rows spaced 20 cm apart. The seeding rate was arranged as 500 seeds m^{-2} and 20.20.0 composed fertilizer was used which include 50 kg ha^{-1} pure nitrogen (N) and 50 kg ha^{-1} pure phosphor (P_2O_5) was with the sowing. In addition to this, 60 kg ha^{-1} pure N as urea fertilizer (46% N) at the tillering stage and 50 kg ha^{-1} pure N as ammonium nitrate fertilizer (33% N) at the stem elongation stage were also given. Moreover, chemical control method was applied between tillering and stems elongation stages in spring considering the maturation condition and density of the prevalent weeds such as ryegrass (*Lolium multiflorum*), creeping thistle (*Cirsium arvense*), finger speedwell (*Veronica triphyllos*), wild mustard (*Sinapis arvensis*). All plots were harvested with a HEGE-160 combine harvester on the July 2015 during the maturity.

2.3. Measurement of grain yield and physiological traits

Grain Yield (t ha^{-1}): After 0.5 m pieces were cut from the beginnings and endings of the plots at the maturity (Zadoks Growth Stage (ZGS) 93; Zadoks et al 1974), and obtained values were transformed into t ha^{-1}. In addition, all the physiological traits presented below were a measured at the post-anthesis periods of the plants (ZGS 69; Zadoks et at 1974).

Membrane Thermostability (MT-%): It was fixed as (%) in the fully-developed flag leaves according to Reynolds et al (2001).

Canopy Temperature (CT- ^{o}C): It was measured with a portable infrared thermometer (Extech Mini IR Thermometer Modell 42500) as ^{o}C (Reynolds et al 2001). It was taken as two measurements per plot during the day between (11:00h to 14:00h).

Leaf Chlorophyll Content (LCC-SPAD): It was measured with "Konica Minolta SPAD-502 Plus" portable chlorophyll meter in the fully-developed flag leaves and determined as "SPAD value" (Pask et al 2012). It was taken three averages of five leaves per plot, and they were done from 11:00h to 14:00h.

Stomatal Conductance (SC-mmol m^{-2} s^{-1}): It was measured with a portable leaf porometer (Decagon SC-1 Leaf Porometer) and determined as mmol m^{-2} s^{-1} by calculating the average (Pask et al 2012). It was taken three readings on different, randomly chosen leaves from each plot. Readings were done from 11:00h to 14:00h.

2.4. Statistical analysis

All the data obtained from this experiment were subjected to variance analysis (ANOVA) using MSTAT-C statistical software, and mean values were compared using Duncan's Multiple Range Test (Steel & Torrie 1960).

3. Results and Discussion

In the experiment, obtained data for the GY were combined and analyzed to compare the GY performance of bread wheat genotypes under the NS and LS (heat-temperature stress). Whereas, the physiological traits associated with heat tolerance mechanisms which MT, CT, LCC and SC were determined under the LS conditions (Table 3).

Grain yield (t ha^{-1}): The effect of sowing date for the GY was not statistically significant, but genotype and sowing date x genotype interaction was statistically significant ($P \leq 0.01$) in terms of the GY (Table 2). Although the difference between sowing dates for the GY was not statistically significant, the mean of GY which was determined as 5.76 t ha^{-1} in the NS, decreased around 7.8% in the LS and was determined as 5.31 t ha^{-1}. It was taken more rainfall in the LS than NS during heading-maturity stages. However, as it can be understood from Table 1, plants were exposed 169.4 ^{o}C higher temperature during heading-maturity stages in the LS comparing to the NS. This situation caused stress, early senescence and decrease in grain filling stage. Thus, in the LS, the lower GY (5.31 t ha^{-1}) was obtained with the influence of high-temperature stress. Similarly, Mohammadi et al (2004) found out that the high-temperature stress at the post-anthesis stage of wheat decreases the grain filling duration, and the grain and head weight, but it does not influence of grain number per spike. Din et al (2010) discovered that the GY decreased 53.75% at the LS conditions. Modhej et al (2015) revealed that the high-temperature stress occurred at the LS conditions as decreases in the GY 30%, and 1000 grain weight.

