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ORIGINAL ARTICLE

Freeze Injure and Antioxidant Enzyme Activity of Grapevine (*Vitis Vinifera*) Under Bio-Boron Fertilizer Applications

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Abstract

This study was conducted to determine the effects of plant growth promoting bacteria (PGPR) and boron (Bio-B) on the plant freeze injury, antioxidant enzyme activity and fruit yield of grapevine cultivar. The experimental plot was a completely randomized design with 5 replicates. As a trial model Bio-B and control were used as fertilizer agent. Bio-B fertilizer has been applied in different ways as soil + foliar, soil and foliar application methods to grapevine plants. Data through 2 years trials results showed that the use of Bio-B significantly decreased freeze injury and increased antioxidant enzyme activity of grapevine leaf. The highest damage rate at control group occurred with –20 °C and 94.89% ratio. In the same temperature degree at grape plant, the damage ratio of the Bio-B application from soil decreased by 21.55%; the application from leaf by 25.53% and with the application from soil + leaf by 26.24%. In addition to this, compared with control, in the CAT, POD and SOD enzyme activities increases occurred with the ratios as 28.57%, 22.05% and 39.25%, respectively. Generally, as results of this study under field conditions, Bio-B fertilizer application decreased freeze injure and increased antioxidant enzyme activity of the grapevine plant.

Keywords Grapevine · PGPR · Boron · Cold · Antioxidant enzyme

Einfluss von Bio-Bor-Düngern auf die Frostschädigung und antioxidative Enzymaktivität bei Weinreben

Schlüsselwörter Weinrebe · PGPR · Bor · Kälte · Antioxidative Enzyme

Introduction

Berry plants are one of the most important garden plants in terms of production side in the world. The economical importance of berry plants increase day by day due to its different wide usage fields as fresh or dried consumption, usage as wine and liqueur. On the basis of different application results, opposite to some fields yield increase, important quality decreases may be occurred. Specially, under conditions as low temperature, aridity and diseases, productivity and quality may decrease at important levels (Kranner et al. 2010). Due to global climate change, the variations at change of temperature can have more decreasing effect of the decreasing productivity potential of grape plant. The maturity degree of grapes under cold climate conditions have critical importance on the quality of wine grapes (Schwab 2005). For this reason, the applications for resistance of cold stress increase show big importance of the growing products. Cold damage, generally was occurred negative effects on green parts and developing parts of plant tissues and causes serious product losses (Rowland et al. 2013). Severity of freeze injury was effected by application of cultivars in olive (Barranco et al. 2005), blueberry (Rowland et al. 2013) and sweet cherry (Kappel 2010). Specially, in the spring frosts, the early breakings of grape plant buds, tenders the plant and prevents the seconder buds frame (Smiley et al. 2008).

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Stress conditions cause increase of reactive oxygen types (ROS), and oxidative stress (Asada 2006). Ultimate stress conditions cause the increase of ROSs at damaging levels (Aroca et al. 2003) and damage cell structures that contain nucleic acid and protein (Murata et al. 2012). Under these stress conditions, plants secrete antioxidant enzymes to prevent the negative effect of ROS and protect the cell against to protein denaturation and DNA damages (Allakhverdiev et al. 2008; Elavarthi and Martin 2010; Nascimento and Fett-Neto 2010). Grape plant can be damaged from cold stress in very close periods and the product growth can be effected negatively (Fennel 2004; Amarowicz and Weidner 2009; Waśkiewicz et al. 2014). Grape plant changes its physiologic parameters and tries to increase its resistance against to cold stress in cold stress conditions (Takahashi et al. 2013; Easlon et al. 2013).

As known soil living and regulating the plant growth-promoting rhizobacteria (PGPR) provide crucial additives to plant growth and development (Hayat et al. 2010; Tokala et al. 2002). PGPRs produce ACC-Deaminase enzyme and due to this prevent the ethylene generation, also increase resistance to stress conditions via producing hormone and siderophore, increasing the availability of plant nutrients and increase the intake of them by plants and producing antioxidant enzymes (Santner et al. 2009; Shao et al. 2009; Hayat et al. 2010; Des Marais and Juenger 2010). In some studies, it was determined that *Burkholderia phytofirmans* bacteria on grape plant and *Pseudomonas fluorescens, Pantoea agglomerans, Mycobacterium* sp. bacteria on wheat plant, (Egamberdiyeva and Hoflich 2003) increase resistance of plants against to stress conditions (Barka et al. 2006). Under stress conditions the antioxidant enzyme activity of plants decrease, however PGPR added plants antioxidant enzyme activity increased in some plants (Omar et al. 2009; Sandhya et al. 2010).

