MEDITERRANEAN AGRICULTURAL SCIENCES

Volume: 35
Number: 1
Year: April 2022

E-ISSN: 2528-9675

e-ISSN 2528-9675

MEDITERRANEAN AGRICULTURAL SCIENCES

Previous Name: Akdeniz University Journal of the Faculty of Agriculture

The peer reviewed scientific journal of Akdeniz University Faculty of Agriculture

Three issues are published per year in April, August and December

Abbreviation of the journal: Mediterr Agric Sci

Owner on behalf of Akdeniz University, Faculty of Agriculture

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Web site: www.dergipark.org.tr/en/pub/mediterranean

Publisher

Akdeniz University, Faculty of Agriculture
07058 Antalya, Turkey

Phone: +90 242 310 2412
Fax: +90 242 310 2479

Subscription

Open access journal

Online access free of charge
www.dergipark.org.tr/en/pub/mediterranean

Cover design
Assoc. Prof. Dr. Suleyman OZDERIN

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MEDITERRANEAN AGRICULTURAL SCIENCES is indexed and abstracted in **CABI** data bases (**CAB Direct**), **TUBITAK-ULAKBIM** (National Data Bases-Data Base of Life Sciences), **CLARIVATE ANALYTICS, SCIENCE MASTER JOURNAL LIST** (Zoological Records) and **DRJI** (Directory of Research Journals Indexing).

e-ISSN 2528-9675

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES

The journal was published under the name “Akdeniz University Journal of the Faculty of Agriculture” and with the number ISSN 1301-2215 until 2016.

Volume: **35** Number: **1** Year: April **2022**

e-ISSN 2528-9675

MEDITERRANEAN AGRICULTURAL SCIENCES

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MEDITERRANEAN AGRICULTURAL SCIENCES
(2022) 35(1): 1-6
DOI: 10.29136/mediterranean.1020532

Effects of iron fertilization on plant growth, yield components and quality traits of industrial tomatoes

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ARTICLE INFO

Received: November 8, 2021
Received in revised form: December 13, 2021
Accepted: December 14, 2021

Keywords:

FeEDDHA
Marketable yield
Paste yield
Titratable acidity

ABSTRACT

This study was conducted in order to investigate the effects of different iron applications on the yield and fruit quality traits of industrial tomatoes. Experiments were conducted in a randomized block design with three replications under field conditions. The H-5803 and Delfo hybrid industrial tomato cultivars were used as the plant material and experimental treatments included 0 (control), 1.0, 2.0, 3.0, 4.0 kg ha^{-1} FeEDDHA (6% Fe) applications. The greatest increases in plant growth parameters (fruit length and width), marketable and paste yields were achieved with 2.0 and 3.0 kg ha^{-1} FeEDDHA. Iron treatments had significant effects on fruit weight, width, and lengths, and the greatest values were obtained from 2.0 and 3.0 kg ha^{-1}. Increasing iron treatments also increased fruit quality traits (dry matter, soluble solids, total acidity). A significant relationship, however, was not observed between iron treatments and fruit pH values. In terms of plant nutrition, fertilizer cost, and yield increases, 2.0 kg ha^{-1} FeEDDHA treatment could be recommended as a useful fertilization strategy in tomato cultivation.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the most commonly cultivated vegetable worldwide under field and greenhouse conditions (Kallo 1986). Tomato is consumed in different forms including bulk-paste, puree, ketchup, tomato juice, fresh and dry tomato. Several researchers have investigated the effects of different plant nutrients on tomato yield and quality and indicated that some of these nutrients play a key role in tomato cultivation (Dorais et al. 2001). Among these nutrients, micronutrients, especially iron, were reported as the key element. Iron plays a significant role in tomato nutrition, development, fruit yield, and quality of tomato. Iron acts as a cofactor for about 140 enzymes catalyzing biochemical reactions. Besides, iron plays an important role in chlorophyll synthesis, chloroplast development, transpiration function, electron transfer, and various other metabolic processes (Mengel et al. 1994; Chohura et al. 2009).

Previous studies have demonstrated that world and Turkish soils have various nutritional problems related to micronutrients and soils were mostly identified as being poor in micronutrients. Such deficiencies have various negative impacts on plants, as well as on humans and animals through the food chain. Iron deficiency is encountered in 27% of Turkish soils (Eyüpoğlu et al. 1998). Iron (Fe) deficiency in alkaline and/or calcareous soils result in a common nutritional disorder in plants grown in these soils because of the low solubility of Fe (Lindsay 1991). High pH, high bicarbonate ion concentrations in soils and irrigation waters, and high Ca^{+2}, Mg^{+2}, PO_4^{-3}, Cu^{+2}, Mn^{+2}, and Zn^{+2} concentrations of the soils significantly reduce the availability of iron in soils (Havlin et al. 1999; Kaçar and Katkat 2018).

Iron-containing fertilizers are used to improve the bioavailability of iron in soils. These fertilizers improve plant root development, positively influence plant iron uptakes and thus increase plant yield and quality (Chen and Aviad 1990; Padem and Öcal 1998). FeEDDHA efficiency is high in soils with different pH levels and iron-deficient plants (Kaçar and Katkat 2018). Sanchez-Sanchez et al. (2002, 2005) applied FeEDDHA compounds in soil and reported increased leaf iron concentrations and improved fruit quality parameters in tomato and citrus species. In another study, FeEDDHA was reported as the most efficient fertilizer in the prevention of chlorosis (Karaman 2003). Iron compounds are commonly applied through irrigation lines and such practices (fertigation) yield highly positive outcomes. Especially FeEDDHA could successfully be applied through drip lines (Kaçar and Katkat 2018).

Although iron is a very abundant nutrient in soils, chlorosis is very common in plants as a result of its deficiency. Finding high iron in soil analysis does not mean that the plant will not suffer from iron deficiency. Furthermore, high total iron concentrations in plant leaves do not guarantee that plants have adequate iron. Factors that cause iron deficiency in plants are those that prevent the absorption of iron from the soil through roots, its transport and metabolism within the plant. Differences between cultural processes and plant species are also among the factors that cause iron chlorosis. Fe-EDDHA is the

recommended form of Fe to correct iron deficiency in calcareous soils (Lindsay 1984; Loué 1986; Benntt et al. 1988; Aktaş 1994; Shalau 2010; Kaçar and Katkat 2018). In the present study the effects of supplementary iron fertilization (FeEDDHA form) in the soil, on plant growth parameters of two commonly grown industrial tomato cultivars were investigated under field conditions and changes in fruit characteristics, fruit, and tomato paste yields with different iron treatments were assessed.

2. Material and Method

2.1. Experimental site and plant material

The experiments were conducted in the experimental fields of Mustafakemalpaşa Vocational School of Bursa Uludağ University (40°, 02' N, 28°, 23' E; altitude of 22 m) in the May-August growing season of the years 2020 and 2021. Commonly cultivated industrial-type hybrid tomato cultivars of 'H-5803' and 'Delfo' *(Lycopersicon esculentum* Mill.) were used as the plant materials for the experiments. Tomato seedlings were supplied from a commercial seedling company (Marmara Seedling Product. Agri. Industry Trade Co. Bursa, Turkey).

2.2. Soil and climate parameters of the experimental site

Experimental soils were clay-loam (sand: 23.6%; silt: 43.6%; clay: 32.8%) in texture with an average soil depth of 90 cm, nonsaline (0.49 dS m^{-1}) with slightly alkaline reaction (pH= 7.9) and high lime content (11.9%). Soils were rich in available potassium (283.0 mg kg^{-1}), low in phosphorus (11.8 mg kg^{-1}), poor in organic matter (1.8%), moderate in total nitrogen (0.17%). Available iron content was 7.70 mg kg^{-1}, volumetric water content was 38.3% at field capacity (0.03 MPa) and 23.2% at permanent wilting point (1.5 MPa); bulk density was identified as 1.41 g cm^{-3}.

In this region, the summers are generally hot and dry with precipitation in the winters. Climate data throughout the tomato growing season (May-August) of the years 2020 and 2021 and long-term (1928–2018) averages are provided in Table 1.

2.3. Experimental design and growth conditions

The experiments were conducted in a randomized block design with three replications. Each replicate (plot) was composed of one plant row and each row had 60 tomato plants. About 1.5 m spacing was provided between the plots and two plant rows were provided to prevent interactions with the surrounding environment. Treatments were randomly assigned to the plots. Before iron fertilization, nitrogen (ammonium sulphate), phosphorus (diammonium phosphate), and potassium (potassium sulphate) (150 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5 and 50 kg ha^{-1} K_2O) were applied as basic fertilizers. All phosphorus and potassium fertilizers and half of the nitrogenous fertilizer were applied before planting at soil tillage and the rest of the nitrogen was applied at the small-fruit stage (Şalk et al. 2008). Tomato seedlings were manually planted on 14th of May in the first year (2020) and 20th of May in the second year (2021) at 30 x150 cm (*within-row spacing and between-row spacing)* spacing. Herbicide treatments were not applied and manual weed control was practiced with a hand hoe. Standard cultural practices were conducted throughout the growing season. Plants were irrigated from groundwater resources and applied through drip lines.

2.4. Iron fertilizer treatment

Five different iron doses [1.0 kg ha^{-1} (Fe_1), 2.0 kg ha^{-1} (Fe_2), 3.0 kg ha^{-1} (Fe_3) and 4.0 kg ha^{-1} (Fe_4)] were applied to the tomato plants. No iron fertilizer was applied to the control plots (Fe_0). The FeEDDHA with high availability at high pH conditions was used as iron fertilizer. FeEDDHA was supplied from a commercial dealer (Hunter Fe, Tarsa Agriculture, Industry and Trade Co, Antalya, Turkey). It contains 6% metallic iron (in iron chelate EDDHA form). It is in granular form and soluble in water. The iron fertilizer was dissolved in water, and then homogeneously applied to the soil manually. The first treatment was applied at the beginning of flowering, the second treatment was applied at the full-bloom stage and the last treatment was applied at the veraison stage of the fruits (Demir 2017).

2.5. Harvest, measurement and weighing, fruits analysis

Full-red fruits were harvested 5 times between 26 July and 25 August of the first year (2020) and 6 times between 4 August and 1 September of the second year (2021).

After each harvest, fruit diameter and length, single fruit weight, marketable yield, tomato paste yield, fruit dry matter, soluble solids contents, and fruit total acidity were determined. Following the last harvest, the average of all the parameters was calculated.

Before the harvest, plant height (*PH, cm*) and plant diameter (*PD, cm*) were measured in all treatment groups with the use of a tape measure.

Fruit weight *(FW, g)* was calculated by dividing the weight of all harvested fruits by the total number of fruits.

Fruit diameter *(FD, cm)* was measured from the middle cross-section of 30 fruits, and the average of them was taken. Fruit length *(FL, cm)* was measured with the use of a digital caliper.

For marketable yield *(MY, t ha^{-1});* fruits were harvested at the full-red stage and classified as marketable or non-marketable fruits (fruits with mechanical, physiologic, and/or phytosanitary damages) (Campos et al. 2006). Following each harvest, marketable fruits were weighed and expressed in t ha^{-1} by considering 30 x150 cm spacing (Kuşçu et al. 2016).

For tomato paste yield *(PY, t ha^{-1});* harvested tomatoes were washed and peeled. They were chopped, heated up to 85-90^{0}C (hot-processing method) and passed through a pulper. The resultant pulp was evaporated in open cookers until they reached soluble solids content of 28% (28^{0}Brix) (Cemeroğlu et al. 2003).

Following each harvest, three randomly selected fruits were washed with distilled water; seeds were removed, and ground. Dry matter (*DM, %*) was determined by oven drying at 70°C for 2 days. The soluble solids content *(SSC, ^{0}Brix)* of the fruit juice was measured with the use of a refractometer (Abbe-type refractometer, model 60/DR) (Tigchelaar 1986). Total acidity *(TA, %, in citric acid)* was determined by titration of the fruit juice with 0.1 N NaOH (Anonymous 1968).

2.6. Statistical analyses

Experimental results were subjected to analysis of variance (ANOVA) with the use of statistical software (IBM® SPSS® Statistics for Windows, Version 20.0, Copyright, 2011, IBM

Corp, Armonk, NY). Significant means (based on the F test) were compared with the use of Duncan's multiple range tests.

3. Results and Discussion

Different iron (FeEDDA) doses were applied to two different industrial tomato cultivars (H-5803 and Delfo) grown under field conditions and the relationships between the treatments and plant growth parameters PH and PD were investigated. As can be inferred from Table 2, significant differences were observed in PH and PDs among the treatments. The greatest PH in 'H-5803' and 'Delfo' cultivars were observed in Fe_2 and Fe_3 treatments respectively. Plant diameters significantly increased with the applications doses. In terms of plant diameters, Fe_2 was identified as the most efficient treatment. Similar to the present findings, Mohamadi et al. (2021) indicated that tomato PHs could significantly be increased with iron applications and combined iron and phosphorus treatments could even further increase PHs. Roosta and Mohsenian (2015) reported the greatest vegetative growth of eggplant was in iron-treated plants.

Iron treatments had significant effects on MYs of tomato plants and such effects varied with the iron doses (Table 2). Complying with the present findings, significant yield increases were reported in soybean (Schenkeveld et al. 2008), spinach (Zengin et al. 2010; Yılmaz et al. 2012), and tomato (Asri and Sönmez 2010) with iron treatments. Besides, Schenkeveld et al. (2008) investigated the effects of different iron compounds and indicated FeEDDHA as the most effective source of iron. In comparison to the control treatment, the greatest increase in MY was achieved with Fe_2 (21.62%) treatment in the 'H-5803' cultivar and with Fe_2 (32.22%) and Fe_3 (24.09%) treatments in 'Delfo' cultivar. The other treatments had limited effects on yield. For instance, in both cultivars, in comparison to the control, Fe_1 treatment did not have significant effects on yield. On the other hand, the degree of response of tomato cultivars to high-dose applications varied. For example, Fe_4 high dose treatment increased yield by 0.16% in 'H-5803' cultivar and decreased by 10.63% in 'Delfo' cultivar.

Iron deficiency has significant effects on agricultural practices of various regions and significantly limits the yield potential of field crops and vegetables (Hansen et al. 2006). According to Kobayashi et al. (2005), iron reduces crop yields in low-solubility calcareous and high-pH soils. Civelek (2006) conducted a study with soybean and reported significant increases in soybean yields with FeEDDHA treatments as compared to the control. In the present study, tomato plants were fertilized with iron fertilizers at different doses in the form of FeEDDHA, and the results are provided in Table 2. Variance analysis results revealed that iron treatments at different doses significantly increased PY. The greatest increase in PY in both cultivars was achieved with Fe_2 and Fe_3 treatments. In comparison to the control, Fe_2 and Fe_3 treatments increased PY respectively by 28.46 and 24.38% in the H-5803 cultivar and by 35.09 and 28.62% in 'Delfo' cultivar. The effects of low (Fe_1) and high (Fe_4) dose treatments on PY varied with the cultivars. Low dose treatment slightly increased the PY of both tomato cultivars (H-5803 and Delfo) (1.28 and 5.69%). On the other hand, Fe_4 treatment increased the PY of the 'H-5803' cultivar by 18.35% but reduced the PY of 'Delfo' cultivar by 5.69%.

The effects of different iron doses on tomato fruit characteristics (fruit length, width and weight) were found to be significant (Table 3). Increasing iron doses positively influenced fruit characteristics and fruit dimensions increased with increasing iron doses. The longest fruits were obtained from Fe_3 (H-5803) and Fe_2 (Delfo) treatments and the widest fruits were obtained from Fe_2 (H-5803) and Fe_4 (Delfo) treatments. Single fruit weights were influenced the most from Fe_2 and Fe_3 doses.

Table 1. Average weather conditions during the experimental period in 2020 and 2021

	Mean temperature °C			Precipitation (mm)		
Months	2020	2021	1928-2018	2020	2021	1928-2018
May	17.7	19.3	17.7	51.1	20.2	49.8
June	22.0	21.4	22.1	34.4	69.6	33.8
July	24.5	26.0	24.5	22.3	24.7	21.3
August	24.3	26.4	24.3	18.6	0.2	16.4

Table 2. The effect of iron applications (FeEDDHA) on some growth parameters and yield components in tomatoes

Treatments	PH (cm)	PD (cm)	MY (t ha^{-1})	PY (t ha^{-1})
		H-5803		
Fe_0	112.83 c	116.50 b	61.41 c	11.76 c
Fe_1	114.43 bc	116.33 b	61.90 c (0.81%)^X	11.91 bc (1.28%)^X
Fe_2	124.87 a	129.00 a	74.86 a (21.62%)	15.10 a (28.46%)
Fe_3	120.62 ab	121.83 b	73.80 ab (19.36%)	14.62 a (24.38%)
Fe_4	117.52 bc	119.33 b	61.50 bc (0.16%)	13.91 ab (18.35%)
		DELFO		
Fe_0	106.77 c	109.94 b	79.14 b	17.07 bc
Fe_1	105.60 c	112.16 b	83.42 b (5.39%)^X	18.04 b (5.69%)^X
Fe_2	113.09 b	121.66 a	104.67 a (32.22%)	23.06 a (35.09%)
Fe_3	120.14 a	120.24 a	98.93 a (24.09%)	21.96 a (28.62%)
Fe_4	106.99 c	117.07 ab	71.01 c (-10.53%)	16.10 c (-5.69%)

Different letters in each column represent significant differences at $P<0.05$ according to Duncan's multiple distribution tests. As: Fe_0 control "no iron application", Fe_1 1.0 kg ha^{-1}, (Fe_2) 2.0 kg ha^{-1}, Fe_3 3.0 kg ha^{-1} and Fe_4 4.0 kg ha^{-1}, PH plant height, PD plant diameter, MY marketable yield, PY paste yield, ^{X}% change from the control (Fe_0-Fe_1)/Fe_0)x100).

Table 3. The effect of iron applications (FeEDDHA) on fruit characteristics and quality in tomato

Treatments	FH (cm)	FD (cm)	FW (g)	SSC (^{0}Brix)	pH	DM (%)	TA (%)
			H-5803				
Fe_0	5.76 c	5.30 c	92.62 c	5.33 d	4.38	5.43 e	0.35 c
Fe_1	5.90 bc	5.85 ab	98.17 b	5.44 c	4.33	5.57 d	0.39 c
Fe_2	6.05 ac	6.02 a	107.73 a	5.82 b	4.31	5.92 c	0.49 b
Fe_3	6.22 a	5.90 a	105.20 a	5.85 b	4.37	6.06 b	0.48 b
Fe_4	6.19 ab	5.55 bc	88.31 d	5.99 a	4.30	6.21 a	0.57 a
			DELFO				
Fe_0	5.86 c	4.88 c	102.40 b	6.04 c	4.40	6.21 c	0.32 d
Fe_1	6.06 ac	5.07 bc	102.21 b	6.07 c	4.39	6.22 c	0.31 d
Fe_2	6.38 a	5.20 ab	109.97 a	6.17 b	4.40	6.39 b	0.38 c
Fe_3	6.32 ab	5.18 ab	106.85 a	6.22 b	4.38	6.47 b	0.44 b
Fe_4	5.97 bc	5.38 a	91.31 c	6.35 a	4.37	6.57 a	0.54 a

Different letters in each column represent significant differences at $P<0.05$ according to Duncan's multiple distribution tests. As: Fe_0 control "no iron application", Fe_1 1.0 kg ha^{-1}, (Fe_2) 2.0 kg ha^{-1}, Fe_3 3.0 kg ha^{-1} and Fe_4 4.0 kg ha^{-1}, FH fruit length, FD fruit diameter, FW fruit weight, SSC soluble solids content, DM dry matter, TA titratable acidity.

In previous studies, it was reported that iron treatments increased the number of fruits, fruit size, and weight in tomatoes (Houimli et al. 2015; Sakya and Sulandjari 2019) and tuber weights in potatoes (Hadi et al. 2015). Chaurasia et al. (2005) pointed out the significance of foliar fertilizer applications and indicated that foliar treatments significantly increased the number of fruits and fruit dimensions (diameter and length) in tomatoes.

The soluble solids content is an important parameter in tomato paste production (Gould 1992). High dry matter and soluble solids content are desired in the tomato paste industry. Since high soluble solids content reduces the energy required to evaporate the juice from the fruit and shortens the process duration, it increases productivity in tomato paste production (DePascale et al. 2001; Johnstone et al. 2005; Patane and Cosentino 2010; Turhan 2020). As can be inferred from Table 3, the soluble solids content of tomato fruits was significantly influenced by the iron fertilizer treatments. The lowest soluble solids content was obtained from the control plants. In the control treatment, soluble solids content was identified as 5.33% in the 'H-5803'cultivar and 6.04% in the 'Delfo' cultivar. Increasing iron doses significantly increased the fruit soluble solids content and the greatest values were obtained from Fe_4 treatments. In Fe_4 treatment, soluble solids content was identified as 5.35% in 'H-5803' cultivar and 6.35% in 'Delfo' cultivar. Effects of different iron doses on fruit pH values were found to be nonsignificant. In other words, iron treatments did not any have positive or negative effects on fruit pH values (Table 3). As it was insoluble solids, some researchers indicated that tomato fruit quality parameters could be improved with micronutrient mixtures in which iron is included (Rahi et al. 2020). It was reported that different iron doses (Fetrilon-13 chelate) positively influenced grape soluble solids and total dry matter contents, pH and titratable acidity as compared to the control (Çoban et al. 2002).

The fruit dry matter contents were also significantly influenced by the iron treatments. There were significant positive relationships between iron doses and dry matter contents. Present findings on dry matter content comply with the findings of Asri and Sönmez (2010) which reported increasing tomato fruit dry matter contents with increasing potassium and iron treatments. In the present study, increasing dry matter contents were observed with increasing iron doses and the greatest values were obtained from F_4 treatments. On the other hand, in both cultivars (H-5803 and Delfo), the lowest dry matter contents were obtained from the control plants (Table 3).

Total acidity is an important quality parameter for tomato fruits. Organic acids give a sour taste to fruits and influence sweetness perception, thus influencing taste (Azodanlou et al. 2003). Micronutrients play a significant role in the fruit quality of tomatoes (Habashy et al. 2008). It was reported that the combined treatment of micronutrients (Zn+B+Fe) with potassium humate increased fruit acidity (Rahi et al. 2020). Similar findings were also observed in tomato plants treated with different iron doses. As can be inferred from Table 3, the lowest total acidity values were obtained from the control and Fe_1 treatments. The acidity values increased with increasing iron doses and the highest values were obtained from Fe_4 treatments.

4. Conclusions

The present findings revealed that soil iron treatments in FeEDDHA form had highly positive effects on tomato growth and development, FD and FL, thus FW, fruit dry matter, soluble solids content, and fruit titratable acidity. These findings also revealed that the marketable fruit yield and PY of industrial tomatoes could be improved with iron treatments. In terms of efficacy and economy, 2.0 kg ha^{-1} Fe treatment was found to be marked . The present findings proved that the inclusion of iron into fertilizer combinations might offer various advantages in crop and vegetable cultivation.

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Research Article

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MEDITERRANEAN AGRICULTURAL SCIENCES
(2022) 35(1): 7-14
DOI: 10.29136/mediterranean.1061475

Comparison of spinach cultivation in floating hydroponic system and soil in glasshouse and open field conditions

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ARTICLE INFO

Received: January 22, 2022
Received in revised form: February 22, 2022
Accepted: March 1, 2022

Keywords:

Floating hydroponic culture
Soil growing
Nitrate
Spinacia oleracea

ABSTRACT

The objective of this study was to investigate the effect of glasshouse and outdoor conditions on the growth of spinach plants in floating hydroponic culture and soil. In the floating hydroponic culture, the plants were grown in a plastic tank (120x50x30 cm) and a volume of 80 L in a glasshouse and open field. Each seed was inserted at 13x5 cm in rock wool in styrofoam and then placed in the nutrient solution. There was no significant change in EC values measured in the glasshouse and outside, and the pH of the solutions in the outdoor environment was higher (except in late December) than those in the glasshouse. The earliest and late harvests were made in floating hydroponic culture in the glasshouse and outdoor cultivation at 64 and 97 days, respectively. The highest yield was 1.54 kg m^{-2} in open field cultivation, it was followed by 1.45 kg m^{-2} in the greenhouse and 1.32 kg m^{-2} in the open field in floating hydroponic culture, respectively. Despite the high yield that can be obtained from floating hydroponic culture cultivation in the glasshouse and outside, the fact that there is a lower marketable amount is a negative aspect. However, the floating hydroponic culture could be preferred to soil cultivation due to many advantages such as production 2-3 times a year, low labor costs, and less pesticide use. Spinach cultivation in the open field does not have any problems in terms of nitrate, but nitrate accumulation can be a problem in hydroponic culture.

1. Introduction

Growing the same crop for many years in greenhouses (monoculture) triggers soil fatigue, soil salinity, diseases, and pest formation. Therefore, in recent years short vegetation period plants such as lettuce, spinach, chard, leek, green onions, green garlic, all kinds of green leafy aromatic vegetables, parsley, cress, arugula, mint, basil have been grown in floating hydroponic systems (Çelikel 2002; Ergün 2011). The hydroponic system has important advantages compared to cultivation in the soil such as the ability to adjust the structure of the growing medium, better drainage, fewer risks of weed and soil-borne pathogens, and low worker wages. Besides the reusability of the nutrient solution, it is easier to control parameters such as temperature, light intensity, light quality, application time, nutrient composition and density, and the amount of gas given to the roots (Marr 1994). The impact of the increase of the human population can be seen with more land needed for housing and decreasing land for agriculture, especially in urban areas. One solution for farming in urban areas is by utilizing rooftop farming by hydroponic cultivation. Water and electricity consumption for one growing period was 300.63 L and 31.816 kW, respectively in the floating raft fertigation system for spinach growing. The efficiency of water use was determined as 99.6 kg m^{-3} (Fadhlillah et al. 2019).

