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Araştırma Makalesi/*Research Article (Original Paper)*

Determining the Yield and Morpho-Physiological Responses of *'Fortuna'* Strawberry cv. of Using Different Irrigation Levels with Bio-stimulant Application

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Abstract: In the current study, the response of *cv. Fortuna* grown under high tunnel conditions to water deficiency and bio-stimulant application were inspected by evaluating the morphological (crown number, whole crown diameter and leaf area) and physiological (stomatal conductance and leaf water potential) parameters along with the yield. The amounts of applied irrigation water were 1.00 (control) and 0.50 times water evaporation from surface measured by a standard Class A pan. In current study, when compare to drought conditions, application of bio-stimulant resulted in the increase of crown number (25%), yield (13%), mean fruit weight (11%), leaf area (9%), stomatal conductance (8%), leaf water potential (6%), whole crown diameter (% 5) and fruit number (3%). Moreover; it could be stated that *cv. Fortuna* responded to water deficiency, besides stomatal closure, by reduction of leaf area, fruit number, crown number, whole crown diameter, leaf water potential (Lwp), mean fruit weight and yield. When the interactions between bio-stimulant applications and limited-watered conditions (IR_{50}) are examined in terms of their morpho-physiological responses, it has been found there are clearly abundant variation for stomatal conductance (12%), leaf area (4%), Lwp (5%) and mean fruit weight (8%). All these results show that bio-stimulant applications should be combined with optimal irrigation water, besides, obtaining information about plant morpho-physiological responses under drought conditions can contribute to improving of plant adaptations under multiple stress conditions for strawberry.

Keywords: Leaf area, Lwp, Stomatal conductance, Water stress,

Farklı Sulama Seviyeleri ve Biyo-uyarıcı Uygulamalarına Fortuna Çilek Çeşidinin Verim Morfo- Fizyolojik Tepkilerinin Belirlenmesi

Öz: Mevcut çalışmada; yüksel tünel koşullarına altında *Fortuna* çilek çeşidinin su eksikliğine ve biostimulant uygulamasına verdiği tepkiler verim, morfolojik (yaprak alanı, gövde sayısı ve tüm gövde kalınlığı) ve fizyolojik (stoma iletkenliği ve yaprak su potansiyeli) parametreler incelenerek değerlendirilmiştir. Uygulanan sulama suyu miktarları, standart bir Class A panın yüzeyinden buharlaşan suyun 1.00 ve 0.50 katı olarak belirlenmiştir. Mevcut çalışmada, su eksikliği koşulları kıyaslandığında, biostimulant uygulamaları gövde sayısını (% 25), verimi (% 13), ortalama meyve ağırlığını (% 11), yaprak alanını (% 9), stoma iletkenliğinin (% 8), yaprak su potansiyelini (% 6), tüm gövde kalınlığını (% 5) ve meyve sayısını (% 3) arttırmıştır. Ek olarak; *Fortuna* çeşidinin su eksikliği koşullarına verdiği tepkiler: stomalarını kapatmasının yanı sıra, yaprak alanında, meyve sayısında, gövde sayısında, tüm gövde kalınlığında, yaprak su potansiyelinde, ortalama meyve ağırlığında ve verimde düşüşler meydana getirmesi olarak belirtilebilir. Biostimulant uygulamaları ve su kısıntısı uygulanan koşullar (Ir_{50}) arasındaki etkileşimler, morfo-fizyolojik tepkileri açısından incelendiğinde, stoma iletkenliği (% 12), yaprak alanı (% 4), yaprak su potansiyeli (% 5) ve ortalama meyve ağırlığı (% 8) için önemli miktarlarda farklılıklar olduğu belirlenmiştir. Tüm bu sonuçlar; biostimulant uygulamalarının en uygun miktarda sulama suyuyla birleştirilmesi gerektiğini ve ayrıca kuraklık koşulları altında bitki morfo-fizyolojik tepkileri hakkında bilgi elde edilmesinin, çilek bitkisine yönelik çoklu stres koşulları altında bitki adaptasyonlarının geliştirilmesine katkıda bulunabileceğini göstermektedir.

