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ORIGINAL ARTICLE

Effects of Bio-Bor Fertilizer Applications on Fruit Yield, Antioxidant Enzyme Activity and Freeze Injury of Strawberry

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Abstract Deficiency of Boron (B) is widespread in the many parts of region of Turkey. So, the effects of boron and plant growth promoting bacteria (Bio-B) on the fruit yield, antioxidant enzyme activity and plant freeze injury of strawberry cv. Fern were investigated under field conditions between 2013 and 2014. The experimental plot was a completely randomized design with 4 replicates. Control and Bio-B were used as fertilizer agent in the experiment. Bio-B fertilizer was applied in three methods as soil, foliar and soil + foliar application methods to strawberry plants. Data through 2 years showed that the use of Bio-B significantly increased fruit yield, antioxidant enzyme activity and decreased freeze injury of strawberry leaf. Soil + foliar applications of Bio-B fertilizer increased to fruit yield compared to the control by 55.91 %. However, foliar application of Bio-B fertilizer increased to catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) enzyme activity compared with the control treatment 41.86 %, 48.99 %, and 26.59 %, respectively and decreased freeze injury of strawberry leaves 27.41 %. Overall, the results of this study suggest that Bio-B fertilizer application have the potential to increase the yield, antioxidant enzyme activity and decreased freeze injury of strawberry plants under field conditions.

Keywords Strawberry · Boron · B · PGPR · Boron deficiency · Cold · Antioxidant enzyme

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Auswirkungen von Bio-Bor Düngeanwendungen auf Fruchtertrag, antioxidative Enzym-Aktivität und Frostschäden bei Erdbeeren

Zusammenfassung Bormangel (B) ist in vielen Regionen der Türkei weit verbreitet. Demzufolge wurden die Auswirkungen der Ausbringung von Bor und wachstumsfördernden Bakterien (Bio-B) auf den Fruchtertrag, die antioxidative Enzym-Aktivität und auf Frostschäden bei Erdbeerpflanzen der Sorte „Fern“ in den Jahren 2013 und 2014 unter Feldbedingungen untersucht. Als Versuchsanordnung wurde eine vollständig randomisierte Anlage mit 4 Wiederholungen gewählt. Die Düngevarianten in diesem Versuch waren die Kontrolle und die Applikation von Bio-B. Der Bio-B-Dünger wurde den Erdbeerpflanzen in 3 Ausbringungsvarianten verabreicht: über den Boden, über das Blatt und über Boden und Blatt. Die Auswertungen während der beiden Versuchsjahre zeigten, dass die Anwendung von Bio-B den Fruchtertrag und die antioxidative Enzym-Aktivität erhöhte und Frostschäden an den Blättern verminderte. Die Anwendung des Bio-B-Düngers über Boden und Blatt erhöhte den Fruchtertrag, verglichen mit der Kontrolle, um 55,91 %. Die Blattapplikation allein verbesserte, verglichen mit der Kontrolle, die Enzymaktivität von Katalase (CAT), Peroxidase (POD) und Superoxid Dismutase (SOD) jeweils um 41,86 %, 48,99 % und 26,59 % und verminderte den Frostschaden an den Erdbeer-Blättern um 27,41 %. Insgesamt lässt sich anhand der Ergebnisse dieser Studie feststellen, dass die Anwendung von Bio-B-Dünger grundsätzlich die Erntemenge und die antioxidative Enzym-Aktivität erhöhen und die Frostschäden an Erdbeer-Blättern vermindern kann.

Schlüsselwörter Erdbeere · Bor · B · PGPR · Bormangel · Frostschaden · Antioxidative Enzym-Aktivität

Introduction

Producing fruits in the field conditions may be challenging due to extreme temperatures such as very low temperature during the growing season. The low temperature limited fruit yield, and fruit quality. So it is necessary to protect plants from the cold, either plants should be grown in a controlled greenhouse conditions, or cold resistance of plants to be increased. Increasing the cold resistance of plants is the most appropriate and effective method.

