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Mechanism of resistance against lime stress by plant growth-promoting Rhizobacteria in Vitis

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Abstract
Plant growth-promoting rhizobacteria (PGPR) have gained world wide importance and acceptance. PGPR are the soil bacteria inhabiting on the root surface and are directly or indirectly involved in promoting plant growth and development. It has documented the increased health and productivity of plants by the application of PGPR under both normal and stressed conditions. In this study, it was aimed to determine the effects of PGPR on 1103 Paulsen grown in 0%, 10 and 25 lime concentrations. Content of chlorophyll, leaf number, shoot length, shoot weight, degree of membrane injurity, proline, total phenolic compound and lipid peroxidation were determined in the plants. The longest shoots were found on inoculated plants at 0% (26, 00 cm) and 10% (17,67 cm) lime concentration. Membrane damage (78,607%) was the highest on 25% lime medium and non inoculated plants. Maximum proline content was determined from inoculated plants and 10% to 25% lime medium. Maximum total phenolic content (4,087 mg/g) was obtained from inoculated plants at 25% lime. The highest lipid peroxidation (7.861 µmol/g) was observed with non inoculated, and 25% lime medium. As a conclusion, all criterias were affected by different $CaCO_3$ concentrations and injurity symptoms result from $CaCO_3$ generally reduced with the PGPR treatment.

Keywords: lime, PGPR, grapevine, proline, phenolic compound

1. Introduction
The deficiencies in the nutrient intake of plant necessitate fertilization in the cultivation. Incorrect fertilization can cause to problems like soil salinization and stressful medium in the plants. In recent years, it has been determined that a wide variety of stressful mediums involving plants can be alleviated/inhibited by providing biological approaches, or plant can be provided to gain resistance through its defense system. One of the applications is bacterial applications, having the ability to alter the physiology and metabolism of plants as well as ensuring plants' a range of defensive proteins to be synthesised, and helping plants in respect of avoiding a variety of environmental stress factors and even partly overcoming them. These bacteria used in the agriculture as biological warfare agents or biofertilizer are called as "plant growth- promoting rhizobacteria (PGPR)".

PGPR have direct and indirect influence on the plant development. The antibiotic or siderophore secretions of bacteria and the control of pathogenic microorganisms indirectly promote plant development. The synthesis of plant hormones like indole acetic acid (Xie *et al*., 1996) [37] provide tensional reduction in the nitrogen fixation (Christiansen-Weneger, 1992) [7], and in root membranes (Bashan and Levanony, 1991) [4]. Besides, PGPR make many bacteria and organic and inorganic substances included by plant's rhizosphere useful for plants. Microorganisms increase the uptake of elements such as iron and zinc, as well as having the feature of phosphate solving (Cakmakci *et al*., 1999; Sahin *et al*., 2004) [8, 34]. In addition, it can indirectly promote the plant development by acting against pathogenic microorganisms with the production of siderophore, 1,3 glucanase, chitinase, antibiotic and cyanite (Dobbelaere *et al*., 2003) [10]. One of the most important effects of PGPR is regarding enzyme synthesis. Especially with the synthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase-like enzymes, plant hormone levels have been regulated. Especially due to the low content of ethylenes; plants, to which the bacterium exhibiting ACC deaminase activity are implemented, develops proportionally more roots, and become more resistant to stress conditions (Safronova *et al*., 2006) [30]. It has been seen that researchers have been carried out in every aspect intending to determine effects of PGPR on both intake of nutrients and negative stressed mediums. This research was carried out with the aim of determining the effects of PGPR applications on 1103 P rootstock growing in mediums containing lime in different levels.

2. Material and Methods
In the study, 1103 Paulsen rootstock was used as a plant material. 1103 P is a rootstock growing strongly, having damp bottom layer and adapting to clay-limy soils, and resisting against 17-18% active lime. The rootstocks were supplied from Gaziosmanpasa University, Faculty of Agriculture (Tokat/TURKEY). The rootstocks supplied were recut as 35-45 cm in length. A mixture of soil: perlite: turf (1:1:1) was used as the growth medium of the rootstocks and these mediums, after being sterilized, were placed in the 2 L volume polyethylene bags so as to each bag contain 1.5 k. The rootstocks were taken to the controlled growth rooms at 24±1°C and they were regularly irrigated. Approximately 15 days after planting, PGPR were applied to the root zone as 5 mL. The PGPR used in the research were supplied from "ROA Biotechnology" (Antalya/TURKEY) company as a solution. After 2 weeks, lime applications were performed. Different concentrations of lime ($CaCO_3$), including 0% (control), 10% and 25%, were applied. The cuttings were improved for about 3 months in these conditions and the removal procedure was performed subsequent to the formation of roots and shoots and their analyzes were carried out.