Anahtar kelimeler: Yaprak alanı, Lwp, Stomatal iletkenlik, Su stresi

Introduction

Strawberries are one of the most economically important products to Turkey. Turkey is the pioneer for this crop's cultivation in Europe with production of 400.167 metric tons in 2017 (TUIK 2018). Leading practices for increasing efficiency consist of low cost and optimum economic rate combined with high tunnel system (Ferrato 2003; Kapur

2018). Strawberry has a shallow root system, wide leaf area, and considerable water content of fruits, for this reason, it uses too much water (Klamkowski and Treder 2006). Cultivation of strawberry needs a convenient program of irrigation to obtain the most water use efficiency (Treder 2009; Klamkowski 2015). Drought is one of the most commonly environmental stress that influence adversely plant growth, economic income, and environmentally sustainable productivity (Ghaderi 2015) of all crops in especially Mediterranean region. In conjunction with climate changes, the mean global temperatures are expected to rise over the next few decades (Houghton 2001; European Environment Agency 2004; Grant 2012) and consequently it will be resulted with increasing demand for water. Experts envisage that Turkey if some measures are not taken about global warming would be among water poor countries in coming decades. Water is one of the main essential abiotic factors that regulate plant growth and its deficiency shifts the morphological, physiological features of plants (Tiwari 2013). Reductions in world water resources require pay particular attention to irrigation strategies and improve ways of water use efficiency enhance. Reduce existing irrigation causes dramatically physiologic modifications in whole vegetative cell (Klamkowski 2008; Kapur 2018). Water stress end up with stomatal closure, decline in leaf tissues water potential (Starck 1995; Klamkowski and Treder 2006). Furthermore, the physiological responses in these plants cause morphological modifications. Changes in plant physiological status as well as changes in leaf development in water deficient conditions have been previously reported (Nautiyal et al. 1994; Palliotti et al. 2001; Klamkowski and Treder 2008). Similarly; a rapid decline in Lwp was found when subjected to deficit irrigation (Blanke and Cooke 2006; Grant 2012), and yield reduced as a result of water deficiency at effective root-zone depth (Save 1993; Yuan 2004; Liu 2007; Li 2010; Grant 2012). Moreover; Grant et al. (2010) have mentioned that there was a variation amongst ten cultivars for physiological and morphological features both when well-watered and subjected to deficit irrigation. In that study, especially with a significant interaction found was between stomatal conductance and Lwp.

One of the current challenge in agricultural practice and research today is how to cope with the abiotic stresses, particularly drought, in an economically and at the same time environmentally sustainable approach. To increase the yield and quality, it is vital that detect the optimum irrigation amount and use newest implement for agriculture such as bio-stimulant application. The application has been accepted by the scientific community as increasingly agricultural production material (Bulgari 2015). The bio-stimulants have been friend of nature, natural ingredients that can support mineral nutrient uptake, plant development, plant adaptation to another climatic conditions and tolerance to abiotic stresses. (Vernieri et al. 2006; Spinelli 2010). Similarly; Kunichi (2010) and Bulgari (2015) stated that bio-stimulants are of interest for sustainable agriculture because their applications provide a number of physiological processing opportunities which build utilization of nutrient, promote vegetative growth, allow the fertilizers reduction. Another study illuminated that the application of bio-stimulant, in strawberry, increased the yield (27%), leaf chlorophyll content (11%), vegetative development (10%), photosynthetic activity, stomata density (6.5%), fruit weight (Spinelli et al. 2010, Bulgari 2015). To date, inadequate numbers of researches have been conducted on different strawberry genotypes when subjected to severe drought conditions. Moreover; there isn’t any study under the Mediterranean conditions in Fortuna variety. Understanding of plant responses to tolerance mechanisms for drought may contribute to the development of adaptation to extreme conditions of plant (Klamkowski 2015). Purpose of the research was to identify on impacts of water deficit combined with bio-stimulant application on the physiological parameters and vegetative growth of *cv. Fortuna*. Stomatal conductance, Lwp, leaf area, whole stem thickness and number of stem were observed.