Strawberry fruit and leaves are affected by climatic conditions and agricultural factors. The major problems in strawberry production in Turkey are mainly connected with extreme temperature (low temperature) and unsuitable soil conditions (nutrient deficiency). Strawberry leaves were not significantly damaged when temperature decreases to –3 °C, but significant damage occurred in leaves were temperature decreases to –5 °C (Maughan 2013; Maughan et al. 2015). Strawberry blossoms are occurred at below –1 °C (Maas 1998; Maughan 2013). After this temperature, pistils in flower damaged or kill. It is depending on the severity cold conditions, deformation, low yield and quality may occur in fruits. For strawberries plants optimum root growth night temperature is 12 °C (Wang and Camp 2000).

Soils in Turkey usually have high pH and high levels of lime. Therefore, micro nutrient element such as B deficiency frequently have been shown in different fruit crops. Boron plays an important role in sugar transport, cell division, membrane functioning, regulation of plant hormone levels, generative growth, and root elongation of plants (Marschner 1995). Especially, in semi arid regions, the soils are having low organic content and deficiency of micronutrient (e. g. B deficiency) usually occurs during the growth period under low rainfall (Wojcik and Lewandowski 2003).

It has been reported that soil or foliar application of B increased fruit yield of strawberry (Bragg et al. 2008; Esringu et al. 2011), sunflower (Oyinlola 2007), chickpea (Ceyhan et al. 2007), brussels sprout (Turan et al. 2009), tomato, pepper, cucumber (Dursun et al. 2010), tomato (Davis et al. 2003).

In chilling temperature, B uptake and transport into growing shoots are strongly damage, and so plant B use efficiency in the growing tissues may be decreased. Reduction in temperature conduces to plant B deficiency and so the internal B requirement for shoot growth will increase. Therefore, determination of relationship between the boron and low temperature are required in cell walls which plays an important role in leaves tolerance to freezing temperature conditions (Ye 2004).

PGPR (Plant growth Promoting Bacteria) known as helpful microorganisms that used to improve plant growth, plant yield, crop quality and uptake of plant nutrition from soil by plant (Esitken et al. 2005). These microorganisms can produce plant hormone such as auxins (Egamberdiyeva 2007; Mia et al. 2012; Gunes et al. 2014), and inhibitor for ethylene production from plant tissue (Glick et al. 1995). Applications of PGPR significantly have increased fruit yield, and plant growth of strawberry (Gunes et al. 2009; Esitken et al. 2010; Karlidag et al. 2013), apple (Aslantas et al. 2007; Karlidag et al. 2007), raspberry (Orhan et al. 2006), apricot (Esitken et al. 2005), sweet cherry (Esitken et al. 2006), cabbage (Turan et al. 2014).

The objectives of this study were (1) to evaluate the yield response of strawberry to Bio-B fertilizer; (2) to determine the effects of Bio-B addition on the antioxidant enzyme activity of strawberry plant; and (3) to determine the effects of Bio-B addition on freeze injury of strawberry plant leaves under semi arid climate field conditions.

Materials and methods

Background information for the study site

This study was conducted at the Konya, Turkey (1100 m) during the summer periods (late May–late September) of 2013–2014. This region soils were classified as an Entisol with parent materials mostly consisting of marn and lacustrin transported material (Soil Survey Staff 2006). The experimental region has a semi-arid climate. The mean maximum temperature was 22–34 °C while the mean minimum temperature was 4–8 °C during the growing period.

Trial design

This experiment was conducted in randomized block design with soil, foliar and soil + foliar application as main plot and two Bio-B (control and PGPR + 10 % Boron) fertilizer applications as subplot in four replicates. Field experiments were carried out on "Fern" day-neutral strawberry cultivar. In plots of 20 plants giving a total of 320 strawberry plants were planted on raised bed at a spacing of 0.30 m–0.35 m on a clay loam soil with black plastic mulch in the beginning of May 2013. For soil application of Bio-B was performed using a dipping method. For foliar application, floral and foliar plant organs were sprayed Bio-B until run off, 15 days intervals during growing period (June to October). Control plants were sprayed with sterile water and dipped into sterile water. Based on analysis results, 100 kg ha^{-1} N, and 160 kg P_2O_5 ha^{-1} was applied to this area, and because of optimal concentrations of available K in the soil, this element was not applied during the experiment.