For optimum product yield and plant growth, the physiologic changes and the intake of nutrients are presenting big importance under stress or negative soil conditions (Munns and Tester 2008). The intake of plant nutrients at optimum levels provide to plants in plant growth and yield increase and provides resistance to plant specially under low temperature conditions (Heidari and Jamshid 2010; Khoshgoftarmanesh et al. 2010).

Boron is one of the important plant nutrient for plant growth and fruit quality (Fortunati 2006). B effects important properties in plants as sugar transport, cell division, root and flower generation. For these reasons, lack of B causes some problems as abnormal dimension fruit generation and low fruit generation in grape plant (Christensen et al. 2006). Specially, high lime and low organic matter contented soils, B deficiency is seen widely (Demir and Serindag 2006; Turan et al. 2009; Dursun et al. 2010). In the lack of B, the growth of grape subsides and due to this the cold damage surface increases and the amount of products decreases (Csikász-Krizsics and Diofási 2007; Angin et al. 2008; Turan et al. 2010).

Purpose of this study was to evaluate the effects of Bio-Bor fertilizer methods on freeze injure and antioxidant enzyme activity of grapevine in field conditions.

Materials and Methods

Trial Design

This trial was found in Erzincan, Üzümlü at the date of 19.05.2013, with Baran system presented vineyard and Karaerik grape species. According to depend on full chance trial design was conducted with traditional cultivation system (Baran) used vineyard in average age of 20 and 10 m^2 parcels. In the said parcels, 4 Bio-B applications were conducted as control, soil, leaf and soil + leaf at totally 3 repeated 12 parcels. The soil application was made in one time. In each baran 10 vines tocks and totally in 6 barans 60 vinestock were used. In trial fields, to sustain the normal growth of grape, in autumn as base fertilizer 8 kg/ha^{-1} TSP, 5 kg/ha^{-1} K_2SO_4, 30 kg $MgSO_4$, 4 kg $ZnSO_4$ were applied close to plant roots with the 60–70 cm distance and 20 cm depth with rotovator.

Before budding period; 46 kg/ha^{-1} from 10-20-20-6AS + 1Zn fertilizer, in flowering period 18 kg/ha^{-1} from 33% Ammonium nitrate and in grain size period; 14 kg/ha^{-1} from 33% Ammonium nitrate were applied.

In grape plant Bio-B fertilizer was divided in 3 equal pieces and autumn application was made in firs leaf seen period and before flowering period. The soil application was made in spring period via mixing into soil. The soil + leaf application was made in soil and leaf application periods to the grape plants with taking care of soil and leaf applications.

Bacterial Strain, and Environments

Bacteria culture was grown on nutrient agar (NA), and hold in nutrient broth (NB) with 15% glycerol at –80 °C for long lived storage. Received a colony was transferred to flasks containing NB, and on a rotating shaker (150 rpm) for 48 h at 27 °C. The bacterial suspensions were then diluted in pure water and to a final amount of 10^8 CFU ml^{-1} (Esitken et al. 2010).

Soil Analysis

Soil samples were taken (0–30 cm, 30 subsamples) to determine some soil properties. After the soil samples have been taken, exchangeable cations was determined

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

Soil Properties	Units	Means
Clay	%	37.25 ± 1.44
Silt	%	26.45 ± 1.77
Sand	%	36.30 ± 2.11
Cation exchangeable capacity	Cmol$_c$/kg	26.23 ± 2.13
pH (1:2 soil:water)	–	7.77 ± 0.21
Organic matter	%	1.88 ± 0.16
CaCO$_3$	%	0.63 ± 0.11
Plant available P	mg/kg	3.79 ± 0.68
Exchangeable Ca	Cmol$_c$/kg	18.00 ± 1.22
Exchangeable Mg	Cmol$_c$/kg	2.38 ± 0.31
Exchangeable K	Cmol$_c$/kg	4.47 ± 0.89
Exchangeable Na	Cmol$_c$/kg	0.70 ± 0.13
Available Fe	mg/kg	3.29 ± 0.45
Available Mn	mg/kg	4.44 ± 1.01
Available Zn	mg/kg	3.15 ± 0.75
Available Cu	mg/kg	6.54 ± 1.25
Available B	mg/kg	0.35 ± 0.09
Electric conductivity	dS/m	0.98 ± 0.08

using ammonium acetate (Thomas 1982) and cation exchange capacities (CEC) were determined using sodium acetate—ammonium acetate (Sumner and Miller 1996). Total *N* was determined by the Kjeldahl method (Bremner 1996), plant-available *P* was determined by using the sodium bicarbonate method (Olsen et al. 1954). Electrical conductivity (EC) was measured in saturation extracts (Rhoades 1996). Calcium carbonate and soil pH were determined as a method by McLean (1982). Soil organic matter was determined using the Smith-Weldon method (Nelson and Sommers 1982). Available Fe, Mn, Zn, and Cu in the soils were determined by DTPA methods (Lindsay and Norvell 1978). Available B was analyzed using the azomethine-H (Wolf 1974). These soil characterization data are presented in Table 1.