Materials and methods

Study was applied in the field of the Cukurova University inside high tunnel system from 20th September in 2016 to June 15th in 2017. In the current study, inside the high tunnel temperature and relative humidity values are given Table 1. The soils were classified as heavy clay texture by Dingil (2010). The distances between intra-row was 40 cm, and planted in raised beds of a height of 0.30 m along with covered by 0.05 mm thick grey polyethylene mulch. Once planting, every single treatment was exposed to equal amount irrigation water until the plants were reached 3 trifoliate. Fertilizers were applied identically to all plants until the end of the trial via drip irrigation system. The applied fertilizers are given in Table 2 together with their quantities and dates. The trial was carried out as 2 (irrigation levels) × 2 (bio-stimulant use) factorial designs, in split plot design at 4 replicates, totaling 16 plots.

Table 1. The average temperature and relative humidity for inside the high tunnel during growing season

Data	Jan.	Feb.	Mar.	Apr.	May	June
Inside high tunnel temp., °C	12.3	14.6	16.9	21.3	23.3	29.3
Inside high tunnel relative humidity, %	68.6	68.4	67.6	69.1	67.1	68.1

While different irrigation levels were sub plot, bio-stimulant applications were designed as main plot. There are two irrigation regimes which the irrigation amounts given were 0.5 (as Ir_{50}), 1.0 times (Ir_{100}) the pan evaporation measured from the Class A pan placed in the central of the high tunnel. ComCat (certified by the BCS Öko-Garantie GMBH, Nurnberg, Germany) was selected as a bio-stimulant. ComCat contains alginic acid (18%), organic substance (67%),

K_2O (1.5%), and gibberellic acid (250 ppm). The ComCat was implemented as 40 gr inside 30 L water da^{-1} at each application through foliar spraying at four times. Bio-stimulant applications were carried out at approximately three week's intervals starting from about two months (first flowering) after the planting of strawberry seedlings. Irrigation was applied by using drip irrigation throughout the trial and duration for irrigation system was calculated by using Eq (1).

$$T=(Pc \times Ep \times A \times Kcp) / (q \times n) \quad (1)$$

where, T: duration of irrigation (hours) (leaching was not considered), Pc: plant cover (%) (showed variation according to plant growth), A: trial area (m^2), Ep: the cumulative evaporation at irrigation interval (mm) in Class A pan, Kcp: coefficient for crop-pan which is accepted 0.7 during the trial as mentioned in Kanber (2006), q: emitters flow rate and n: :number of emitters. Different irrigation levels were introduced in 20 January 2017. From the date of planting to the different irrigation level applications, 179 mm water was applied to each subject evenly. After passing to different irrigation level applications, a total of 41 irrigations were applied. While Ir 100 was given a total of 397 mm, Ir 50 was given 288 mm irrigation water. In order to determine the morpho-physiological response of strawberry samplings were harvested on 2th June (pre-harvest) in the trial. Evaluation of leaf area, crown number, whole crown diameter, midday Lwp and stomatal conductance were investigated to illustrate the morpho-physiological reactions of strawberry at sampling time. Lwp was measured with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) at midday and stomatal conductance was measured with Leaf porometer (Decagon Devices) at midday. Leaf area was measured with leaf area meter (model 3050A; Li-Cor Lincoln, NE, USA). Mature strawberry fruits were harvested twice a week from mid-March to mid-June. The yield of the fruits was calculated by proportioning the total fruit weight harvested from ten randomly selected plants to the number of selected plants for each treatment. Average berry weight was calculated as the ratio of yield per application to the total number of fruits at each harvest time.

