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Influence of arbuscular mycorrhizae and plant growth promoting rhizobacteria on proline content, membrane permeability and growth of strawberry (*Fragaria* × *ananassa* Duch.) under salt stress

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Summary

Salinity is one of the most important factors negatively effecting the yield in crop species. In this study the effect of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizae on proline content, membrane permeability and growth of strawberry cv. 'San Andreas' were studied under different salt treatments (0, 30 and 60 mM/L NaCl). The leaf area was measured 0, 15, 30, 45 and 60 days after saline solution applications on the plants. The results showed that increasing concentrations of NaCl decreased all growth parameters. Increased salt concentration led to increased proline level compared to the control. Bacterial application at 60 mM/L NaCl concentration provided the highest ameliorative effect and therefore determined the most effective protection of the plant against salt stress. It was observed that the anthocyanin content increased in line with the increasing salt concentration. In general, the salt applied on the plants causes an increase in membrane permeability and thus disrupts membrane stability and becomes a significant factor damaging the plant. Membrane permeability increased at applications with 30 mM/L and 60 mM/L NaCl. Our results revealed that bacteria application can have an ameliorative effect that helps the plant to tolerate the negative effects of salt stress by increasing proline and anthocyanin levels.

Introduction

Strawberries (*Fragaria* × *ananassa* Duch.) dominate the world berry production and are cultivated in Europe, Asia, North and South America with a big commercial importance (Alkan Torun et al., 2014). The total strawberry production of the world is more than 4.516.000 tons with USA taking the first places as highest strawberry producing country (1.367.000 tons), followed by Mexico (360.426 tons), and Turkey (353.173 tons), respectively) (FAO, 2012).

Strawberry cultivation is suitable for both greenhouse and open field condition and it has high capability to adapt to diverse ecologic conditions. Strawberries are relatively low salt tolerant plants and one of the main problem for their cultivation is salinity that restrict plant growth (Keutgen and Pawelzik, 2009). In particular in greenhouse conditions, salinization is serious problem due to the fact that a certain area of space is used continuously and intensively with intense use of salt included fertilizers. It is reported that soil and water salinity decrease the usefulness of ground water and thus affect plant growth negatively (Tanji, 1990).

One of the major effects of salinity on plants is the ethylene accumulation in their roots, which decrease root growth and finally reduce the yield of crops. PGPRs are able to produce ACC-deaminase in plants rhizosphere and they can consume pre-produced ethylene (ACC) and convert it to α-ketobutyrate and ammonium, so they are able to reduce ethylene level in plants and hence, increase their growth (Penrose and Glick, 2003). Arbuscular mycorrhizal (AM) fungi were reported by several researchers as enhancer of root systems and they are known to support stronger, healthier, higher-yielding plants through increased nutrient acquisition (Miller et al., 2010), reduce levels of water stress (Auge, 2001), and increase phytohormone production (Shaul-Keinan et al., 2002). Hence, mycorrhizal plants are able to increase their tolerance to salinity stress due to their high soil exploration conferred by their hypha structure and growth (Miransari et al., 2008).

Anthocyanins are a large class of water soluble pigments in the flavonoid group found in all plant tissues, are largely responsible for coloration in higher plants (Iwashina, 2000). These substances accumulate in different plant tissues under the influence of various environmental stimuli (Gould et al., 2000). Previously, it is reported that anthocyanins in plant tissue is increased with elevated salt stress (Chalker-Scott, 1999).

It is known that under salt stress conditions, plants increase cellular osmotic pressure by producing secondary metabolites, various chemicals and particularly stress proteins (such as proline, etc.), and thus they sustain their existence by balancing the high osmotic pressure that emerges in the nutrient medium. Physiological reactions of plant species cultivated in media with increasing salinity vary and to avoid negative effects of the osmotic pressure due to increasing salinity, plants increase their proline content. Plants and species capable of generating more proline are more likely to resist the stress and grow healthily (Edreva, 1998).

In this study, the ameliorative effects of some rhizobacteria and mychorriza applications on growth and the biochemical changes of strawberry plants under salt stress were investigated.

