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Effects of Proline Applications on Yield and Quality Parameters in Kapija Pepper Grown Under Different Irrigation Levels-2

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Abstract

Turkey is among the countries at risk in terms of global warming, therefore precautions for drought are necessary. Effects of drought stress to the fatty acid composition of the kapija pepper is unknown. This research wasdone at the research and practice field of Çanakkale 18 Mart University to determine the effects of different irrigation levels and proline applications on yield, chlorophyll and some fatty acid compositions of pepper (Capsicum annum var. conoides Mill.) cv. Yalova Yağlik-28 variety. This experiment was laid out in a factorial setted randomized block design with 3 replications. Plants were irrigated with 3 different irrigation levels (Kcp1: 0.5, Kcp2: 1, Kcp3: 1,5) and also proline was applied to all subjects except the control group. Linoleic and alpha linolenic acids were found to bes major acids and the effects of deficit irrigation and proline applications on the fatty acid composition of Yalova Yağlik-28 *(Capsicum annum* L.) were found statistically significant.

Keywords: *Capsicum annuum* L., Irrigation level, Proline

Introduction

Pepper is a cultivated plant which grows annual in warm climates and perennial in tropical climates. Bailey, classifies the peppers as cherry peppers, conical peppers, red fascicular peppers, long sharp peppers and bell peppers and mentions the need for optimal water request and that the roots are sensitive to excess amounts of water. Therefore the amount of water that peppers need have to be given without interruption (Vural, 2000).

Pepper (*Capsicum annuum* L.) is one of the most susceptible crops to drought stress and moderately susceptiple to salt stress (Alvino et al., 1994; Ayers and Westcot, 1989; Meiri and Shalhevet, 1973; Rhoades et al., 1992).

From the terrains classified according to stress factors, drought stress has the highest percent (%26) followed by the mineral stress (%20) and cold stress (%15). Other stress factors have 29 percent (Blum, 1986).

In water deficit conditions closed stomata can't allow the intake of CO_2 and electrons which have to take part in the reduction of CO_2, interacting with O_2 and create 'Active O_2 Radicals' like superoxide (O^-) (Oztekin, 2009).

Five drought sensitive and drought resistant chickpea cultivars studied to determine proline accumulation, protein profile and DNA polymorphism in water deficit conditions by Ahire et al. (2005). Consequently drought resistant chickpea cultivars had a higher proline accumulation than drought sensitive cultivars.

In plant tissues, the most abundant saturated fatty acids are palmitic and stearic, and the most common unsaturated fatty acids are oleic, linoleic, and linolenic (Murphy, 1993).

Fatty acids which have one or more double bonds between carbon atoms are known as poly unsaturated fatty acids. The most important of these fatty acids are Linoleic acid; [C18:2 (n-6 omega)], α-linolenic acid; [C18:3 (n-3 omega)], arachidonic acid; [C20:4 (n-6 omega)], Eikosapentaenoic acid; [C20:5 (n-3 omega)] and Dokosahekzaenoic acid; [C22:6 (n-3 omega)] (Gogus and Smith, 2010; Holub, 2002).

α-linolenic acid (C18:3) is part of the n-3 series, and it is known to be present in many plants (Ghafoorunissa & Pangrekar, 1993; Pereira *et al*., 2001; Liu *et al*., 2002).

Perez-Galvez et. al. (1999) determined that fatty acid compositions in the pericarp of two pepper varieties (*Capsicum annuum* L. cv. Jaranda and Jariza*)* were; linolenic (29.93%), linoleic (27.15%), palmitic (18.22%), oleic (8.48%), myristic (7.16%) and stearic (4.73%), from major to minor respectively on Jaranda and linolenic (30.27%), linoleic (25.18%), palmitic (18.72%), myristic (8.27%), oleic (7.19%) and stearic (4.90%) on Jariza variety.

Fatty acid compositions were approximate in three pepper varieties (Arnoia, Fresno de la and Los Valles-Benavente) and in three ripening stages. Linoleic acid was the most abundant fatty acid followed by linolenic and palmitic acids which contain more than 80 percent of total fatty acids (Martinez et al., 2006).

Drought stressed *Arabidopsis thaliana* (Ecotype Columbia) leaves represented 61% of total fatty acid content at a 47.5% relative water content.