Table 2. The amount, types and dates of fertilizer applied in the growing season

Application time	Content of Fertilizer	Amount
23/11/2016	% 60 phosphate-containing fertilizer	700 gr da^{-1}
29/11/2016	% 9 Iron containing fertilizer	400 ml/ 100 l^{-1}
29/11/2016	12% organic matter, 22% humic and pulvic acid, 3% potassium oxide.	1 l da^{-1}
08/12/2016	% 9 Iron containing fertilizer	400 ml/ 100 l^{-1}
23/01/2017	12% organic matter, 22% humic and pulvic acid, 3% potassium oxide.	1 l da^{-1}
23/01/2017	% 3 nitrogen, % 30 potassium	700 gr da^{-1}
13/02/2017	12% organic matter, 22% humic and pulvic acid, 3% potassium oxide.	1 l da^{-1}
13/02/2017	Kodefol 710 (11-6-44+ME)	800 gr da^{-1}
20/02/2017	18+18+18 +(3 MgO) + ($8So_3$) + TE	2 kg da^{-1}
15/03/2017	% 3 nitrogen, % 30 potassium	700 gr da^{-1}
15/03/2017	Micro combined (% 0.4 B, % 1.5 Cu, % 4 Fe, % 3.5 Mn, % 4.0 Zn)	700 gr da^{-1}
25/03/2017	% 51 phosphorus, % 34 potassium	2 kg da^{-1}
31/03/2017	18+18+18 +(3 MgO) + ($8So_3$) + TE	2 kg da^{-1}
31/03/2017	12% organic matter, 22% humic and pulvic acid, 3% potassium oxide.	2 l da^{-1}
07/04/2017	Crop Master (5% organic matter, 0.03% amino acid, 0.01% alginic acid, 75 ppm gibberellin)	700 ml da^{-1}
07/04/2017	% 51 phosphorus, % 34 potassium	2 kg da^{-1}
20/04/2017	% 21 Boron	250 g da^{-1}
20/04/2017	% 9 Iron containing fertilizer	2 kg da^{-1}
09/05/2017	Micro combined (% 0.4 B, % 1.5 Cu, % 4 Fe, % 3.5 Mn, % 4.0 Zn)	700 gr da^{-1}
09/05/2017	% 60 phosphate-containing fertilizer	350 gr da^{-1}
12/05/2017	Calcium Nitrate	1 l da^{-1}
23/05/2017	% 3 nitrogen, % 30 potassium	700 gr da^{-1}
23/05/2017	Micro combined (% 2.72 Fe, % 2.72 Mn ve % 2.72 Zn)	700 gr da^{-1}
31/05/2017	% 3 nitrogen, % 30 potassium	700 gr da^{-1}
31/05/2017	% 21 Boron	150 g da^{-1}

The experiment was carried out in four replications in split plot with two factors randomized complete block design. The data's were interpreted via JMP (statistical program). The data's were controlled with ANOVA to specify the effects of bio-stimulant and different irrigation regimes on total yield and at pre-harvest on examined some morpho-physiological parameters.