In the research, the mean of the GY (for the genotypes) varied between 4.35 to 6.34 t ha^{-1}. The highest GY was obtained from CIMMYT-HTN 2014/15-5 (6.34 t ha^{-1}). It was followed by Basribey (6.22 t ha^{-1}), CIMMYT-HTN 2014/15-1 (6.21 t ha^{-1}), CIMMYT-HTN 2014/15-4 (6.20 t ha^{-1}) and Nota (6.19 t ha^{-1}), but the lowest GY was observed in CIMMYT-HTN 2014/15-7 (4.35 t ha^{-1}) and it was followed by CIMMYT-HTN 2014/15-10 (4.43 t ha^{-1}) (Table 2). In the research, it has been realized that the lines obtained from the CIMMYT and having high heat tolerance have high the GY.

The highest GY in the NS was obtained from CIMMYT-HTN 2013/14-4492 (6.87 t ha^{-1}). It was followed by CIMMYT-HTN 2013/14-4489 (6.85 t ha^{-1}), Nota (6.76 t ha^{-1}), CIMMYT-HTN 2014/15-5 (6.73 t ha^{-1}), CIMMYT-HTN 2013/14-4488 (6.63 t ha^{-1}), Basribey (6.46 t ha^{-1}), Yubileynaya 100 (6.39 t ha^{-1}), Tina and Flamura 85 (6.09 t ha^{-1}), and CIMMYT-HTN 2013/14-4490 (6.06 t ha^{-1}) (Table 2). The GY performances of bread wheat genotypes which were used for the LS date, they were exposed to high-temperature stress at the post-anthesis stage were lower than normal sowing date. The genotype

Table 2- Mean values and statistically significance groups for the GY (t ha^{-1})

Genotype	*Sowing date*		*Change rate (%)*	*Mean*
	N	*L*		
CIMMYT-HTN 2014/15-1	6.37 a-f	6.06 a-ı	-4.87	6.21 ab
CIMMYT-HTN 2013/14-4489	6.85 a	5.19 j-t	-24.23	6.02 a-d
CIMMYT-HTN 2014/15-5	6.73 abc	5.95 b-j	-11.59	6.34 a
CIMMYT-HTN 2013/14-4492	6.87 a	5.26 ı-t	-23.44	6.06 abc
CIMMYT-HTN 2014/15-8	5.63 e-o	3.88 w	-31.08	4.75 hıj
Basribey	6.46 a-e	5.99 b-j	-7.28	6.22 ab
CIMMYT-HTN 2014/15-4	6.27 a-g	6.14 a-h	-2.07	6.20 ab
CIMMYT-HTN 2014/15-6	5.46 g-s	5.36 h-t	-1.83	5.41 efg
Esperia	5.29 h-t	5.66 e-n	6.99	5.47 d-g
CIMMYT-HTN 2013/14-4488	6.63 a-d	5.06 k-u	-23.68	5.84 a-f
CIMMYT-HTN 2013/14-4490	6.06 a-ı	5.91 c-j	-2.48	5.98 a-e
Syrena	5.04 k-u	5.55 f-r	10.12	5.29 fgh
CIMMYT-HTN 2014/15-2	5.93 b-j	5.87 d-k	-1.01	5.90 a-e
CIMMYT-HTN 2014/15-9	5.72 e-n	5.64 e-o	-1.40	5.68 b-f
Krasunia	5.65 e-n	5.56 f-q	-1.59	5.60 c-f
Nota	6.76 ab	5.62 e-p	-16.86	6.19 ab
Gelibolu	5.83 d-m	4.74 q-v	-18.70	5.28 fgh
Tina	6.09 a-ı	4.71 r-v	-22.76	5.40 efg
Dropia	4.65 s-v	5.02 l-u	7.96	4.83 hıj
CIMMYT-HTN 2014/15-7	4.67 s-v	4.03 vw	-13.70	4.35 j
CIMMYT-HTN 2013/14-4464	5.62 e-p	5.95 b-j	5.87	5.78 a-f
Yubileynaya 100	6.39 a-f	5.38 e-n	-15.80	5.88 a-e
Kate A1	5.94 b-j	5.53 f-v	-6.90	5.73 b-f
Saraybosna	5.00 m-u	4.72 q-v	-5.60	4.86 hıj
CIMMYT-HTN 2014/15-10	4.59 t-w	4.28 uvw	-6.75	4.43 ıj
CIMMYT-HTN 2014/15-3	5.15 j-t	5.89 c-k	14.37	5.52 c-f
Golia	4.78 p-v	5.14 j-t	7.53	4.96 ghı
Tekirdağ	4.87 n-u	4.79 o-v	1.64	4.83 hıj
Pehlivan	5.53 f-r	5.64 e-o	1.99	5.58 c-f
Flamura-85	6.09 a-ı	4.94 n-u	-18.88	5.51 c-f
Mean	5.76	5.31	-7.81	
MSE	17.86148			