Polar lipid contents and lipid composition of *Arabidopsis thaliana* leaf membranes were stable under water stress with an enhance in fatty acid unsaturation (Gigon et al., 2004).

Drought tolerant plants reduce the effects of stress through cellular mechanisms, however drought sensitive plants undergo irrecoverable cell damage (Vieira da Silva et al., 1974).

Rape plants (Brassica napus) decreased linolenic acids mainly in chloroplast monogalactosyldiacylgylcerol (MGDG) and decreased linoleic acids in phospholipid fractions in drought stress (Dakhma et al., 1995).

Drought tolerant (Tifway) and drought sensitive (C299) bermudagrass genotypes were grown under control and drought conditions. Unsaturated fatty acids, particularly linoleic, linolenic reduced and palmitic, stearic acids increased in both genotypes under water stress conditions while Tifway had more unsaturated fatty acids, mainly linoleic acids and less stearic and palmitic acids (Huang et al., 2011).

This research has been laid out in order to determine the yield and some quality parameters of the red pepper (*Capsicum annuum var. conoides Mill.*), an important export product of Turkey-Çanakkale which had been planted in different irrigation regimes and in an exogenous proline application.

Materials and Methods

This research was conducted at the research and practice field of Çanakkale 18 Mart University. The fertilization program was implemented according to soil analysis and results have been taken from 0-20 cm depth as represented in the terrain before the beginning of the trial. Seedlings of the Yalova Yaglik-28, Kapija pepper (*Capsicum annuum* L.) were used as plant material.

Irrigations were performed by the measuring of evaporation from a class a pan evaporation container and by applying three different pan coefficients (Kcp1: 0.5, Kcp2: 1, Kcp3: 1.5) with drip irrigation and constant pressure lateral pipes (4 liters/hour) (Yildirim, 1996).

The plants were divided into three groups for exogenous proline applications and sprayed after the 30 days from the seedling planting date. 12 mM proline applications were sprayed to all plants by spraying them from the upper and lower parts of leaves.

This experiment was laid out on a randomized block split plot design with 3 replications, consisting of 5 rows and 7 plants in each row and a total of 35 plants for each subject. The research was completed with (35*6*3) 630 plants and 18 parcels. The 2 outer rows on each side were left as edge effect and the analysis and measurements were performed in 10 randomly selected plants for each replication.

Subjects of the research:

1. Kcp1:0.5, No Proline Application
2. Kcp1:0.5, 12mM Proline (30th day from seedling planting)
3. Kcp2:1, No Proline Application
4. Kcp2:1, 12mM Proline (30th day from seedling planting)
5. Kcp3:1.5, No Proline Application
6. Kcp3:1.5, 12mM Proline (30th day from seedling planting)

Physical And Chemical Analysis

Fresh Fruit Weight (g)

The fruit weight (g) was determined by measuring the scales and the means calculated, with precision.

Yield per Plant (g/plant)

The yield per plant (g/plant) was determined by collecting all the fruit weights from yield plants when using 0.33 of meter row and 1.20 meter inter row spacing.

Fruit Number

The fruit number determined by counting the fruits and means calculated.

Total Chlorophyll (µg/100cm^2)

The chlorophyll amount in the leafs was determined with the spectrometric method (Holden, 1976).

Non-diseased, matured and homogenous 6th leafs were collected from the bottom of all applications. The leafs were carried to the laboratory with containers. 4 g of discs cut out from the leafs were extracted in %90 acetone and a solution distilled by wattman no 2 filter paper. The solution was used for the absorption spectra from 663, 645 and 652 nm by using a UV-1800 spectrophotometer and the amount of total chlorophyll, chlorophyll a and b determined as µg/100cm^2 with correction.

Analysis of Fatty Acid Composition

For detecting the total lipid values; the samples were homogenised and extracted in methanol/chloroform (2:1; v:v) according to Folch et al.

The fatty acid composition was analyzed by using a GC-FİD (Gas Chromatography, Flame İonization Detector). For this reason; aliquots of the extracted samples were taken. The lipid fraction was saponified with a 0.5 N methanolic NaOH solution. The fatty acids were methylated with BF_3:MeOH and the resulting esters were analyzed.