2.1 Physical analyzes
Shoot weight were stated in g and measured with an analytical balance with precision of 0,0001g. Shoot lengths were stated in cm measuring with a ruler. All of the leaves developed on the shoots were counted and average number of leaves per shoot stated in terms of item.

2.2 Biochemical analysis
The membrane damage was determined by measuring the excess amount of electrolytes excreted from plant cells under stressed conditions (Fan and Blake, 1994) [12]. The chlorophyll analysis were measured by SPAD method using the Konica Minolta SPAD-502 Plus device. Proline analysis was performed according to Bates *et al*. (1973) [5] and it was determined as µmol prolin/g (fresh weight, FW). Extraction of total phenolic compounds was performed according to Kiselev *et al*. (2007) [16], and analyzes were performed according to the procedure of Singleton and Rossi (1965) [33]. Amounts of total phenolic compound were given in mg/g in terms of gallic acid equivalent (GAE). Lipid peroxidation was determined according to Zhang *et al.* (2007) [39] as µmol/g (FW). The study was carried out with 3 repetitive and 4 plants per each repetitive. Data were analyzed using the statistical software package, SPSS 20.0. The difference between the practices was determined by Duncan's multiple comparison test.

3. Results and Discussion
The results of this study, carried out in order to determine the effects of the PGPR applications on 1103 P rootstocks growing in the mediums containing lime in different levels, are given below. The effects of PGPR applications on the physical characteristics of 1103 P rootstocks were investigated (Table 1). It was seen that there are differences between the applications in terms of shoot length and the lime concentrations. According to these values, the longest shoots were found in the non-inoculated plants with 10% lime concentration (18,17 cm) and in the inoculated plants containing 0% and 10% lime concentration (26,00 and 17,67 cm). In both groups, the shoot length decreased in the high concentrations of the lime. Similarly, Ozdemir (2005) [26] cultivated Yalova Incisi, 140 Ru and 1103 P genotypes in the mediums containing 10, 30, 50% lime, and as a result of the research, the longest shoots was obtained in the mediums containing 10% lime. Regarding the values of the shoot weight, similar to the shoot length, the shoots appear to be heavier in the environments, limeless or containing relatively lower lime. In this regard, the heaviest shoots were determined in the plants, non inoculated and containing 10% lime and inoculated plants growing in limeless medium. Regarding the number of leaves, in the results of our research, any difference wasn't observed statistically among the application groups. However, it was numerically observed that the number of leaves was higher in the inoculated plants growing in the mediums, limeless and containing 10% lime. It has also been determined by many studies that PGPR applications have positive effects on both growth and developmental characteristics of plants growing in both normal conditions and stressed mediums (Barnawal *et al*. 2014; Martínez *et al.*, 2015) [3, 23].

Table 1: Effects of PGPR applications on some physical properties of 1103 P rootstock growing in the different lime levels.

	$CaCO_3$ (%)	Physical characteristics		
		Shoot Length (cm)	Shoot weight (g)	Number of leaves (peace)
Control	0	15,72 b*	1,191 bc	4,00
	10	18,17 ab	1,844 ab	4,00
	25	9,67 b	0,394 c	3,50
Inoculated	0	26,00 a	2,493 a	5,33
	10	17,67 ab	1,221 bc	5,00
	25	13,67 b	1,339 bc	4,50

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

Table 2: Effects of PGPR applications on some biochemical characteristics of 1103 P rootstock growing in the different lime levels.