Material and methods

Plant material and site description

The day-neutral strawberry cultivar 'San Andreas' was used in this study. First class health cold-stored frigo plants (seedlings) were planted in pots (25 × 18 × 20 cm) filled with perlite: turf media (1:1) (Sahin et al., 2002) at May 6th 2013. The experiment was established with 1 cultivar × 3 salt concentrations (0, 30, 60 mM/L NaCl) × 4 treatments (control, mychorriza, bacteria and mychorizza + bacteria) × 3 repetitions × 20 seedlings in each repetition = 720 seedlings in factorial randomized block design. The experiment was conducted at the Bozok University Gedikhasan experimental farm.

Bacterial application

The bacteria used in the study were isolated from rhizosphere soils of a total 56 tea growing orchards located different agroclimatic regions in Rize and Trabzon provinces in Black Sea region in Turkey and were identificated according to their fatty acid methyl esters (FAMEs) analysis conducted in Sherlock Microbial Identification System and confirmed with BIOLOG system. Among bacteria

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isolated two bacteria of them (*Bacillus cereus RCP 3/1 + Rhizobium radiobacter RCR 11/2*) were selected for their ability to grow in a saline culture medium (10% sodium chloride (NaCl). The phosphate dissolving and nitrogen fixing capacities of bacteria were determined previously (Tab. 1) (CAKMAKCI et al., 2010). For this experiment, the bacterial strains were grown on nutrient agar. A single colony was transferred to 250-mL flasks containing nutrient broth and grown aerobically in flasks on a rotating shaker (95 rpm) for 24 h at 27 °C. Inoculation of bacterial treatments was performed using a dipping method in which plant roots in celled trays were inoculated with the bacterial suspensions of the concentration of 10^8 colony-forming units/mL in sterile water for 30 min before planting. Control plants were dipped into sterile water.

Mychorriza application

In mychorriza application, preparates in commercial powder form containing 9 different Glomus fungi were used. In the preparation fungi types and ratios were; G*lomus intraradices* (21%), *Glomus aggregatum (*20%), *Glomus mosseage (*20%), *Glomus clarum (*1%), *Glomus monosporus* (1%), *Glomus deserticola (*1%), G*lomus brasilianum (*1%), *Glomus etunicatum (*1%), and *Gigaspora margarita (*1%). Mychorrizal applications were performed by inoculating the plant roots by fungi in the solution prepared by mixing 10-liter water and 250 gr powder in packages before planting.

Salt application

40 days after planting (when 3-4 leaves were formed, 17.06.2013) salt solution including 0, 30 and 60 mM/L NaCl were applied with an amount of 100 ml, 2 times a week. Also, in order to ensure plants are nourished, 100 gram of 12-2-14 fertilizer solution (11.7% nitrate, 0.3% ammonium, 2% phosphoric acid, 14% potassium, 6% calcium, 3% magnesium and trace elements) per pot was applied three times a week.

Growth parameters

Growth parameters were measured after plants rooted out on 0, 15th, 30th, 45th and 60th days after salt application on the plants. Leaves surface area (LSA) were measured in cm^2 by ADC BioScientific Area Meter AM300. The roots and shoot dry weight (RDW and SDW) were determined in a drying cabin immediately after their fresh weights were determined. They were kept in drying cabin until their weights did not change at 65 °C and then, their weights were measured. Weighing operations were performed by balances sensitive to 0.001 g.

Proline analysis

Proline content was determined according to BATES et al. (1973). Leaves were ground and homogenized in sulfosalicylic acid. The homogenate was filtered through filter paper. After the addition of ninhydrin reagent, the mixture was heated to 100 °C for one hour. The reaction was then stopped in ice. The mixture was extracted with 4 ml toluene, and the sample was vigorously shaken for 15 s. Sample absorbance of the toluene layer was read at 520 nm. Proline concentration was determined by using a calibration curve and expressed as μM per 100 mg fresh weight (FW).

Anthocyanin content

The ACM-200 plus Anthocyanin Content Meter provides a fast estimate of anthocyanin content on the intact leaves of plants. The non-destructive technique allows researchers to monitor anthocyanin without the costly and time consuming extraction. For each plant, measurements were taken at four locations on each leaf; two on each side of the mid rib on all fully expanded leaves and then averaged (KHAN et al., 2003).