During the experimental period (2013–2014) data were collected. Growth promoting effects of bacterial-boron treatments were evaluated by determining cumulative yield (g $plant^{-1}$), antioxidant enzyme activity, and determination of freezing injury.

Bacterial strain, culture conditions and media

Preparing of Bio-B fertilizer PGPR strains (mixing of PGPR) were obtained from Yeditepe University, Dept. of Genetics and Bioengineering (Dr. Fikrettin Sahin, personal communication). Bacteria were grown on nutrient agar (NA) for routine use, and maintained in nutrient broth (NB) with 15 % glycerol at –80 °C for long-term storage. A single colony was transferred to 500 ml flasks containing NB, and grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27 °C (Merck KGaA, Germany). The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^9 CFU ml^{-1}, and 90 L ha^{-1} the resulting suspensions were used to treat strawberry plants (Esitken et al. 2010).

Soil analysis

Soil samples were taken over one depths (0–30 cm, 20 subsamples) to determine baseline soil properties. Soil samples were air-dried, crushed, and passed through a 2 mm sieve prior to chemical analysis. Cation exchange capacity (CEC) was determined using sodium acetate (buffered at pH 8.2) and ammonium acetate (buffered at pH 7.0) according to Sumner and Miller (1996). The Kjeldahl method (Bremner 1996) was used to determine total N while plant-available P was determined by using the sodium bicarbonate method of Olsen et al. (1954). Electrical conductivity (EC) was measured in saturation extracts according to Rhoades (1996). Soil pH was determined in 1:2 extracts, and calcium carbonate concentrations were determined according to McLean (1982). Soil organic matter was determined using the Smith-Weldon method according to Nelson and Sommers (1982). Ammonium acetate buffered at pH 7 (Thomas 1982) was used to determine exchangeable cations. Available Fe, Mn, Zn, and Cu in the soils were determined by Diethylene Triamine Pentaacetic Acid (DTPA) extraction methods (Lindsay and Norvell 1978). Available B was analyzed for extractable B using the azomethine-H extraction of Wolf (1974) and a UV/VIS (Aqumat) spectrophotometer (Thermo Electron Spectroscopy LTD, Cambridge, UK). These soil characterization data are presented in Tab. 2.

Tab. 1 Chemical properties of the experimental field soils before the experiment (mean ± standard deviation, n = 20)

Soil Properties	Units	Means
Clay	%	39.12 ± 1.23
Silt	%	28.23 ± 1.10
Sand	%	32.65 ± 2.43
Cation exchangeable capacity	$Cmol_c$/kg	20.44 ± 1.98
pH (1:2 soil:water)		7.34 ± 0.36
Organic matter	%	2.36 ± 0.23
$CaCO_3$	%	0.63 ± 0.11
Plant available P	mg/kg	7.68 ± 1.22
Exchangeable Ca	$Cmol_c$/kg	14.35 ± 1.57
Exchangeable Mg	$Cmol_c$/kg	2.12 ± 0.45
Exchangeable K	$Cmol_c$/kg	3.42 ± 0.73
Exchangeable Na	$Cmol_c$/kg	0.44 ± 0.08
Available Fe	mg/kg	3.46 ± 0.67
Available Mn	mg/kg	7.46 ± 1.13
Available Zn	mg/kg	4.54 ± 0.88
Available Cu	mg/kg	6.24 ± 1.13
Available B	mg/kg	0.22 ± 0.07
Electric conductivity	dS/m	0.87 ± 0.05

Plant sampling and determination of freezing injury

Of the 20 plants per plot, 5 plants were sampled. Fully developed mid-shoot leaves were sampled in August in the two years of the study. In order to determine the determination of freezing injury of leaves, freezing injuries were determined in strawberry leaves allowed to freeze spontaneously using a modification of Marentez et al. (1993) method. Fresh leaves were cut into 2 cm lengths and rinsed in six changes of H_2O. The removing of cellular proteins was determined in a spectrophotometer at 280 nm. Leaves (0.2 g) were placed in each of tubes, and it were positioned in a freezing bath. After equilibration at –1 °C for 30 min, the temperature was lowered stepwise by 1 °C intervals from –1 to –20 °C. The tubes were allowed to equilibrate at each temperature for 15 min. The tubes were then removed, and 4 mL of cold H_2O was added in each tube containing the frozen leaves. These tubes were stored at 4 °C for 24 h. Ion leakage was calculated as the conductivity of the frozen sample divided by the conductivity of

