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Determination of the Effects of Plant Growth-Promoting Rhizobacteria Applications on Kober 5 BB American Grapevine Rootstock in Lead Stress

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are free-living soil bacteria that can affect plant growth by the synthesis of phytohormones and vitamins, inhibiting plant ethylene synthesis, enhancing stress resistance, improving nutrient uptake and mineralising organic phosphate. In this study, it was aimed to determine the effects of PGPR application on Kober 5 BB grapevine rootstock under the lead stress. Different lead concentrations (0; 10, 25 and 50 ppm $Pb(NO_3)_2$) were applied to plants. Some morphological characteristics (shoot length, shoot weight, average number of leaf per shoot) and biochemical characteristics (chlorophyll, degree of membrane injurity, proline, total phenolic compound and lipid peroxidation) were tested in the study. As a conclusion, the damage by lead stress generally reduced with the PGPR treatment.

Keywords: American Grapevine Rootstock, Lead, PGPR, Proline, Total Phenolic Compound.

1. Introduction

Heavy metals are substances that accumulate more gradually in environments such as soil, water and air, threaten the life of all living things, and reach high concentrations by transporting through food chains from one organism to another, and sustain its harm for years (Yucel et al. 2010). These substances, which are known to cause serious damage to humans and animals, cause different harmful effects on plants. First of all, the quantity and quality of the product decrease, and in the advanced cases it even causes the death of the plant.

One of the most important heavy metals that contain toxic substances and cause environmental pollution is lead. Lead is absorbed from the medium by plants and accumulated in different organs of plants (Kabata Pendias 1984). Lead, which is not needed for plants, causes stress with excessive accumulation in plant tissues and organs, adversely affects the cell turgor and cell wall structure, slows stoma movements and reduces leaf area (Miranda and Ilangovan 1996). Roots are more likely to be exposed to lead, which reduces root development and negatively affects the intake of the nutritional elements due to the imbalances in cation-anion intake (Sharma and Dubey 2005). In addition, lead and many other heavy metals promote the formation of free radicals, harming plant tissues and synthesizing "stress ethylene" that inhibits plant growth. One of the most effective strategies for decreasing stress ethylene formed by abiotic stress conditions is the formation of ACC deaminase activity. Here bacteria, which have the ability to produce ACC deaminase, and promote root elongation and development by reducing the amount of ethylene in the plant roots, come into prominence (Penrose and Glick 2001; Safronova et al. 2006). It has been determined that the stress of the heavy metals such as nickel, lead, zinc, copper, cadmium, cobalt and arsenic (Nie et al. 2002; Glick 2003; Reed et al. 2005; Farwell et al. 2006; Yang et al. 2011) can be reduced by using a kind of bacteria: Plant Growth Promoting Rhizobacteria (PGPR).

The aim of this study is to investigate the effects of PGPR applications on 5 BB American grapevine rootstocks grown in mediums containing $Pb(NO_3)_2$ at different concentrations. Physical (shoot length, shoot weight, average number of leaf per shoot) and biochemical (chlorophyll, degree of membrane injurity, proline, total phenolic compound and lipid peroxidation) analyzes were conducted in plants grown in $Pb(NO_3)_2$ containing mediums (0, 10, 25 and 50 ppm) to determine the potential of PGPR in terms of reducing heavy metal stress.

2. Material and Method

In this study, Kober 5 BB American grapevine rootstock was used as plant material. 5 BB is a rootstock that accommodates with humid and clayey soils, and is a strong rootstock that resist up to 20% active lime and nematodes. Plant materials were obtained from Gaziosmanpasa University, Faculty of Agriculture, Department of Horticulture (Tokat/TURKEY). The rootstocks were recut to a length of 35-45 cm and the edges to be under the ground taken to get ready to be planted. A mixture of sterilized soil: perlite: turf (1: 1: 1) was used as the growing medium, and 1.5 k of the mixture was placed in 2 L volume polyethylene bags each.

To ensure the adequate development of roots and shoots, rootstocks taken into controlled growing rooms with 24±1°C temperature, and regularly irrigated. Approximately 15 days after the planting, 5 mL PGPR inoculation were applied to the root zone. PGPR used in the research are *Arthrobacter*-type bacteria that are obtained from the "ROA Biotechnology" (Antalya/TURKEY) company in the form of ready-to-use solution. It is known that the relevant PGPR shows ACC deaminase activity, mitigates ethylene-induced damage in stressed plants, and promotes plant growth as well as nutrition intake (Barnawal et al. 2014).