Plant Sampling and Determination of Freezing Injury

To determine the effetcs of freezing injury of leaves, fully developed mid-shoot leaves were sampled and then freezing injuries were determined using a modification method of Marentez et al. (1993). Fresh grapevine leaves were cut into 2 cm lengths and rinsed in pure water. Leaves (0.5 g) were placed in tubes, and then were positioned in a freezing bath. After stabilization at –1 °C for 30 min, the temperature was lowered that stepwise by 1 °C times from –1 to –20 °C. The tubes were removed, and 4 mL of cold püre water was added in the tube. These tubes were stored at 4 °C for 24 h (Turan et al. 2007).

Antioxidant Enzymes Activity

Antioxidant enzyme analysis were done at 4 °C. Cells of plant leaves (500 mg) were homogenized in phosphate buffer (a mortar with 3 ml of 50 mM at pH 7). Frozen cell samples were ground to a fine powder with liquid nitrogen and extruted with ice cold phosphate buffer (pH 7.8, 0.1 mM EDTA), 1 mM phenylmethane-sulphonylfluoride (PMSF) and 0.5% polyvinylpyrrolidone (PVP). The POD, SOD, and CAT enzyme activities in grapevine leaves were measured spectrophotometrically (Dhindsa et al. 1981; Sairam and Srivastava 2002).

Statistical Analysis

These results were subjected to analysis of variance and significant means were compared by Duncan's test method. As a result mean differences were considered significant if $p \leq 0.05$.

Results

Effects on Grapevine Yield

The effects of Bio-B application on grape plant production were examined. Due to this determination Bio-B application effect on grape productivity was found statistically important with ($p < 0.01$) and the highest grape yield was obtained from soil + leaf Bio-B application group as a result of average of two years' trial period (Table 2). The lowest fruit production was obtained from control application. Compared with control group the soil + leaf Bio-B application provide 16.3% increase.

In grape fruit quality parameters were evaluated and the most grain size, height and grain wet weight and seed count were obtained with soil + leaf Bio-B application.

Effects on Freezing Injury of Grapevine Plant

In the taken plant samples according to result of analysis, the grape plant damage ratios were determined in different temperatures and evaluated on the average of two-year data. As a result of evaluation, depend on Bio-B applications decreases were occurred in cold damage ratios and this difference occurred statistically important level with ($p < 0.01$). In control group, the highest damage ratio occurred at –20 °C and 94.89% (Table 3). At the same temperature, the soil Bio-B application to grape plant were showed decreases in damage ratio with 21.55%; from leaf application 25.53% and soil + leaf application 26.24%. From these values the most efficient application was determined in soil + leaf Bio-B application (Fig. 1.).

Table 2 Yield and quality parameters of grapevine

Bio-B Applications		Yield	Fruit width	Fruit size	Fruit wet weight	Fruit dry weight	Kernel number
		kg da^{-1}	cm		g		Piece
1. Year	Control	5047c	2.22a	2.63	8.12b	1.91c	1.73b
	Soil	6730b	2.24a	2.66	8.05b	2.14b	2.27a
	Foliar	6676b	2.11b	2.64	7.26c	2.22a	2.33a
	Foliar + Soil	7182a	2.26a	2.67	8.31a	2.28a	2.27a
2. Year	Control	6754b	2.44b	2.79b	7.54c	2.12b	2.18c
	Soil	6912a	2.40b	2.75b	8.04c	3.14a	2.67a
	Foliar	7011a	2.42b	2.86ab	8.68b	1.87c	2.33b
	Foliar + Soil	6543c	2.74a	2.92a	9.55a	2.34b	2.67a
Means	Control	*5901b*	*2.33b*	*2.71b*	*7.83b*	*2.02c*	*1.96b*
	Soil	*6821a*	*2.32b*	*2.71b*	*8.05b*	*2.64a*	*2.47a*
	Foliar	*6844a*	*2.27b*	*2.75ab*	*7.97b*	*2.05c*	*2.33a*
	Foliar + Soil	*6863a*	*2.50a*	*2.80a*	*8.93a*	*2.31b*	*2.47a*