Electrolyte leakage (membrane permeability)

For measurement of electrolyte leakage, 10 leaf discs (10 mm in diameter) from the young fully expanded leaves from two plants per replicate were placed in 50 mL glass vials, rinsed with distilled water to remove electrolytes released during leaf disc excision. Vials were then filled with 30 mL of distilled water and allowed to stand in the dark for 24 h at room temperature. Electrical conductivity (EC1) of the bathing solution was determined at the end of the incubation period. Vials were heated in a temperature-controlled water bath at 95 ºC for 20 min and then cooled to room temperature and the electrical conductivity (EC2) was again measured. Electrolyte leakage (membrane permeability – MP) was calculated as a percentage of EC1/EC2 (SHI et al., 2006).

Data analysis

The experiment was arranged in a randomized blocks in factorial design with three replications. Data were tested by SPSS 20.0 for Windows program. The differences between the means were compared using the Duncan test ($P < 5\%$).

Results and discussion

Salt stress is complex and has toxic effects on plants and lead to metabolic changes (LATRACH et al., 2014). Strawberry is considered as a NaCl salinity sensitive species and it has been shown to reduce number of runners, runner length, leaf number, fresh and dry root weight (SAIED et al., 2005).

Within the scope of our study, it was determined that effects of salt concentrations, applications, and uprooting days on leaf area are statistically significant ($p < 0.05$, Fig. 1). Leaf areas were observed to decrease with the increasing concentrations of salt. While the leaf area with 60 mM/L NaCl concentration was determined to be 33.37 cm^2, with 0 mM/L NaCl concentration leaf area was measured as 38.43 cm^2. In terms of applications, it was observed that mycorrhiza and bacteria applications have positive impact on leaf area.

Tab. 1: Laboratory test results on the used isolates

MIS Results	Oxidase Test	Catalase Test	N-free media development	Sucrose Test	NBRIP-BPB media development	Amylase Test
Bacillus cereus RCP3/1	-	W+	S+	-	S+	W+
Rhizobium radiobacter 11/2	S+	+	S+	-	+	-

S+ = Strong positive; W+ = weak positive

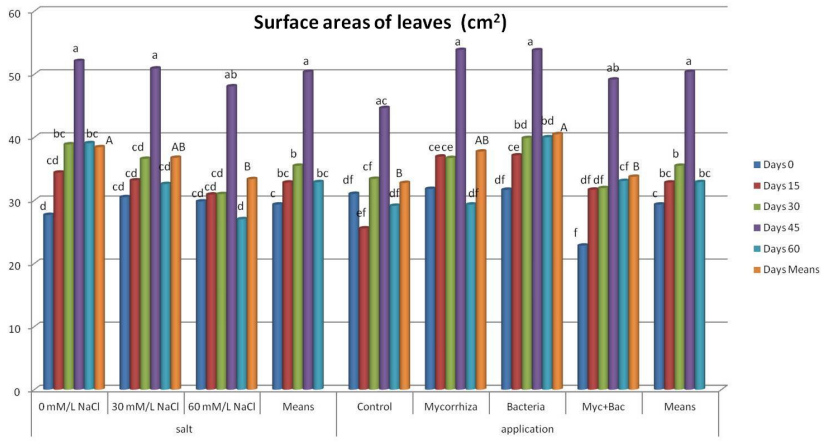

Fig. 1: Surface areas of leaves of strawberry 'San Andreas' plants under salt (0, 30 and 60 mM/L NaCl) stress and applications (control, mycorrhiza, bacteria and myc+bac) for the periods of 0, 15, 30, 45 and 60 days.

While leaf area increased up to 40.50 cm^2 in plants that were subjected to bacteria application, the plants of the control group, had an average leaf area of 32.76 cm^2. In terms of uprooting days, while the largest leaf area was determined at the plants uprooted on the 45th day, it was observed that leaf areas decrease at the uprootings of the 60th day (Fig. 1). In salt × application interaction while the largest leaf area was measured at plants with 30 mM/L NaCl concentration and bacteria application, the smallest areas were determined at the plants subjected to Mycorrhiza+Bacteria combined application (Tab. 2). Bacteria application at 0 mM/L NaCl concentration on the plants uprooted on the 45th day gave the highest average leaf area value (Fig. 1). Salinity stress is reduction in the rate of leaf surface expansion leading to cessation of expansion as salt concentration increases (WANG and NIL, 2000). Salt stress also results in a considerable decrease in the dry weights of leaves, stems, and roots (CHARTZOULAKIS and KLAPAKI, 2000). While the effects of salt concentration, applications and salt × application interaction were determined to be statistically insignificant on offshoot dry weight, the effects of uprooting dates and day × salt × application interaction on the same were determined to be significant. Examining the uprooting dates showed that the average shoot dry weight reached its highest value on the plants uprooted on the 45th day, while the weight started to decrease on the uprootings of the 60th day (Tab. 3). As for the day × salt × application interaction, mycorrhiza+bacteria application at 0 mM/L NaCl concentration on the plants uprooted on the 45th day gave the highest average value (Tab. 3).