$Pb(NO_3)_2$ applications were conducted 2 weeks after the PGPR inoculation at four different concentrations (0 (control), 10, 25 and 50 ppm). Cuttings were taken for about 2 months of growing periods under these conditions. Following the development of plants, physical and biochemical analysis were made.

2.1. Physical Analyzes

2.1.1. Shoot weight: The weight of the shoot was measured with a 0.0001g sensitive analytical balance and indicated in grams (g).
2.1.2. Shoot length: The length of the shoot was measured with the aid of a ruler and indicated in centimeters (cm).
2.1.3. Average number of leaf per shoot: All leaves grown on shoots are counted and indicated with numbers.

2.2. Biochemical analyzes

2.2.1. Determination of degree of membrane injurity: Membrane damage was determined with measuring the amount of excess electrolyte excreted from the plant cells under stress conditions (Fan and Blake 1994).
2.2.2. Determination of chlorophyll content: Chlorophyll analysis was calculated with SPAD method using Konica Minolta SPAD-502 Plus chlorophyll meter.
2.2.3. Determination of proline content: Proline analysis of the samples was done according to Bates et al. (1973). The proline concentration was determined in µmol/g (fresh weight, FW).
2.2.4. Determination of total phenolic compound: The total phenolic compound extraction were made using the Kiselev et al. (2007) method. Analyzes were carried out according to Singleton and Rossi (1965), and total phenolic compound content were given in mg/g as gallic acid equivalent (GAE).
2.2.5. Determination of lipid peroxidation: It was determined with the principle of measuring the amount of malondialdehyde, the last product of lipid peroxidation, according to Zhang et al. (2007), and the results are given in µmol/g (FW).

The study was set up according to the randomized plot design with 3 replications in two different applications (PGPR inoculated and uninoculated) and four different concentrations of $Pb(NO_3)_2$, with each replication consisting of 4 plants. The SPSS 20.0 software package was used to statistics analyzes results obtained from the research, and the differences between the treatments were determined by Duncan's multiple range test.

3. Results and Discussion

Results of this study are as follows to determine the effects of PGPR applications on Kober 5BB rootstocks in mediums containing $Pb(NO_3)_2$ at different concentrations.

Table 1. Effects of PGPR applications on some physical features of 5 BB rootstocks in different $Pb(NO_3)_2$ mediums

	$Pb(NO_3)_2$ (ppm)	**Physical Characteristics**		
		Shoot Length (cm)	**Shoot weight (g)**	**Number of leaves (peace)**
Noninocule	**0**	19,83 a	1,174 ab	4,866 b
	10	13,50 bcd	0,969 bc	3,167 c
	25	10,67 cd	0,559 c	2,887 c
	50	9,17 d	0,552 c	3,167 c
Inocule	**0**	21,67 a	1,474 a	6,500 a
	10	16,83 ab	1,137 ab	5,277 ab
	25	14,67 bc	0,996 abc	3,333 c
	50	12,00 bcd	1,056 ab	2,667 c

*Mean followed by same letter are not significantly different ($P<0.05$).

In Table 1, where the values obtained in terms of physical features are presented, it is seen that plants formed longer shoots when they are in lead-free mediums and PGPR inoculated mediums with containing the lowest dose of lead at 10 ppm. Therefore, in terms of shoot length, it is understood that PGPR application can be effective in 10 ppm dose, which is the lowest dose. Similar to our study, Hassan et al. (2014) also conducted a research to determine the efficiency of 6 different PGPR strains on maize plants grown under lead stress. It has been determined that PGPR applications increase the length of the shoots as a result of their research.

The weight of the shoots grown on the plant is also an effective criterion to measure stress. Heavier shoots indicate that substance accumulation is also more. In our study, it seems that PGPR application is effective on shoot weight of rootstocks in all lead concentrations. However, in the mediums that PGPR is not applied, the highest shoot weight was obtained only in lead-free ones. As a matter of fact, Ogar et al. (2015) also found biologic agents such as PGPR significantly increase shoot and root dry weights against zinc and lead stress of *Dichondra repens* and alfalfa plants. Meanwhile Kiran et al. (2015) conducted a study to determine the effect of lead stress on some morphological and biochemical features of lettuce. In their research, they gave 0, 150 and 300 ppm doses of lead with irrigation water to 30 day plants, and as a result they indicated that lead application decreased biomass, stem and root length, and leaf area values in line with increasing doses.