Results and discussion

After planting, same amounts of irrigation water was applied until the different irrigations applications from beginning of the trial to the end. A total as 397 and 288 mm irrigation were given to Ir_{100} and $Ir_{50,}$ respectively. The works which conducted formerly with drip irrigation in strawberry has reported the use of irrigation water in immense variety from 250 mm to 825 mm (Yuan 2004; Lozano 2016; Strand 2008; Kanber1986; Kumar 2011; Trout 2004; Kapur 2018). Consequently, in this research the irrigation amounts were in conformity with those of Yuan (2004) and Kanber (2006) who both planed on the same growing conditions detected amount of irrigation ranging from respectively 254 to 414 mm and 424 mm. The sampling time was carried out on 256th days after planting. The Leaf area, Crown number and Whole crown diameter values per plant are presented in Table 3. These results clearly indicate that all plant development parameters with productivity were negatively influenced from water deficiency. Decrease in plant growth is one of the reactions of plants against drought (Boyer 1970; Hsiao 1973; Tiwari 2013 and Kapur 2018) as well. In a similar way, Liu (2007), Ghaderi (2015), Grant (2010) and Grant (2012) found decreases in Leaf area as a consequence of water stress. Consistent with these studies, significant reductions in leaf area were found in our study under water stress and found statistically significant in these differences. Furthermore, bio-stimulant applications have been statistically significant to affecting the leaf area positively. Although bio-stimulant and different irrigation levels did not statistically affect both the whole crown diameter and crown number values, irrigation levels with bio-stimulant were found to bring about significant increase in both crown number and whole crown diameter values (Table 3). Under water stress conditions (Ir_{50}), bio-stimulant treatments have been found to generally decrease the negative effects of plant development parameters. Some studies are harmony with this research in which bio-stimulants induced plant vegetative development and endurance to some abiotic stresses like water stress. (Battacharyya 2015). In Another study, the application of bio-stimulant induced vegetative growth by 10% in strawberry (Spinelli et al. 2010).

Table 3. Impact of different irrigation regimes and bio-stimulant applications on morpho-physiological responses at pre-harvest for *'Fortuna' cv.*

	Irrigation regime		Average of Application
Leaf Area (cm^2 plant^{-1})	**50**	**100**	
Bio-stimulant	1930	2774	2352 a
Control	1854	2462	2158 b
Average of irrigation regime	1892 b	2618 a	
LSDapp*= 193	LSDırr***= 193	LSDappxırr= N.S	
Crown number (crown number plant^{-1})	**50**	**100**	
Bio-stimulant	3.67	4.67	4.17
Control	3.00	3.67	3.33
Average of irrigation regime	3.33	4.17	
LSDapp= N.S	LSDırr= N.S	LSDappxırr= N.S	
Whole Crown diameter (mm)	**50**	**100**	
Bio-stimulant	30.4	33.6	32.0
Control	28.8	32.3	30.5
Average of irrigation regime	29.6	33.0	
LSDapp= N.S	LSDırr= N.S	LSDappxır= N.S	
Lwp (bar)	**50**	**100**	
Bio-stimulant	-19.0	-14.7	-16.8 a
Control	-20.0	-15.7	-17.8 b
Average of irrigation regime	-19.5 b	-15.2 a	
LSDapp*= 0.80	LSDırr***= 0.80	LSDappxırr= N.S	
Stomatal Conductance (mmol m^{-2}s^{-1})	**50**	**100**	
Bio-stimulant	516	677	596 a
Control	462	642	552 b
Average of irrigation regime	489 b	659 a	
LSDapp*= 34.9	LSDırr***= 34.9	LSDappxırr= N.S	

(1): Differences between the means were showed with different letters
(2): N.S.: Not Significant, ***: p<0.01; *: p<0.05

Water stress affects unfavorably the soil moisture, so soil water potential, Lwp and stomatal conductance are negatively influenced as well. For this reason, Lwp and stomatal closure are the most important parameters to evaluate drought on studies of plant responses for water deficiency. Water stress caused decreased in Lwp from -15.2 bar in control plants (Ir_{100}) to -19.5 bar in water restricted plants (Table 2). Similar results which showed lower value of water potential have also been reported by Klamkowski and Treder (2006) in strawberry. Bio-stimulant also enhanced Lwp. Application of bio-stimulant resulted in the increase of Lwp by 5% under drought conditions, and these difference found statistically significant as well.