Spinach was harvested 52 days after planting in the autumn and 37 days in the floating hydroponic culture in spring. The highest yields in autumn were in 'Olympia' with 2093 g m^{-2} and in spring were in F91-415' with 1649 g·m^{-2}. 'F91-415' for autumn greenhouse production, and 'F91-415' and 'Padre' for spring production were recommended (Brandenberger et al. 2007).

The study aims to determine growing potential, earliness, and yield in an unheated glasshouse and outside in a floating hydroponic system and soil in winter in the ecological conditions in Antalya.

2. Material and Methods

The research was carried out in a glasshouse and an open field in the Research and Application Station of the Faculty of Agriculture, Akdeniz University, Antalya in winter (Figure 1). The glasshouse is 5 m wide, 6 m long, and 2 m side height with a double side ventilation system. The land is located at 36° 54 028' north latitude, 30° 38 810' east longitude, 1.5 km from the sea, and 38 m altitude. According to the soil analysis made at the Western Mediterranean Agricultural Research Institute; the soil type in the glasshouse and outside is clay loam with low organic matter (2.69%) and a pH of 8.23.

Matador (*Spinacia oleracea* var. Matador) spinach cultivar was used as the plant material. In floating hydroponic culture, the plants were grown in plastic tanks (120x50x30 cm) and a volume of 80 L. Styrofoam seedling trays (120x50x4.9 cm) was used and rock wool (Belagro Substrate) was placed in

styrofoam trays at 13x5 cm intervals for seed sowing (Figure 1). Oxygen was provided to the nutrient solutions through the aquarium air motor and air stones. Cooper (1988), the nutrient solution was used (Table 1), and the pH and EC of the solution were kept around 6.0-6.5 and 1.5-1.7, respectively.

Table 1. Cooper's nutrient solutions.

Elements	Amount mg l^{-1}
N	236
P	60
K	300
Ca	185
Mg	50
S	68
Fe (EDTA)	12
B	0.3
Mn	2
Zn	0.1
Cu	0.1
Mo	0.2

Spinach seeds were directly sown in 13x5 cm in rock wool in Styrofoam on December 21, and then placed in a greenhouse and outside in plastic tanks including a nutrient solution. The tank outside was covered with a round roof with plastic to protect it from external environmental factors such as rain (Figure 2). Changes in pH, EC, and temperature of the growing solution in the greenhouse were recorded. For cultivation in the glasshouse, the same soil as the open field was filled into 120x50x30 cm boxes with a 10 cm space at the top, and spinach seeds were sown in the soil by hand at 13x5 cm (Figure 3).

For open field cultivation in the soil, the soil was ploughed at a depth of 30 cm and, then smoothed, and made ready for seed sowing. Spinach seeds were sown by hand at a depth of 1 cm, with a row spacing of 13x5 cm (Figure 3). Cultural treatments such as irrigation, fertilization, plant protection, and harvesting were carried out in a timely and appropriate manner. Yield, plant height, root length, marketable plant weight (Ercan and Bayyurt 2014) chlorophyll content "Konica Minolta SPAD-502" (Geravandi et al. 2011), K, Ca, Mg, Fe, Z, Mn, Cu and nitrate accumulation (Kacar and İnal 2008) and P (Kacar and Kovancı 1982) content, vividness and color conditions of the leaves (Minolta Chorometer Reflectance) were determined.

The study was conducted in a split plots trial design in three replications, and 16 seeds were used in each replication. Glasshouse and open field growing factors were located in the main plot; cultivation technique was located in the subplots. Statistical analysis was carried out in the Statistical Package for the Social Science (SPSS, version 17), and the Tukey test was used to determine the differences between means (ns: nonsignificant, *: significant at $P \leq 0.05$).

Figure 1. Glass (left) glasshouse in which the research was conducted (left), and Styrofoam trays with rock wool in which spinach seeds were sown (right).

Figure 2. Spinach growing in floating culture in the glasshouse (left) and open field (right).

Figure 3. Spinach growing in the soil in the glasshouse (left) and open field (right).

3. Results

3.1. Temperature changes

The temperature values measured inside the glasshouse were higher than in the outdoor environment. The temperature in the glasshouse ranged from 14-20°C from December 21 to February 14, then it slightly increased, and was 25°C on February 23 at harvest time. The outdoor temperature changed more than inside the glasshouse and dropped to 8°C in mid-January. The maximum outdoor temperature was measured as 22-23°C in March (Figure 4).

The solution temperatures inside and outside were found to be lower than the glasshouse inside temperature during the experiment period. However, the solution temperatures inside and outside, except for early December and January, were measured at almost the same levels as the outside temperature. Only the outside temperature was determined to be lower than the solution temperatures in mid-January (Figure 4).

3.2. EC and pH changes

There was not much change in EC values measured in the glasshouse and outside. Although solution EC's were tried to be kept between 1.5-1.7, the measured values were higher. EC increase in the glasshouse was higher than in the outdoor environment, and the highest EC increase in solutions in the greenhouse was detected in February during harvest (Figure 5). Although 0.1 N nitric acid (HNO_3) was continuously given to the environment to keep the pH value constant between 6-6.5 in the solutions, the pH could not be kept at the desired values. Except for the period when the experiment started, the pH of the solutions in the outdoor environment was recorded to be higher than those in the greenhouse. The highest pH rise occurred at the beginning of the trial (7.34 in the greenhouse and 7.32 in outside), in January (7.1 in outside), and early February (7.13 in outside) (Figure 6).

3.3. Plant growth and yield values

The spinaches were harvested when they had five to six true leaves. The parameters measured during the harvest of spinach grown in the glasshouse and outside in floating hydroponic culture and soil had changed significantly ($P \leq 0.05$) (Table 2).

The earliest and latest harvest was made with 64 days on February 23 and with 97 days on March 28 in the floating hydroponic culture in the glasshouse and open field, respectively. However, the spinach grown in the glasshouse in the soil was harvested (72 days) earlier than grown in the floating hydroponic culture (83 days) outside (Table 2).

The highest yields were obtained with 1.54 kg m^{-2} in the open field and with 1.45 kg m^{-2} in floating hydroponic culture in the greenhouse. It was followed by floating hydroponic culture outside with 1.32 kg m^{-2} and soil in the glasshouse with 1.07 kg m^{-2}. After removing non-market value outer leaves (the choice was made based on personal experience), the yield was highest 1.18 kg m^{-2} in the open field, followed by the soil in the greenhouse with 0.94 kg m^{-2}, floating hydroponic culture outside with 0.87 kg m^{-2} and floating hydroponic culture in the glasshouse with 0.81 kg m^{-2} (Table 2).

The growing systems also affected the plant height. The length of spinach grown in floating hydroponic culture and soil was determined longer than that grown in the glasshouses. The length of spinach grown in the soil in the open field and glasshouse was higher than grown in floating hydroponic culture, and the length in the soil in the open field with 47.94 cm was measured to be twice as long as the other treatments. The shortest root length was in floating hydroponic culture in the glasshouse with 18.86 cm (Table 2).

The root length of spinach grown in floating hydroponic culture in the glasshouse (36.10 cm) and outdoor (30.02 cm) was measured as longer than spinach grown in soil. The root length of spinach grown in soil outside and glasshouse were 27.79 and 19.10 cm, respectively (Table 2).

While the most marketable spinach was in the open field with 1.18 kg m^{-2}, the amount in other applications were found to be close to each other. The marketable amount was determined as 0.94 kg m^{-2} in the soil in the glasshouse, 0.87 kg m^{-2} in the floating hydroponic culture outside, and 0.81 kg m^{-2} in the floating hydroponic culture in the glasshouse. The amount of marketable spinach was determined as 87.85% in the soil in the glasshouse, 76.62% in the soil in the open field, 65.91% in floating hydroponic culture outdoor, and 55.86% in floating hydroponic culture in the glasshouse (Table 2).

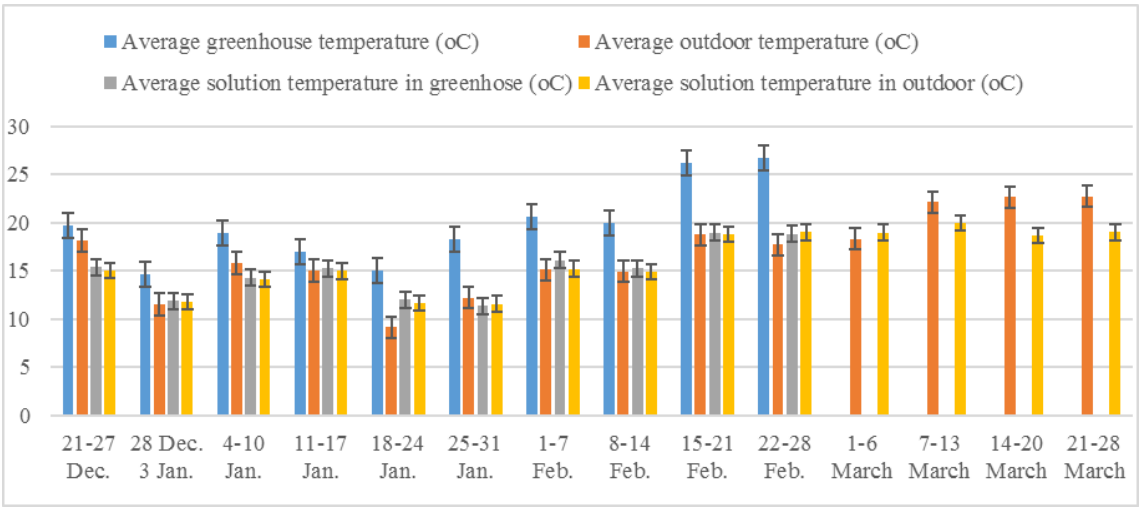

Figure 4. Average temperature values determined in the glasshouse, outdoor environment, and solutions.

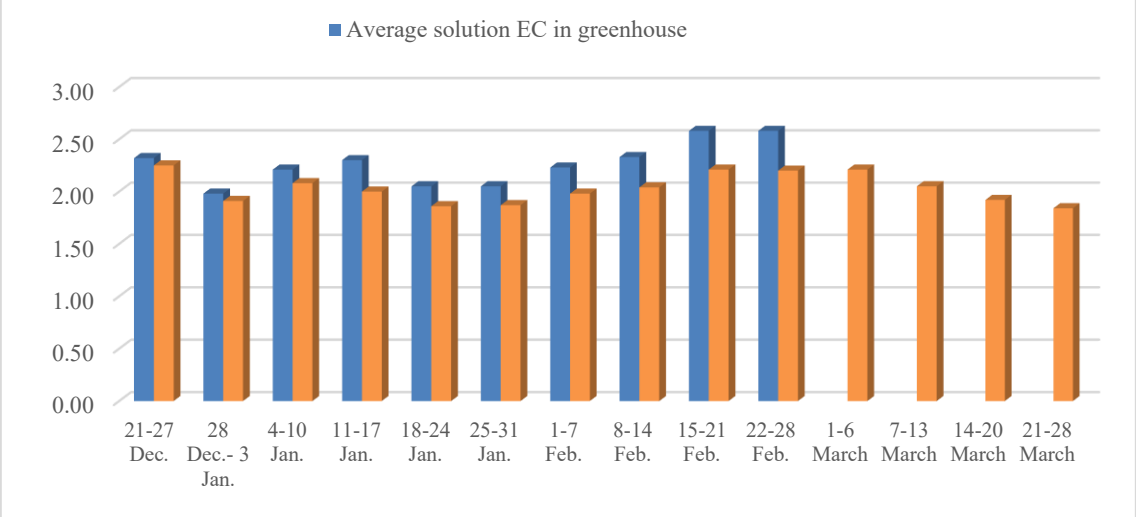

Figure 5. Average EC values determined in solutions in the glasshouse and outdoor environment.

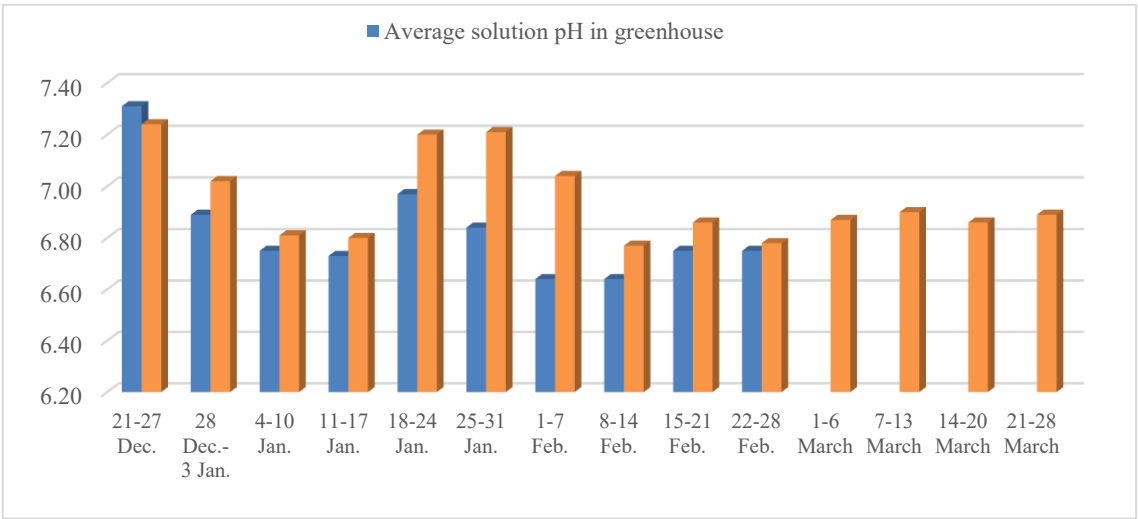

Figure 6. Average pH values determined in solutions in the glasshouse and outdoor environment.

The chlorophyll levels in the leaves of spinach grown in floating hydroponic culture in the glasshouse (71.16 mg kg^{-1}) and outdoor (68.31 mg kg^{-1}) were found close to each other, and their levels were determined to be higher than those grown in the soil. The chlorophyll content was 64.58 mg kg^{-1} in the soil in the open field and was 54.30 mg kg^{-1} in the soil in the glasshouse (Table 2).

The nitrate accumulation in the leaves of spinach grown in floating hydroponic culture (3111 mg kg^{-1}) and soil (2069 mg kg^{-1}) in the glasshouse was found to have considerably higher levels than those grown in the open field. The lowest nitrate accumulation was in the open field with 87 mg kg^{-1} (Table 2).

3.4. Macro and microelement contents in leaves

The contents of N, Ca, Mg, P, K, Fe, Mn, Zn, and Cu in the leaves in floating hydroponic culture and soil outside and greenhouse were significantly changed ($P \leq 0.05$). The level of N in leaves in floating hydroponic culture (3.80 mg kg^{-1}) and soil (3.67 mg kg^{-1}) in the glasshouse were almost the same, and the highest N content was determined in the soil in the open field with 4.30%. P and K levels in leaves in floating hydroponic culture and soil outside were lower than those grown in the glasshouses. The highest P and K contents were detected in floating hydroponic culture and soil in the glasshouse with 0.90% and 12.37%, respectively. The highest Ca and Mg levels in leaves were determined in the soil in the glasshouse with 1.80% and 1.10% respectively while the lowest Ca 0.37% and Mg 0.57% were in the soil outside. The Ca and Mg levels in floating hydroponic culture in the glasshouse and outside were detected at about the same level (Table 3).

Fe levels of leaves with 303.67 mg kg^{-1} in the glasshouse and with 284.33 mg kg^{-1} in the open field in soil were higher than those grown in floating hydroponic culture. The amount of Fe in floating hydroponic culture in the glasshouse was found to be higher than for outside. The lowest Fe content was in floating hydroponic culture outside with 80.33 mg kg^{-1}. Mn levels in leaves were found in the glasshouse in soil with 62.67 mg kg^{-1} and floating hydroponic culture with 61.00 mg kg^{-1}. The highest Mn level was determined in the soil in an open field with 83.00 mg kg^{-1}. The highest Zn with 158.67 mg kg^{-1} and Cu with 19.00 mg kg^{-1} were detected in floating hydroponic culture in the glasshouse, while the lowest Zn with 75.00 mg kg^{-1} and Cu with 10.00 mg kg^{-1} were found in soil in the glasshouse (Table 3).

There was not much change in EC values measured in the glasshouse and outside. Although solution EC's were tried to be kept between 1.5-1.7, the measured values were higher. EC increase in the glasshouse was higher than in the outdoor environment, and the highest EC increase in solutions in the greenhouse was detected in February during harvesting (Figure 5).

3.5. Leaf color analysis

The brightest colored leaves were measured in the soil in the glasshouse and floating hydroponic culture outdoors. Th e brighter green color of leaves was mostly determined in the floating hydroponic culture in the glasshouse however, the green color was less intense outside in soil and floating hydroponic culture. The green color was detected more in the plants grown in greenhouses and yellowing in the plants grown in the open (Table 4).

Table 2. Yield and biomass values of spinach grown in floating culture and soil in the greenhouse and outdoor environment

Applications		Days between seed sow and harvest (day)	Yield (kg m^{-2})	Plant height (cm)	Root length (cm)	Marketable plant weight/plant (kg)	Chlorophyll content (mg g^{-1})	Nitrate accumulation in leaf (mg kg^{-1})
Green-house	Floating culture	64^{a*}	1.45^{a}	18.86^{c}	36.10^{a}	0.81^{c} (44.14% loss)	71.16^{a}	3112^{a}
	Soil	72^{b}	1.07^{c}	22.77^{b}	19.10^{d}	0.94^{b} (12.15% loss)	54.30^{c}	2069^{b}
Outdoor	Floating culture	83^{c}	1.32^{ab}	20.21^{c}	30.02^{b}	0.87^{bc} (34.09% loss)	68.31^{a}	621^{c}
	Soil	97^{d}	1.54^{a}	47.94^{a}	27.79^{c}	1.18^{a} (23.38% loss)	64.58^{b}	87^{d}

Means within each column followed by the same letters are significantly different according to the Tukey test (*significant at $P \leq 0.05$).

Table 3. Macro and microelement contents detected in spinach leaves in floating culture and soil in the glasshouse and outside.

Applications		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Greenhouse	Floating culture	3.80^{b*}	0.90^{a}	9.00^{b}	0.73^{b}	0.80^{b}	90.67^{c}	61.00^{b}	158.67^{a}	19.00^{a}
	Soil	3.67^{b}	0.73^{b}	12.37^{a}	1.80^{a}	1.10^{a}	303.67^{a}	62.67^{b}	75.00^{d}	10.00^{c}
Outdoor	Floating culture	3.00^{c}	0.53^{c}	3.47^{c}	0.73^{b}	0.70^{b}	80.33^{d}	32.00^{c}	94.33^{c}	13.67^{b}
	Soil	4.30^{a}	0.53^{c}	3.33^{c}	0.37^{c}	0.57^{c}	284.33^{b}	83.00^{a}	100.67^{b}	10.67^{c}

Means within each column followed by the same letters are significantly different according to the Tukey test (*significant at $P \leq 0.05$).

Table 4. Average L, a, and b values of leaves measured in the glasshouse and outside in floating culture and soil (L: brightness, a: green color, b: yellow color).

Applications		L	a	b
Greenhouse	Floating culture	42.63^{b*}	-11.96^{a}	14.81^{c}
	Soil	46.53^{a}	-14.48^{b}	20.32^{b}
Outdoor	Floating culture	44.93^{a}	-16.13^{c}	23.17^{a}
	Soil	42.06^{b}	-16.76^{c}	22.99^{a}

Means within each column followed by the same letters are significantly different according to the Tukey test (*significant at $P \leq 0.05$).

4. Discussion and Conclusion

Protected cultivation of spinach in floating hydroponic is an alternative way of year round vegetable production and has a much faster economic return (Brandenberger et al. 2007). EC and pH values of the solution affect the cultivation, and the appropriate EC value is 1 and 3 dS m^{-1}, and pH is 5.5 and 7. Solution pH in aquaponics systems is a compromise between microbial and plant demands (Cerozi and Fitzsimmons 2016). A recent study determined that pH 6.0 was optimal for plant growth and nitrogen utilization efficiency in aquaponics at the expense of increased N_2O emission due to high denitrification (Zou et al. 2016). In the experiment, although it was tried to keep solution EC between 1.5-1.7 and pH 6.0-6.5, solution EC varied between 1.7-2.5, and pH was between 6.64-7.31. Other researchers reported similar increases in EC and pH in the solution in hydroponic culture (Öztekin et al. 2018; Leal et al. 2020). On the other hand, fewer changes of EC and pH values of the solutions in the floating hydroponic culture in the glasshouse and outside can be explained by the fact that there is no significant difference between the solution temperatures.

While the harvest period for spinach grown in autumn and winter in soil cultivation can be extended by up to 150-180 days, harvesting takes up to 60 days in spring planting (Anonymous 2011). Spinach has a good development below 26°C and the best yield and quality characteristics are obtained at 22°C (Lee and Takakura 1995; Brandenberger et al. 2007). The germination and growing period of spinach in hydroponics and aquaponics were earlier than the soil growing method (Ranawade et al. 2017). Similarly, in the research, spinach in floating hydroponic culture in the glasshouse (64 days) came to harvest 29 days before the outside soil (93 days). However, the fact that spinach grown in the soil in the glasshouse (72 days) came to harvest before the floating hydroponic culture outside (83 days) shows that spinach cultivation in floating hydroponic culture outside does not affect the earliness. The earlier harvest of spinach in floating hydroponic culture in the glasshouse can be explained by the higher temperature of glasshouse than outside. During the experiment period (December 21 to February 15), while the temperature inside the glasshouse varied between 14-24°C the outside temperature decreased to 8°C in January and the temperature varied between 8-23°C. However, the solution temperatures in the glasshouse and outside did not change much except for December and early January. This can be explained by the high specific heat of the water. Because water can absorb large amounts of heat energy and releases heat energy slowly (Kacar et al. 2002).

In the cultivation of spinach in soil, the yield per decare depending on the climate, soil structure, growing season, harvest type and variety could be 1.1-1.5 tons (Cocetta et al. 2007), 0.86-1.8 tons (Engindeniz 2008), 1.5-3.0 tons (Anonymous 2011), 1.01-4.54 tons (Sensoy et al. 2011) and 0.40 tons (Ranawade et al. 2017). In floating culture, in winter 1.25 tons, in early spring 1.99 tons (Öztekin et al. 2018), and in glasshouses between 1.88 and 2.09 tons (Brandenberger et al. 2007) of spinach per decare can be produced. In the research, the yield value of 1.45 tons da^{-1} obtained from floating hydroponic culture in the greenhouse during the winter period was similar to other research results. The fact that 1.45 tons of yield obtained from the floating hydroponic culture in the glasshouse in winter were close to 1.54 tons obtained from the soil outside showed that spinach can be grown economically in floating hydroponic culture in winter in the ecological conditions in Antalya.

While color, length, vitality, and the number of leaves is important factors for the sale of spinach, the amount of marketable quantity from the produced part is also substantial for economical gain. The most important factor is how much of the spinach produced can be marketed. In floating hydroponic culture, the marketable produce in the glasshouse and outdoor was 55.86% (yield decreases from 1.45 kg m^{-2} to 0.81 kg m^{-2}) and 65.91% (yield decreases from 1.32 kg m^{-2} to 0.87 kg m^{-2}) respectively. On the other hand, the marketable amount in the open field was 87.85% (yield decreases from 1.54 kg m^{-2} to 1.18 kg m^{-2}) and 76.62% in the soil in the glasshouse (yield decreases from 1.07 kg m^{-2} to 0.94 kg m^{-2}). The decrease in the marketable amount in floating hydroponic culture in the glasshouse and outside can be explained by the plant being constantly in water, and at high temperatures in the glasshouse. Although the marketable amount is low in floating hydroponic cultivation in a glass greenhouse and outdoor compared to outdoor cultivation in the soil, this situation should not be perceived as negative. It is more advantageous compared to cultivation in the soil due to being able to cultivate 2-3 times in a year, low labor costs. Besides, due to the high temperatures in the glass greenhouse in the Mediterranean coastal zone during the summer months, outdoor floating cultivation may be seen to be more suitable during hot periods.

Although plant height in the soil (especially open field) was taller than those grown in floating hydroponic culture, the root lengths were shorter in the soil. Similarly, the height of the spinach in the soil (23 cm) was determined to be taller than the floating hydroponic culture (18 cm) (Ranawade et al. 2017). The results indicate that floating hydroponic culture makes spinach shorter in height. This can be explained by the fact that the N level determined in the leaves of spinach grown in the soil in the open fields was higher than in the floating culture.

The nutrient content of spinach in leaves varies according to the growing medium. Research results differed from those of other researchers (Shah et al. 2009; Vandam et al. 2017; Öztekin et al. 2018), and in the study, N, Ca, and Mg levels were lower in spinach grown in winter whereas P, and Fe levels were found to be higher. In the spinach leaves, K, Ca, Mg, and Fe contents in the soil and P, Zn, and Cu in the floating hydroponic culture in glass greenhouse were found to be higher, but N and Mn were found more frequently in the open field.