GY, grain yield; MSE, mean squared error; N, normal; L, late

that had the highest GY in the LS date was CIMMYT-HTN 2014/15-4 (6.14 t ha^{-1}). This line was followed by CIMMYT-HTN 2014/15-1 (6.06 t ha^{-1}), Basribey (5.99 t ha^{-1}), CIMMYT-HTN 2013/14-4464 (5.95 t ha^{-1}), CIMMYT-HTN 2013/14-4490 (5.91 t ha^{-1}), CIMMYT-HTN 2014/15-3 (5.89 t ha^{-1}), CIMMYT-HTN 2014/15-2 (5.87 t ha^{-1}) (Table 2). Basribey cultivar and CIMMYT-HTN 2014/15-5, CIMMYT-HTN 2014/15-4, CIMMYT-HTN 2013/14-4490 were the highest GY both in NS and LS (Table 2).

Membrane thermostability (MT-%): There were significant differences (P≤0.01) among the mean of bread wheat cultivars for the MT (Table 3). That is one of the methods that are recommended in the

Table 3- Means and significance groups of genotypes' some examined traits

Genotype	*MT (%)*	*CT (°C)*	*LCC (SPAD)*	*SC (mmol m^{-2} s^{-1})*
Nota	50.270 e	20.000 a-d	43.233 efg	58.133 b-g
Kate A1	44.670 h	19.333 b-e	48.867 a-f	79.200 bcd
Basribey	39.960 m	20.667 ab	44.367 c-g	72.500 b-f
Gelibolu	30.930 uv	21.000 ab	46.367 a-f	32.733 efg
Esperia	20.000 A	20.667 ab	50.233 a-e	93.500 bc
Saraybosna	33.670 t	20.667 ab	53.000 ab	166.800 a
Syrena	49.530 f	20.000 a-d	48.633 a-f	33.133 efg
Flamura 85	30.930 uv	20.000 a-d	49.667 a-e	55.000 b-g
Krasunia	35.000 s	22.000 a	49.367 a-e	95.633 ab
Dropia	37.130 o	21.000 ab	50.733 a-d	78.300 b-e
Tina	26.660 y	20.000 a-d	50.233 a-e	64.100 b-g
Golia	31.040 u	21.333 ab	47.300 a-f	32.733 efg
Tekirdağ	10.580 B	21.000 ab	45.667 c-g	93.933 bc
Pehlivan	42.830 i	20.333 abc	41.833 fg	38.000 d-g
Yubileynaya 100	54.230 d	21.000 ab	50.900 a-d	55.633 b-g
CIMMYT-HTN 2014/15-1	44.590 h	20.333 abc	49.367 a-e	61.567 b-g
CIMMYT-HTN 2014/15-2	66.250 a	19.333 b-e	49.367 a-e	56.600 b-g
CIMMYT-HTN 2014/15-3	37.380 n	20.000 a-d	45.500 c-g	55.900 b-g
CIMMYT-HTN 2014/15-4	40.970 l	19.333 b-e	46.100 b-f	33.400 efg
CIMMYT-HTN 2014/15-5	58.010 b	17.667 e	48.733 a-f	46.133 d-g
CIMMYT-HTN 2014/15-6	50.300 e	19.333 b-e	51.467 ac	39.533 d-g
CIMMYT-HTN 2014/15-7	46.110 g	19.333 b-e	49.800 a-e	52.667 b-g
CIMMYT-HTN 2014/15-8	43.010 i	18.000 de	44.333 c-g	51.167 c-g
CIMMYT-HTN 2014/15-9	56.820 c	17.667 e	38.300 g	79.100 bcd
CIMMYT-HTN 2014/15-10	36.660 p	18.333 cde	53.300 a	28.767 fg
CIMMYT-HTN 2013/14-4464	23.960 z	20.667 ab	45.700 c-f	43.633 d-g
CIMMYT-HTN 2013/14-4488	26.890 y	20.333 abc	44.033 d-g	55.433 b-g
CIMMYT-HTN 2013/14-4489	30.670 v	21.000 ab	48.700 a-f	78.500 bcd
CIMMYT-HTN 2013/14-4490	35.670 r	21.000 ab	47.600 a-f	43.867 d-g
CIMMYT-HTN 2013/14 4492	41.730 k	21.000 ab	50.333 a-e	71.267 b-g
MSE	0.022	1.292	12.820	466.613

MT, membrane thermostability; CT, canopy temperature; LCC, leaf chlorophyll content (LCC); SC, stomatal conductance (SC); MSE, mean squared error

tolerant plant selection to stress condition(s). The method used for the identification of membrane permeability depends on the principle of the determination of the number of ions that leak into the apoplastic fluid from cytoplasm as a result of function disorder in cell membrane caused by injury (Gusta et al 2003). The study revealed that the MT increased in stress conditions, which means that the genotypes with lower cell injury are more tolerant to heat stress conditions.