When water availability was limited, stomatal closure is one of the earliest reactions of the plants (Klamkowski 2015). Stomata principally promote to control water relations mechanism in plants (Hetherington and Woodwar 2003; Spinelli 2010). In current research, there is superior stomata activities in well irrigated plants compared to the deficit irrigated plants, with a significant interaction. Water stress caused decreased in stomatal conductance from 659 mmol $m^{-2}s^{-1}$ in well irrigated plants to 489 $m^{-2}s^{-1}$ in water restricted plants (Table 3). And similar observations were obtained for strawberry (Grant et al. 2010; Ghaderi and Siosemardeh 2011; Klamkowski et al. 2015). Moreover, higher stomatal conductance values of the applied bio-stimulant plants compared to control plants with a significant interaction as well (Table 2) and similar observations were obtained by Spinelli et al. (2010) who found the application of bio-stimulant enhanced stomata density by 6.5% in strawberry.

The optimal yield with quality is the main purpose of agriculture. (Yuan et all. 2004). At this study; strawberries harvesting period was around 92 days from 16th March to 15th June. Table 4 shows accumulated strawberry fruit yield per plant. These results clearly indicate that all the yield parameters are adversely affected by water deficiency. There are important differences in total berry yield, number of fruit and mean fruit weight between under water restricted plants (Ir_{50}) and well-watered plants (Table 4). The more irrigation water is given the more large fruit heaviness is generated. Moreover; it is observed that irrigation has increased the yield of strawberries, especially by increasing the average weight of the strawberries, which is in great harmony with former studies (Rennquist et al. 1982a, 1982b; Save et al. 1993; Krüger et al. 1999; Yuan et al. 2004). Although bio-stimulant applications didn't statistically affect both yield and fruit number values, these applications were found to bring about significant increase in both yield and fruit number values by 13%, 3%, respectively. And similar observations were obtained by Spinelli et al. (2010) who found the application of bio-stimulant enhanced the yield by 27% and fruit weight in strawberry.

Table 4. Impact of different irrigation regimes and bio-stimulant applications on yield for *'Fortuna' cv.*

	IRRIGATION REGIME		**Average of Application**
Yield (gr/plant)	**50**	**100**	
Bio-stimulant	819	1133	976
Control	693	1028	861
Average of irrigation regime	756 b	1080 a	
LSDapp= N.S	LSDırr***= 173		LSDappxırr= N.S
Fruit Number (berries per plant)	**50**	**100**	
Bio-stimulant	46.8	51.3	49.0
Control	42.9	52.0	47.5
Average of irrigation regime	44.8 b	51.7 a	
LSDapp= N.S	LSDırr*= 6.56		LSDappxırr= N.S
Mean Fruit Weight (gr)	**50**	**100**	
Bio-stimulant	17.5	22.1	19.8 a
Control	16.2	19.7	17.9 b
Average of irrigation regime	16.9 b	20.9 a	
LSDapp*= 1.26	LSDırr***= 1.26		LSDappxır= N.S

(1): Differences between the means were showed with different letters
(2): N.S.: Not Significant, ***: $p<0.01$; * : $p<0.05$

Conclusions

Bio-stimulant application in drought resistance of *cv. Fortuna* have been investigated in Mediterranean conditions. According to current studies results, water stress had several adverse effects on *cv. Fortuna* because all morpho-physiological parameters negatively influenced from water deficiency. These results indicate that IR_{50} might have been insufficient to meet the demands of well irrigated plants. The use of bio-stimulant has been found to reduce the effects of water stress under drought conditions. This means that when water availability was limited, the use of bio-stimulant is providing to have greater drought resistance of the Mediterranean conditions for *cv. Fortuna.* Besides; the best results were obtained as 1133 g / plant from bio-stimulant x irrigation (Ir_{100}) interaction. And therefore, further studies are needed to make it clear that different mechanism underlying the mode of action of bio-stimulant. So, using bio-stimulant should be conjunctive with current agricultural applications, along with detected optimal irrigation water, for generate new planting approaches that aim to maximize the potential of a strawberry plant. There is also inadequate knowledge on response from strawberry cultivars exposed to long-term drought (Klamkowski 2015). Obtaining information about plant morpho-physiological responses under drought conditions can contribute to improving of plant adaptations under multiple stress conditions.

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