The amount of nitrate in plants varies greatly depending on the type of plant, genetic structure, plant age, plant parts, environmental factors, applied N form, and agricultural processing methods (Xiang et al. 2020). Low nitrate contents are desired indirectly consumed from edible leaf vegetables. Because nitrate is converted to nitrite by mouth bacteria and conveyed to the human stomach, excess nitrate adversely affects human health (Kara 1993; Özdestan and Üren 2010). Vegetables such as beets, celery, lettuce, spinach, and radishes contain high levels of nitrates (>1000 mg kg^{-1}) (Anonymous 2019). The maximum limit of the nitrate amount in lettuce leaves is determined as 3500-4500 mg kg^{-1}, and 2500 mg kg^{-1} in cabbage, carrot, and celery tubers in Switzerland, the Netherlands, and Austria (Bergmann 1992). The maximum nitrate level in spinach is 3500 mg kg^{-1} according to the Turkish Food Codex Regulation published in Turkey in 2008 (Ayaz and Yurttagül 2006). In the spinach leaves in the experiment, the nitrate accumulation in floating hydroponic culture and soil in a glass greenhouse and outside were determined to be below these levels. Nitrate accumulation was detected at 1800 mg kg^{-1} in the spinach leaves in floating hydroponic culture during the winter

period (Öztekin et al. 2018) and 3610 mg kg^{-1} in hydroponic culture in glass greenhouses (Lenzi et al. 2011). Parallel to the increase in the nutrient solution contained in the hydroponic culture in the plastic greenhouse, nitrate accumulation increased from 750 mg kg^{-1} to 1200 mg kg^{-1} (Cocetta et al. 2007) and the nitrate accumulation increased parallel to the increase in the amount of salt in the solution in hydroponic culture (Cocetta et al. 2007; Leal et al. 2020). The results show that spinach cultivation in the open field does not have many problems in terms of nitrate, but nitrate accumulation in glass greenhouse can be a problem in floating hydroponic culture and soil cultivation. Therefore, the content of the solution to be prepared becomes important in preventing nitrate accumulation in plants grown in a glass greenhouse.

The chlorophyll content varied according to the growing environment and the period in which it was grown. The chlorophyll content of spinach grown in the greenhouse in winter was 118 mg kg^{-1} (Öztekin et al. 2018), and the chlorophyll content did not change in spinach leaves in different nutrient applications in the plastic greenhouse (Cocetta et al. 2007). According to the results of the research, the detection of more chlorophyll in the leaves of spinach grown in floating hydroponic culture in the greenhouse and outside shows that hydroponic culture can promote chlorophyll content in spinach compared to soil cultivation.

Although spinach leaves have the more intense green color in the greenhouse in floating hydroponic culture, the fact that brightly colored leaves are grown in the greenhouse in soil and outside in floating hydroponic culture indicates that there may not be a linear relationship between chlorophyll content and brightness. The fact that the green color of the leaves is more intense when grown in the greenhouse, whereas the yellowing is more prominent in those grown outside can be explained by the low temperature of the external environment. The green color and yellowing of the leaves may vary by year (Brandenberger et al. 2007).

Protected cultivation is intensively utilized in the Mediterranean region of Turkey. Protected cultivation is mostly done in soil, however, in recent years, the cultivation of most vegetables, especially tomatoes, has been started in soilless culture. In addition, there has been a rapid increase in the use of the hydroponic system for the cultivation of plants with a short vegetative life. In this study, the cultivation of spinach in floating hydroponic culture was examined as an alternative and it was revealed that spinach could be grown in both greenhouse and outside floating hydroponic culture in the conditions in Antalya. Due to the portability of the floating hydroponic culture system, it seems that it will be possible to grow spinach in the open field in summer. Despite these positive conditions, the low marketable amount of spinach grown in floating hydroponic culture and high nitrate accumulation in glass greenhouse have been identified as a negative aspect.

Acknowledgments

This work was part of the Master Science experiment and financially supported by the Scientific Projects Unit of Akdeniz University with the project no FYL-2015-412, 2015-2016. The authors would like to thank the previous graduate advisor, Prof. Dr. Nurgul Ercan.

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Research Article

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MEDITERRANEAN AGRICULTURAL SCIENCES
(2022) 35(1): 15-19
DOI: 10.29136/mediterranean.1040263

Thrips species associated with cereals of the Lakes Region of Turkey with new records

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ARTICLE INFO

Received: December 23, 2021
Received in revised form: February 5, 2022
Accepted: February 24, 2022

Keywords:

Thrips
Wheat
Barley
Oat
Pest

ABSTRACT

This study was carried out to determine the Thysanoptera species in the cereal production areas in the Lakes Region of Turkey, in 2016 and 2017. Thrips samples were collected at two different periods of cereal crops, before and after the spike stage. Destructive sampling and strike methods were used to obtain thrips species. The individuals were mounted on slides with Canada balsam for morphological characterisation. As a result of this study, a total of 51 species were identified including 9 species belonging to Tubulifera and 42 species belonging to Terebrantia. The most abundant species were *Haplothrips tritici* (Kurdjumov 1912) (58.25%), *Collembolothrips mediterraneus* Priesner 1935 (8.29%), *Thrips angusticeps* Uzel 1895 (6.61%), and *Limothrips cerealium* Haliday 1836 (4.31%). The most common species were *H. tritici*, *T. angusticeps*, *L. cerealium*, *Aeolothrips intermedius* Bagnall, 1934 and *Aptinothrips stylifer* Trybom, 1894. Three species were first recorded for Turkish fauna: *Tylothrips osborni* (Hinds), *Mycterothrips sanubari* Alavi Modarres Awal Fekrat & Minaei and *Tenothrips hispanicus* (Bagnall).

1. Introduction

The Lakes Region includes Afyonkarahisar, Antalya, Burdur, Konya, and Isparta in the west of the Mediterranean Region. Due to the coexistence of water and terrestrial ecosystems, it has a wide variety of fauna and flora. Cereals provide an important contribution to the Turkish economy in agricultural production, nutrition, domestic and foreign trade (Kızılaslan 2004). While Turkish cereal production in 2019 was 64588157 tonne, the Lakes Region cereal production was 1723972 tons, of which 441275 and 435768 tons are wheat and barley respectively (TUIK 2020). There are many important pests belonging to different insect groups, which cause economic loss in cereal crops are the main food source for humanity. Among these groups, thrips (Thysanoptera) are with about 6000 species (classified into two suborders, Terebrantia and Tubulifera), which are not well known by the farmers (Smith-Pardo and O'Donnell 2015). Thrips usually feed under the sheath of the flag leaves, on the stem, on the leaves and spikes. In the case of intensive contamination, adults and nymphs cause damage in the form of silvery staining as they feed on tissues and suck cell contents (Larsson 2005; Gaafar and Volkmar 2010). It has been reported that thrips feeding in fresh wheat grains may cause deformation, shedding and shrinkage in the grains (Lewis 1973). The economic damage threshold for thrips (*Limothrips* spp. and *Haplothrips* spp.) in cereals in Europe has been reported to be 5-10 thrips/spike (Seidel et al. 1983) and more than 30 nymphs and adults/spikes (Freier et al. 1982; Cuthbertson 1989; Parrella and Lewis 1997). Numerous faunistic studies have been conducted to reveal the insect biodiversity in the Lakes Region. However, there are just a few studies on determining Thysanoptera species found on cereals (Tunç et al. 2012a, 2012b; Demirözer and Bilginturan 2014). The aim of this study is to determine the Thysanoptera species in the areas where cereal production is extensive, in the Lakes Region, and to ascertain the distribution and incidence rates of these species in detail.

2. Materials and methods

Strike (Bacci et al. 2008) and destructive sampling methods (Demirözer and Bilginturan 2014) were used to collect thrips individuals from provinces and districts dependent on the cereal production capacity in the Lakes Region. Samplings were carried out in 2 periods; before spike (period starting from 15-20 cm plant height until spike formation) and after spike stage, in commercial cereal production fields of the Lakes Region, in March-June 2016-2017.

The number of fields to be sampled was determined depending on the cereal cultivated area capacity of each province of the Lakes Region including Afyonkarahisar (Dinar, Dazkırı), Antalya (Korkuteli), Burdur, Konya (Beyşehir), and Isparta. For this purpose, the scale included cultivated area (decare)/number of sampling locations was used (under 5 decares <5 sampling locations; 100-250 decares range, 10-15 sampling locations; on 250 decares, 15-25 sampling locations) (Uzun 2020) (Table 1).

Table 1. The locations where sampling was carried out in 2016 and 2017 in the grain production areas of the Lakes Region, the number of fields sampled and survey date

Province	District	Number of sampled fields in 2016	Survey date (BS/AS)	Number of sampled fields in 2017	Survey date (BS/AS)
Isparta	Yalvaç	13	27.04.2016/12.06.2016	6	26.04.2017/8.06.2017
	Şarkikaraağaç	7	11.05.2016/12.06.2016	4	26.04.2017/8.06.2017
	Gelendost	9	6.04.2016/18.06.2016	5	26.04.2017/8.06.2017
	Keçiborlu	5	7.05.2016/15.06.2016	3	20.04.2017/18.05.2017
	Gönen	3	7.05.2016/15.06.2016	3	20.04.2017/18.05.2017
	Merkez	3	27.05.2016/22.06.2016	3	4.05.2017/11.06.2017
	Senirkent	5	23.04.2016/19.06.2016	4	20.04.2017/18.05.2017
	Atabey	3	27.05.2016/22.06.2016	3	27.04.2017/7.06.2017
	Eğirdir	3	6.04.2016/18.06.2016	3	27.04.2017/7.06.2017
	Aksu	3	12.05.2016/25.06.2016	3	4.05.2017/11.06.2017
	Sütçüler	3	12.05.2016/25.06.2016	3	4.05.2017/11.06.2017
	Uluborlu	5	23.04.2016/19.06.2016	4	20.04.2017/18.05.2017
	Yenişarbademli	3	12.05.2016/25.06.2016	-	-
Burdur	Bucak	5	26.05.2016/21.06.2016	4	23.04.2017/17.05.2017
	Gölhisar	4	25.05.2016/16.06.2016	4	11.05.2017/15.06.2017
	Merkez	4	18.05.2016/17.06.2016	3	10.05.2017/14.06.2017
	Tefenni	3	18.05.2016/17.06.2016	3	10.05.2017/14.06.2017
	Yeşilova	4	18.05.2016/17.06.2016	3	10.05.2017/14.06.2017
	Kemer	3	26.05.2016/21.06.2016	3	11.05.2017/15.06.2017
	Çavdır	4	25.05.2016/16.06.2016	3	11.05.2017/15.06.2017
	Karamanlı	3	18.05.2016/17.06.2016	3	10.05.2017/14.06.2017
	Çeltikçi	3	26.05.2016/21.06.2016	3	13.05.2017/25.05.2017
	Altınyayla	3	25.05.2016/16.06.2016	3	11.05.2017/15.06.2017
	Ağlasun	3	26.05.2016/21.06.2016	3	13.05.2017/25.05.2017
Antalya	Korkuteli	16	20.04.2016/5.06.2016	9	23.04.2017/17.05.2017
Konya	Beyşehir	9	14.05.2016/18.06.2016	7	27.04.2017/7.06.2017
Afyon	Dazkırı	6	15.05.2016/15.06.2016	3	14.05.2017/3.06.2017
	Dinar	4	15.05.2016/15.06.2016	3	14.05.2017/3.06.2017
Total	**28**	**139**		**101**	

-: Absent

2. 1. Destructive sampling method

In both periods (BS and AS), the part of the plant remaining above of the soil was cut from randomly selected plants that were taken as 100-150 number depending on the size of the production area and then the label information was placed on the polyethylene bags (5 L) in which a paper towel was placed. The plant materials were brought to the laboratory and kept in a refrigerator at 4°C for 1 hour to slow down the movement of the thrips individuals. Adult individuals were collected with the help of a mouth aspirator and fine brush.

2.2. Strike method

Sampling was also made in the two periods mentioned above. For a sampling area, a total of 50-100 strokes were made into a white tub in both plant growth periods (Bacci et al. 2008). Individuals falling into the white container were taken to the Falcon tubes (50 ml) with the help of a mouth aspirator and brought to the laboratory with their label information.

Thrips individuals obtained by both sampling methods were separated under stereo-microscope (Leica S8 APO, 80X) and transferred to eppendorf tubes containing 70% ethyl alcohol for morphological diagnosis.

2.3. Morphological identification

Thrips specimens were separated under a binocular microscope (Nicon Eclipse E100, 40X) and slides of specimens were prepared according to Gibb and Oseto (2006). Morphological identification of prepared thrips slides was made by various keys, such as Mound et al. (1976) and zur Strassen (2003). All identifications were confirmed by the third author.

3. Results

Thrips species were collected from 240 points in total and the majority of these points (76%) came from Burdur (30.8%) and from Isparta (45.4%). In addition, 41% of thrips individuals in the Lakes Region were detected in Isparta and 29% in Burdur. Other sampling areas remained below 14%.

As a result of this study, a total of 51 thrips species, 42 species belonging to Terebrantia, and 9 species belonging to Tubulifera were identified (Table 2). It was determined that the most abundant species were *H. tritici* (58.25%), *C. mediterraneus* (8.29%), *T. angusticeps* (6.61%), and *L. cerealium* (4.31%). In addition, the most common species were also *H. tritici* (94%), *T. angusticeps* (59%), *L. cerealium* (44%), *A. intermedius* (38%) and *A. stylifer* (37%). According to the 2016 data, 58.6% of thrips individuals collected from cereal production areas were Tubulifera and 41.3% were species related to Terebrantia. In 2017, the second year of the study, it was determined that 62.4% of the thrips species collected consisted of Tubulifera and 37.5% were species belonging to Terebrantia. The incidence rates of Thysanoptera species in the cereal production areas of Lakes Region are listed as Isparta (41.19%), Burdur (29.98%), Antalya (13.35%), Konya (10.05%), and Afyonkarahisar (5.44%).

Table 2. Thysanoptera species identified in the cereal production areas of the Lakes Region

Sub-order	Family	Genus	Species	Host	
				2016	2017
Terebrantia	Aeolothripidae	*Aeolothrips*	*Aeolothrips collaris* Priesner 1919•	Barley, wheat, oat	-
			Aeolothrips intermedius Bagnall 1934•	Barley, wheat, oat	Barley, wheat, oat
			Aeolothrips priesneri Knechtel 1923•	Wheat, oat	-
		Rhipidothrips	*Rhipidothrips brunneus* Williams 1913•	Barley, wheat, oat	Barley, wheat, oat
			Rhipidothrips gratiosus Uzel 1895•	Barley, wheat, oat	Barley, wheat, oat
	Melanthripidae	*Melanthrips*	*Melanthrips pallidior* Priesner 1919▲	Barley, wheat, oat	Barley, wheat
			Melanthrips trifasciatus Priesner 1961▲	-	Wheat
	Thripidae	*Anaphothrips*	*Anaphothrips obscurus* (Muller 1776)▲	Barley, wheat, oat	Barley, wheat, oat
		Aptinothrips	*Aptinothrips stylifer* Trybom 1894▲	Barley, wheat, oat	Barley, wheat, oat
			Aptinothrips elegans mediterranea▲	Barley, wheat, oat	Barley, wheat, oat
		Chirothrips	*Chirothrips aculeatus* Bagnall 1927▲	Oat	Barley
			Chirothrips africanus Priesner 1932▲	Barley, wheat, oat	-
			Chirothrips manicatus Haliday 1836▲	Wheat	-
		Collembolothrips	*Collembolothrips mediterraneus* Priesner 1935▲	Barley, wheat, oat	Barley, wheat, oat
		Frankliniella	*Frankliniella intonsa* (Trybom 1895)▲	Barley, wheat, oat	Barley, wheat
			Frankliniella occidentalis (Pergande 1895)▲	Barley, wheat, oat	Barley, wheat, oat
			Frankliniella pallida (Uzel 1895)▲	Barley, wheat, oat	-
			Frankliniella tenuicornis (Uzel 1895)▲	Barley, wheat, oat	-
		Isoneurothrips	*Isoneurothrips australis* Bagnall 1915▲	Wheat	-
		Limothrips	*Limothrips angulicornis* Jablonowski 1894▲	Barley, wheat, oat	Barley, wheat
			Limothrips cerealium Haliday 1836▲	Barley, wheat, oat	Barley, wheat, oat
			Limothrips denticornis Haliday 1836▲	Wheat	-
			Limothrips transcaucasicus Sawenko 1944▲	Barley, wheat, oat	Barley, wheat
		Mycterothrips	*Mycterothrips sanubari* Alavi Modarres Awal Fekrat & Minaei*,▲	Wheat	-
		Neohydatothrips	*Neohydatothrips gracilicornis* (Williams 1916) ▲	Barley, wheat, oat	Barley, wheat
		Odontothrips	*Odontothrips aemulans* Priesner 1924▲	Wheat	
		Oxythrips	*Oxythrips ajugae* Uzel 1895▲	Wheat, oat	-
			Oxythrips priesneri Pelikan 1957▲	Barley, wheat	-
			Oxythrips uncinatus Priesner 1940▲	Barley	-
		Pezothrips	*Pezothrips bactrianus* (Pelikan 1968)▲	Barley	-
		Sitothrips	*Sitothrips arabicus* Priesner 1931▲	Barley, wheat, oat	Barley, wheat
		Tenothrips	*Tenothrips anatolicus* (Priesner 1961)▲	Barley	-
			Tenothrips hispanicus (Bagnall) *,▲	Wheat	-
		Thermothrips	*Thermothrips mohelensis* Pelikan 1949▲	Wheat	-
		Thrips	*Thrips angusticeps* Uzel 1895▲	Barley, wheat, oat	Barley, wheat, oat
			Thrips dubius Priesner 1927▲	Barley, wheat	-
			Thrips flavus Schrank 1776▲	Barley, wheat, oat	-
			Thrips hawaiiensis (Morgan 1913) ▲	Barley	-
			Thrips linarius Uzel 1895▲	Oat	-
			Thrips major Uzel 1895▲	Barley, oat	-
			Thrips meridionalis (Priesner 1926) ▲	Barley, wheat, oat	Barley, wheat, oat
			Thrips pillichi Priesner 1924▲	Barley, wheat	-
			Thrips hawaiiensis (Morgan 1913)▲	Barley	-
Tubulifera	Phlaeothripidae	*Haplothrips*	*Haplothrips acanthoscelis* (Karny 1910)▲	Barley, wheat	-
			Haplothrips bolacophilus Priesner 1938▲	Barley, wheat, oat	-
			Haplothrips distinguendus (Uzel 1895)▲	Barley, wheat, oat	Barley, wheat
			Haplothrips flavicinctus (Karny 1910)▲	Barley	Wheat
			Haplothrips knechteli Priesner 1923▲	Barley, wheat	Barley, wheat
			Haplothrips reuteri (Karny 1907)▲	Barley	-
			Haplothrips tritici (Kurdjumov 1912)▲	Barley, wheat, oat	Barley, wheat, oat
		Neoheegeria	*Neoheegeria dalmatica* Schmutz 1909▲	Barley	-
		Tylothrips	*Tylothrips osborni* (Hinds)*,■	Wheat	-

-: Absent/ *new record for Turkey ■Mycophagus ▲Phytophagous • Predator.

4. Discussion

The first systematic study for Thysanoptera fauna species in Turkey was carried out by Bagnall (1934). Thysanoptera's first inventory in Turkey was completed by Lodos (1993) and reported 154 thrips species in a catalogue. Tunç et al. (2012b) identified a total of 74 thrips species, 45 of which were determined for the first time in this region, within the scope of their study between 1990-1992, in order to determine the Thysanoptera fauna in the Lake Region. The most common and abundant species in this study were *Thrips meridionalis* Priesner 1926 (92-437 individuals), *Thrips tabaci* Lindeman 1889 (137-412 individuals), *Haplothrips reuteri* Karny 1907 (130-489 individuals) on different host groups. *Frankliniella tenuicornis* Uzel 1895, *Limothrips denticornis* Haliday 1836, *Limothrips transcaucasis* Sawenko, 1944 also were determined on barley, *Chirothrips manicatus* Haliday 1836 on barley and oats, *H. tritici* on wheat, barley and oats. Demirözer and Bilginturan (2014) determined the insect species found in the cereal production areas of the Lakes Region and identified 8 species including *H. tritici*, *F. tenuicornis*, *Aeolothrips collaris* Priesner, *A. intermedius*, *T. angusticeps*, *L. denticornis* and *Sitothrips arabicus* Priesner. The above-mentioned species were also found in our study. In studies conducted on cereal production areas in different countries in Europe, the most common or abundant species were reported to be *L. cerealium*, *L. denticornis*, *F. tenuicornis*, *Anaphothrips obscurus* (Muller), *T. angusticeps*, *Haplothrips aculeatus* (Fabricius) and *H. tritici* (Johansen 1938; Franssen and Huisman 1958; Franssen and Mantel 1963, 1965; Holtmann 1963; Lattauschke and Wetzel 1985; Larsson 2005; Šmatas 2009; Šmatas et al. 2013; Virteiu et al. 2015; Parnea et al. 2018). *Haplothrips tritici* (94%), *T. angusticeps* (59%), *L. cerealium* (44%) were the most common species in our study. However, *H. tritici* was the most abundant species (58.25%). In the current study three species, *Tylothrips osborni* (Hinds), *Mycterothrips sanubari* Alavi Modarres Awal Fekrat & Minaei and *T. hispanicus* (Bagnall) were the first time recorded for Turkish Thysanoptera fauna. *Tylothrips osborni* was reported from Massachusetts, New York State, Illinois, Florida, Cuba, Panama, Trinidad, Spain, Italy, and Germany (Mound 1977; Goldarazena and Mound 1998; de Marzo and Ravazi 2007; Ulitzka 2013, 2021). *Tenothrips hispanicus* has also been identified in Iran, Spain, Italy, Corsica, Greece, Albania, Rumania, Hungary, Georgia, Transcaucasia, Morocco (Bhatti 2003; Afsharizadeh Bami and Minaei 2020). *Mycterothrips sanubari* was recorded in Iran (Alavi et al. 2013). In addition, of the 51 species obtained, *T. osborni* is mycophagus, *A. collaris*, *A. intermedius*, *A. priesneri*, *R. brunneus*, and *R. gratiosus* are predator species. The remaining species are phytophagous which constitute 88.2% of all species.

In comparison to the PhD dissertation of the first author (Uzun 2020), the report of *Oxythrips retamae* (Priesner) and *Thrips herricki* could not be verified at present, due to insufficient materials. Furthermore, reports of *Neoheegeria sinaitica* Priesner, *Hoplothrips caespitis* (Uzel) and *Pezothrips frontalis* (Uzel) (Uzun 2020) are misidentifications of *Neoheegeria dalmatica* Schmutz, *Tylothrips osborni* (Hinds) and *Tenothrips hispanicus* (Bagnall) respectively. Also, although *Mycterothrips sanubari* Alavi Modarres Awal Fekrat & Minaei was not identified in the previous study (Uzun 2020), it is included in the current study. Additionally, *Aptinothrips stylifer* Trybom was considered to be the first record for Turkish insect fauna by Uzun (2020), however, it has been reported the first time in Turkey without specifying the coordinates by Tunç and Hastenpflug-Vesmanis (2016).

5. Conclusion

The results of this study have contributed to the Thysanoptera database of Turkey. Among 51 species that were identified in this research, there are some that have economic importance and need careful consideration.

Acknowledgement

We thank Professor Doctor Ekrem ATAKAN (Cukurova University, Faculty of Agriculture, Department of Plant Protection, Turkey) for morphological diagnosis of some of the Terebrantia species. We are grateful to the Scientific Research Projects Coordination Unit of Süleyman Demirel University, Turkey (project number 4642-D2-16) that supported this study.

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Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2022) 35(1): 21-26
DOI: 10.29136/mediterranean.1012466

The analysis of competitiveness of Mediterranean countries in the world citrus trade

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ARTICLE INFO

Received: October 20, 2021
Received in revised form: November 29, 2021
Accepted: January 27, 2022

Keywords:

Mediterranean basin
Competition power
Citrus fruit
İnternational trade

ABSTRACT

With their low calorie and rich nutrient content, citrus fruits are an important element of the daily diets of individuals living in Mediterranean countries. Although the origin of citrus fruits lies in Southeast Asia, today the Mediterranean Basin comes to the fore in terms of production and trade. In this study, the competitiveness of the countries in the Mediterranean Basin, which has a coast to the Mediterranean, in the world citrus trade between 2005-2019 was competitiveness demonstrated. for this research, Balassa's relative comparative advantages, revealed symmetric comparative advantage and Lafay indexes are used. As a result of the study, the rate of increase in the total production and exports of the can countries in the Mediterranean Basin is realized under the world average. However, the noticeable dynamic increase in the production and exports of the developing countries in the region ensures the continuity of the competitive power of the Mediterranean Basin. To increase the market competitiveness of the region, it is suggested that state policies make citrus production activities an important issue.