Tab. 2 Antioxidant enzyme activity of strawberry leaves

	Bio-B Applications	CAT	POD	SOD
		Enzyme unit gr leaf^{-1}		
	Control	44 ± 2 c	984 ± 36 b	322 ± 26 c
	Soil	52 ± 3 b	1015 ± 45 b	354 ± 20 bc
1. Year	Foliar	60 ± 4 a	1432 ± 52 a	412 ± 14 a
	Foliar + Soil	54 ± 2 b	1045 ± 75 b	388 ± 20 b
	Control	42 ± 3 d	1012 ± 102 c	340 ± 25 d
	Soil	54 ± 3 c	1036 ± 88 c	369 ± 18 c
2. Year	Foliar	62 ± 6 a	1542 ± 123 a	425 ± 16 a
	Foliar + Soil	58 ± 4 b	1136 ± 101b	400 ± 23b
	Control	*43* ± 3 *d*	*998* ± 91 *b*	*331* ± 30 *b*
	Soil	*53* ± 5 *c*	*1026* ± 85 *b*	*362* ± 19 *b*
Means	Foliar	*61* ± 4 *a*	*1487* ± 112 *a*	*419* ± 21 *a*
	Foliar + Soil	*56* ± 2 *b*	*1091* ± 95 *b*	*394* ± 25 *ab*

the same sample after it was boiled for 3 min (Turan et al. 2007a).

Antioxidant Enzymes Analysis

All operations were done at 4 °C. Cells (500 mg) of plant leaves were homogenized in a mortar with 3 ml of 50 mM phosphate buffer at pH 7. Homogenates were filtered through two layers of Miracloth and the filtrate was centrifuged at 15,000 g for 15 min, at 4 °C. The resulting supernatant was stored at –80 °C. For antioxidant enzyme assays, frozen cell samples were ground to a fine powder with liquid nitrogen and extracted with ice-cold 0.1 mM phosphate buffer, pH 7.8, containing 1 mM ethylenediaminete-traaceticacid (EDTA), 1 mM phenylmethanesulphonylfluoride (PMSF) and 0.5 % polyvinylpyrrolidone (PVP). The CAT, POD, and SOD enzyme activities in the apoplastic fractions were measured spectrophotometrically. The CAT activity was measured by monitoring the decrease in absorbance al 240 nm in 50 mM phosphate buffer (pH 7.5) containing 20 mM H_2O_2. One unit of CAT activity was defined as the amount of enzyme that used 1 µmol H2O2/min. The POD activity was measured by monitoring the increase in absorbance at 470 nm in 50 mM phosphate buffer (pH 5.5) containing 1 mM guaiacol and 0.5 mM H_2O_2. The SOD activity in apoplastic fractions was estimated by recording the decrease in optical density of nitroblue tetrazolium dye by the enzyme (Dhindsa et al. 1981). The absorbance was recorded at 560 nm, and one unit of enzyme activity was taken as that amount of enzyme, which reduced the absorbance reading to 50 % in comparison with tubes lacking enzyme (Sairam and Srivastava 2002).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and significant means were compared by Duncan's multiple range test method. Mean differences were considered significant if $p \leq 0.05$.

Results

Effects of Bio-B applications on strawberry yield

Two years of trials (2013 and 2014) under growing conditions showed that bacterial treatments including Bio-B applications significantly affected all parameters tested in this study (Fig. 1). The results showed that yield significantly increased ($p < 0.001$) by bacterial treatments such as soil, foliar and soil + foliar compared with control (Fig. 1). Significant yield increase was obtained with soil + foliar Bio-B (24500 kg ha^{-1}) treatments as compared with the foliar (22350 kg ha^{-1}), the soil (20250 kg ha^{-1}), and control (18560 kg ha^{-1}). The average percentage of yield increase was 55.9 %, 48.3 % and 15.9 % when Bio-B application was applied, respectively.