In this study, mean MT values had a large variation between 10.580 to 66.250% (Table 3). This might be a result of different genetic structures of the genotypes used in the research. The highest MT value was detected in CIMMYT-HTN 2014/15-2 (66.250%). It was followed by CIMMYT-

HTN 2014/15-5, CIMMYT-HTN 2014/15-9 and Yubileynaya 100 (58.010%, 58.200% and 54.230%, respectively). It can be said that the heat-temperature stress tolerance of these genotypes for the MT values is higher than others. In the study, the lowest MT value was taken from Tekirdağ (10.580%) and Esperia (20.000%) cultivars. Our findings on genotypes have similar with the reports of Sikder et al (1999), Blum et al (2001), Hasan et al (2007), Yıldırım et al (2009), Khan et al (2013) and Khan et al (2015) who revealed that the MT of wheat genotypes that are exposed to high-temperature stress caused by LS which depends on the genotype.

Canopy temperature (CT- °C): In the variance analysis made for the CT, differences between the genotypes have been found as P≤0.01 significant (Table 3). The high CT causes the increase in respiration of the plants and consequently decreases the net photosynthesis rate. For this reason, the genotypes which have the lower CT under the same ecological conditions are advantageous considering plant development.

The CT in all genotypes was varied between (17.667 °C to 22.000 °C) (Table 3). Accordingly, differences occur that reach to 4.333 °C between CT of the wheat genotypes that grown under the same ecological conditions. This can be a result of different reactions of tested genotypes to the high-temperature stress caused by late sowing. Similar to our findings, Ray & Ahmad (2015) revealed that CTs of wheat genotypes at the post-anthesis stage are different. Also, the researchers revealed that CT can be used as a selection criterion under the high-temperature stress conditions.

In our study, it was found that CIMMYT-HTN 2014/15-9 and CIMMYT-HTN 2014/15-5 (17.667 °C) had the lowest CT among bread wheat genotypes. These lines were followed by CIMMYT-HTN 2014/15-8 (18.000 °C), CIMMYT-HTN 2014/15-10 (18.333 °C), CIMMYT-HTN 2014/15-7, CIMMYT-HTN 2014/15-6, CIMMYT-HTN 2014/15-4, CIMMYT-HTN 2014/15-2 and Kate A-1 (19.333 °C). The results revealed that the CIMMYT origin genotypes have the lower CT and more tolerant to the high temperatures. Our findings in accordance with Sikder & Paul (2010) who are found that high heat tolerant wheats have the lower CT than those of the sensitive. On the other hand, the highest CT was measured in Krasunia (22.000 °C), Golia (21.333 °C), Dropia (21.000 °C), Tekirdağ (21.000 °C), CIMMYT-HTN 2013/14-4490 (21.000 °C), CIMMYT-HTN 2013/14-4492 (21.000 °C), CIMMYT-HTN 2013/14-4489 (21.000 °C), Yubileynaya 100 (21.000 °C) and Gelibolu (21.000 °C). In the light of these results, it was realized that Krasunia, Dropia, Tekirdağ, Gelibolu, Esperia cultivars' CT were affected by high temperatures. Besides, CIMMYT-HTN 2013/14-4490, CIMMYT-HTN 2013/14-4492, CIMMYT-HTN 2013/14-4489 and CIMMYT-HTN 2013/14-4464 lines are believed to have a lower tolerance in terms of the CT.