1. Introduction

Citrus is the general name given to high value-added fruits such as oranges, tangerines, lemons, grapefruit, and bergamot, which are the most commonly grown. Citrus fruits are rich in vitamin C, minerals, and carotenoids, in addition to being consumed as food due to their abundances. Owing to their phenol content, the essential oils obtained from the shell, leaves, and flowers allow them to be used in the perfumery sector (Duarte et al. 2016; Ozer and Köksal 2016).

Citrus fruits require a lot of water, the optimum growing temperature is 23-30°C. Citrus fruits, which start to bear fruit at the end of three years, are economically efficient from the fifth year. The yield varies from year to year, the most fruitful tree is the orange species. Few of the citrus flowers bear fruit is given, the longest flowering period is lemon species (FAO 2020).

Citrus fruits, which have a history of about 20 million years, have their origin in Southeast Asia, and their spread to the Mediterranean and other regions goes back to before Christ. The Mediterranean climate has provided a suitable environment for the cultivation of many agricultural products, and in particular citrus fruits (Barcikowska et al. 2020). Citrus fruits have spread to the American continent, especially through Spanish and Portuguese explorers, with the effect of geographical discoveries since the 15th century, and since this date, its production, processing, and trade have significantly (Liu et al. 2012This situation has continued until today (2019), it has been the most trade fresh fruit and vegetable product group in the world. (TradeMap 2020).

There has been a lot of research done in literature on the citrus economy of the countries in the Mediterranean Basin. Some of this research was conducted on the citrus production of the countries in the Mediterranean Basin and the general structure of the sector (D'Onghia and Lacirignola 2009), and the political effects of the increase in citrus production in the Mediterranean Basin countries located in the European continent (Schimmenti et al. 2013). In country-based research, Turkey's; competitiveness of citrus exports in the European Union market (D'Onghia and Lacirignola 2009), the factors affecting the export of citrus using a gravity model analysis (Ozer and Koksal 2016), and competitive analysis of citrus exports (Fidan 2009; Kadanalı 2019), quality assurance in citrus exports in Morocco (Oubahou and Otmani 2000), economic analysis of Egypt's orange exports (Ali 2014) and competitiveness (Hassanain and Gabr 2020), the competitiveness of Spain's orange sector in Europe (Ben-Amor and De-Miguel 2020) and the impact of citrus exports on the economy in Tunisia (Bakari 2018) were investigated.

In the first part of this study, the place and importance of citrus production and export of the world and Mediterranean countries in the world economy are mentioned. In the second stage, the competitiveness of the Mediterranean Basin countries in the citrus exports of the Mediterranean Basin countries in the last 15 years (2005-2019) was analyzed comparatively. In the last part of the study, some policy recommendations for the improvement of citrus production were suggested based on research findings.

2. Materials and Methods

The main material of the study is composed of secondary data on citrus exports of the countries in the Mediterranean Basin for the period 2005-2019. The Basin surrounding the Mediterranean consists of 21 countries which are located in Europe, Asia, and Africa (United Nations 2020). Since no data on citrus exports from Malta, Libya and Monaco could be found, the research was conducted on data from 18 countries. The data of 8 countries with a citrus export value of $ 100,000 in 2019 and above from 18 countries within the scope of the research are analyzed in detail (TradeMap 2020).

The research data were obtained from databases on TradeMap and FAOSTAT (Food and Agriculture Organization Corporate Statistical Database). Export data of countries in the World and Mediterranean Basin in US dollars, the TradeMap database were based on the harmonised product classification with code digit "Commodity Description and Code System HS-4" according to the product definition of 0805 "Citrus fruit, fresh or dried". According to the HS-6 Code system, citrus fruits are orange, tangerine, grapefruit, lemon, and lime (TradeMap 2020).

For this research, to determine the comparative advantages of citrus exports of countries in the Mediterranean Basin, Balassa's revealed comparative advantages (RCA) and revealed symmetric comparative advantages (RSCA) index were used. Also, the Lafay index has been applied to determine the effects of citrus fruits on the trade balance.

The RCA index which was first put forward by Liesner in 1958, was named the Balassa index because it was developed by Balassa in 1965. An index, in general, is determined by the strong or weak sectors by measuring the share of one or a group of goods of a country in total exports of the same country (Ben-Amor and De Miguel 2020).

$$RCA = \frac{X_{ij} / X_j}{X_{iw} / X_w} \qquad (1)$$

X_{ij}= Exports of good i of j country (Citrus exports of Mediterranean Basin countries)

X_j= Exports of all goods of j country (Total exports of Mediterranean Basin countries)

X_{iw}= Total world exports of good i (Total World citrus export)

X_w= Total world's exports in year t is defined.

The use of the RCA index is limited by the fact that it cannot explain the competitiveness in trade based on the concept of performance and related variables, does not take imports as a basis, ignores international trade policies, and is asymmetric because it takes zero and infinite values (Rosatto et al. 2018). Dalum et al. (1998) introduced the RSCA index to correct the asymmetry intended for the advantage or disadvantage of the RCA index. It takes values between -1 and +1, indicates a comparative disadvantage if the index value is between -1 and 0, and comparative advantage between 0 and +1 (Rosatto et al. 2018). RSCA Index equation;

$$RSCA_{ij} = (RCA_{ij} - 1) / (RCA_{ij} + 1) \qquad (2)$$

In addition to the measurement of competitiveness, the effects of a country on the balance of foreign trade in a product were taken into account with the index proposed by Lafay (1992) and it is determined whether the product is a net importer or exporter by comparing it with the theoretical trade balance. In the calculation of the Lafay index, due to the consideration of imports, can more powerful information value, takes macroeconomic distortions into account, and better analyzes competitiveness on a product basis (Burianová and Belová 2012).

$$LFI_j^i = \left[\frac{X_j^i - M_j^i}{X_j^i + M_j^i} - \frac{\sum_{j=1}^{N}(X_j^i - M_j^i)}{\sum_{j=1}^{N}(X_j^i + M_j^i)}\right] \frac{X_j^i + M_j^i}{\sum_{j=1}^{N}(X_j^i + M_j^i)} \qquad (3)$$

X^i_j= export of good "i" in period "t" of country "j";

M^i_j= import of good "i" in period "t" of country "j";

$\sum X^i_j$= total export on period "t" of country "j";

$\sum M^i_j$= total import on period "t" of country "j" is expressed.

3. Results and Discussion

3.1. Citrus production and export of Mediterranean countries

The Mediterranean Basin differs from the global system not only with its geographical borders within the vast land but also its historical, cultural and economic characteristics (Galchina et al. 2019). Besides these features, climatic, topographic and geological conditions of the basin, especially citrus fruits it provides high product productivity in perennial plants such as olives, almonds and grapes. (Cayuela et al. 2017).

From the time of the Roman Empire in the Mediterranean Basin, citrus fruits cultivation has become the main product in agricultural fields with the developments in agricultural technology since the 20th century (Duarte et al. 2016). The Mediterranean Basin realised 18% of the world citrus production at the end of 2019 (FAOSTAT 2020). In this period, although the Mediterranean Basin increased its citrus production by 29.17%, the rate of increase remained below the world average. When examined based on countries, the highest increase rate occurred in Algeria, Morocco, and Syria, respectively (Table 1). The National Agricultural Development Program (UNDP) initiated by Algeria in the 2000s and Morocco's support for developing citrus cultivated areas and irrigation systems contributed to this increase (Laoubi et al. 2010; Verner et al. 2018).

Table 1. Citrus production of Mediterranean countries (Thousand ton)

Country	Years		Change (%)	Country	Years		Change (%)
	2005	2019			2005	2019	
Spain	5310716	6008570	13.14	Algeria	627406	1583492	152.39
Egypt	2993030	4632701	54.78	Syria	610460	1177911	92.97
Turkey	2910000	4299185	47.74	Greece	1153189	1085080	-5.91
Italy	3488889	2864970	-17.88	Others	2159713	1529498	-29.18
Morocco	1320400	2604291	99.96	Total	19963313	25785698	29.17

Source: FAOSTAT (2020)

While the Mediterranean Basin had 57% of the world citrus exports in 2005, at the end of 2019 this rate had decreased to 46% (TradeMap 2020). In the period examined, although the citrus exports in the Mediterranean Basin increased by more than 50%, its share in the world decreased due to being below the world average. In the period covering the research, Egypt realised the highest increase in exports by eight times, and an increase was recorded in all of the leading countries (Table 2). One of the main reasons for this rapid increase in Egypt was that the target market, the EU, provided a preferential trade opportunity in orange exports (Spreen et al. 2020).

When the production and export shares of the Mediterranean countries are examined, Spain leads the production and exports. Spain's export share is more than twice its share in production. The main reasons for this are that the Valencia and Castellón regions, which realise 60% of the citrus production in Spain, contain a logistics network that can quickly reach large-scale distribution and the privilege of trade provided by the EU membership (Rose 2020).

In the developing countries of the Mediterranean Basin, the citrus sector is effectively supported by governments for its productive and economic contribution (Schimmenti et al. 2013). The demand for products in certain standards of the EU, which is the largest market in citrus exports of North African countries, ensured that breeding work became a state policy in these countries. Breeding studies have contributed to citrus fruits to provide product quality and unique features and have become an indicator that it will contribute to competitiveness (Oubahou and Otmani 2000; D'Onghia and Lacirignola 2009).

3.2. Measurement of competitiveness in citrus trade of the Mediterranean countries

With the effect of globalisation, countries are making intense efforts to increase their competitiveness and get a larger share of the world trade. The economic literature suggests the use of indices to measure competitiveness. Although global competition indexes are generally applied at a national level, studies on the impact of exports on competitiveness and performance have also gained the attention of researchers (Dobrovic et al. 2018; Buchalter 2019).

The RCA index, although it causes various discussions from time to time since it cannot explain some macroeconomic factors (Oelgemöller 2013; Rosatto et al. 2018), is a widely used competitiveness index, as it determines the strengths and weaknesses of the sectors and goods in international trade.. RCA index values made during the research period, which reveal the competitive power according to the citrus export performance of Mediterranean countries, are presented in Table 3. Morocco was the most competitive country according to index values of RCA, followed by Egypt, Spain, and Greece. Except for Italy, France, and Israel, the average RCA index value of other countries are over four, and these countries have high competitive power. In the period examined, only three of the eight countries with the highest citrus exports have increased in the average RCA index. The Mediterranean Basin, in general, has maintained its competitive power although there has been a slight decline in the RCA index averages. Egypt has realised the highest increase in index value with 186.35%, followed by Israel with 25.83% and Italy with 12.38%. Morocco has the highest index value, lack of competitiveness, trade pressures and inability to keep up with changing market conditions limited its potential and index decreased by half. (Schimmenti et al. 2013). However, the fact that Spainish owned markets supply the production deficiencies from Morocco has contributed to the continuation of Morocco's export power (Ben-Amor and De Miguel 2020).

The results regarding RSCA index values, which eliminate the asymmetric structure of the RCA Index, are presented in Table 4. Except for Italy and France, citrus exports of leading Mediterranean Basin countries, RSCA index values are taken to be positive. The country with the highest index value is Morocco, followed by Egypt and Spain. The total RSCA index value of other Mediterranean countries is close to zero, while all Mediterranean countries have a high RSCA index value of 0.66 in the competition index. According to this result, the fact that the leading citrus exporters from the Mediterranean countries have high RSCA shows that both these countries and the Mediterranean Basin have a high comparative advantage and a high share of citrus exports in total exports. Naseer et al. (2019) reported that three of the five countries with the highest RSCA index in the world during the 2007-2016 period are countries located in the Mediterranean Basin such as Spain, Turkey and Morocco.

Lafay index values, which reveal the balance of citrus fruits in foreign trade, are given in Table 5. During this period, leading countries other than France and Italy had a comparative advantage over the Lafay index. Moreover, Egypt realised the highest increase in the Lafay index value with 224% followed by Israel with a 46% increase. Egypt has the highest average Lafay index value with 0.87 in the 15 years, followed by Morocco (0.81) and Spain (0.66). Ben-Amor and De Miguel (2020) reported that the countries with the highest market share in the citrus market of Mediterranean countries in the period 1994-2013 were Spain, Egypt, Greece, and Morocco, respectively.

Table 2. Citrus export values of Mediterranean countries (Thousand $)

Country	Years		Change (%)	Country	Years		Change (%)
	2005	2019			2005	2019	
Spain	2669238	3580279	34.13	Greece	122508	204452	66.89
Turkey	404844	751172	85.55	Israel	95310	180391	89.27
Egypt	82511	748286	806.89	France	86074	115188	33.82
Morocco	341394	496432	45.41	Others	87476	68667	-21.50
Italy	143526	253556	76.66	Total	4032881	6398423	58.66

Source: TRADEMAP (2020)

Table 3. RCA index values in citrus exports of Mediterranean countries

Years	Spain	Turkey	Egypt	Morocco	Italy	Greece	Israel	France	Other country	All country
2005	20.52	8.17	11.49	45.23	0.57	10.41	3.30	0.29	1.35	4.77
2006	21.37	8.93	8.77	35.77	0.58	10.41	2.78	0.25	1.37	4.82
2007	21.08	7.63	10.51	29.08	0.61	11.26	3.31	0.27	1.20	4.75
2008	19.55	6.67	23.93	31.67	0.76	10.91	2.52	0.27	1.58	4.82
2009	18.63	9.30	26.52	26.80	0.64	11.68	3.49	0.24	2.25	5.08
2010	18.31	10.06	26.22	28.60	0.83	13.09	3.79	0.32	2.50	5.25
2011	17.62	11.99	27.32	34.47	0.74	12.10	3.46	0.26	1.18	5.31
2012	18.84	9.07	25.67	25.82	0.64	9.64	4.62	0.32	1.18	5.24
2013	17.83	8.84	26.69	26.67	0.67	9.80	4.08	0.30	1.03	5.17
2014	17.38	8.57	25.70	22.82	0.65	8.98	4.27	0.32	1.26	5.05
2015	17.23	7.51	31.16	22.94	0.62	7.96	3.65	0.32	0.88	4.98
2016	14.84	7.44	29.72	18.79	0.71	11.52	3.57	0.30	1.00	4.65
2017	14.91	6.88	30.46	19.02	0.64	7.23	4.22	0.34	0.69	4.60
2018	13.89	6.85	33.77	19.64	0.70	7.61	4.19	0.29	0.70	4.53
2019	14.30	5.91	32.89	22.78	0.64	7.27	4.16	0.28	0.75	4.59
Mean	17.75	8.26	24.72	27.34	0.67	9.99	3.69	0.29	1.26	4.91
%	-30.33	-27.61	186.35	-49.63	12.38	-30.22	25.83	-4.86	-44.67	-3.84

Table 4. RSCA index values in citrus exports of Mediterranean countries

Years	Spain	Turkey	Egypt	Morocco	Italy	Greece	Israel	France	Other country	All country
2005	0.91	0.78	0.84	0.96	-0.27	0.82	0.53	-0.55	0.15	0.65
2006	0.91	0.80	0.80	0.95	-0.27	0.82	0.47	-0.60	0.16	0.66
2007	0.91	0.77	0.83	0.93	-0.24	0.84	0.54	-0.57	0.09	0.65
2008	0.90	0.74	0.92	0.94	-0.14	0.83	0.43	-0.57	0.22	0.66
2009	0.90	0.81	0.93	0.93	-0.22	0.84	0.55	-0.61	0.38	0.67
2010	0.90	0.82	0.93	0.93	-0.09	0.86	0.58	-0.52	0.43	0.68
2011	0.89	0.85	0.93	0.94	-0.15	0.85	0.55	-0.59	0.08	0.68
2012	0.90	0.80	0.93	0.93	-0.22	0.81	0.64	-0.52	0.08	0.68
2013	0.89	0.80	0.93	0.93	-0.20	0.81	0.61	-0.54	0.01	0.68
2014	0.89	0.79	0.93	0.92	-0.21	0.80	0.62	-0.52	0.12	0.67
2015	0.89	0.76	0.94	0.92	-0.23	0.78	0.57	-0.52	-0.06	0.67
2016	0.87	0.76	0.93	0.90	-0.17	0.84	0.56	-0.54	0.00	0.65
2017	0.87	0.75	0.94	0.90	-0.22	0.76	0.62	-0.49	-0.18	0.64
2018	0.87	0.75	0.94	0.90	-0.18	0.77	0.61	-0.55	-0.18	0.64
2019	0.87	0.71	0.94	0.92	-0.22	0.76	0.61	-0.56	-0.14	0.64
Mean	0.89	0.78	0.91	0.93	-0.20	0.81	0.57	0.29	1.26	4.91
%	-4.17	-9.12	12.04	-4.27	19.85	-8.07	14.49	-2.20	-195.92	-1.71

Table 5. Lafay index values in citrus exports of Mediterranean countries

Years	Spain	Turkey	Egypt	Morocco	Italy	Greece	Israel	France	Other Country	All country
2005	0.80	0.23	0.36	1.00	0.00	0.58	0.11	-0.07	0	0.11
2006	0.75	0.22	0.25	0.89	-0.01	0.49	0.08	-0.07	-0.01	0.10
2007	0.77	0.20	0.31	0.72	-0.01	0.60	0.11	-0.07	-0.02	0.09
2008	0.73	0.19	0.76	0.85	-0.01	0.59	0.08	-0.07	-0.03	0.09
2009	0.84	0.34	1.10	0.87	-0.02	0.76	0.14	-0.07	0.02	0.15
2010	0.73	0.32	0.98	0.88	0.00	0.69	0.14	-0.07	0.02	0.14
2011	0.61	0.33	0.89	0.83	-0.01	0.51	0.12	-0.05	-0.03	0.12
2012	0.62	0.26	0.80	0.69	-0.02	0.38	0.16	-0.06	-0.04	0.11
2013	0.61	0.26	0.88	0.76	-0.02	0.40	0.15	-0.07	-0.04	0.11
2014	0.60	0.26	0.83	0.66	-0.02	0.37	0.15	-0.06	-0.04	0.11
2015	0.66	0.26	1.11	0.77	-0.03	0.35	0.14	-0.08	-0.04	0.13
2016	0.59	0.28	1.19	0.69	-0.02	0.57	0.15	-0.09	-0.02	0.13
2017	0.58	0.24	1.15	0.67	-0.03	0.33	0.17	-0.08	-0.04	0.11
2018	0.53	0.24	1.22	0.68	-0.02	0.34	0.17	-0.07	-0.04	0.10
2019	0.53	0.21	1.16	0.77	-0.02	0.31	0.17	-0.07	-0.04	0.11
Mean	0.66	0.25	0.87	0.81	-0.01	0.48	0.14	-0.07	-0.05	0.11
%	-33.17	-9.12	-4.27	19.85	-8.07	14.49	-2.20	-195.92	-1.71	0.00

As a result of the research it was revealed that the countries located in the Mediterranean Basin have high competitive power in citrus exports. In the analysis, Egypt, which achieved the biggest increase proportionally and in quantity in citrus production and exports, also achieved the highest increase in the competitiveness indexes. The most important reason for this is that Egypt supplies oranges at a more affordable price, in the competitive Saudi Arabia, Russia and Holland markets (Hassanain and Gabr 2020). Spain and Morocco respectively followed Egypt as the countries with the highest competitive index. The low share of Turkey's citrus exports in total exports has resulted in a low competitiveness index. Seleka and Obi (2018) reported that Spain and Morocco were the countries that increased their citrus competitiveness the most between 1961 and 2013. Italy and France have less competitive power than other countries. Although the citrus exports of these two countries increased, the increase in the exports of other sectors led to a decrease in competitiveness (TradeMap 2020). Since the research period did not cover the pandemic process, price changes and competitiveness effects could not be determined during this period.

4. Conclusion

As a result of the research, it has been observed that there was a slight decrease in the competition index values of citrus exports of the countries in the Mediterranean Basin during the period examined. However, the research results showed that in world citrus exports, the competitive structures of the countries in the Mediterranean Basin are still strong and that they carry on their important positions in exports. EU countries, whose citrus exports are low in their total exports, have a low competitiveness index and it is expected that this rate will continue to decrease. Although Spain and Turkey come first and sixth in the world, respectively, the high share of citrus exports of countries such as Egypt and Morocco in total exports has led these two countries to come to the fore in the comparative advantage indices.

Mediterranean countries, especially in Europe, because of their proximity to export markets and the advantage of EU member basin countries such as Spain, Italy, and Greece in international trade contributes to the increase of competitive power in citrus fruits. Countries outside other countries than these, European Union provides opportunity to preferential trade opportunity and Spain has met from this country the products in need of the market. And also these countries adopt as a state policy to carry out breeding activities in citrus varieties by their institutions or organizations, shows that the competitiveness can be maintained in citrus exports. Export leads to economic and social development. For this reason, it should be planned in a way that will continue to contribute to the development of North African countries with a high competitive index in citrus exports. To increase the added value provided by citrus exports, there should be an importance placed on macroeconomic policies that will ensure the stability of the value of the developing countries' currencies.

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Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2022) 35(1): 27-31
DOI: 10.29136/mediterranean.1051392

The effects of drought, salt and combined stresses on ion exchanges of eggplant (*Solanum melongena* L.) seedlings

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ARTICLE INFO

Received: January 3, 2022
Received in revised form: March 2, 2022
Accepted: March 2, 2022

Keywords:

Abiotic stress
Combined stress
Eggplant
Ca^{2+}
K^{+}

ABSTRACT

In this study, ion exchanges in eggplant plants exposed to drought, salt, and combined stress were researched. While drought-stressed plants were irrigated at 60% FC, salt-stressed plants were irrigated with water containing 50 mM sodium chloride (NaCl). Plants under combined stress were irrigated with water containing 50 mM sodium chloride (NaCl) at 60% FC. The plants remained under stress conditions for 90 days, after which they were harvested and evaluated for their ion content. Ca^{2+}, K^{+} and Mg^{2+} contents in the shoot and root decreased significantly under drought, salt, and combined stresses. The most severe losses were detected in plants grown under combined stress. However, while Na accumulations increased under stress, these increases were more pronounced in the root under combined stress. K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios in the shoot and root under salt and combined stress were found to be lower than those under drought stress. In all stress conditions, especially K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios in the root showed significant decreases compared to the control. These findings showed that when drought and salt stress conditions were separately applied, Ca^{2+}, K^{+} and Mg^{2+} accumulations in the shoot were less. On the other hand, the combination of drought and salt increased the ion losses in each stress factor more.

1. Introduction

Drought and salinity are considered the most important abiotic stress factors that negatively affect plant production. Agricultural drought is expressed as the lack of sufficient moisture for the plant to grow and develop in the root zone. Salinity is another source of abiotic stress that negatively affects plant growth and crop yield, especially in arid and semi-arid regions (Kiran et al. 2019). Insufficient precipitation, high evaporation, natural salt rocks, saline irrigation water and insufficient drainage lead to soil salinity. Many products of economic importance can be significantly affected by drought and salinity. Morpho-physiological and biochemical changes occur in plants that are faced with drought and salinity problems (Munns and Tester 2008), as a result, plant growth is inhibited, and many losses, such as yield and quality decline, may occur as a result of metabolic damage (Abobatta 2019). Although there is a strong relationship between soil moisture and macro and micro nutrients that affect plant growth, the mobility of mineral elements and their uptake by the plant are prevented in soils with low soil moisture (Al-Kaisi et al. 2013). As the salt content in the soil increases, the plant's water uptake from the soil decreases, and the ion toxicity and nutrient uptake resulting from the decrease in the osmotic potential of the soil solution negatively affect plant growth (Parvaiz and Satyawati 2008). In addition, the uptake of nutrients by the roots and their transmission to the shoots are reduced due to active transport and membrane permeability, which deteriorates with the effect of physiological drought caused by salinity (Alam 1999). Salty conditions; Ca^{2+}, Na^{+}, K^{+}, Mg^{2+} and Cl^{-} can cause ion toxicity, nutrient imbalances may occur due to the competition of Na^{+} and Cl^{-} with nutrients such as K^{+}, Ca^{2+} and NO_3^{-}. High Na^{+} and Cl^{-} accumulations in plants under salinity stress may impair nutrient ion activities by causing high Na^{+} Na^{+}/Ca^{+2} and Na^{+}/K^{+} ratios. (Singh et al. 2014). As a result, changes may occur in the physicochemical and metabolic properties of the plant (Zhao et al. 2021).

Eggplant, which is widely grown in open and greenhouse agriculture in our country; although it varies genotypically, it is a type of vegetable that is generally moderately sensitive to salinity and drought (Ghaemi and Rafiee 2016; Brenes et al. 2020). In this plant species, drought and salinity stresses can be seen together and serious yield losses can occur. The effect of ion exchanges is also very important in the emergence of these losses. The aim of this study is to determine the effects on drought and salt stress alone and in combination on the ion exchanges of eggplant plant.

2. Materials and methods

2.1. Plant material and setting up the trial

The study was carried out in greenhouse conditions where temperature and humidity conditions (day-night temperature: 18-22-26/22-26°C, relative humidity 50-55%) are automatically provided. Eggplant (*Solanum melongena* L.) seeds of the Kemer variety were germinated in a medium containing vermiculite and perlite (1:1, v/v) (March 18). 30 days after planting (Day after planting-DAS), the seedlings reaching 3-4 true leaves were transferred to pots (diameter: 25 cm, depth: 22 cm) with

medium textured soil (electrical conductivity (EC): 1.28 dS m^{-1}, soil reaction (pH): 7.75, soil organic matter: 0.54%, soil reaction (pH): 7.75, soil organic matter: 0.54%, available phosphorus: 3.60%, total nitrogen: 0.18%, available potassium 0.86%). It was ensured that there were 8 seedlings in each pot. For each matter, 3 pots were used and a total of 48 pots were studied in 4 replications. Before planting, chemical fertilizers were applied to the seedlings according to the soil analysis results (100 mg kg^{-1} N, 25 g kg^{-1} P and 100 mg kg^{-1} K). All pots were watered at field capacity (FC) until stress treatments began. The amount of irrigation water was determined by considering the pot weight. Pots were weighed every three days and the missing water was made up to FC level. For the determination of FC; first, the sample pots were kept in a container filled with water until the saturation point was reached for 48 hours. Afterwards, the pots (covered with plastic covers) were kept for the gravity water to drain and then weighed and accepted as 100% FC.