Table 3 Freeze injury rate of grapevine leaves

Bio-B Applications		0 ˚C	–5 ˚C	–10 ˚C	–15 ˚C	–20 ˚C
		%				
1. Year	Control	54.12a	60.34a	72.31a	89.34a	95.45a
	Soil	34.25b	41.23b	49.88b	56.76b	64.35b
	Foliar	29.76c	38.72c	46.51c	53.24b	60.11b
	Foliar + Soil	31.28c	40.24b	46.90c	55.49b	61.29b
2. Year	Control	55.46a	62.32a	71.21a	88.67a	94.32a
	Soil	47.65b	52.31b	60.23b	77.56b	84.53b
	Foliar	45.33b	49.87c	58.79bc	72.13c	81.21c
	Foliar + Soil	44.32b	48.56c	55.11c	69.80d	78.69d
Means	Control	***54.79a***	***61.33a***	***71.76a***	***89.01a***	***94.89a***
	Soil	***40.95b***	***46.77b***	***55.06b***	***67.16b***	***74.44b***
	Foliar	***37.55c***	***44.30c***	***52.65c***	***62.69c***	***70.66c***
	Foliar + Soil	***37.80c***	***44.40c***	***51.01c***	***62.65c***	***69.99c***

Fig. 1 Effects of Bio-B applications on freeze injury rate of grapevine plants

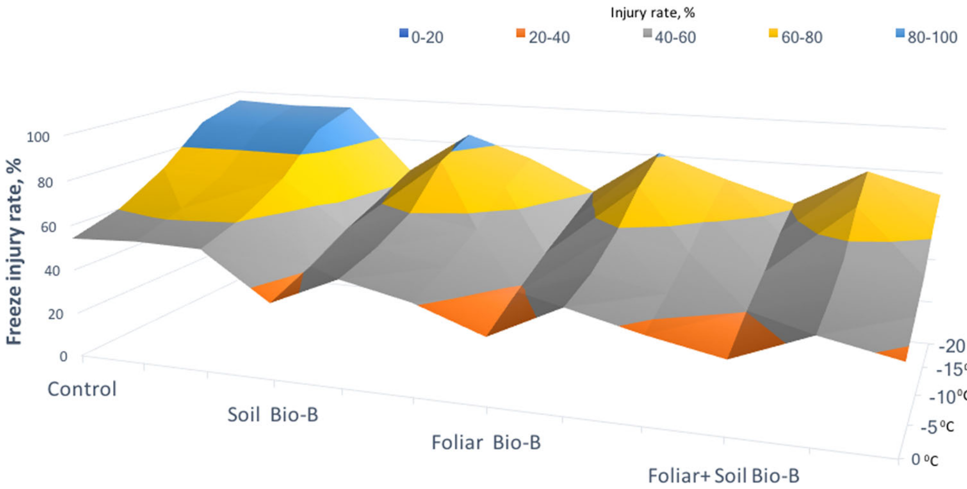

Table 4 Antioxidant enzyme activity of grapevine leaves

Bio-B Applications		CAT	POD	SOD
		EU g leaf^{-1}		
1. Year	Control	57c	1655c	413c
	Soil	68b	1872b	489b
	Foliar	74a	2013a	573a
	Foliar + Soil	69b	1988a	512ab
2. Year	Control	55c	1546c	387d
	Soil	63b	1712b	423c
	Foliar	70a	1895a	540a
	Foliar + Soil	66b	1811a	493b
Means	Control	***56c***	***1601c***	***400d***
	Soil	***66b***	***1792b***	***456c***
	Foliar	***72a***	***1954a***	***557a***
	Foliar + Soil	***68ab***	***1900a***	***503b***

Effects on Antioxidant Enzyme Activity

The grape plant antioxidant enzyme activity was examined and Bio-B application effected POD, CAT, and SOD enzyme activity, statistically at important level ($p < 0.01$). At the evaluation of two-year data, the highest CAT, POD and SOD enzyme activities were determined in the leaf Bio-B application (Table 4). Compared to control group, increases occurred in SOD, POD, and CAT enzyme activity with the ratios of 28.57%, 22.05% and 39.25%, respectively with leaf Bio-B application.