One of the physical features examined in the study is the number of leaves on the shoot, and the effect of PGPR in those terms is also shown in Table 1. It is observed that PGPR application increases the number of leaves in lead-free mediums. However, more leaves were grown in the groups treated with PGPR than those not treated in 10 ppm dose lead. Likewise, Zengin and Munzuroglu (2004) stated that exposing to lead negatively affects root, stem and leaf growth in plants. Yolcu et al. (2012) used 12 different bacterial strains in a study conducted to determine the effects of PGPR on Hungarian vetch (*Vicia pannonica Crantz.*) in terms of yield and some quality criterion. In the study, they stated that bacteria have positive effects on the some values such as plant height and number of leaves.

The effects of PGPR applications on some biochemical features of 5 BB American grapevine rootstocks grown in different lead concentrations were also examined in our research (Table 2).

Table 2. Effects of PGPR applications on some biochemical features of 5 BB rootstocks in different $Pb(NO_3)_2$ mediums

	$Pb(NO_3)_2$ (ppm)	**Biochemical characteristics**				
		Chlorophyll (SPAD)	**Degree of membrane injurity (%)**	**Proline (µmol/g)**	**Total phenolic compound (GAE) (mg/g)**	**Lipid peroxidation (µmol/g)**
Noninocule	**0**	19,333 ab	56,685 d	0,173 c	1,397 e	8,480 cd
	10	17,699 bcd	85,940 ab	0,170 cd	2,001 d	9,510 cd
	25	15,350 cd	87,977 ab	0,160 cd	1,296 ef	11,200 c
	50	14,593 d	90,135 a	0,152 d	1,910 d	18,455 a
PGPR	**0**	22,067 a	45,618 e	0,294 a	1,112 f	4,712 e
	10	19,767 ab	60,361 cd	0,278 b	2,481 c	6,814 de
	25	18,467 abc	67,684 c	0,164 cd	4,044 a	9,931 cd
	50	16,150 bcd	78,234 b	0,192 c	3,716 b	15,269 b

*Mean followed by same letter are not significantly different ($P<0.05$).

When the effect of PGPR application on the chlorophyll content of 5 BB American grapevine rootstocks under lead stress is examined, it is observed that the inoculated plants have the highest amount of chlorophyll even at 10 and 25 ppm doses. In the uninoculated plants, only the lead-free plants contained high chlorophyll and the others were found to be adversely affected by lead stress. Similarly, Janmohammadi et al. (2013) and Hassan et al. (2014) also found that PGPR applications increased chlorophyll content in plants under lead stress.

In stressed plants, preserving the membrane integrity is a serious indicator of protecting the plant from stress. It is also known that heavy metals cause membrane damage (Janmohammadi et al. 2013). In this study, it was observed that membrane damage is at the highest levels in the uninoculated plants in all lead concentrations except for the control group. Therefore, the highest damage occurred in plants that are not treated PGPR under lead stress. However, even in the highest lead concentration in PGPR applications, plant membranes appear to be less damaged than uninoculated plants in lead-containing mediums. The least damage was detected in the membranes (45.618%) of plants that are applied PGPR and are lead-free.

The plants have developed different mechanisms in order to protect their cells in adverse conditions. Proline accumulation is one of these mechanisms and is effective as an osmotic preservative (Gratao et al. 2015; Kolupaev et al. 2016). Lead, as a heavy metal, is an abiotic stress factor that causes serious damages on plants. Therefore, in the research to determine proline accumulation in plants under lead stress and effects of PGPR applications on this stress, the highest proline accumulation was found in plants that are stress-free but applied PGPR (0.294 µmol/g). Hassan et al. (2014) also determined the efficacy of six different PGPR strains in maize plants grown under lead stress in their study. As a result of the research, they determined that PGPR applications increase the proline amount.