2.2. Drought, salt and combined stress treatments

Drought, salt, and combined stress treatments were initiated at 37 DAS and lasted up to 90 DAS. Plants belonging to the drought stress treatment were kept at 60% FC, while control plants were irrigated at FC level. For this, 60% of the water given to the control pots was given to the pots in which drought stress was applied. Moisture lacking in the control was completed to FC. In the salt stress treatment, plants were irrigated with water containing 50mM sodium chloride (NaCl) during the study. For this, the missing moisture in the pot was completed to FC with water containing 50 mM NaCl. The plants in the combined stress application were irrigated with water containing 50mM NaCl at 60% FC. That is, 60% of the water supplied to the control was given as water containing 50 mM NaCl.

2.3. Mineral element analysis

At the end of the study (90 DAS), the plants removed from each application were divided into shoot and root parts. After washing with tap water and distilled water, they were dried at 65°C until they reached a constant weight and were then ground. For K, Ca, Mg and Na analysis, 250 mg of the ground leaf sample was first burned with nitric acid (HNO_3) in a microwave device and then the samples were transferred to a 50 ml erlenmeyer (Kacar and Inal 2008). Total K^+ and Na^+ in the obtained extracts were determined by reading with a Jenway PFP 7 Flamephotometer device. Ca^{2+} and Mg^{2+} were measured by reading with a Varian 720-ES ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) (Kacar and Inal 2008).

2.4. Statistical analysis

The study was carried out in 4 replications according to the randomised plot design. The obtained data were subjected to analysis of variance. SPSS 11.0 software program was used for statistical evaluations. Principal component analysis (PCA) was performed using the XLSTAT program (Addisonsoft XLSTAT, Paris) to determine relationships between the parameters.

3. Results and Discussion

The responses of eggplant plants in terms of Ca^{2+}, K^+, Mg^{2+} and Na^+ contents in the shoot and roots were found to be statistically significant ($P<0.01$) (Table 1 and Table 2). Ca^{2+}, K^+ and Mg^{2+} concentrations in root and shoot decreased importantly in the drought, salt and combined stresses compared to the control. The highest values in terms of Ca^{2+} concentration; while it was determined under drought and salt stresses (1.58% and 1.66%), combined stress (1.48%) gave the lowest value. Root Ca^{2+} content in the roots was similar to shoot Ca^{2+} content in the shoots under stress conditions, with the lowest Ca^{2+} accumulation under combined stress conditions (0.76%). Ca^{2+} accumulation was higher under drought and salt stress (0.89% and 0.84%). Yuan-Yuan et al. (2009) stated that the deterioration of chloroplasts under drought stress may decrease Ca^{2+} accumulation. It is also known that salt stress causes Na^+ accumulation in plants and decreases the Ca^{2+} ratio (Nengfei et al. 2010). It has also been emphasized in previous

Table 1. Effect of drought, salt and combined stress on Ca^{2+} and K^+ contents of shoot and root (mean±SE) in *S. melongena*. Duncan test ($P<0.01$) was used for differentiation among treatments mean ($n= 4$)

Treatment	Ca^{2+} (%)		K^+ (%)	
	Shoot	Root	Shoot	Root
Control	1.96±0.05^a	1.17±0.04^a	1.56±0.04^a	1.70±0.05^a
Drought stress	1.58±0.02^b	0.89±0.04^b	1.40±0.03^b	0.99±0.06^b
Salt stress	1.66±0.04^b	0.84±0.02^b	1.38±0.05^b	0.73±0.05^c
Combined stress	1.48±0.08^c	0.76±0.04^c	0.97±0.03^c	0.56±0.02^d
Significance of treatments	**	**	**	**

***P 0.01*

Table 2. Effect of drought, salt and combined stress on Mg^{2+} and Na^+ contents of shoot and root (mean±SE) in *S. melongena*. Duncan test ($P<0.01$) was used for differentiation among treatments mean ($n= 4$)

Treatment	Mg^{2+} (%)		Na^+ (%)	
	Shoot	Root	Shoot	Root
Control	0.46±0.01^a	0.41±0.01^a	0.49±0.02^c	0.10±0.01^d
Drought stress	0.39±0.01^b	0.33±0.01^c	0.50±0.01^c	0.35±0.02^c
Salt stress	0.40±0.01^b	0.36±0.01^b	0.62±0.04^b	1.14±0.10^b
Combined stress	0.37±0.01^c	0.31±0.01^d	0.72±0.03^a	1.53±0.07^a
Significance of treatments	**	**	**	**

***p 0.01*

studies that NaCl reduces the plant's Ca^{2+} uptake in the plants and transport, causes Ca^{2+} deficiency and ion imbalance in the plant (Kiran et al. 2015; Hand et al. 2017). In this study, only drought and salt stress decreased Ca^{2+} concentrations in the shoot and roots. The fact that Na^+ accumulation was higher, especially in the root, in the combined stress environment led to a decrease in the uptake of Ca^{2+} in the root and its transmission to the stem. Thus, the combined effect of drought and salt stress heightened Ca^{2+} losses.

When the change in K^+ concentrations in the trunk under stress conditions was examined; The highest K^+ accumulation occurred under drought and salt stress (1.40% and 1.38%). This was followed by the combined stress with a value of 0.97 (Table 1). Roots accumulated more K^+ under drought stress than combined and salt stress (0.99%). This was followed by salt and combined stress with a value of (0.73% and 0.56%, respectively) (Table 1). Potassium is of great importance for the osmotic potential of the cell to increase and water intake to take place. Nasri et al. (2008) determined that there was a decrease in K^+ concentration in the plant body as a result of drought stress in watermelon; It has been reported that potassium is effective in the opening and closing of stomata, photosynthetic effect and maintaining water balance. In the study, K ion losses in the root are relatively higher than in the trunk under all stress conditions. It has been reported that there is an antagonistic relationship between Na^+ and K^+, and that K^+ uptake can be prevented due to competition with Na^+ (Levitt 1980). Also, it has been reported that salt stress may cause a decrease in K^+ uptake in shoot and/or root in different plant species (Yong et al. 2014; Turhan et al. 2020), and that more K^+ may accumulate in the shoot than in the root (Jalali-Honarmand et al. 2014). In addition, the increase in Na^+ and the limitation in the uptake of K^+ under salt stress conditions, K^+ uptake is limited in the combined stress environment due to the inability to take water into the shoot under drought stress.

While drought, salt stress and combined stress caused decreases in the amount of Mg^{2+} in shoot and root, statistical differences emerged between applications. When the values measured in terms of Mg^{2+} amount in the shoot were examined, the highest Mg^{2+} values were determined in plants under salt and drought stress (0.49% and 0.39%, respectively) (Table 2). Plants under combined stress faced the highest Mg^{2+} loss (0.37%) (Table 2). Similarly, Mg^{2+} losses in the root were highest under combined stress (0.31%) (Table 2). Plant roots under salt stress were able to preserve the amount of Mg^{2+}, keeping the Mg^{2+} loss at a limited level (36%) (Table 2). Mg^{2+} is part of photosynthesis as a chlorophyll component and activator. Decreased water uptake and delivery due to drought and salinity also led to less Mg^{2+} accumulation under combined stress. In addition, the decrease in Mg^{2+} concentration in the shoot may be the reason for the deterioration in photosynthesis. Similar results were also reported by Liu et al. (2020) under salt stress conditions. On the other hand, under combined stress; the reduction in Mg^{2+} content appeared more severely under the combined stress due to the reduction in water potential and ion toxicity.

Na^+ concentration in shoots and roots of eggplant plants under all stress treatments increased compared to the control (Table 2). Eggplant plants under stress applications, showed similar responses in terms of Na^+ concentration in shoot and root. Accordingly, the plants that limited Na^+ uptake to their shoots and roots the most were those under drought stress (0.50% and 0.35%, respectively) (Table 2). However, Na^+ accumulation was higher in shoots and roots under salt and combined stress (for salt stress; 0.62% and 1.14, for combined stress; 0.72% and 1.53) (Table 2). It has been emphasised by many researchers that there may be an increase in Na^+ amounts in root under drought and/or salt stress conditions (Chen et al. 2011; Hand et al. 2017; Dugasa et al. 2019). In the study, Na^+ accumulation in the trunk was found to be high under all stress conditions. Thus; it is seen that the development of plants that accumulate Na^+, which they take with their roots, at toxic level by being transmitted to the shoot, is adversely affected (Alian et al. 2000).

Plants need to continue to be fed with K^+ and/or Ca^{2+} while keeping their Na^+ intake limited; this is an important feature that contributes to the high salt stress tolerance of plants. In this respect, K^+/Na^+ and Ca^{2+}/Na^+ ratios are considered to be an indicator of the preferences between K^+ and Ca^{2+} with Na^+. In the study, the changes in K^+/Na^+ and Ca^{2+}/Na^+ ratios were found to be significant ($P<0.01$) (Table 3). In the shoot, the highest K^+/Na^+ ratio was determined under drought and salt stress (2.80 and 2.23 respectively), and the lowest ratio was determined under combined stress (1.35) (Table 3). In terms of K^+/Na^+ ratio in the root, the highest ratio was again determined in drought stress (2.82), while they remained relatively low (0.65 and 0.37 respectively) under salt and combined stress (Table 3). The fact that Na^+ ion accumulation was higher than K^+ under salt stress caused a decrease in the K^+/Na^+ ratio. This was especially evident in the combined stress environment and roots. Due to the similarity of their ionic diameters and electrical charges, the Na^+ ion competed with the K ion and prevented the uptake of this ion. High K^+/Na^+ ratios are used as a reliable parameter for the determination of drought and/or salt tolerance in crop plants such as tomato (Dasgan et al. 2002), tobacco (Ahmed et al. 2013), wheat (Kumar et al. 2018). However, in plants under stress Ca^{2+}/Na^+ ratios were higher in the shoot than in the root. The highest rate was determined in the trunk under drought stress (3.16). Similarly, the highest Ca^{2+}/Na^+ ratio in the root occurred under drought stress (2.54). The lowest Ca^{2+}/Na^+ ratios were determined in shoot (2.06) under the combined stress conditions and in root (0.74 and 0.50 respectively) under the salt and combined stress conditions. Na^+ ions under the salt and combined

Table 3. Effect of drought, salt and combined stress on K^+/Na^+ and Ca^{2+}/Na^+ of shoot and root (mean±SE) in *S. melongena*. Duncan test ($P<0.01$) was used for differentiation among treatments mean ($n= 4$)

Treatment	K^+/Na^+		Ca^{2+}/Na^+	
	Shoot	Root	Shoot	Root
Control	3.17±0.13[a]	17.00±0.98[a]	3.99±0.22[a]	11.70±1.00[a]
Drought Stress	2.80±0.15[b]	2.82±0.20[b]	3.16±0.14[b]	2.54±0.16[b]
Salt stress	2.23±0.19[c]	0.65±0.06[c]	2.68±0.18[c]	0.74±0.09[c]
Combined stress	1.35±0.08[d]	0.37±0.01[c]	2.06±0.17[d]	0.50±0.04[c]
Significance of treatments	**	**	**	**

***P 0.01*

stress also decreased in Ca^{2+} ion concentrations such as K^+ in the root. This led to significant decreases in the Ca^{2+}/Na^+ ratio. Drought and, indirectly, the decrease in water potential under salt stress adversely affected the transport of Ca ions in the xylem (Kiegle et al. 2000). Our findings are similar to Sahin et al. (2018).

Principal component analysis (PCA) was carried out to determine the connection between the ion contents determined in the shoot and root of the plants. The relationships between the variables are shown with a biplot (Figure 1). According to biplot analysis, two principal components accounted for 97.74% of the total variation. PCA shows that there is a significant and positive relationship between drought stress and K^+-Shoot and K^+/Na^+-Shoot (as shown in the ellipse in Figure 1). As a matter of fact, plants tend to accumulate K^+ in their shoots as opposed to Na^+. Kumar et al., (2018) reported that plant tissues tend to accumulate enough K^+, which can reduce the toxic effects of Na^+. A similar correlation was observed between drought stress and Ca^{2+}/Na^+-Shoot. Combined stress (DS+SS) according to PCA had significant positive correlations with Na^+-Root and Na^+-Shoot. The short distance to the Na^+-Root and Na^+-Shoot variables of the combined stress (DS+SS,) indicates the strong increase in Na^+ accumulation in the root and shoot under combined stress conditions. Indeed, strong increases in Na^+ accumulation in plant roots and leaves have been reported under combined applications of drought and salt stress (Dugasa et al. 2019). Regarding the correlations between the examined variables; a strong negative correlation was observed between Na^+-Root and K^+/Na^+-Shoot (a 180 degree angle). More Na^+ accumulation in the root under stress caused a decrease in the K^+ concentration in the shoot and a decrease in the K^+/Na^+ ratio due to the increase in Na^+ conduction. This indicates that plant tissues may sometimes not tend to accumulate enough K^+ that can effectively reduce the toxic effects of Na^+. There was also a strong positive correlation between Mg^{2+}-Root and Mg^{2+}-Shoot (a rather narrow angle). A similar correlation was observed between Ca^{2+}-Shoot and Ca^{2+}-Root. As a matter of fact, it is known that Mg^{2+} and Ca^{2+} accumulations in the shoot are related to their concentrations in the root. Kumar et al. (2018) reported a similar relationship. In addition, there were strong and positive correlations between K^+/Na^+-Root and Ca^{2+}/Na^+-Root. This may be due to increases in both K^+/Na^+-Root and Ca^{2+}/Na^+-Root due to low Na^+ concentrations compared to high K^+ and Ca^{2+} concentrations in the roots of stressed plants.

4. Conclusions

The results of the study revealed that ion exchanges can differ significantly in eggplant plants under drought and salt stress. While the K^+, Ca^{2+} and Mg^{2+} accumulations in shoot and root decreased under the salinity and drought stress, the Na^+ accumulations increased significantly. However, the combined application of salinity and drought strengthened the negative effects of individual stress factors. The significant decrease in K^+/Na^+ and Ca^{2+}/Na^+ ratios with the combined effect of drought and salt stress indicated that eggplant is sensitive to the combined effects of salt and drought stress. Eggplant cultivation will be significantly restricted under drought and salinity conditions, which are highly likely to be seen together. This study, which examines the changes in the ion uptake mechanism in eggplant, is important for determining valid strategies that may be effective in improving the growth of eggplants under combined stress conditions. However, in order to better understand the ion uptake mechanism under combined stress conditions, it is recommended to examine the eggplant plant in its advanced developmental stage and under continuous/temporary stress conditions.

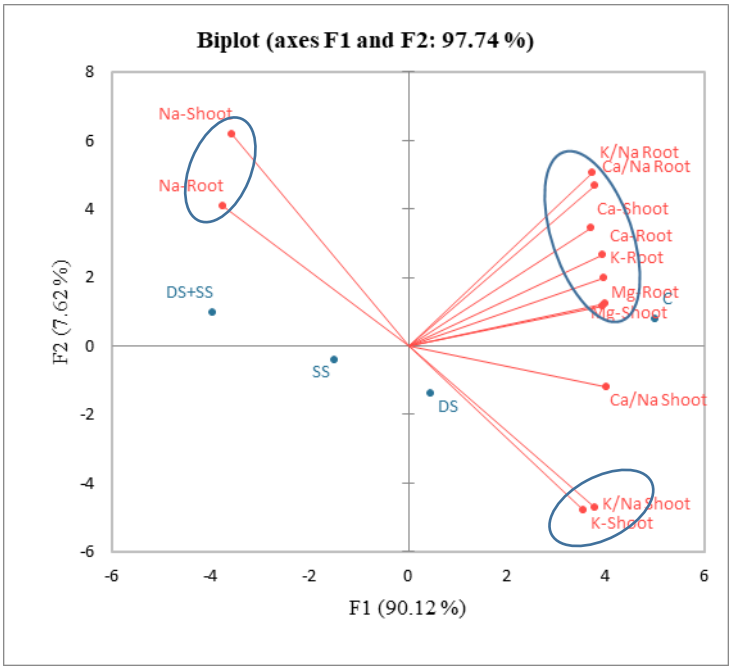

Figure 1. Biplot on ion contents in shoot and root of eggplant plants under drought (DS), salt (SS) and combined (DS+SS) stress.

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Research Article

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MEDITERRANEAN
AGRICULTURAL SCIENCES
(2022) 35(1): 33-38
DOI: 10.29136/mediterranean.874521

The effects of different harvest periods to bio-active compounds in wheat

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ARTICLE INFO

Received: February 12, 2021
Received in revised form: December 1, 2021
Accepted: December 2, 2021

Keywords:

Antioxidant
Anthocyanin
Harvest
Phenolic compound
Wheat

ABSTRACT

Natural antioxidants, especially plant phenolics, such as anthocyanins, are reliable and have a history of food use; they are also bio-active so consumption of plant extracts from natural sources is increasing day by day. The aim of this study is to detect the effects of different harvest periods on some growth parameters and bio-active compounds in wheat. The study was conducted in the 2015-2016 growing season in Konya. Seeds of Bezostaja 1, AN 110 and AT 053 genotypes were used. Growth parameters and bio-active compounds were determined on the grains of spike samples obtained at 6 different harvest periods. Whist all of the three genotypes of the trial had the highest TA (Total Anthocyanin) levels at 1st harvest; genotype AN 110 had the highest TAnt. (Total Antioxidant) content at 1st, and Bezostaja 1 and AT 053 had the highest TAnt. levels at the 6th sampling. Bezostaja 1 had the highest TPC (Total Phenolic Content) at 1st sampling; AN 110 had the highest TPC at 3rd and AT 053 at the 4th harvest period. Total antioxidant values decreased until the 3rd sampling then increased at the 4th, 5th and 6th harvest periods. Whilst the values of GM (Grain Moisture), SFW (Spike Fresh Weight), TA and TPC traits decreased on going maturity stages; values of GDW (Grain Dry Weight), SDW (Spike Dry Weight) and TGW (Thousand Grain Weight) features increased linearly. According to the results some of the growth parameters and bio-active compounds of wheat grains that were harvested earlier were higher.

1. Introduction

Wheat is one of the main compounds of human nutrition especially in developing countries. Wheat varieties have some characteristic properties that determine their usage purposes. For example if the gluten content of wheat in hard endosperm is higher, that allows it's use in making bread and cakes. In wheat, quality is related to grain gluten content, grain colour and nutrition. Colour is one of the important quality traits in pasta production and results of genotypic factors most of the time (Adom et al. 2003; Marconi and Carcea 2001; Hentschel et al. 2002). Anthocyanins are chemical molecules that are responsible for blue, purple, red and orange colours in plants (Havrlentova et al. 2014). Anthocyanins are compounds that have high antioxidant activities, anti-inflammatory and many positive effects on human health. They are bio-active compounds that are found in high levels in fruits, vegetables and cereals and have many health benefits (Abdel Aal et al. 2018; Shipp and Abdel–Aal 2010). Wheat grain and its' derivatives include phytochemicals that have high antioxidant activities such as phenolic acids, carotenoids and tocopherols (Yu et al. 2013). Wheat antioxidants are also important in bread making. Nowadays people prefer abstaining from white flour for a healthier lifestyle. It is known that consuming whole wheat grain effects human health positively because of the synergetic effects of phytochemicals (Arshad et al. 2017). Natural antioxidants are consumed more than artificial ones because of their positive effects on physiological systems. Antioxidants neutralize free radicals, detoxify ROS (reactive oxygen species), act as metal chelators and inhibit oxidative enzymes. Reactive oxygen species trigger uncontrolled cell development and cause DNA damage (Harris and Kris–Etherton 2010; Arshad et al. 2017; Flight and Clifton 2006). Cereals have come to the attention of researchers with their phenolic compounds that have strong antioxidant effects and health benefits. Concentrations of phenolic compounds in cereals depend on the variety and the parts of the grain. Phenolic compounds are generally classified as phenolic acids, flavonoids, stilbenes, coumarins, lignans and tannins. These compounds exist in the grain as sugar derivatives and form complexes with organic acids, amins, lipids and other phenols (Liu 2007; Zilic et al. 2011; Zilic 2016). Nowadays the positive effects of bio-active compounds on human health has encouraged producers to develop varieties with higher antioxidants and proteins. The suitability of bio-active compounds of wheat is affected by treatments before harvest, milling and storage conditions (Cheng et al. 2006; Arshad et al. 2017). Harvesting time also effects bio-active compounds (Merendino et al. 2006; Paradiso et al. 2006). Changes of bio-active compounds according to different harvest periods were investigated in this study.

2. Materials and Methods

2.1. Materials

In the trial Bezostaja 1 patented wheat variety and two wheat lines that were developed by Prof. Dr. Ali TOPAL from

SUAF (Selcuk University Agriculture Faculty) Crop Science Department were used.

2.2. Methods

The study was conducted in the 2015–2016 growing seasons in Konya/Turkey (32°, 31' N, 37°, 52' E) at SUAF Crop Science Department, Prof. Dr. Abdulkadir AKÇIN trial area. The trial was established according to "Factorial Experimental Design in Randomised Blocks" with three replications. Seeds were sown in the winter in parcels that have 4 rows; each was 2 m long. Distance between two rows was 20 cm. There were 500 seeds in each square meter. Irrigation cycles were completed during the sowing, steam elongation and spike formation stages respectively. Fertilisation was given according to the following calculation; 6 kg da^{-1} P_2O_5 (sowing), 10 kg da^{-1} N (1/3 in sowing, 2/3 in spring). Spike formation data was recorded during spike formation at 50% of each parcel (first week of May). Six samplings (6th June, 13th June, 20th June, 28th June, 4th July, 11th July in 2016) were done at different harvest periods at one week intervals. The relative humidity value of June was 48.2% and 41.6% in July in 2016 (MGM 2017). Eleven spike samples were taken from each parcel at each sampling time. These spikes were transferred to the laboratory immediately in plastic bags. The following parameters were detected in spike and grain samples.

GFW (Grain Fresh Weight): Grains were separated from fresh spikes and weighed. The mean of eleven values was recorded as gram "GFW".

GDW (Grain Dry Weight): Spikes were left at room conditions for 7 days then oven dried at 35ºC for 48 hours. Grains were separated from spikes and were weighed. The mean of eleven values was recorded as gram "GDW".

SFW (Spike Fresh Weight): Spike samples were put in plastic bags and moved to the laboratory immediately. Spike samples were weighed with precision scales. The mean of eleven values was recorded as gram "SFW".

SDW (Spike Dry Weight): Fresh spike samples were left at room conditions for 7 days then oven dried at 35ºC for 48 hours. Spike samples were weighed with precision scales; values were recorded as gram "SDW".

GM (Grain Moisture): Grains that were separated from fresh spikes were weighed then oven dried and re-weighed. Values obtained from these stages were subtracted. Moisture values were recorded as per cent "GM".

TGW (Thousand Grain Weight): Dry grains in each spike were counted and weighed. Means were calculated. Values were used by the following formulae and recorded as gram "TGW".

$$TGW\ (g) = (1000 \times Grain\ Weight\ per\ Spike)/Grain\ Number\ per\ Spike$$

TA (Total Anthocyanin): Total anthocyanin analysis was performed according to Leticia et al. (2009). 0.1 g grain sample was homogenized with 5 ml proponal:HCl:water solution. The extract was boiled after centrifuge and left at room conditions for 24 hours. After the last centrifuge ABSs of the supernatants at 535-650 nm were detected with a spectrophotometer.

TAnt. (Total Antioxidants): Grain total antioxidant activity was determined according to Khampas et al. (2013) by the spectrophotometric method. 0.5 ml phenolic extract was homogenised with 5 ml DPPH (60mM) solution. Supernatants were left at room conditions for 30 minutes after vortex; then ABS values were detected at 517 nm by spectrophotometer.

Values were obtained with the following formulae and recorded as % "TAnt."

$$Scavenging\ rate\ (\%) = [(A_0 - A_1)/A_0] * 100$$

TPC (Total Phenolic Content): Total phenolic content of grains was detected according to Ma et al. (2016). 2.0 g grain sample was homogenised in 16 ml methanol including 1% HCl. Homogenate was centrifuged and supernatant was stored at +4ºC. 5 ml Folin–Ciocalteu solution was added to 0.5 ml phenolic extract and the solution was neutralized with 4 ml sodium carbonate (75 g l^{-1}) than left for two hours at room conditions. Absorbances of the supernatants were determined at 765 nm with a spectrophotometer.

2.3. Statistical analysis

All data shown are the mean values (n=3). Data were statistically analysed by the analysis of variance (ANOVA) with MSTAT-C software using Duncan' s multiple range test at the level of significance $P<0.05$.