Effects of Bio-B applications on antioxidant enzyme activity of strawberry plants

Bio-B applications significantly affected enzyme activity such as CAT, POD and SOD.

Two years of trials showed that enzyme activity significantly increased ($p < 0.001$) by bacterial treatments such as soil, leaves and soil + leaves compared with control (Fig. 1). Significant CAT, POD and SOD increase was obtained with foliar Bio-B (61, 1487, 419 enzyme unit gr leaf^{-1}) treatments as compared with the soil + foliar (56, 1091, 394 enzyme unit gr leaf^{-1}), the soil (53, 1026, 362 enzyme unit gr leaf^{-1}), and control (43, 998, 331 enzyme unit gr leaf^{-1}). The average percentage of CAT, POD and SOD increase was 41.9 %, 48.9 % and 26.6 % when Bio-B application was applied, respectively (Tab. 2).

Effects of Bio-B applications on freezing injury of strawberry plants

Two years of trials (2013 and 2014) under different temperature (0, –5, –10, –15 and –20 °C) conditions showed that Bio-B applications significantly affected freezing in-

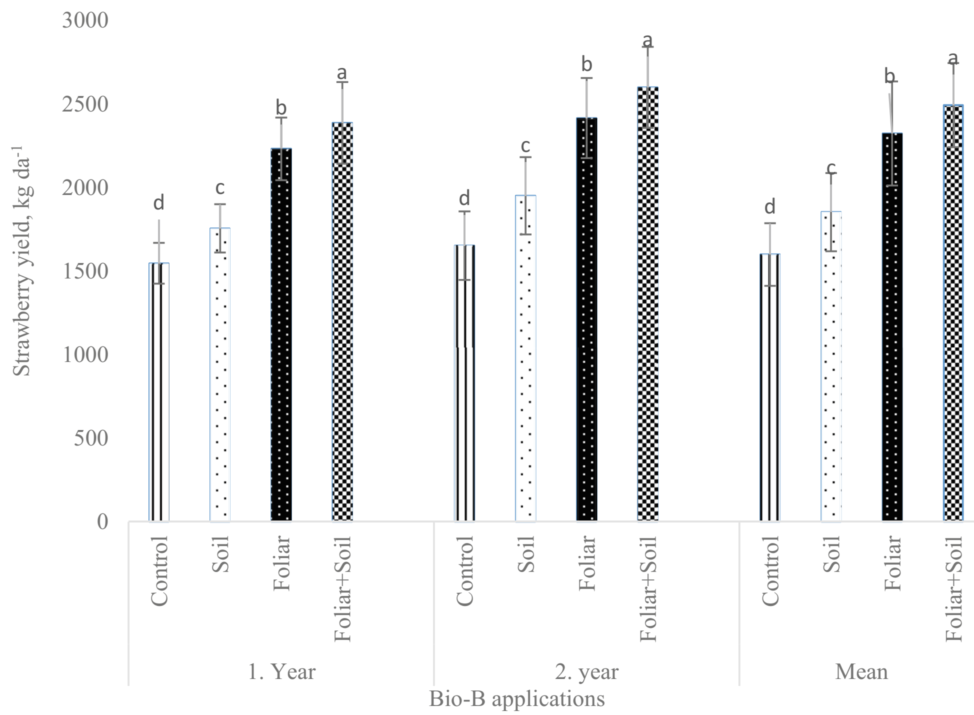

Fig. 1 Effects of Bio-B applications on strawberry yield

Tab. 3 Freeze injury rate of strawberry leaves in %

	Bio-B Applications	0 °C %	–5 °C	–10 °C	–15 °C	–20 °C
	Control	60.13 ± 5.44 a	67.75 ± 6.13 a	79.23 ± 7.44 a	88.11 ± 6.12 a	96.54 ± 8.67 a
	Soil	33.24 ± 2.44 b	37.68 ± 2.67 b	44.12 ± 2.45 b	50.12 ± 3.22 b	57.68 ± 4.55 bc
1. Year	Foliar	29.89 ± 3.11 c	34.35 ± 3.24 c	40.23 ± 3.42 c	46.57 ± 2.87 c	54.44 ± 3.44 c
	Foliar + Soil	32.24 ± 2.75 b	36.90 ± 4.54 b	39.80 ± 3.11 c	48.13 ± 4.55 bc	59.13 ± 5.23 b
	Control	62.53 ± 6.34 a	68.52 ± 7.12 a	80.24 ± 7.88 a	87.54 ± 5.66 a	97.12 ± 6.12a
	Soil	54.41 ± 5.46 b	60.12 ± 5.40 b	70.15 ± 6.54 b	82.14 ± 7.11 b	88.65 ± 4.35 b
2. Year	Foliar	50.15 ± 4.77 c	56.49 ± 3.56 c	67.54 ± 6.98 c	80.22 ± 4.35 c	86.14 ± 7.11 c
	Foliar + Soil	49.65 ± 5.11 c	54.11 ± 4.35 c	66.59 ± 5.44 c	76.53 ± 6.11 d	85.97 ± 5.44 c
	Control	*61.33* ± 7.44 *a*	*68.14* ± 5.44 *a*	*79.74* ± 6.12 *a*	*87.83* ± 5.46 *a*	*96.83* ± 8.76 *a*
	Soil	*43.83* ± 3.24 *b*	*48.90* ± 4.35 *b*	*57.14* ± 4.35 *b*	*66.13* ± 7.13 *b*	*73.17* ± 6.56 *b*
Means	Foliar	*40.02* ± 4.30 *c*	*45.42* ± 3.88 *c*	*53.89* ± 5.22 *c*	*63.40* ± 4.35 *c*	*70.29* ± 3.45 *c*