Leaf chlorophyll content (LCC-SPAD): The mean of the LCC differences of the genotypes which are exposed to heat stress in late sowing were determined as P≤0.01 significant (Table 3). The LCC values were changed between (38.300 to 53.300 SPAD) and had been a large variation (Table 3). This might be a result of different genetic structures of the wheat genotypes used in the research. Similar to our findings, Javed et al (2014) revealed that the LCC of cultivars in high-temperature stress were significantly different. In our study, the highest LCC was detected in CIMMYT-HTN 2014/15-10 (53.300 SPAD). It was followed by Saraybosna (53.000 SPAD), CIMMYT-HTN 2014/15-6 (51.467 SPAD), Yubileynaya 100 (50.990 SPAD) and Dropia (50.773 SPAD). These results revealed that CIMMYT origin lines which are known to be tolerant to the high-temperature since they have the high LCC. Our findings are similar to the findings of Reynolds et al (1996), Nawaz et al (2013) and Feng et al (2014). The lowest LCC among the genotypes was found in CIMMYT-HTN 2014/15-9 with (38.300 SPAD). It was followed by Pehlivan (41.833 SPAD) and Nota (43.233 SPAD). In a general evaluation considering the LCC of other genotypes, it was observed that some lines which are known to be tolerant to the high-temperature(s) (CIMMYT-HTN 2013/14-4488, CIMMYT-HTN 2014/15-8, CIMMYT-HTN 2014/15-3 and CIMMYT-HTN 2013/14-4464) had

the very low LCC (44.033, 44.333, 45.500, 45.700 SPAD, respectively) (Table 3). On the other hand, it can be mentioned that there was not a certain correlation between the LCC and high-temperature stress and this trait depends on genotype, ecology, and their interactions and used growing techniques.

Stomatal conductance (SC- mmol m^{-2} s^{-1}): The SC is known as a physiological selection criterion used in estimating gas exchange such as CO_2 absorption thoroughly to the leaves and water loss with transpiration depending on stomata pore (Pask et al 2012). Munjal & Rana (2003) revealed that the low of CT and high SC during the grain filling period of bread wheat genotypes under the high-temperature(s) can be basic morpho-physiological criteria for the high GY. According to the results of variance analysis in the study, the difference between the genotypes has been statistically ($P \leq 0.01$) found significant for the SC (Table 3). The SC in the bread wheats was shown a large variation between (25.200 to 166.800 mmol m^{-2} s^{-1}). This variation shows that bread wheat genotypes do not respond equally to heat- temperature stress in terms of the SC. The highest SC values were detected in Saraybosna (166.800 mmol m^{-2} s^{-1}), Krasunia (95.633 mmol m^{-2} s^{-1}), Tekirdağ (93.933 mmol m^{-2} s^{-1}), Esperia (93.500 mmol m^{-2} s^{-1}) cultivars (Table 3). Golia cultivar (25.200 mmol m^{-2} s^{-1}) had the lowest stomatal conductance. This cultivar was followed by CIMMYT-HTN 2014/15-10 (28.767 mmol m^{-2} s^{-1}), Gelibolu (32.733 mmol m^{-2} s^{-1}), Syrena with (33.133 mmol m^{-2} s^{-1}) and CIMMYT-HTN 2014/15-4 (33.400 mmol m^{-2} s^{-1}). It has been realized that the flag leaf SC values of tested genotypes were found as low during the post-anthesis period. This may be caused by the senescence of flag leaves in the post-anthesis period in which measurements have been made. Similar to our findings, Bahar et al (2009) reported that SC value of durum wheats' (*Triticum durum* Desf.) 294 mmol m^{-2} s^{-1} at the early milk stage, and it decreased to 225 mmol m^{-2} s^{-1} at the end of the milk stage and to 167 mmol m^{-2} s^{-1} at the early dough stage, and this resulted from senescence of flag leaf after anthesis.

4. Conclusions

As a summary, Dropia, Nota, CIMMYT-HTN 2014/15-2, CIMMYT-HTN 2014/15-6, CIMMYT-HTN 2014/15-10 were found as prominent for all investigated traits, except for the GY. It is possible that to use of these genotypes as genitor(s) or progenitor(s) in the wheat breeding programs for the heat-temperature stress tolerance. The (5) genotypes (Basribey, CIMMYT-HTN 2013/14-4490, CIMMYT-HTN 2014/15-1, CIMMYT-HTN 2014/15-4, CIMMYT-HTN 2014/15-5, respectively) were higher in terms of the GY at the NS and LS. On the other hand, these genotypes were found as the prominent for the examined heat tolerance parameters. In order to develop the heat tolerant and to get higher of the GY, a comprehensive wheat breeding program can be suggested which includes multiple crossing among them with the selection method considered for the heat tolerance, yield and yield components like the physiological parameters such as MT, CT, LCC and SC against to the high-temperature stress in wheat.

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