Shimadzu GC (Shimadzu, GC-2014, Japan) was used for the determination of the composition of the fatty acids of the samples. GC solution software was used in order to control the system. A capillary column TRB-WaxOmega with dimensions of 30 m* 0.25 mm İ.D*0.25 µm film thickness (Teknokroma, Spain) was used for the chromatography of the fatty acid methyl esters. GC conditions were performed, such as injection:Split 1:10 at 260ºC, 2.0 μL; carrier gas: nitrogen 0.8 mL min^{-1}; oven program: the initial temperature of 70 ºC for 2 min, 4 ºC min^{-1} from 70 ºC to 150 ºC, hold at 150 ºC for 10 min, 3 ºC min^{-1} from 150 ºC to 180 ºC, hold at 180 ºC for 12 min, 2 ºC min^{-1} from 180 ºC to 200 ºC, hold at 200 ºC for 15, 2 ºC min^{-1} from 200 ºC to 220 ºC, hold at 220 ºC for 10 and then finally raised to 240 ºC at the rate of 10 ºC min^{-1} and hold at 240 ºC for 10 min.

The peak identification of the fatty acids in the analyzed samples was carried out by comparing it to the retention times and the spectra of known standards used a Supelco 37 Component FAMEs (fatty acid methyl esters) Mix. The instrument was calibrated with 0.1, 0.5, 1.00, 2.50 and 5.00 mg/L concentrations using a standard solution of Supelco 37 Component FAMEs (Folch et al., 1957).

The statistical analysis was conducted using the SAS.9.1.3. Portable Computer Pocket Program and controlled by a LSD test. Biplot used this for comment to fatty acid conclusions.

Results and Discussion

As it can been seen in Table 1, the highest fruit weights were obtained from Kcp3 level of irrigation and more fruit weight was obtained by increasing the level of irrigation (Demirel et al., 2012). With Proline applications at Kcp=0.5 the irrigation level increased the fruit weight whereas statistically the same fruit weight was obtained with the proline application at Kcp=1 and Kcp=1.5 level of irrigations.

As represented in Table 2, Kcp=1 and Kcp=1.5 level of irrigations had the same fruit number values which were more than Kcp=0.5 level of irrigation. Plants with Kcp=0.5 level of irrigation with proline application had statistically higher values than plants with no proline application in this irrigation level. Proline applications at Kcp=1 and Kcp=1.5 level of irrigation had no statistical difference regarding the fruit number. Ünlükara et al. (2015) determined that a higher reduction of the amount of water decreased the fruit number whereas it remained unchanged or even increased at a proper reduction of water.

The yield per plant decreased from Kcp=1.5 level of irrigation to Kcp=1 and Kcp=0.5 level of irrigation and from major to minor respectively (Anjum et al., 2011) and the proline applications at Kcp=0.5 level of irrigation reduced the impact of water degradation on yield per plant (Table 3).

At Table 4, the contents of the total chlorophyll were expressed as chlorophyll a and chlorophyll b. The irrigation level encanced the amount of the total chlorophylls. The proline application enhanced the total chlorophyll while this enhancement was more pronounced at Kcp=0.5 level of irrigation. It was reported that drought stress decreased the chlorophyll content (Olıveıra Neto et al., 2009; Chegah et al., 2013; Moustakas et al., 2011).

A decline was determined at chlorophyll a contents with a lower level of irrigation. Proline applications caused a statistical difference at Kcp=0.5 and

Kcp=1 level of irrigations whereas no difference was determined at Kcp=1.5 level of irrigation (Table 5).

As shown in Table 6, Kcp=1.5 and Kcp=1 level of irrigations had similar and more chlorophyll b contents than Kcp=0.5 level of irrigation and proline applications increased the chlorophyll b content at Kcp=0.5 level of irrigation.

It was reported that exogenous proline positively affected yield and physiological parameters on salt (Öztekin, 2009) and drought stress (Ali et al., 2007).