	Foliar + Soil	*40.95* ± 3.98 *c*	*45.51* ± 4.11 *c*	*53.20* ± 3.22 *c*	*62.33* ± 5.66 *c*	*72.55* ± 4.55 *b*

jury tested in this study (Tab. 3). The results showed that freezing injury significantly decreased ($p < 0.001$) by bacterial treatments such as soil, foliar and soil + foliar compared with control. Significant freezing injury decrease for 0, –5, –10, –15 and –20 °C was obtained with soil + foliar Bio-B (40.95, 45.51, 53.20, 62.33, and 72.55 %) treatments as compared with the foliar (40.02, 45.42, 53.89, 63.40, and 70.29 %), the soil (43.83, 48.90, 57.14, 66.13, and 73.17 %), and control (61.33, 68.14, 79.74, 87.83 and 96.83 %). The average percentage of freezing injury decreasing ratio was 27.41 %, 25.07 % and 24.43 % when Bio-B application was applied, respectively.

Discussion

As a result of this work, it was determined that Bio-B applications (soil, foliar and foliar + soil applications) significantly increased to yield of strawberry fruit. The improved fruit yield and antioxidant enzyme activity in response to all inoculants compared with the control indicates the beneficial role of these rhizobacteria. The improving effect of application with PGPR on yield of plant were reported earlier by Yildirim et al. (2008, 2011), Gunes et al. (2009), Karlidag et al. (2011), and Turan et al. (2012). In this study, Bio-B application (especially PGPR) may increased nutrient content, uptake and boron translocations of strawberry plant, and increase to tolerance of stress conditions (Turan et al. 2014). PGPRs have been reported to stimulate nutrient content in tomato, radish, lettuce, and strawberry (Turan et al. 2007b; Yildirim et al. 2008, 2011; Gunes et al. 2009; Karlidag et al. 2011).

Availability of nutrient elements in soil can be a major constraint to plant growth in extremely low in nutrients and temperatures. Exudates of root and PGPR increase that availability of nutrient in soil. And these microorganisms assist take up nutrient and transported to the other plant organs (Bottini et al. 2004; Turan et al. 2012). It was reported that some application of PGPR, also used in the present study, increased yield of plants such as wheat (Bulut 2013), cabbage (Turan et al. 2014), strawberry (Gunes et al. 2009).