3. Results

In the trial GDW, GFW, GM, SDW, SFW, TA, TAnt. and TPC traits of cultivar Bezostaja 1 and lines AN 110, AT 053 were reviewed at six different harvest periods. Variance analysis results of each trait and mean values are presented in Table 1 and Table 2 respectively. In the trial, while values of GFW, GM, SFW, TA and TPC features decreased, compared to previous samplings; GDW, TGW and SDW properties increased linearly. Fluctuations were observed in TAnt. among sampling times (Table 2, Table 3). Whilst Bezostaja 1 variety was investigated according to GDW values; the highest GDW levels were observed at the 4th and 5th sampling times in accordance with what had been expected. Grain dry weight values increased linearly, until the 5th sampling. In the last sampling time, GDW value decreased by 13% compared to the previous one. Grain dry weight value of line AN 110 increased at the 2th sampling; decreased by 33% at the 3rd sampling compared to the previous one and increased by 34% at the 4th sampling compared to the 3rd one as well. A linear increase was observed in variety AT 053 compared to two other genotypes in GDW. A 3% decrease was observed at the 5th sampling and a 5% increase was observed at the next sampling time. Line AT 053 had the highest GDW value at the final sampling. Although TGW values of Bezostaja 1 increased linearly up to the 4th sampling, values decreased in the 5th and 6th sampling periods (Table 2). The TGW values of Bezostaja 1 decreased by 14.06% and became 51.53 g then moisture loss continued and grain weight became 49.32 g at the 6th sampling. Thousand grain weight values of line AN 110 increased linearly up to the 6th harvesting time and then decreased by 12.21% compared to the previous one. A similar situation was observed in variety AT 053 as well; TGW value decreased by 7.39% compared to the previous sampling time (Table 2). Spike dry weight value of line AN 110 was 3.910 g at the 5th sampling and became 2.800 g at the last sampling by losing weight of 28.38%. Increasing SFW values of each genotype at the first time stopped by the 4th, 5th and 6th samplings. Spike fresh weight value (3.490 g) of variety Bezostaja 1, decreased by 34% at the 5th sampling. Similarly increasing SFW value till the 4th sampling of

Table 1. Variance analysis results of each trait

Source of Variation	DF	GDW (g)	GFW (g)	GM (%)	TGW (g)	SDW (g)	SFW (g)	TA (mg kg^{-1} C3G)	TAnt. (%)	TPC (mg kg^{-1} GAE)
Replication	2	0.000	0.001	7.436	18.134	0.284	0.443	1.124	0.000	31. 818
Genotype	2	0.020**	0.029**	229.243**	1634.616**	4.869**	21.670**	18.480**	168.061**	10916.514**
Error	4	0.000	0.002	11.255	43.952	0.060	0.036	0.789	0.921	4.477
Sampling Time	5	0.001**	0.075**	3744.715**	2662.561**	3.073**	6.200**	619.243**	233.283**	79142.211**
Genotype * Sampling Time	10	0.002	0.005	64.142**	61.536**	0.253**	0.842**	33.053**	99.330**	60787.355**
Error	30	0.037	0.016	13.863	20.336	0.032	0.156	0.295	2.114	143.308
Total	53	…	…	…	…	…	…	…	…	…
CV (%)		8.88	12.95	9.24	11.07	7.74	9.70	6.27	4.99	1.48

** ***P*<0.01**

DF (Degree of Freedom), CV (Coefficient of Variation), GDW (Grain Dry Weight), GFW (Grain Fresh Weight), GM (Grain Moisture), TGW (Thousand Grain Weight), SDW (Spike Dry Weight), SFW (Spike Fresh Weight), TA (Total Anthocyanin), TAnt. (Total Antioxidant), TPC (Total Phenolic Content).

Table 2. Mean values of each genotype obtained from different sampling times

Genotypes	ST	GDW (g)	GFW (g)	GM (%)	TGW (g)	SDW (g)	SFW (g)	TA (mg kg^{-1} C3G)	TAnt. (%)	TPC (mg kg^{-1} GAE)
Bezostaja	**1**	0.193 fg	0.563 cde	65.74 a	17.44 ı	0.820 h	2.360 ı	20.540 b	26.70 fg	322.3 a
	2	0.240 ef	0.573 bcd	57.25 bcd	29.06 h	1.530 g	3.590 fg	8.260 e	24.30 gh	183.0 b
	3	0.337 bcd	0.633 abc	47.09 ef	45.10 efg	1.880 f	3.560 fg	4.650 gh	18.37 ı	122.5 ı
	4	0.369 abcd	0.623 abc	37.38 g	60.22 bc	2.000 ef	3.490 gh	3.840 hı	23.45 h	130.7 h
	5	0.369 abcd	0.359 f	12.04 ı	51.53 de	2.070 ef	2.280 ı	5.510 fg	27.15 f	122.8 ı
	6	0.318 d	0.420 f	11.26 ı	49.32 def	2.160 ef	2.430 ı	2.760 j	38.14 bc	113.8 j
AN 110	**1**	0.164 g	0.429 f	62.01 ab	6.25 j	1.580 g	4.160 def	32.850 a	44.78 a	147.0 f
	2	0.248 ef	0.599 abc	58.89 bc	17.66 ı	2.240 e	5.430 b	10.850 d	26.14 fg	140.1 g
	3	0.227 ef	0.461 def	48.22 ef	27.31 h	2.780 cd	6.110 a	5.430 fg	24.87 fgh	186.4 b
	4	0.344 abcd	0.629 abc	45.48 f	39.65 g	3.330 b	6.090 a	2.770 j	22.75 h	144.9 f
	5	0.339 bcd	0.453 ef	26.23 h	49.32 def	3.910 a	5.330 bc	1.020 k	22.65 h	112.8 j
	6	0.325 cd	0.439 f	25.56 h	43.29 fg	2.800 cd	3.750 efg	4.710 gh	30.51 e	55.88 l
053	**1**	0.257 e	0.702 a	63.03 ab	19.67 ı	1.550 g	4.190 def	21.030 b	31.50 e	172.0 c
	2	0.313 d	0.685 ab	54.73 cd	29.55 h	1.950 ef	4.300 de	11.870 c	26.28 fg	161.5 d
	3	0.318 d	0.660 abc	51.71 de	53.35 cd	2.770 cd	5.770 ab	5.510 fg	26.28 fg	151.2 e
	4	0.389 ab	0.578 bcd	37.79 g	62.87 ab	2.920 c	4.690 cd	5.820 f	34.20 d	173.2 c
	5	0.376 abc	0.419 f	10.66 ı	68.27 a	2.580 d	2.880 hı	3.610 ıj	36.45 cd	111.4 j
	6	0.397 a	0.443 f	10.05 ı	63.22 ab	2.600 d	2.890 hi	5.010 fg	39.70 b	104.4 k
LSD (0.05)		0.052	0.117	6.209	7.520	0.298	0.658	0.905	2.424	3.645

GDW (Grain Dry Weight), GFW (Grain Fresh Weight), GM (Grain Moisture), TGW (Thousand Grain Weight), SDW (Spike Dry Weight), SFW (Spike Fresh Weight), TA (Total Anthocyanin), TAnt. (Total Antioxidant), TPC (Total Phenolic Content).

Table 3. Mean values of each traits at each sampling time

Sampling Time	GDW (g)	GFW (g)	GM (%)	TGW (g)	SDW (g)	SFW (g)	TA (mg kg^{-1} C3G)	TAnt. (%)	TPC (mg kg^{-1} GAE)
06.06.16	0.204 c	0.565 a	63.589 a	14.448 d	1.310 d	3.562 c	24.800 a	34.321 b	213.774 a
13.06.16	0.267 b	0.619 a	56.950 b	25.417 c	1.900 c	4.433 b	10.320 b	25.563 d	161.516 b
20.06.16	0.294 b	0.585 a	49.000 c	41.912 b	2.471 b	5.140 a	5.190 c	23.164 e	153.343 c
28.06.16	0.367 a	0.610 a	40.209 c	54.239 a	2.744 a	4.751 b	4.137 d	26.793 d	149.577 d
04.07.16	0.359 a	0.432 b	16.303 d	56.366 a	2.844 a	3.017 d	3.374 e	28.742 c	115.656 e
11.07.16	0.347 a	0.414 b	15.617 d	51.937 a	2.510 b	3.490 c	4.151 d	36.111 a	91.354 f

GDW (Grain Dry Weight), GFW (Grain Fresh Weight), GM (Grain Moisture), TGW (Thousand Grain Weight), SDW (Spike Dry Weight), SFW (Spike Fresh Weight), TA (Total Anthocyanin), TAnt. (Total Antioxidant), TPC (Total Phenolic Content).

variety AN 110 decreased at 5th sampling time (Table 2). A similar situation was observed for variety AT 053 as well (Table 2). While Bezostaja 1 investigated according to TA levels it was observed that the highest TA was obtained from materials of the first sampling time. The most prominent decrease was observed at the second harvesting time. The total anthocyanin content of grains obtained during the second sampling, decreased 58.78% compared to the previous sampling time. Decrease of TA levels of variety Bezostaja 1 continued linearly at all other harvesting times (Table 2). The highest TA level of AN 110 was obtained from the first harvesting time, similar to Bezostaja 1 (Table 2). The total anthocyanin level of line AN 110, decreased by 60% at the second harvesting period. This critical decline continued at other harvesting times as well. In addition the TA level of AN

110 increased to 78% at the last harvesting time. While TA values obtained from line AT 053 were investigated it was observed that the highest TA was detected at the first sampling, similar to the other two genotypes. Total anthocyanin level (21.03 mg kg^{-1} C3G) of line AT 053 decreased linearly at sampling times (Table 2). At the last sampling time the TA level increased by 29.94% as line AN 110. A wide variation was also observed for TAnt. levels of all genotypes of the trial. While a linear decrease was observed at the first three sampling times of variety Bezostaja 1, a 21% increase was observed at the 4th sampling time and this situation continued until the last harvesting time. The highest antioxidant values were obtained from the last harvesting time. Total antioxidant content of line AN 110 decreased linearly up to the last harvesting time; a 25.76% increase was observed at the last harvesting time compared to the previous one. Whilst line AT 053 was investigated according to TAnt. content, a 16.57% decrease was observed at the second sampling. Changes of the TAnt. content were not observed at the 3rd sampling. A 23.15% increase was observed at the 4th sampling period compared to the previous one; this situation continued linearly up to the last sampling time. Line AT 053 had the highest antioxidant level at the last sampling time. While genotypes of the trial were investigated according to TPC levels, it was observed that variety Bezostaja 1 had the highest TPC at the first sampling time. Total phenolic content decreased up to the 4th sampling. A little increase (6.27%) was observed at the 4th sampling, then the decrease continued up to the last sampling. Decreasing TPC of line AN 110 at the 2nd sampling time; a 24.83% increase was observed compared to the previous one at the 3rd sampling period. Total phenolic content that started decreasing during previous samplings became the lowest at the last sampling. Fluctuations were observed in TPC feature of line AT 053 as well. Total phenolic content of grain samples decreased up to the 4th sampling. An increase of 30.05% was observed at the 4th sampling, the decrease continued at two other sampling times. The lowest TPC was detected at the last sampling period (Table 2).

4. Discussion

Phenolics are subunits with high biological activities in cereals; consumption of these antioxidants decreases risk of cardiovascular diseases and some types of cancer. Nowadays including high radical scavenging antioxidants, increases the popularity of coloured wheat varieties (Lutsey et al. 2007). Anthocyanins are water soluble natural colourants that belong to the flavonoid class of phenolic phytochemicals (Liu 2004). In the trial it was observed that TA and TPC levels of wheat grains decrease parallel to each other at later maturity stages (Table 3) supported by previous literature a significant and positive relation was observed between TA and TPC traits as well (Table 4). Zofajova et al. (2012) determined TA levels changed between 2.37 and 291.07 mg kg^{-1} C3G; in the same study genotype ANK 28 had the highest anthocyanin level at the 3rd maturity stage and it had no anthocyanins at the 6th one. In this research a significant and negative correlation was observed between TA and SDW features (Table 4). The decrease of anthocyanins' from water soluble flavonoids at the next stages of harvest were found to be significant and it is thought that the decrease of the anthocyanin level can be related to moisture loss. The positive relation between anthocyanin levels and grain properties in this study also supports this induction as well (Table 4). Kenievel et al. (2009) reported that starch content increased during the on-going harvest period. The speed of dry matter accumulation in endosperm is higher than dry matter accumulation in *alueron* and *pericarp* thus accumulation speeds of anthocyanins in endosperm become slower. Wheat grains and fractions have high antioxidant activities, phenolic compounds, many phytochemicals, carotenoids and tocopherols. Phytochemicals and antioxidants in wheat support the immune system and prevent many diseases. Consumption of whole wheat grain decrease the risk of cardiovascular diseases and types of cancer (Arshad et al. 2017). Among the genotypes of the trial a wide variation was observed according to antioxidant contents (Table 2). Total antioxidant levels decreased up to the 4th sampling time, though increased in the next one. The increase of TAnt. level at the 4th maturity stage continued up to the next maturity stages. Saha et al. (2018) reported that they determined TAnt. levels of wheat extracts between 0.39%-80.10%. While maturity of bio-active compounds in wheat was investigated; it was observed that TA, TAnt. and TPC levels were higher at early maturity stages parallel with higher moisture levels. In the literature there are many studies whose results are also compatible with this study. De Gara et al. (2003) and Paradiso et al. (2006) reported that wheat grains with higher moisture content (70%) have higher antioxidant activities compared to wheat grains with lower moisture levels. It was declared that TAnt. levels were higher at early maturity stages and increase 2–3 weeks after flowering in the same study. Wheat is generally consumed as an energy source besides being rich in fibre, minerals, antioxidant compounds and bio-active phytochemicals. Phytochemicals are phenolic compounds and synthesis under stress conditions as secondary metabolites (Levakova and Bartoza 2017). Some phytochemicals are bound and cannot be digested by human enzymes. These kinds of compounds take part in the digestion process in colons through fermentation. This fermentation process occurs in colons

Table 4. Correlation coefficients of all traits with each other

	GDW	GFW	GM	TGW	SDW	SFW	TA	TAnt.
GFW	-0.44	...	...	...	...	...	...	...
GM	-0.83*	0.86**	...	...	...	...	...	...
TGW	0.98**	-051	-0.86**	...	...	...	...	...
SDW	0.96**	-0.39	-0.78*	0.98**	...	...	...	...
SFW	-0.05	0.79*	0.51	-0.07	0.06	...	...	...
TA	-0.40	0.32	0.72	-0.90**	-0.94**	-0.19	...	...
TAnt.	-0.12	-0.62	-0.30	-0.11	-0.30	-0.74*	0.35	...
TPC	-0.84*	0.72	0.93**	-0.85*	-0.80*	0.26	086**	-0.12

*P<0.05, **P<0.01

and has many benefits to human health. In many epidemiologic studies researchers reported that consuming whole grains prevents chronic diseases such as colon cancer, gastro intestinal cancers and breast cancer (Liu 2007; Gabor et al. 2006; Narwal et al. 2014). According to the results of this study anthocyanin levels, obtained during earlier sampling stages, were higher and then decreased during the on-going harvest period. A similar situation was observed for total phenolics as well (Table 3). Ma et al. (2016) determined bounded TPC between 603.10 µg g^{-1}-917.20 µg g^{-1}, and free TPC between 67.94 µg g^{-1}-113.66 µg g^{-1} in wheat samples. A significant and positive correlation was also observed between TAnt. and TPC traits in this study (Table 4). A lot of literature has recorded higher levels of TPC in grains at early maturity stages; TPC levels decrease at on-going maturity stages for wheat grain (McCallum and Walker 1990; Shao et al. 2014; Ma et al. 2016). Lewis et al. (1999) reported that sucrose is necessary for polyphenol biosynthesis; it is also necessary for starch synthesis. Starch bio-synthesis accelarates at early and mid-stages of grain filling. At these stages starch bio-synthesis is faster than polyphenol bio-synthesis so polyphenol synthesis decreases at on-going stages of grain filling periods in wheat (Ma et al. 2016). This situation causes the increase of the starch level and a decrease in anthocyanin contents. The findings of this study support this literature as well.

5. Conclusion

In this study it was observed that the antioxidant capacity of wheat grains were higher at earlier harvest periods. These results have highlighted the possibility of using early harvested wheat grains as a source of bio-active compounds. The findings may indicate that early selection can be applied for traits that do not have regular linear decreases in the negative direction, such as the TA character. The output of the current work indicates the necessity and/or possibility of developing/producing materials rich in these kinds of bio-active compounds.

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MEDITERRANEAN AGRICULTURAL SCIENCES
(2022) 35(1): 39-45
DOI: 10.29136/mediterranean.995382

Genotypic structure of four cattle breeds raised in Turkey by loci related to several diseases*

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ARTICLE INFO

Received: September 14, 2021
Received in revised form: November 10, 2021
Accepted: January 18, 2022

Keywords:

ARMS-PCR
Candidate genes
Indigenous population
Microsatellite
PCR-RFLP

ABSTRACT

This study aims to reveal the genotypic structure of four cattle breeds; Holstein (HS), Turkish Grey Steppe (TGS), Anatolian Black (AB) and East Anatolian Red (EAR), raised in Turkey in terms of CD14, MBL, ITGB6, SLC11A1 and TLR2 genes and to evaluate their usefulness in Marker Assisted Selection (MAS). It also assesses whether the loci associated with resistance to diseases are suitable for phylogenetic analysis. Desired alleles and/or genotypes were detected in native Turkish cattle breeds at different frequencies in terms of polymorphisms of CD14, MBL, ITGB6, SLC11A1 and TLR2 genes which were previously reported to be associated with mastitis, foot-and-mouth disease and tuberculosis. These variations offer opportunities to improve selection strategies against diseases in the future. These results preliminary indicate that associated studies between these variations and disease resistance in native Turkish cattle breeds should be conducted. On the other hand, phylogenetic tree constructed based on genetic distance clearly separated native Turkish cattle breeds from HS breed. The gene regions related to diseases can be used to distinguish native cattle breeds from exotic ones.

1. Introduction

Cattle breeding is mainly centred on exotic breeds in Turkey in which Holstein Friesian and its crossbreeds are the most commonly raised breed with approximately 12 million individuals. The comparative population size of native Turkish cattle breeds is low due to their lower economical yields. According to official data, the current population of Anatolian Black is represented by 650000 heads, while East Anatolian Red and Turkish Grey Steppe are estimated at 135000 and 25000 heads, respectively (HBS 2019, TUIK 2019). On the other hand, since native Turkish cattle breeds are highly resistant to harsh environmental conditions and some diseases (Yilmaz et al. 2012; Demir and Balcioglu 2019), genetic diversity in these breeds must be identified and conserved to meet the current production level under various environmental conditions as well as for adaptation to the potential changes in breeding purposes (Mahmoudi et al 2010; Ramadan et al 2012).

It is well known that economic production in livestock breeding depends on raising healthy animals with high breeding value. Health problems may cause economic losses in livestock breeding. Although, preventative vaccines and drugs are available for numerous diseases, there are increasing consumer concerns about drug residue in animal-derived products. Also, the economic burden may not be affordable for smallholder farmers. Therefore, alternative methods, such as obtaining animals genetically resistant to diseases, are being sought by researchers and some breeders (Morris 2007). Thanks to the advances in DNA technologies in the last decades, several gene regions including *CD14, MBL, ITGB6, SLC11A1* and *TLR2* have been reported to be associated with various diseases such as mastitis, foot-and-mouth disease and tuberculosis in cattle (Kumar et al. 2014; Sadana et al. 2015; Singh et al. 2015). According to polymorphisms of gene regions, individuals resistant to diseases can be easily detected by using fast developing molecular tools. The selection of animals with resistant genes as breeding material and its use in MAS programs can make significant contributions to getting these diseases under control. In this regard, this study aims to investigate polymorphisms of CD14, MBL, ITGB6, SLC11A1 and TLR2 genes which were reported to be associated with resistance/susceptibility to mastitis, foot-and-mouth disease and tuberculosis in four cattle breeds raised in Turkey and to evaluate their possible usefulness in MAS in the future. Additionally, these polymorphisms were tested to see whether they are suitable for phylogenetic analysis according to the genetic origins of the cattle breeds studied.

2. Materials and Methods

2.1. Ethic Statement

This research was approved by the Akdeniz University Animal Experiments Ethics Committee, Antalya, Turkey (Protocol No: B.30.2.AKD.0.05.07.00/1).

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2.2. Sample collection and DNA extraction

A total of 210 animals from HS (n= 64), AB (n= 54), TGS (n= 48) and EAR (n= 44) cattle breeds were chosen from different representative farms. HS samples were obtained from the dairy farm of the Faculty of Agriculture, Akdeniz University; Antalya, Turkey; AB samples from 4 different farms located in Eskisehir and Antalya province, Turkey; TGS samples from 3 different farms in Balıkesir province, Turkey; and EAR samples from breeders in Erzurum province. Genomic DNA was extracted from blood samples by using the salting-out method described by Miller et al (1988).

2.3. Determination of polymorphisms of candidate genes

In this study, the polymorphisms of CD14 and MBL1 (Exon 2; 2534G>A and 2651G>A), SLC11A1 (5411G>A and 7400G>A) and TLR2 genes were detected by PCR-RFLP; the polymorphisms of ITGB6 receptor (5'UTR region 29G>A; 2145T>C) gene by ARMS-PCR; and the variation in the repetitive region of the SLC11A1 gene by the microsatellite marker technique. Some descriptive information about candidate genes is given in Table 1.

2.4. PCR-RFLP analysis

To determine SNPs associated with diseases, gene regions were amplified by PCR using specific primers (Table 1). The PCR reaction mixture consisted of in total 30 µL including 10X PCR buffer (3 µl, pH 9.0); 5 pmol each primer; 1 U Taq DNA polymerase; 10 mM $MgCl_2$; 7.5 µl dNTPs and deionized water. PCR amplification was carried out as follows; the first denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 45 seconds, annealing (Table 1) for 45 seconds, extension at 72°C for 50 seconds and the last extension at 72°C for 5 minutes.

Then, PCR products were digested by restriction enzymes to detect genotypes (Table 2). PCR products were digested by using 9 µL PCR products, 9 µL H2O, 1.1 mL enzyme buffer and 0.3-0.5 µL (10 U μl^{-1}) restriction enzyme.

Agarose gel electrophoresis was applied to visualise the possible genotypes for PCR-RFLP process (Table 3).

2.5. Microsatellite analysis

In order to determine the size of the repetitive region of the SLC11A1 gene associated with resistance to tuberculosis, PCR was performed with primers in Table 1. The PCR program and PCR components were the same as RFLP analysis. The lengths of PCR products were determined by using 96 automated capillary electrophoresis systems (Advanced Analytical Technologies-AATI, Ames, Iowa, USA). After capillary electrophoresis process, PROSize® 2.0 version 1.3.1.1 (Advanced Analytical Technologies, Inc., Ames, IA, USA) was used to visualise the bands.

Table 1. Some descriptive information on the gene regions studied

Diseases	Gene	Method	Primers	Annealing Temp. (°C)	References
Mastitis	CD14	PCR-RFLP	F: CTTCCTGTTATAGCCCCTTTCC R: CACGATACGTTACGGAGACTGA	60	Kumar et al. (2014)
	MBL1Exon2 (2651G>A)	PCR-RFLP	F: GGTGGCAAATGTTGGCTA R: GTCTTCTGAGCATCCTCCA	54	Wang et al. (2011)
	MBL1Exon2 (2534G>A)	PCR-RFLP	F: GTATCCTTCTCAAATACAAAAGAC R: CCCCTGTCTCTATGCTAGAC	54	Yuan et al. (2013)
Foot-and-Mouth Disease	ITGB6 5'UTR (29G>A)	ARMS-PCR	Outer F: CTTTCCCTAGCCTGCCTTCT Outer R: GTTCAATCCCCATCCGTTT Inner F: ATCATGTTGGAGTTGCTCATG Inner R: GGTAAAGAAGAAAAGCTGTGATT	-	Singh et al. (2014)
	ITGB6 (2145T>C)	ARMS-PCR	Outer F: TGCATAATAAAACTCAATAC Outer R: ATTCATCAGCCACCTTTTTG Inner F: CAGATTTCTCAAAGGATAGCTT Inner R: CTTGCAGAGAACAGGAAACAG	-	Singh et al. (2015)
Tuberculosis	SLC11A1	Microsatellite	F: GTGGAATGAGTGGGCACAGT R: TCTCCGTCTGCTGTGCAT	55	Kadarmideen et al. (2011)
	SLC11A1 (7400C>G)	PCR-RFLP	F: TGTGCTTCACATCTCCTTCCTA R: AGCACATTGAGCAGGTCGTT	60	Liu et al. (2017)
	SLC11A1 (5411G>A)	PCR-RFLP	F: TGAGGATCAGTGAGGGAAAAGA R: AAACTGCTTGCATATTCCCAAC	58	Liu et al. (2017)
	TLR2	PCR-RFLP	F: TTAACCTCCATCCCCTCTGG R: TAAAGGGACCTGAACCAGG	55	Sadana et al. (2015)

Table 2. Digestion conditions and enzymes used in RFLP process

Gene	Restrictions Enzyme	Enzyme Catalog Number	Digestion time (hour)	Digestion temperature
CD14	*Hinf*I	Thermo ER0801	3	37°C
MBL1, Exon 2 (2651G>A)	*Sty*I	Thermo ER0411	3	37°C
MBL1, Exon 2 (2534G>A)	*Mae*II (Tail)	Thermo ER1142	3	65°C
SLC11A1 (7400C>G)	*Pst*I	Thermo ER0611	3	37°C
SLC11A1 (5411G>A)	*Mae*II (Tail)	Thermo ER1142	3	65°C
TLR2	*Eco*RV (Eco32I)	Thermo ER0301	3	37°C

Table 3. Possible band sizes after RFLP process

Gene	PCR products (bp)	Digestion products (bp)
CD14	832	CC: 377, 272,183 CD: 377, 272, 225, 183, 47 DD: 377, 225, 183,47
MBL1, Exon 2 (2651G>A)	162	GG: 162 GA:162, 141, 21 AA: 141,21
MBL1, Exon 2 (2534G>A)	217	GG: 194,23 GA: 217, 194, 23 AA: 217
SLC11A1 (7400C>G)	936	GG: 633, 303 CG: 709, 633, 303, 227 CC:709;227
SLC11A1 (5411G>A)	998	GG: 631, 226, 141 AG: 631, 367, 226, 141 AA: 631,367
TLR2	245	CC: 245 CA: 245, 182, 63 AA: 182,63

2.6. Statistical analysis

The POPGENE 1.31 (Yeh et al. 1997) program was used to generate the phylogenetic tree by determining the gene and genotype frequencies. In addition, Hardy-Weinberg equilibrium was checked by using the chi-square (χ^2) statistic in each population (Hartl and Clark 1989).