The biplot comparison metot (Figure 1) was used to compare the effects of drought and proline applications and to identify differences on some fatty acid compositions of pepper. The biplot displays 76% of the information in the standardized data of the 3 irrigation levels and the proline applications for 4 fatty acids. Relative vector lengths of fatty acids didn't show an apparent difference, implying that all fatty acid factors equally contributed to total variation shown in the biplot graphic. Subjects that have higher palmitic, stearic and linoleic acid contents had lesser alfa linolenic acid contents as seen in biplot graphic (Figure 1), which shows a negative association between alfa linolenic acid and other fatty acids. The proline application to Kcp=0.5 elevated the alfa linolenic acid content as suggested by (Ali et al., 2013), and slightly decreased the stearic acid content, whereas the stearic acid continually decreased with higher levels of irrigations (Kcp=1.5). The alfa linolenic acid content increased and the stearic acid decreased in 3 (Kcp=1) and 5 (Kcp=1.5) compared to 1 (Kcp=0.5). These results were obtained by Junior et al., 2008; Hamrouni et al., 2001; Guerfel et al., 2008 on drought and Zhang et al., 2005 on salt stress. The alfa linolenic acid content decreased in 6 compared to 5 while 6 had the highest linolenic acid content among all treatments. The effect of the proline application was more obvious between treatments 1 and 2 compared to well watered treatments (Figure 1 and Table 7).

Table 1. *Effects of Different Levels of Irrigation and Proline Applications on the Fresh Fruit Weight*

	NP	PR	Kcp mean
Kcp1=0.5	48.67 D	52.08 C	50.37 C
Kcp2=1	67.71 B	68.05 B	67.88 B
Kcp3=1.5	75.26 A	75.47 A	75.36 A
P mean	63.88 B	65.20 A	

Shown are NP: No proline application, PR: Proline application; LSD values for P $\leq$0.01 PR×Kcp=1.9223; Kcp=2.6259; PR=0.9066

Table 2. *Effects of Different Levels of Irrigation and Proline Applications on the Fruit Number*

	NP	PR	Kcp mean
Kcp1=0.5	8.16 C	8.46 B	8.31 B
Kcp2=1	9.8 A	9.8 A	9.8 A
Kcp3=1.5	9.8 A	9.8 A	9.8 A
P mean	9.25 B	9.35 B	

Shown are NP: No proline application, PR: Proline application; LSD values for P ≤0.01 PR×Kcp=0.2485; Kcp=0.3315; P ≤0.05 PR=0.0816

Table 3. *Effects of Different Levels of Irrigation and Proline Applications on the Yield per Plant*

	NP	PR	Kcp mean
Kcp1=0.5	397.48 C	441.02 D	419.25 C
Kcp2=1	663.67 B	666.93 B	665.3 B
Kcp3=1.5	737.59 A	739.7 A	738.64 A
P mean	599.58 B	615.88 A	

Shown are NP: No proline application, PR: Proline application; LSD values for P ≤0.01 PR×Kcp=34.29; Kcp=48.257; PR=14.925

Table 4. *Effects of Different Levels of Irrigation and Proline Applications on the Total Chlorophyll*

	NP	PR	Kcp mean
Kcp1=0.5	26.02 E	28.67 D	27.34 C
Kcp2=1	33.34 C	33.58 CB	33.46 B
Kcp3=1.5	35.04 AB	35.13 A	35.08 A
P mean	31.47 B	32.46 A	

Shown are NP: No proline application, PR: Proline application; LSD values for P ≤0.01 PR×Kcp=1.5289; Kcp=1.4181; P≤0.05 PR=0.7222

Table 5. *Effects of Different Levels of Irrigation and Proline Applications on Chlorophyll a*

	NP	PR	Kcp mean
Kcp1=0.5	19.65 D	21.47 C	20.56 C
Kcp2=1	25.06 B	25.19 BA	25.12 B
Kcp3=1.5	26.44 A	26.45 A	26.45 A
P mean	23.71 B	24.37 A	

Shown are NP: No proline application, PR: Proline application; LSD values for P ≤0.01 PR×Kcp=1.2695; Kcp=1.1622; P≤0.05 PR=0.6035

Table 6. *Effects of Different Levels of Irrigation and Proline Applications on Chlorophyll b*

	NP	PR	Kcp mean
Kcp1=0.5	6.38 C	7.21 B	6.80 B
Kcp2=1	8.3 A	8.42 A	8.36 A
Kcp3=1.5	8.61 A	8.69 A	8.65 A
P mean	7.76 B	8.11 A	