Temperatures variations are the highest in spring months and these situations may lead to an increased risk of frost damage to strawberry buds and flowers (Salas et al. 2014). Longed cold winters and late spring frosts can have a negative effect on the growth and development of plants, which can result in significant decrease in fruit yield (Vij and Tyagi 2007; Shokaeva 2008; Badek et al. 2014). Nutrient contents of strawberry plants affect freeze acclimation. Deficient or high nutrient levels in plant will inhibit acclimation. İn this study, Bio-B application, especially from foliar applications decreased freezing injury of strawberry plant leaves and this results have been suitable to the other studies. Because, Bio-B fertilizer contents of plant growth promoting bacteria and boron nutrient. Optimum levels of nutrient contents promote acclimation (Fisher 2004). Taulavuori et al. (2005), and Turan (2007a), stated that mineral application to plants reduced frost injury. So night and day temperature effect of strawberry fruit yield, nutrient composition and the other physiological parameters (Sonsteby and Heide 2008). In deficient nutrient, freezing tolerance has been decreased in some plants. Especially, boron nutrient of plant (e. g. Norway spruce) increased to freezing tolerance plant shoot and leaves (Raisanen et al. 2009). These literatures support us study results.

Freezing damage in low temperature, especially buds has been damage depend on B deficiency in plant tissue (Pietiläinen 1984; Raisanen et al. 2009). When the buds were deformed because of B deficiency, they were unable to cold strong very well (Raisanen et al. 2009). But this situation was not shown that B fertilization could improve the tolerance to freezing of plant organs (Bassil et al. 2004). Because, boron may affect the plasma membrane directly or indirectly and B deficiency may increase the sensitive of cells to freezing injury in other organs (Huang et al. 2005). İn this study, due to be done under field conditions, strawberry plants were affected the night/day temperatures variations. The other study results showed that strawberry fruit stored at 10 °C or 5 °C showed higher antioxidant capacity, total phenolics, and anthocyanins than those stored at 0 °C (Zavala et al. 2004). Applied Bio-B fertilizer increased plant resistance to temperature variations and the amount of fruit increased. Application of Bio-B also increased antioxidants enzyme activity such as oxidase, peroxidase, and superoxide dismutase enzyme in plant. These antioxidant enzymes are an indicator that increasing resistance of plants to stress conditions (Kaya et al. 2013, 2015). The ability of plants to tolerate low temperatures depends on the degree of indurate they have arrived. In this study, the freezing tolerance which is known to increase to antioxidants enzyme activity. The level of carbohydrate, amino acid and secondary metabolism (e. g. antioxidant enzyme activity) is positively correlated with cold tolerance (Chen and Li 2002; Hannah et al. 2006; Kaplan et al. 2007; Janska et al. 2010). Also, boron and PGPR may tend to be slow growing and store sugars in underground tissue for cold adapted (Guy 1999; Janska et al. 2010). Similarly, antioxidant enzyme activities have been reported to increase under cold and the other stress conditions whereas use of PGPR can help growth by reduce the negative effects of freeze stress by supporting the concentration of antioxidant enzyme (Biemelt et al. 2000; Kang and Saltveit 2001).

The other similar study, on cold acclimation and tolerance to frost showed that the physiological, biochemical, and molecular mechanisms developed by plants increased tolerance to stress conditions (Miura and Furumoto 2013; Badek et al. 2014). For some plant species (e. g. cucumber, sunflower) root chilling at 10–17 °C reduce boron uptake and use efficiency in the shoot. Boron deficiency make worse freeze injuries in leaf and significantly decreased about 50–100 % yield of plant species (Huang et al. 2005). İn this study, 61–96 % of plant tissue damaged by freezing temperature 0 and –2 °C, respectively, in laboratory conditions.

Conclusion

In this study, results showed that optimum plant nutrition especially boron decreased the risk of freeze injury due to plant ion balance and membrane changes. The type of fertilizers application methods, plant species, age, and phenology of the plants influence the results. Overall, the results of this study suggest that Bio-B fertilizer application have the potential to increase the yield, antioxidant enzyme activity and decreased freeze injury of strawberry plant under field conditions.

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