One of the most important secondary metabolite groups synthesized in plants under different environmental factors is phenolic compounds, which have high antioxidant functions. In plants, it is known that phenylpropanoid metabolism and the amount of phenolic compounds increase under stress (Tewari et al. 2006; Quan et al. 2008; Rodriguez-Serrano et al. 2009). In our research, the highest amount of total phenolic compounds (4.044 mg/g) was reached in PGPR inoculated plants treated with 25 ppm dose lead. This value was also followed by plants grown at the highest lead concentration (50 ppm) that are applied PGPR (3.716 mg/g). In their study Pazoki (2015), studying the effects of PGPR and mycorrhizas in wheat plants grown in lead-containing mediums at different concentrations, reported that PGPR applications increased phenolic compounds by about 17.9%. They also emphasized that the effects of PGPR are greater than those of mycorrhizas.

Another damage of heavy metals is that removal of hydrogen from unsaturated fatty acids through reactive oxygen species, which cause to peroxidation in lipids (Dey et al. 2007; Zhou et al. 2009; Colak ve Dogan 2011). The main criterion for lipid peroxidation is the determination of the amount of malondialdehyde (MDA), the product of oxidation. Lipid peroxidation was also investigated in our study, and it was observed that the highest damage was observed in plants that did not treated PGPR at a dose of 50 ppm lead (18.455 µmol/g). This positive effect of PGPRs on lipid peroxidation in stressed plants has also been determined by previous studies under different stress conditions on different plants. For example, Wang et al. (2011) stated that PGPR applications to arsenic rich soils reduced arsenic-induced lipid peroxidation in plants. Haneef et al. (2014) also reported that the amount of MDA in isabgol (*Planta ovata*) plants under cadmium stress was high, whereas in PGPR and mycorrhiza applied plants this value decreased. Similarly Kiran et al. (2015) found that lead had an effect on the amount of plant's MDA in their investigations to determine the effect of lead stress in head lettuce, while the highest MDA values were determined at 150 ppm lead treatment (9.36 ± 0.36 µmol/g). Again, lead stress has been reported to increase the amount of MDA in wheat (Colak ve Dogan 2011) and rough rice (Verma and Dubey 2003).

4. Conclusion

Elements such as manganese, iron, copper and zinc, which are among the heavy metals, are necessary elements for vascular plants (Nedelkoska and Doran 2000). Copper and zinc, for instance, function as cofactors for the structure of proteins and enzymes that play an important role in the growth and development of plants (Steffens 1990). Excessive accumulation of other heavy metals such as lead, mercury and cadmium, on the other hand, cause physiological stress, and decrease growth and development of plants. Heavy metal accumulation in plant tissues negatively affects numerous issues such as nutrient intake, chlorophyll biosynthesis, photosynthesis (Ghani et al. 2010), transpiration (Poschenrieder et al. 1989), membrane integrity (Janmohammadi et al. 2013), enzyme activity (Kiran et al. 2015), and germination (Munzuroglu and Geckil 2002). The PGPR bacteria have been identified to be effective organisms in reducing heavy metal stress in previous studies (Farwell et al. 2006; Safronova et al. 2006), PGPR's effectiveness under lead stress in that regard was investigated in our study as well.

Heavy metal accumulation that arises due to increasing industrialization and industrial wastes is a big environmental pollution problem. When this problem affecting all living things, soil and water resources on the earth is evaluated in terms of plant production, on the one hand it threatens plant life, on the other hand it threatens the food safety. Heavy metals are transported from one organism to another by the food chain, reaching high concentrations and sustaining their damages for many years. Since it is unlikely that the soil can be cleared of pollution, the most effective measure to be taken in this respect is to create an environment in which plants would experience as little damage as possible under such stress. Reduction of stress ethylene synthesized in plants under stress conditions is seen as one of the effective strategies in this respect. At this point it is known that some soil bacteria around the root and/or on the root surface of the plant are effective. PGPR containing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase enzyme contribute to plant growth and development, by reducing the level of plant ethylene especially in different environmental stress conditions. Therefore, the decrease in the level of ethylene helps plants to be more resistant to different environmental stresses. Hence, in this study, which was aimed to investigate the effects of PGPRs on lead stress in vine plant, it was found that the bacteria were effective in alleviating the stress. However, areas that PGPRs are effective are increasing day by day. Studies to be conducted in this area needs to be carried out in multifaceted ways.

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