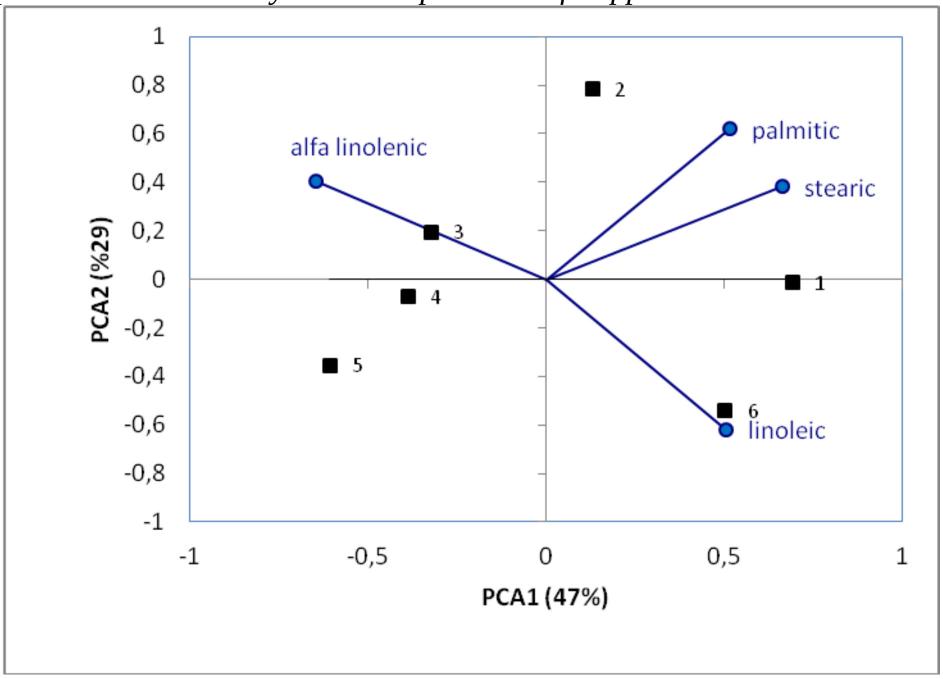
Shown are NP: No proline application, PR: Proline application; LSD values for P ≤0.01 PR×Kcp=0.3995; Kcp=0.3246; PR=0.3016

Table 7. *Effects of Different Levels of Irrigation and Proline Applications on the Fatty Acid Compositions of Pepper (%)*

Subject	Linoleic	Alfa Linolenic	Palmitic	Stearic
1	39.75	19.28	20.87	3.78
2	38.81	25.68	22.68	3.51
3	38.83	25.92	20.13	3.39
4	39.50	26.51	19.30	3.40
5	38.93	23.83	19.49	2.91
6	42.21	22.03	20.93	3.39

Shown are 1: Kcp1=0.5, no proline applied; 2: Kcp1=0.5, proline applied; 3: Kcp2=1, no proline applied; 4: Kcp2=1, proline applied; 5: Kcp3=1.5, no proline applied; 6: Kcp3=1.5, proline applied

Figure 1. *Biplot of Effects of Different Levels of Irrigation and Proline Applications on the Fatty Acid Compositions of Pepper*



Shown are 1: Kcp1=0.5, no proline applied; 2: Kcp1=0.5, proline applied; 3: Kcp2=1, no proline applied; 4: Kcp2=1, proline applied; 5: Kcp3=1.5, no proline applied; 6: Kcp3=1.5, proline applied

Conclusions

According to the results; fruit weight, fruit number, yield per plant, total chlorophyll, chlorophyll a and b values were increased in Kcp=1 and Kcp=1.5 irrigation levels whereas decreased in Kcp=0.5. Fruit weight, fruit number, yield per plant, total chlorophyll, chlorophyll a and b values were increased by the proline applications at Kcp=0.5 irrigation level. This shows that, effects of proline application was more obvious in the deficient (Kcp=0.5) irrigation regime in terms of some (fresh fruit weight, yield, total chlorophyll, chlorophyll a and b) parameters. Kcp=1.5 level of irrigation with proline application had the highest fruit weight, yield and chlorophyll contents. Treatments with Kcp=1 and Kcp=1.5 level of irrigation and Kcp=0.5 level of irrigation with proline application had a higher alfa linolenic and lower stearic acid contents than Kcp=0.5 level of irrigation. The highest linoleic acid content found in proline applied Kcp=1.5 irrigation level while other treatments showed similar linoleic acid content.

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