	CaCO3 (%)	Biochemical characteristics				
		Chlorophyll (SPAD)	Degree of membrane injurity (%)	Proline (µmol/g)	Total phenolic compound (mg/g)	Lipid peroxidation (µmol/g)
Control	0	17,950 ab*	48,435 b	0,203 bc	3,392 b	4,844 b
	10	18,206 ab	56,614 b	0,174 c	3,391 b	5,661 b
	25	16,850 b	78,607 a	0,175 c	3,256 b	7,861 a
Inoculated	0	20,075 ab	46,259 b	0,136 c	3,557 b	4,626 b
	10	23,450 a	43,619 b	0,247 ab	3,378 b	4,362 b
	25	22,700 a	54,952 b	0,286 a	4,087 a	5,495 b

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

As well as physical characteristics, the effects of PGPR applications on the biochemical characteristics of 1103 P rootstocks were investigated (Table 2). It was seen that all the biochemical properties statistically vary according to PGPR and lime applications. PGPR application is more effective in the highest lime concentrations on chlorophyll content. Therefore, it can be said that PGPR application increases the amount of chlorophyll especially in high lime content. Similarly, Ozdemir and Tangolar (2006) [27] also used Yalova Incisi, a variety of grape, 140 Ru and 1103 P rootstocks, having different lime-resistant characteristics.

As a result of the study, it was determined that the chlorophyll concentration decreases with increasing lime content in leaves. Ghorbanpour *et al*. (2013) [13] stated in the study conducted to determine PGPR effects on the antioxidant enzyme and tropane alkaloid production in black henbane *(Hyoscyamus*

niger) plant under drought stress that PGPR applications have increased chlorophyll a and b contents against drought stress.

The damage occured in cell membranes due to the stress cause the permeation of ions to the medium. Therefore, the high degree of membrane damage indicates that the plant is more susceptible to stress. Tipirdamaz and Ellialtioglu (1997) [35] reported that the protection of the cell integrity in environmental stress conditions is a great importance in ensuring the stress tolerance of the plant. In our study, the highest level of membrane damage (78,607%) was determined in the medium, non inoculated and including the highest rate of lime. This situation has been considered as an indication of that PGPR has effects for alleviation of the lime-sourced stress in inoculated plants. Dhanda and Sethi (2002) [9] also stated that lower levels of the membrane damage index is an important indicator of tolerance against stress. It has been reported in the studies carried out similarly in the corn (Marulanda *et al*., 2010) [24], cucumber (Kang *et al.*, 2014) [14], and bean (Mahmoodi *et al*., 2016) [20] that the level of the membrane damage in plants inoculated with PGPR in a stressed medium is lower.

The osmotic stress causes changes in various cellular components such as amino acids (proline etc.), amines (glycine-betaine, polyamines, etc.), sugar and sugar alcohols (trehalose, mannitol etc.). These metabolites are not active for metabolism under normal conditions and accumulate intensely in the cytoplasm during stress (Chen and Murata, 2002) [6]. The most important metabolites, playing an important role in the turgor pressure and ensuring stabilization in proteins and cellular structure, are proline, glycine betaine, polyamines, sugars and poliols (Yancey *et al*., 1982) [38]. Many plants accumulate proline under stress thus adapting osmotically and protecting plasma membranes (Mansour, 1998) [22]. It was determined in this study, in which the content of proline was examined in this respect that the highest proline content has been obtained from mediums, inoculated with PGRP and containing 10% to 25% lime. It has been indicated in studies carried out with different plants that tolerance against stress increases with increasing amount of proline together with increasing stress. Moreover, performing some applications like PGPR for the growing medium are effective in preventing stress. *Vardharajula* et al. (2011) [36] reported that the amount of proline increases under drought stress in corn, which was even higher in PGPR's applications.