3. Results and Discussion

Two alleles (C and D) leading to three different genotypes (CC, CD and DD) were detected in CD14/*Hinf*I polymorphism among the cattle breeds studied (Figure 1). No animals with C allele were observed in HS breed and CC genotype in all populations (Table 4). On the other hand, C allele was present in native Turkish cattle breeds with low frequencies ranging from 0.037 (AB) to 0.133 (TGS). According to polymorphisms of CD14, animals with CC genotype have been reported to be more resistant to mastitis (Kumar et al 2014; Selvan et al 2016). In this study, both desired genotype (CC) and allele (C) for CD14 gene were not detected in HS cattle breed. On the contrary, CD genotype and desired C allele were observed in native Turkish cattle breeds. The frequencies of C allele detected in AB (0.037), TGS (0.133) and EAR (0.091) breeds were lower than the values reported in Sahiwal (0.65) (Kumar et al 2014) and Karan Fries (0.29) cattle breed (Selvan et al 2016). Although it was lower than the values reported in the literature, it is important to detect desired allele in native Turkish cattle breeds in order to provide new opportunities for selection studies to be done for resistance to mastitis. The reason for monomorphism (DD genotype) of HS breed for the CD14 gene can be considered as collecting the samples from a single farm or the small numbers of samples (64). Samples for the HS breed were obtained from one single farm (the dairy farm of the Faculty of Agriculture, Akdeniz University; Antalya, Turkey) however, the animals in the farm were collected a very short time ago (four years ago) from about ten different farms in three different cities in Turkey. The number of samples were sufficient compared to similar studies. Therefore, the results obtained in the study for the CD14 gene were thought to be reflective of the actual case in the HS breed.

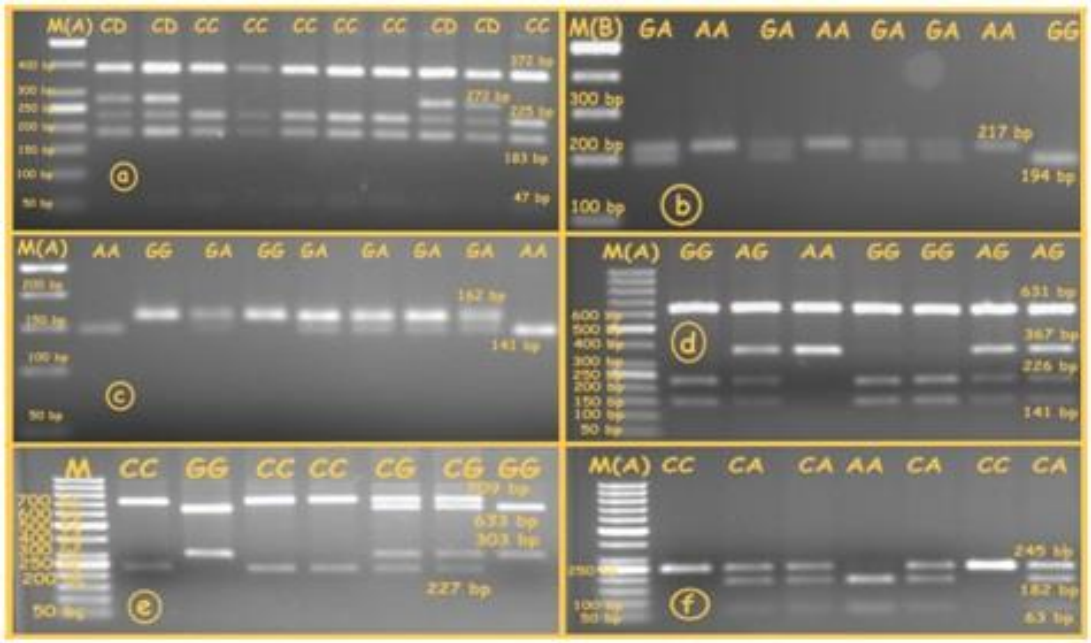

Figure 1. Agarose gel images for PCR-RFLP process. (M(A): Marker A (Thermo 50 bp; Cat. No: SM0371); M(B): Marker B (Thermo 100 bp; Cat. No: SM0241); a) Digestion of CD14 PCR products by *Hinf*I (3% agarose gel); b) Digestion of MBL1 (Ekzon 2, 2534G>A) PCR products by *Mae*II (2% agarose gel); c) Digestion of MBL1 (Ekzon 2, 2651G>A) PCR products by *Sty*I (3.5% agarose gel); d) Digestion of SLC11A1 (5411G>A) PCR products by *Mae*II (3% agarose gel); e) Digestion of SLC11A1 (7400C>G) PCR products by *Pst*I (3% agarose gel); f) Digestion of TLR2 PCR products by *EcoR*V(3% agarose gel)).

Table 4. Allele and genotype frequencies for the genes studied

Gen	Breed	n	Allele Frequencies		Genotype Frequencies			χ^2
			C	D	CC	CD	DD	
CD14	HS	61	0.000	1.000	0.000	0.000	1.000	-
	AB	53	0.037	0.962	0.000	0.075(4)	0.925 (49)	0.081[a]
	TGS	45	0.133	0.867	0.000	0.267(12)	0.733 (33)	1.065[a]
	EAR	44	0.091	0.909	0.000	0.181(8)	0.819 (36)	0.440[a]
MBL1 (Ekzon 2, 2534G>A)	**Breed**	**n**	A	G	AA	AG	GG	χ^2
	HS	61	0.590	0.410	0.327(20)	0.525(32)	0.148(9)	0.434[a]
	AB	54	0.333	0.667	0.093(5)	0.481(26)	0.426(23)	0.375[a]
	TGS	37	0.432	0.568	0.216(8)	0.433(16)	0.351(13)	0.524[a]
	EAR	44	0.262	0.738	0.068(3)	0.387(17)	0.545(24)	0.023[a]
MBL1 (Ekzon 2, 2651G>A)	**Breed**	**n**	A	G	AA	AG	GG	χ^2
	HS	59	0.745	0.255	0.610(36)	0.271(16)	0.119(7)	4.787[b]
	AB	54	0.518	0.482	0.444(24)	0.148(8)	0.408(22)	26.709[b]
	TGS	48	0.552	0.448	0.458(22)	0.188(9)	0.354(17)	18.504[b]
	EAR	44	0.500	0.500	0.477(21)	0.046(2)	0.477(21)	36.363[b]
SLC11A1 (5411G>A)	**Breed**	**n**	G	A	GG	GA	AA	χ^2
	HS	59	0.788	0.212	0.644(38)	0.288(17)	0.068(4)	1.110[a]
	AB	54	0.731	0.269	0.537(29)	0.389(21)	0.074(4)	0.005[a]
	TGS	45	0.655	0.345	0.423(19)	0.467(21)	0.111(5)	0.051[a]
	EAR	44	0.841	0.159	0.682(30)	0.318(14)	0.000	1.575[a]
SLC11A1 (7400G>A)	**Breed**	**n**	G	C	GG	GC	CC	χ^2
	HS	59	0.203	0.797	0.050(3)	0.305(18)	0.645(38)	0.201[a]
	AB	53	0.245	0.755	0.000	0.491(26)	0.509(27)	5.592[b]
	TGS	44	0.284	0.716	0.000	0.569(25)	0.431(19)	6.928[b]
	EAR	44	0.159	0.841	0.000	0.318(14)	0.682(30)	1.575[a]
TLR2	**Breed**	**n**	C	A	CC	CA	AA	χ^2
	HS	54	0.287	0.713	0.093(5)	0.389(21)	0.518(28)	0.134[a]
	AB	54	0.546	0.454	0.315(17)	0.462(25)	0.223(12)	0.235[a]
	TGS	47	0.383	0.617	0.149(7)	0.468(22)	0.383(18)	0.004[a]
	EAR	44	0.557	0.443	0.227(10)	0.659(29)	0.114(5)	4.950[b]
ITGB6 reseptör (5'UTR, 29G>A)	**Breed**	**n**	G	A	GG	GA	AA	χ^2
	HS	61	0.393	0.607	0.213(13)	0.361(22)	0.426(26)	3.640[a]
	AB	54	0.296	0.704	0.093(5)	0.407(22)	0.500(27)	0.029[a]
	TGS	48	0.281	0.719	0.063(3)	0.437(21)	0.500(24)	0.323[a]
	EAR	44	0.477	0.523	0.205(9)	0.545(24)	0.250(11)	0.381[a]
ITGB6 reseptör (5'UTR, 2145T>C)	**Breed**	**n**	T	C	TT	TC	C	χ^2
	HS	62	0.500	0.500	0.000	1.000	0.000	-
	AB	54	0.500	0.500	0.000	1.000	0.000	-
	TGS	48	0.500	0.500	0.000	1.000	0.000	-
	EAR	44	0.500	0.500	0.000	1.000	0.000	-

$\chi^2_{0.05;1}$: 3.84; a: Nonsignificant deviation from H-W equilibrium, b: Significant deviation from H-W equilibrium (P<0.05).

As expected, two alleles (A and G) leading to three different genotypes (AA, AG and GG) were observed in the cattle breeds studied in MBL1/*Mae*II polymorphism (Figure 1). The A allele frequency was ranged from 0.262 (EAR) to 0.590 (HS), whereas the frequency of G allele were between 0.410 (HS) and 738 (EAR) (Table 4). The frequency of GG genotype, which is reported to be resistant to tuberculosis (Yuan et al. 2013) were ranged from 0.148 (HS) to 0.545 (EAR) (Table 4). Yuan et al (2013) reported GG genotype frequency as 0.542 in Holstein, 0.378 in Sanhe and 0.479 in Simmental cattle breeds raised in Chine in which G allele frequency ranged from 0.544 (Sanhe) to 0.633 (Holstein). Although G allele and GG genotype frequencies detected in EAR cattle breed were higher than the values by Yuan et al (2013), similar allele and genotype frequencies were observed in TGS and AB breeds. On the other hand, a higher GG genotype frequency was detected in native Turkish cattle breed than in the HS breed. It was revealed that MBL1 could be used in MAS studies for resistance to mastitis in all breeds.

All the cattle breeds studied were polymorphic and held three different genotypes (AA, AG and GG) in terms of MBL/*Sty*I polymorphism (Figure 1). The A allele frequency varied from 0.500 (EAR) to 0.745 (HS), while frequency of G allele ranged from 0.255 (HS) to 0.500 (EAR) (Table 4). A study conducted by Wang et al (2011) showed that the number of somatic cells in the milk of the animals with AG and GG genotypes was significantly lower (P<0.05) than that of AA genotype according to polymorphisms of MBL1 (Exon 2; 2651G>A) gene. They have stated that the animals with AG and GG genotype were more resistant to mastitis. In the present study, the AA genotype frequency ranged from 0.444 (AB) to 0.610 (HS) and a higher frequency of desired genotypes for

resistant to mastitis (AG and GG) was detected in native Turkish cattle breeds than in the HS breed. There is sufficient genetic variation in local cattle breeds for MBL1 (Exon 2; 2651G>A) gene and it can be used in MAS studies for resistance to mastitis.

Two alleles (G and A) together with three genotypes (GG, GA and AA) were detected in ITGB6 receptor (5'UTR, 29G>A) gene polymorphism by ARMS-PCR across the cattle breeds studied (Figure 2). All breeds were polymorphic in terms of ITGB6 receptor (5'UTR, 29G>A) gene polymorphism, in which G allele frequency ranged from 0.281 (TGS) to 0.477 (EAR), whereas the A allele frequency was between 0.523 (EAR) and 0.719 (TGS) (Table 4). AA genotype, which is more resistant to FMD (Singh et al 2014), and the desired allele A were detected in all breeds. In this study, the frequencies of A allele in HS, AB, TGS and EAR breeds were 0.607, 0.704, 0.719 and 0.523 respectively and were similar to the value reported in HS breed (0.626) raised in India (Singh et al 2014), while they were lower than the values reported in Sahiwal (0.833), Kankrej (1.000) and Ongole (1.000) cattle breed. It is not surprising, since the genetic roots of Sahiwal, Kankrej and Ongole breeds is *Bos indicus*, while the cattle breeds studied originated from *Bos taurus*. It is known that *Bos indicus* is more resistant to FMD than *Bos taurus* (Singh et al 2014; 2015).

All breeds studied were monomorphic (TC) in terms of another mutation (2145T>C) in the ITGB6 receptor gene related to the resistance to FMD (Figure 2). No TT genotype, which is the FMD-resistant genotype (Singh et al 2015), was detected in HS, AB, TGS and EAR breeds (Table 4). Therefore, ITGB6 receptor gene (5'UTR, 2145T>C) cannot be used in MAS studies on Turkish native cattle breeds.

Two alleles (G and A) and three genotypes (GG, GA and AA) were detected in SLC11A1/*Mae*II polymorphism in the cattle populations studied (Figure 1). The lowest and highest G and A allele frequencies were 0.655 (TGS) – 0.841 (EAR) and 0.159 (EAR) – 0.345 (TGS), respectively. In the present study, the frequency of GG genotype is related to resistance to tuberculosis (Liu et al. 2017) ranged from 0.423 (TGS) to 0.682 (EAR) according to SLC11A1 (5411G>A) gene polymorphism. No AA genotype, which is a susceptible genotype for tuberculosis, was detected in EAR breed in which GG and AG genotype frequencies were 0.682 and 0.318, respectively. These values were quite similar to the frequencies of genotype (GG: 0.72; AG: 0.28 and AA: 0.00) obtained by Liu et al (2017) in healthy individuals of HS breed. The fact that all the breeds under analyses in Hardy-Weinberg equilibrium for the corresponding gene indicates that the populations have sufficient genetic variation.

As expected, two alleles (G and C) leading to three different genotypes (GG, GC and CC) were observed in SLC11A1/*Pst*I polymorphism (Figure 1). G allele frequencies ranging from 0.159 (EAR) and 0.284 (TGS) were comparatively lower than C allele frequencies ranging from 0.841 (EAR) and 0.716 (TGS) in all the cattle breeds studied (Table 4). Among the CG and GG genotypes, the genotypes for resistance to tuberculosis for this gene (Liu et al. 2017), GG genotype, was not found in the native breeds (AB, TGS and EAR). GG genotype was identified in the HS breed with a very low frequency (0.050). This value was much lower than the value reported by Liu et al (2017) in the HS breed raised in China. Although the GG genotype in SLC11A1 (7400G>A) gene frequencies of four different cattle breeds raised in Turkey were low, the frequency of CG, the second genotype for resistance to tuberculosis, was found at a moderate level. The frequencies of CG genotype in HS, AB, TGS and EAR breeds were found to be 0.305, 0.491, 0.569 and 0.318 respectively. However, the deviation of Hardy-Weinberg equilibrium of AB and TGS populations for SLC11A (5411G>A) gene may be the sign of lower genetic variation. This could be attributed to the fast reduction in the number of these breeds.

Possessing two alleles (C and A) and three genotypes (CC, CA and AA) (Figure 1), all cattle breeds studied were polymorphic for TLR2/*Eco*RV polymorphism, which were previously reported to be associated with tuberculosis in cattle (Sadana et al. 2015). C allele frequency was between 0.287 (HS) and 0.557 (EAR), whereas A allele frequency ranged from 0.443 (EAR) to 0.713 (HS) (Table 4). HS, AB and TGS were in Hardy-Weinberg equilibrium, while significant deviation was detected in EAR breed for TLR2 gene. The frequencies of tuberculosis-resistant AA genotype (Sadana et al. 2015) in HS, AB, TGS and EAR breeds were 0.518, 0.223, 0.383 and 0.114, respectively. AA genotype frequency (0.260) reported in Sahiwal, a native Indian cattle breed (Sadana et al. 2015), was similar to values detected in AB and TGS. On the other hand, a significantly higher AA genotype frequency was detected in HS breed than in native Turkish cattle breeds indicating that the success rate of MAS studies for tuberculosis is higher in HS breed. Bhaladhare et al (2016) reported CC, CA and AA genotype frequencies as 0.651, 0.269 and 0.080, respectively in native Indian cattle breeds for TLR2 gene polymorphisms. In the present study, the frequencies of the desired genotype (AA) for tuberculosis were higher than the value (0.080) reported by Bhaladhare et al (2016). The underlying reason may be the genetic origins of these cattle breeds.

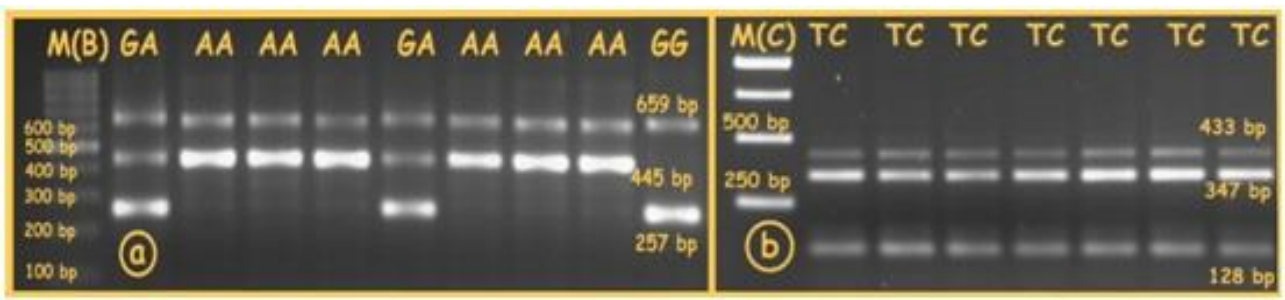

Figure 2. Agarose gel images for ARMS-PCR process. (M(B): Marker B (Thermo 100 bp; Cat. No: SM0241); M(C): Marker C Thermo 1kb; Cat. No: SM0311); a) ITGB6 receptor (5'UTR region, 29G>A; 1.5 % agarose gel) gene b) ITGB6 receptor (5'UTR region, 2145T>C, 2 % agarose gel) gene)

In this study, a total of 9 different repetitive fragments were detected in SLC11A1 gene across the cattle populations studied via microsatellite marker (Table 5). A higher number of alleles was detected in HS (8) than native Turkish cattle breeds (4-6). Observed heterozygosity was between 0.520 (AB) and 0.909 (EAR), while expected heterozygosity ranged from 0.637 (TGS) to 0.840 (HS) (Table 5). Kadarmideen et al. (2011) investigated the repetitive region in the bovine gene of SLC11A1 (solute carrier family 11), using the microsatellite method, and obtained four alleles including 211, 213, 215 and 217 bp length. The animals with the alleles of 211, 215 and 217 bp length were reported to be more resistant to BTB disease ($P<0.001$). In the present study, we found more alleles than Kadarmideen et al (2011). The identification of fragment sizes in the microsatellite marker is very precise. The differences may result from using different devices for fragment analyses. This situation prevented us from reaching exact results when identifying the resistant or susceptible genotypes in our study. Therefore, this locus was only involved determination of phylogenetic relation only.

Table 5. Allele frequencies obtained on microsatellite locus in the SLC11A1 gene.

Allele	HS	AB	TGS	EAR
201	0.020	0.000	0.000	0.000
203	0.030	0.000	0.000	0.000
205	0.098	0.160	0.418	0.523
207	0.206	0.000	0.000	0.000
209	0.245	0.230	0.427	0.204
211	0.156	0.470	0.107	0.182
213	0.000	0.050	0.012	0.091
215	0.137	0.070	0.036	0.000
217	0.108	0.020	0.000	0.000
Na	8	6	5	4
Ne	5.939	3.255	2.701	2.806
Ho	0.745	0.520	0.761	0.909
He	0.840	0.699	0.637	0.651

The phylogenetic tree that was constructed based on disease resistance loci has grouped the four breeds into two different clusters according to their genetic origins (Figure 3). The first branch separated HS breed from native Turkish cattle breeds, while the second branch separated TGS breed from AB and EAR breeds. Similarly, Demir and Balcioglu (2019), who used 20 microsatellite markers, separated native Turkish cattle breeds from HS breed according to phylogenetic analysis. The results of this study indicate that disease resistance loci may be used for the phylogenetic analysis of breeds. Microsatellite markers and next generation sequencing (NGS) analyses are generally used for phylogenetic analyses. These methods, and particularly the NGS analyses, are more informative for phylogenetic analyses, but are also costly procedures that require better technical infrastructure and more time. Therefore, disease resistance loci can be used in breed segregation, if not in ecotype or subtype segregation, when results need to be obtained in a shorter time and for lower costs.

In conclusion, for the MAS studies performed in Turkey for mastitis, CD14 gene is not appropriate for the HS breed but may be used in AB, TGS and EAR breeds. MBL1 (Exon 2; 2534G>A) and MBL1 (Exon 2; 2651G>A) genes may be used in four cattle breeds. However, it should not be forgotten that all populations deviated from the Hardy-Weinberg equilibrium for MBL1 (Exon 2; 2651G>A) gene. ITGB6 receptor gene (5'UTR, 29G>A) is very useful for the MAS studies to be performed on FMD. This gene can be used in MAS for FMD in all four cattle breeds. Since the resistant TT genotype could not be identified in the four breeds under analysis for another mutation in ITGB6 receptor gene (5'UTR, 2145T>C), it is not suitable to be used in MAS studies. SLC11A1 (5411G>A), SLC11A1 (7400G>A) and TLR2 genes can be included in the MAS programs for tuberculosis resistance in HS, AB, TGS and EAR cattle breeds. Additionally, the loci of disease resistance are very suitable for the genetic separation of breeds. Disease resistance loci for cattle breeds separation may be preferred because it is cost effective, quick and offers easy laboratory and statistical analyses.

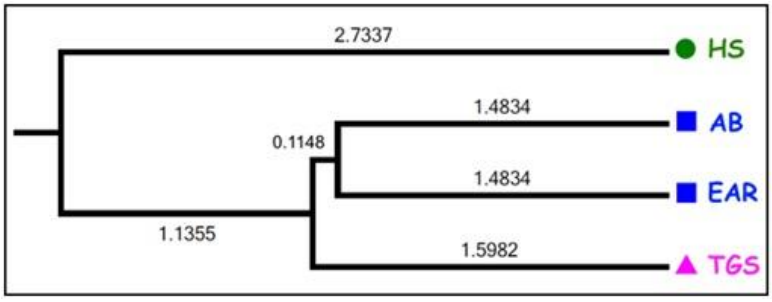

Figure 3. UPGMA dendogram among four cattle breeds raised in Turkey based on the loci of resistance to diseases.

Acknowledgements

This research was financially supported by The Scientific Research Projects Coordination Unit of Akdeniz University with the project number of FYL-2017-2355.

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Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2022) 35(1): 47-54
DOI: 10.29136/mediterranean.1065527

Diagnosing lameness with the Random Forest classification algorithm using thermal cameras and digital colour parameters

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ARTICLE INFO

Received: January 31, 2022
Received in revised form: March 21, 2022
Accepted: March 21, 2022

Keywords:

Image Processing
Classification
Thermal Camera
Lameness
Random Forest

ABSTRACT

Lameness is a serious disease that affects the health and welfare of dairy cattle whilst also causing yield and economic losses. The primary goal of this study is to determine if lameness can be detected early on in herd management using the Random Forest (RF) algorithm and the surface temperatures of the cows' hoof soles, as well as the digital colour parameters generated by processing these thermal camera images. Ages, hoof sole temperatures, and digital colour characteristics of 40 Simmental cattle were used as independent variables in this study, while lameness was evaluated by scoring and employed as a dependent variable after being updated as a binary variable. The parameters ntree= 100 and mtry= 3 were used to develop the RF algorithm for predicting lameness in animals. As a result, the RF algorithm correctly classified 19 of 22 healthy animals and incorrectly classified 3, while it correctly classified 15 of 18 unhealthy animals and incorrectly classified 3. The classification success of the RF algorithm was 85%, sensitivity, specificity and area under the ROC curve (AUC) were 0.864, 0.833, and 0.848±0.059, respectively, and it was successful in detecting lameness. Also, AUC, which is one of the RF algorithm's classification performances, was found to be statistically significant (P<0.05). As a direct consequence it can be stated that the RF algorithm is a suitable classifier in terms of the use of animal hoof sole temperatures and digital colour parameters obtained through image processing in the detection of lameness in herd management.