Discussion

In the study, depend on different application methods of PGPR and Boron including Bio-B fertilizer formulations effects were determined of grape plants on the productivity, antioxidant enzyme content and cold damages ratio. As a result of the study it was determined that Bio-B applications increased the productivity and antioxidant enzyme content and decreased the cold damage ratio on grape plant. PGPRs in Bio-B fertilizer formulations increase the plant nutrients eligibility in soil, good plant growth and as in like tomato, cabbage and strawberry plants (Gunes et al. 2009; Turan et al. 2014), compared with the control (Karlidag et al. 2011). Macro-micro plant elements eligibility in soil effects the parameters as the growth of grape plant as the other plants, metabolic functions, resistance to stress conditions, fruit quality and fruit shape (Ashley 2011).Compared with control group, in grape plant product amount, leaf dimension, flower growth, shoot generation were much more; small fruit generation was less seen via Bio-B application and intake of eligible B from plants thanks to PGPRs in the formulation and similar results were seen with this study (El-Sheikh et al. 2007). The other factor effects the B and PGPR applications efficiency is application type. In general, the Bio-B application from leaf was increased plant productivity more than the soil application. Boron intake was faster and transported in short time to grape plant and provide increase of grape growth via leaf application (Kuepper 2003 and El-Sheikh et al. 2007). Foliar application of Bio-B had been associated with improved number of clusters, and the quality of grapevines as in similar to work (Malakouti 2007; Akbar et al. 2013).

The temperatures under 20 °C and 0 °C degrees effect the plant growth negatively. Exposure to freeze and low temperature increase reactive oxygen species production that damages lipids, proteins, nucleic acid and carbohydrates (Suzuki et al. 2012; Waśkiewicz et al. 2014). The plants increase antioxidant enzyme activities as CAT, POD and SOD to decrease the harmful effects depend on ROS production. (Miller et al. 2010; Habibi 2015). Antioxidant enzyme formation is produced by plants biochemically at cold climate and cold tolerant plants. However, in microclimate regions grape plants, the enzyme production occurs in low level due to weak intolerance of plant under sudden temperature changes and low temperatures. In this state plant can be damaged in high ratios and damage level can be occurred between 80–100% levels (Kumar et al. 2011; Easlon et al. 2013). In this study similar to, plant exposure to different temperature conditions and the cold damage level at 0 °C was 54% in control group; in Bio-B application, the antioxidant enzyme activity increased in plant and cold damage level decreased to 37%. Bio-B application gave tolerance to plant at 0 °C and 31% ratio and showed similar results with the study (Kaur et al. 2011).

Frost and cold damage can show changes depend on plant genotype, environment conditions and grape plant growth conditions and agricultural applications. Specially in early spring, sudden temperature decrease after warm weathers increase the damage level in plants (Gu et al. 2002). Autumn late colds show the same effect and increase damages of plant. As these states, the damage level can be less in healthy and good and balanced fertilized plants in terms of plant nutrients.

In the other studies, a high correlation was found between resistance to frost and antioxidant enzyme activity. In different applications, antioxidant enzyme activity increase was determined in the resistant species to low temperatures (Zhang et al. 2011; Kishimoto et al. 2014). Even in the study, in frost tests, the highest antioxidant enzyme activity was determined in coldest resistant soil + leaf and leaf Bio-B application. In a study of Thurzo et al. (2010), the boron application from leaf increased the color pigments as the chlorophyll and carotenes and fruit production was increased due to photosynthesis and antioxidant enzyme activity.

Under stress conditions PGPR decrease the increasing amount of absisic acid in plant and provide increases amounts of growth promoting hormones as auxin and cytokinin (Cohen et al. 2009). PGPRs will be applicate to grape plant, will increase root and shoot development and will increase the plant tolerance against to low climate degree. (Barka et al. 2000; Compant et al. 2005; Verhagen et al. 2011). Similar to this study, plant production parameters showed increase with Bio-B application and the state provided the increase of tolerance of plant against to low temperatures (Gunes et al. 2016).

In grapevine plant, to increase the tolerance of plant against to frost and cold damages and with the purpose of minimizing the damage level, weather circulation with helicopter (Creasy and Creasy 2009), wind machines (Evans 2000) agricultural applications were used. But due to these methods are so expensive, much less application methods are needed. In the study, the low cost of Bio-B application shows that this method can be used in agricultural applications so smoothly.

Conclusion

This study results showed that the PGPR and boron applications decreased the risk of freeze injury because of plant membrane changes, and ion stability. The types of fertilizers application methods, plant species, age, and physiology of the plants affects the other study results. Overall, the findings of this study recommend that Bio-B have the potential to decreased freeze injury of grapevine plant and increase the yield, antioxidant enzyme activity.

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Conflict of interest C. Köse, A. Güneş, Ö. Kaya, N. Kıtır, M. Turan and F. Şahin declare that they have no competing interests.

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