It was determined that proline accumulation increases by application of PGPR to the broad bean plant under drought stress (Ali *et al*., 2013) [2]. Kumari *et al.* (2015) [17], reported in their study performed to determine the effects of PGPR's applications on salinity stress in soybean that the proline content and lipoxygenase enzyme activity increase in PGRP applied plants, thus increasing the tolerance against salt stress. Singh Gusain *et al.* (2015) [31] stated that the proline accumulation increases with PGPR application in drought stress in rice, and high proline accumulation points out a higher tolerance. The highest proline content was reached in the 10th day of stress (about 1.08 times more). Similarly, the effects of PGPR on basil plant (*Ocimum basilicum* L.) in drought stress were investigated, and it was determined that the proline concentration is higher in PGPR applied plants than the control plants (Agami *et al*., 2016) [1]. When plants meet with biotic and abiotic stresses such as pathogenic attack, UV light, temperature, drought, mechanical injury; they promote phenylpropanoid biosynthesis, with the PAL enzyme to protect themselves, and many secondary metabolites are synthesized in this way. Phenolic compounds are one of them, and they are organic substances comprising many compounds containing a benzene ring. Many studies show that phenolic compounds increase in a stressed medium. In this study, whether PGPR have any effect on this aspect has been investigated. As a matter of fact, as mentioned earlier, PGPR are applications that enable plants directly or indirectly to be less affected from the stressed medium. It is seen as a result of our study that the highest total phenolic content (4,087 mg/g GAE) was determined in the mediums, inoculated with PGPR and containing 25% lime where the highest lime concentration occurs. Similarly, Singh *et al*. (2003) [32], revealed that PGPR and *Sclerotium rolfsii* applications increase the accumulation of phenolic compounds in chickpea. Lavania *et al*. (2006) [18], performed a study in pepper *(Piper betle L.)* to determine the efficiency of PGPR on the root and stem rot disease *(Phytophthora nicotianae).* They reported as a result of the research that PGPR increase the resistance to pathogen introduction promoting the synthesis of phenolic acid. Pejakovic *et al*. (2016) [29] reported that the total phenolic content in the strawberry plants (Fragaria × ananassa Duch.) applied with PGPR 1 and PGPR 2 biovar was higher. It has been reported that isoflavones and total flavonoids are synthesized in higher amounts in soybean plants inoculated with *Azotobacter chroococum*, a PGPR group bacterium (Kiprovski *et al*., 2016) [15].

Plants generally give biochemically and physiologically various reactions when confronted with biotic or abiotic stress that may negatively affect their growth and development. During stress, plants close their stoma, and attempt to provide water use activity in order to reduce water loss. However, the closure of the stoma does not provide the necessary CO_2 fixation. Unused electrons in CO_2 reduction play a role in the reduction of O_2 and cause the formation of free oxygen radicals (Makela *et al*., 1999) [21]. With the effect of these oxygen derivatives, lipids, proteins, and nucleic acids suffer oxidative damage, which cause serious problems in metabolism (Elstner, 1987) [11]. ROS affects membrane lipids, leading to the formation of unsaturated aldehydes and damage to proteins. The final product of lipid peroxidation is MDA and the resulting MDA acts on the ion exchange from the cell membranes, leading to cross-linking of the compounds in the membrane and adverse consequences such as ion permeability and the change of enzyme activity (Niki, 1987) [25]. Hence, lipid peroxidation is considered as an important parameter in measuring stress. In our research, a study was carried out in this regard, and it was determined that the highest value (7.861 µmol/g) is obtained in plants, non inoculated, and in the highest lime concentration. Similar results have also been found in studies carried out on different stress applications and different plant species in this field. Indeed, Singh Gusain *et al*. (2015) [31] reported in the study carried out for determination of the amount of MDA with PGPR application in drought conditions in rice that the amount of MDA in plants subjected to bacterial application, accordingly the stress level is lower.

4. Conclusion

It is known that PGPR are effective in many fields such as biological nitrogen fixation, production of plant hormones such as auxin, gibberellin and cytokinin, the inhibition of

ethylene synthesis by ACC deaminase activity, providing the mineralization of phosphorus compounds, increasing iron uptake through siderophore production, increasing vitamin synthesis and root permeability, and antibiotic production, thus decreasing stress caused by biotic/abiotic factors in the plant (Lemanceau *et al*., 2000; Parmar and Dudarwal, 2000) [19, 28]. In this study, the effectiveness of PGPR for lime application, an abiotic stress, has been intended to be revealed by examining some physical and biochemical properties. It was determined as a result of the research that increasing lime concentrations caused some significant physical and biochemical changes in plant and PGPR applications have been effective in reducing the effectiveness of stress induced by lime. As well as studies, intending to determine effects of PGPR on plants, growing in different stressed mediums, have continued; new bacteria have also been included in this group. Further studies intending to determine the effectiveness of PGPR should be conducted in every aspect, and the resistance against stress factors that threaten plant production and increase day by day should be increased.

5. References

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