It was further determined that the applications, uprooting dates and the day × salt × application interaction had significant effects in terms of root dry weight. While the control and bacteria application caused an increase in root dry weight, mycorrhiza and myc+bac application caused a decrease. In terms of uprooting days, the uprootings of the 30th and the 45th days gave the highest root dry weight (Fig. 3). As for the day × salt × application interaction, control application at 30 mM/L NaCl concentration on the plants uprooted on the 30th day gave the highest average dry root weight (Tab. 3). Shoot dry weight, and root dry weight of strawberry plants were lower at salt stress treatment as compared to non-saline conditions ($p < 0.05$). Similar result has been shown (SAIED et al., 2005). Previous study indicating that the use of mycorrhiza and PGPR that produce ACC (1-aminocyclopropane-1-carboxylate) deaminase (by *Glomus intraradices*) enhanced phytoremediation in saline soils, and the study also showed that the use of PGPR and/or mycorrhiza increase the volume of plants (CHANG, 2007). In another study *Pseudomonas mendocina* Palleroni alone or in combination with either *Glomus intraradices* (Schenk & Smith) or *Glomus mosseae* (Nicol & Gerd) applied on lettuce and revealed that the plants inoculated with *P. mendocina* had higher offshot volume than the control plants at both salinity levels, and that mycorrhiza applications increased offshoot volume only at

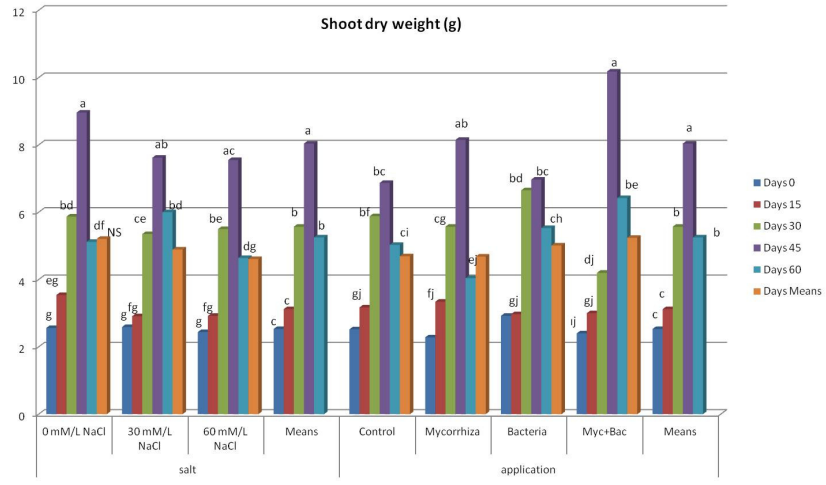

Fig. 2: Shoot dry weights of strawberry 'San Andreas' plants under salt (0, 30 and 60 mM/L NaCl) stress and applications (control, mycorrhiza, bacteria and myc+bac) for the periods of 0, 15, 30, 45 and 60 days.

Tab. 2: Surface areas of leaves, shoot and root dry weights, proline, anthocyanins and membrane permeability in the leaf tissues of strawberry 'San Andreas' plants under salt (0, 30 and 60 mM/L NaCl) stress and applications (control, mycorrhiza, bacteria and myc+bac).