1. Introduction

Cattle breeding has a complex structure due to the numerous factors that influence yield. It is critical, particularly in milk and meat production, to fully reflect the yield potential of cattle to optimize environmental conditions as well as genetic structure (Boztepe et al. 2015). Cattle must be healthy in order to produce the highest yield. The deterioration of the animals' health causes disruptions in herd management, as well as a decrease in productivity and an increase in costs (Thomas et al. 2016).

Foot problems are one of the most serious diseases affecting cattle welfare (Enting et al. 1997; Yaylak.2008). Foot health is a complex phenomenon characterized by an abnormal gait, pain, and discomfort (Werema et al. 2021). Impairment of foot health indirectly affects body conformation, feed and water consumption, reproductive activities and the yield of animals (Leach et al. 2005; Mülling et al. 2006; Whay and Shearer 2017; Akkose and Izci 2017). Early detection and intervention of lameness can reduce economic losses in cattle breeding (Pedersen and Wilson 2021).

Locomotion scoring is widely used in the diagnosis of lameness in cattle. Locomotion scoring is done by an expert, usually by giving points according to the gait and posture of the animals (Werema et al. 2021). Although there are 25 different locomotion scoring methods in the literature, Sprecher et al. (1997)'s method is the most widely used, with scores ranging from 1 to 5. Given the benefits and drawbacks of various locomotion scoring systems, it is clear that reaching a consensus is extremely difficult (Schlageter-Tello et al. 2015). The most significant disadvantage in determining lameness using locomotion scores is the expert's subjective determination. Whilst the success of determining lameness is directly proportional to the specialist's experience and training, the success of a subjective method is debatable. Therefore, it is critical to determine lameness using a quantitative method that is free of subjectivity.

When compared to locomotion scoring, the use of thermal cameras in the detection of lameness is a quantitative method with great potential (Eddy et al. 2001). Its infrared camera absorbs the radiation, producing an image based on the amount of heat on the anatomical region's surface (Alsaaod et al. 2015). It detects the temperature of the region's surface using the thermal image that is created. Thermal images are colour and grayscale, with white or red representing the warmest region and black or blue representing the coldest (Colak et al. 2008). Since the temperature of the area taken with the thermal image depends on the tissue metabolism and blood flow rate, it can be associated with lameness (Bobić et al. 2017). For this reason, the physiological state of cow feet can be examined by detecting surface temperatures using a thermal camera and can be used as

a useful tool to detect lameness in the early stages (Eddy et al. 2001).

The use of data mining algorithms that do not have any prerequisites and can verify with cross-validation in the statistical association of digital colour parameters with lameness will provide more successful results in the diagnosis of lameness. This is due to the fact that data mining algorithms are robust algorithms capable of predicting, classifying, and clustering relationships between variables (Dogan and Turkoglu 2008; Savas et al. 2012).

The present study aimed to determine whether the data obtained by processing images of the hoof soles from Simmental cattle with a thermal camera could be used in the early diagnosis of lameness.

2. Materials and Methods

2.1. Material

This study consists of 40 heads of Simmental cattle with an average age of 5.371±0.510 and 2.182±0.423 lactation numbers from a cattle farm in Konya. In the farm, Simmental cattle were fed *ad-libitum* with TMR containing a mixture of coarse (alfalfa, corn silage, straw) and concentrate feed according to their yield levels.

2.2. Locomotion scoring

According to Sprecher et al (1997), the 5-point scale for detecting lameness in cattle has been revised, and it has been classified into two categories: healthy (score 1-2) and unhealthy (score 3-4-5). Physically healthy animals have a straight to slightly arched back when standing and walking, whereas unhealthy animals have a curved back while standing and walking, with cautious, wide, and reluctant strides.

2.3. Digital image processing and thermal thermography

Because thermal imaging is significantly affected by precipitation, wind, humidity, air flow, and ambient temperature, they were made under the same environmental conditions to avoid being affected by the aforementioned environmental factors (Lahiri et al. 2012). While thermal images were taken, the ambient temperature was 33°C, the humidity was 82.5% and the pressure was 42 mb. Thermal images of the hoof soles of 40 Simmental cattle were taken using the FLIR One Pro thermal camera. This thermal images' Lab (CIE L*, a*, b*), HSB (Hue, Saturation, Brigtness), and Red, Green, Blue (RGB) values were obtained using the Image-j program (Rasband 1997; Coskun and Aytekin 2021). The thermal images of the hoof soles of the healthy and unhealthy cattle are shown in colour in Figure 1, and the colour characteristics in Figure 2 are shown in grayscale.

2.4. Random Forest (RF)

The RF algorithm can be defined as a set of tree-type classifiers. Because it can be used to solve both regression and classification problems, RF is among a popular machine learning algorithm. Decision tree structures consist of roots, nodes, branches and leaves like a tree. This structure's roots and nodes represent decision criteria, leaves represent decision states, and branches form the connections between them (Ercire 2019). The RF algorithm selects different subsets from the dataset and creates different decision trees and makes an individual prediction or classification with each decision tree. If the goal is to classify individuals using the RF algorithm, the individual with the most votes should choose among the predictions, whereas the average of the decision tree predictions should be used when the goal is to make predictions. RF algorithm, branches the nodes using the best among the randomly selected variables at each node, rather than choosing the best branch among all the variables and dividing each node into branches (Breiman 2001).

The RF algorithm employs both the independent variable selection bootstrap method and bagging (Breiman 2001). It creates a tree in the new training set using random independent variables selection, but these trees are not pruned. Therefore, the accuracy of the obtained RF is improved (Breiman 2001; Pal 2003; Archer 2008). The RF algorithm is not only robust but also very fast and resistant to overfitting, allowing it to work with a decision tree as much as desired (Cutler 2007). Figure 3 depicts the stages of the RF algorithm in a classification problem.

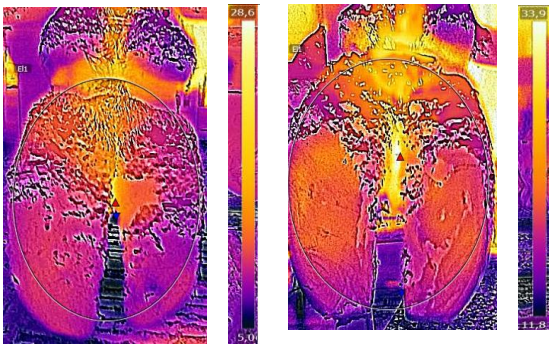

Figure 1. Colour thermal camera images of the hoof sole of healthy and unhealthy cattle.

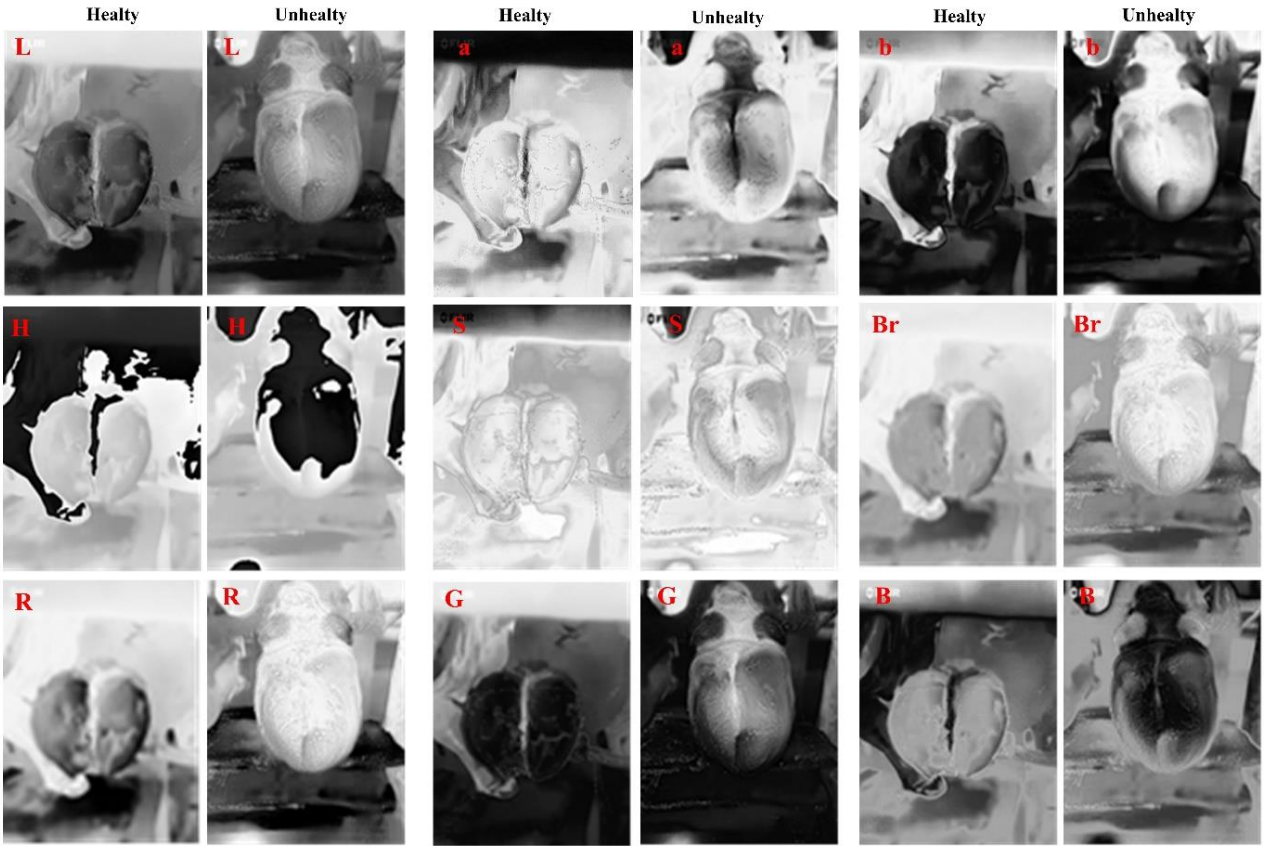

Figure 2. Image processing of grayscale images of healthy and unhealthy cattle based on digital colour parameters.

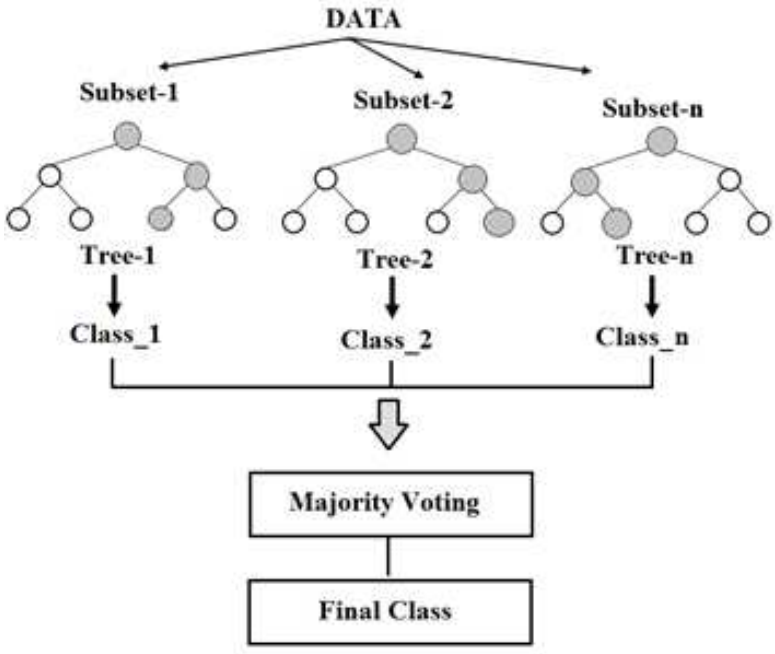

Figure 3. Classification structure of RF (Random Forest) algorithm.

While classifying with the RF algorithm, first of all, 2 parameters must be defined to determine the best splitting. These are the number of variables used at each node (m) and the number of trees to be developed (N). Then errors are tested using out-of-bag data, and trees resembling the CART (Classification and Regression Tree) algorithm are created. Unlike the CART algorithm, pruning is not performed while trees are being created. The computational load is reduced because the trees in the RF algorithm are not trimmed (Breiman 2001; Gislason et al. 2006; Akar and Güngör 2012). At each

node, the bootstrap method selects the m-variable among all variables in each node, and the best branch among these variables is determined by taking the square root of the total number of variables (Gislason et al. 2004; Cutler 2007; Horning 2010). Thus, the number of variables is reduced and the complexity of calculating correlation coefficients between trees is also reduced (Prasad et al. 2006; Liaw and Wiener 2002). Besides this, it allows for the formation of a homogeneous knot structure. In order to test the homogeneity of these nodes, Gini index, Misclassification Error, Entropy, Gain Ratio Criteria, etc. criteria can be used. The Gini index is the most widely used of these criteria (Breiman 2001; Gislason et al. 2006). The Gini index is a measure of the probability that a randomly selected variable will be misclassified. In other words, it can be defined as a measure of the purity of a specific class formed as a result of splitting. L: Assuming a data set consisting of j different classes, the Gini index is calculated as in Equation 1 (Tangirala 2020).

$$\text{Gini Index (L)} = 1 - \sum_{i=1}^{j} p_i^2 \quad (1)$$

In this equation, p_i denotes the likelihood that an object will be classified or included in a specific class. The Gini index, which can take values between 0 and 1, increases as the heterogeneity of the classes rise, and it decreases as the homogeneity of the classes increases. If the Gini index calculated from a child node is smaller than the Gini index calculated from the parent node, it indicates that the branch is successful. When the Gini index reaches zero (only one class remains in each leaf node), the tree stops branching (Watts et al. 2011; Akar and Güngör 2012).

2.5. Statistical method

The temperatures of the hoof soles of 40 Simmental cattle detected by the thermal camera, as well as the numerical color parameters obtained by processing the thermal camera images, were used as independent variables in the statistical model for determining lameness in this study. The animals were classified as either healthy or unhealthy, and the dependent variable was coded as binary responses. The performance of the RF classification algorithm was evaluated using a confusion table (Table 1).

Table 1. Confusion table for the classifier RF algorithm

Observed	Predicted as	
	Unhealthy	Healthy
Unhealthy	w	x
Healthy	y	z

RF: Random Forest

Accuracy = (w+z)/(w+x+y+z)

Sensitivity = w/ (w+x)

Specificity = z/ (y+z)

Error proportion = 1 – ((w+z)/(w+x+y+z))

The expressions w, z, x, and y in the above equations represent true positive, true negative, false negative, false positive numbers, respectively. The area under the ROC (AUC) was calculated using the Equation 2 developed by Hanley and McNeil (1982).

$$se_{AUC} = \sqrt{\frac{AUC(1-AUC)+(n_a-1)(q1-AUC^2)+(n_b-1)(q2-AUC^2)}{n_a n_b}} \quad (2)$$

$n_a = w + y$ and $n_b = x + z$

$q_1 = \frac{AUC}{2-AUC}$ and $q_2 = \frac{2AUC^2}{1+AUC}$

The independent t-test was used to compare the traits examined in both healthy and unhealthy animals. For classification, the RF algorithm with parameters ntree= 100 and mtry= 3 was used, and statistical analysis was performed in R studio using the "randomForest" package (R Core Team 2020). The classification performance of the RF algorithm was determined using the trial version of MedCalc program 19.5.1.

3. Results and Discussion

Several descriptive statistics of thermal camera temperatures and image processing features are presented in Table 2. Using locomotion scoring, it was determined that there were 22 healthy and 18 unhealthy animals in the study. The mean age of healthy and unhealthy animals did not differ statistically (P>0.05). When the thermal temperature average (T_{mean}) of the animals was examined, it was determined that unhealthy animals had a higher temperature mean than healthy animals (P<0.05). Other thermal temperatures traits (T_{min} and T_{max}) were statistically insignificant in distinguishing between healthy and unhealthy animals (P>0.05). While the difference between the a, Hue, Brightness, Red, Green, and Blue trait means obtained from image processing of healthy and unhealthy animals were statistically significant (P<0.05), the difference between the L, b, and Saturation trait means were statistically insignificant (P>0.05).

In the RF algorithm's classification of lameness, 19 out of 22 healthy animals were correctly classified, while 3 animals were predicted to be unhealthy (Table 3). When the accuracy in classifying healthy animals was determined to be 86.36%, the specificity value was determined to be 0.833 (Table 4). In the classification of 18 unhealthy animals, 15 were assigned to the correct class, while three were misclassified and estimated to be healthy. The accurate classification success of unhealthy animals was 83.33% and the sensitivity value was determined as 0.864 (Figure 4). The RF algorithm had an 85% success rate in diagnosing lameness, and the area under the ROC (AUC) was 0.848±0.059, which was found to be statistically significant (P<0.01).

Although the breakpoints of the independent variables of the model could not be determined due to the fact that more than one tree structure was produced in the RF algorithm, the values of the independent variables important used by the algorithm were determined (Figure 5). Green, Hue, Brightness, Red, Blue, L, T_{mean}, a, T_{max}, Saturation, T_{min}, and b, in order of importance were determined to be the most important variables in the diagnosis of lameness.

Table 2. Comparison of healthy and unhealthy animals in terms of considered traits and results of some descriptive statistics.

Variables	Diagnostic	n	Minimum	Maximum	Mean±SE Mean	StDev
Age	Healthy	22	2.80	9.08	6.62±0.415	1.946
	Unhealthy	18	2.84	8.61	5.48±0.454	1.926
T_{max}	Healthy	22	19.60	35.10	27.88±0.945	4.432
	Unhealthy	18	20.70	34.90	29.83±0.915	3.881
T_{min}	Healthy	22	4.60	17.60	11.20±0.711	3.334
	Unhealthy	18	3.40	17.00	10.90±0.966	4.097
T_{mean}*	Healthy	22	11.30	24.80	17.58±0.701[b]	3.289
	Unhealthy	18	13.70	30.60	20.83±1.140[a]	4.820
L	Healthy	22	40.41	48.66	46.15±0.427	2.001
	Unhealthy	18	40.39	51.84	46.52±0.677	2.871
a*	Healthy	22	41.05	49.82	44.33±0.459[a]	2.151
	Unhealthy	18	35.13	46.62	42.16±0.801[b]	3.400
b	Healthy	22	0.15	10.30	3.12±0.623	2.924
	Unhealthy	18	0.36	14.30	4.55±0.947	4.018
Hue*	Healthy	22	66.09	207.16	123.07±7.740[a]	36.300
	Unhealthy	18	30.34	97.85	61.09±5.050[b]	21.440
Saturation	Healthy	22	188.76	220.48	202.03±1.490	6.990
	Unhealthy	18	181.26	218.46	202.68±2.400	10.180
Brightness*	Healthy	22	162.00	228.48	210.64±3.050[b]	14.300
	Unhealthy	18	215.94	238.69	227.80±1.440[a]	6.120
Red*	Healthy	22	129.27	227.54	207.07±4.480[b]	21.020
	Unhealthy	18	214.70	237.88	227.01±1.490[a]	6.310
Green*	Healthy	22	28.45	118.00	92.19±4.540[b]	21.290
	Unhealthy	18	110.20	188.83	136.93±4.920[a]	20.870
Blue*	Healthy	22	52.86	148.75	83.51±4.890[a]	22.950
	Unhealthy	18	35.69	77.12	56.85±2.990[b]	12.680

*P<0.05; a, b.

Table 3. Classification table of the RF algorithm

Dependent Value		Predicted as		
	Observed	Unhealthy	Healthy	Accuracy (%)
Lameness Scoring	Unhealthy	15	3	83.33
	Healthy	3	19	86.36
	General (%)	45.00	55.00	85.00

RF: Random Forest.

Table 4. Classification performances of the RF algorithm for lameness diagnosis test

Dependent Value	Sensitivity	Specificity	AUC	Accuracy	*P*
Lameness Scoring	0.864	0.833	0.848±0.059	0.850	<0.001

RF: Random Forest; AUC: the area under the ROC curve.

Although it has been reported that there was a link between lameness and age in cattle, the current study found no statistically significant difference between the mean of ages of healthy and unhealthy animals (İstek and Durgun 2004; Dembele et al. 2006; Yayla et al. 2012; Yakan 2018). This is thought to be due to differences in shelter structure, breed, yield type, herd projection, care and feeding conditions, and ground structure where the animals spend a significant amount of time.

In this study, although the T_{min}, T_{mean}, and T_{max} temperatures of the hoof soles were all included in the model when determining lameness with IRT, the mean temperature values were the IRT variable that contributed the most to the RF algorithm. Although many studies used different regions and temperatures of the feet, the maximum temperature has been widely used (Rainwater-Lovett et al. 2009; Stokes et al. 2012). The temperature values of different parts of the feet, physical activity, and the viewing angle of the thermal camera were thought to be the cause of this (Nikkhah et al. 2005; Wilhelm et al. 2015; Bobić et al. 2017; Gianesella et al. 2018).

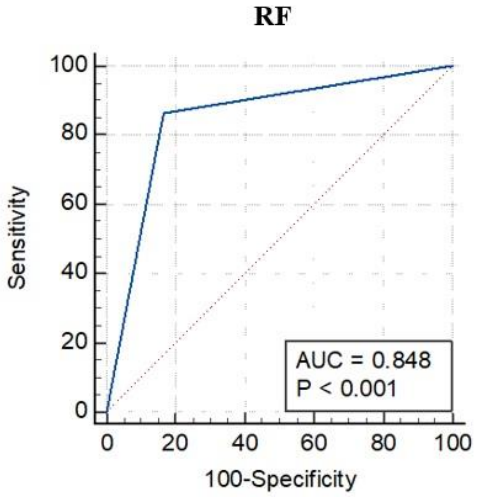

Figure 4. ROC curve of classifier RF (Random Forest) algorithm the diagnosis test.

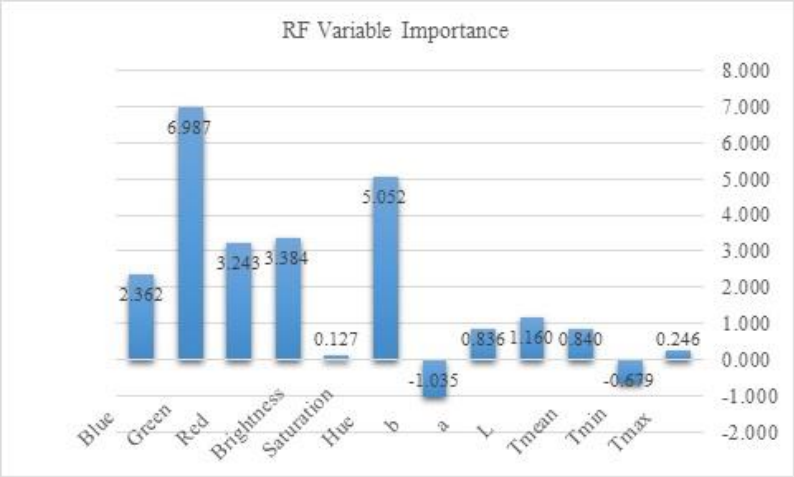

Figure 5. Variable importance of classifier RF (Random Forest) algorithm the diagnosis test.

The IRT variables obtained in the current study are incompatible with the literature because they are limited to one angle of the hind foot sole region. The thermal temperatures of the hind legs were taken in our study because the percentage of lesions on the hind legs of dairy cows is higher than that of heifers and cows (Murray et al. 1996; Chesterton et al. 2008). On the other hand, taking only the thermal temperatures of the hind legs may have reduced the sensitivity of IRT while increasing the skin temperature of the hind legs of animals with forefoot lameness (Werema et al. 2021).

Only applying locomotion scoring is insufficient for detecting lameness in cattle; a supportive element related to locomotion scoring is also required. Because locomotion scoring is subjective, it may change depending on the expert and environmental factors. The results become more robust or reliable by combining infrared thermography (IRT) technology and locomotion scoring (Renn et al. 2014).

The RF algorithm used to estimate lameness contributed the most from digital colour parameters Green (6.987) in our study while a (-1.035) variable contributed the least. The relationship between the digital colour parameters and the lameness of the hoof soles may cause excessive accumulation of dust, mud, and feces during walking, thus increasing the red and green color values.

The cut-off points of the independent variables used in the model could not be determined because the RF algorithm generates more than one tree structure. However, different IRT cut-off points have been determined in the literature using various statistical models, and it has been concluded that thermal cameras can be used to detect lameness (Main et al. 2012; Rodríguez et al. 2016; Lin et al. 2018).

4. Conclusions

In this study, the statistical relationship between lameness and the surface temperature of the hoof soles and digital colour parameters, obtained with the help of an IRT technology, were revealed by using the RF algorithm, which is a data mining algorithm. Because of the RF algorithm's high success rate in the early diagnosis of lameness and the absence of any restrictive conditions, it allows the quick detection of lameness before clinical symptoms appear. Furthermore, when combined with other diagnostic methods, this method is more likely to be successful. As a result, although the use of thermal cameras and

digital color parameters is beneficial for early detection of lameness, increasing the number of animals in the future will contribute to more comprehensive studies, even taking into account each hoof soles and using different data mining algorithms.

Acknowledgement

We would like to thank Assoc. Prof. Dr. İbrahim Aytekin and PhD. Candidate Gizem Coşkun for their assistance in acquiring data with a thermal camera.

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Theses:

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