Salt	Applications				
	Control	Mycorrhiza	Bacteria	Myc+Bac	Means
Surface areas of leaves (cm^2)					
0 mM/L NaCl	34,35 ac	40,55 ac	42,62 ab	36,18 ac	38,43 a
30 mM/L NaCl	31,52 bc	39,83 ac	44,71 a	30,95 c	36,75 ab
60 mM/L NaCl	32,40 bc	32,83 bc	34,16 ac	34,09 ac	33,37 b
Means	32,76 b	37,74 ab	40,50 a	33,74 b	
Shoot dry weights (g)					
0 mM/L NaCl	5,15 ^{NS}	5,12	5,08	5,48	5,21
30 mM/L NaCl	4,64	4,37	5,44	5,13	4,90
60 mM/L NaCl	4,30	4,54	4,50	5,11	4,61
Means	4,70	4,68	5,01	5,24	
Root dry weights (g)					
0 mM/L NaCl	3,37 ^{NS}	3,26	3,57	2,86	3,27
30 mM/L NaCl	3,89	2,69	3,29	3,09	3,24
60 mM/L NaCl	3,42	2,82	2,76	2,78	2,95
Means	3,56	2,92	3,21	2,91	
Proline (µmol/100 mg)					
0 mM/L NaCl	0,582 df	0,558 ef	0,627 df	0,541 f	0,577 c
30 mM/L NaCl	0,835 b-f	0,776 c-f	1,139 b	0,685 c-f	0,858 b
60 mM/L NaCl	1,023 bc	0,933 b-d	1,459 a	0,912 b-e	1,082 a
Means	0,813 b	0,756 b	1,075 a	0,713 b	
Anthocyanins					
0 mM/L NaCl	8,364 cd	7,597 d	8,735 b-d	7,373 d	8,017 c
30 mM/L NaCl	10,013 a-d	9,653 b-d	10,454 a-d	8,871 b-d	9,748 b
60 mM/L NaCl	12,297 ab	11,746 a-c	13,543 a	9,336 b-d	11,731 a
Means	10,224 b	9,665 b	10,911 a	8,527 c	
Membrane Permeability					
0 mM/L NaCl	9,217 bc	8,886 cd	8,538 d	8,496 d	8,784 b
30 mM/L NaCl	9,563 ab	9,349 b	9,438 b	9,262 bc	9,403 a
60 mM/L NaCl	9,914 a	9,404	9,372 b	9,419 b	9,527 a
Means	9,565 a	9,213 b	9,116 b	9,059 b	

*Values within by the same letter are not significantly different at $P < 0.05$ by Duncan
^{NS} Not significant

medium level of salinity (KOHLER et al., 2009).

It is known that under salt stress conditions plants increase cellular osmotic pressure by producing secondary metabolites, various chemicals and particularly stress proteins (such as proline, etc.), and thus they sustain their existence by balancing the high osmotic pressure that emerges in the nutrient medium (EDREVA, 1998).

In present study, the effects of salt concentrations, applications, uprooting days, the interaction between salt and application, and the interaction between day, salt and application were determined to be statistically significant ($p < 0.05$) on proline content. Salt application, in general, caused an increase in the proline content of plants. While the proline content was 1.082 µmol/100 mg at 60 mM/L NaCl concentration, it was determined to be 0.577 µmol/100 mg at 0 mM/L NaCl. Examining the effects of the applications showed that the bacteria application caused the proline content to reach its maximum value. In terms of uprooting days, on the other hand, the highest proline content was determined to be in the first uprooting day, and it decreased on the following uprooting days (Fig. 4). In salt × application interaction, bacteria application at 60 mM/L NaCl concentration provided the highest proline content as for the day × salt × application interaction, bacteria application at 60 mM/L NaCl concentration on the plants uprooted on the 45th day gave the highest average value (Tab. 2, 4). Similarly, a study conducted on tomato (AZIZ et al., 1999) reported that introducing salt concentrations increases plants' proline content. In another study were used six NaCl levels (0, 25, 50, 75, 100 and 150 mM) and two strawberry cultivars, 'Camarosa' and 'Albino'. Elevated salinity level significantly increased leaf proline content of both cultivars. 'Albino' leaves accumulated higher proline compared with 'Camarosa' at salinized and non-salinized treatments (AL-SHORAFA et al., 2014). KEUTGEN

Tab. 3: Surface areas of leaves, shoot and root dry weights in the leaf tissues of strawberry 'San Andreas' plants under salt (0, 30 and 60 mM/L NaCl) stress and applications (control, mycorrhiza, bacteria and myc+bac) for the periods of 0, 15, 30, 45 and 60 days.

Salt	Applications	0	15	30	45	60
Surface areas of leaves (cm^2)						
0 mM/lt NaCl	Control	25,340 f-j	29,510 d-j	29,730 d-j	45,067 a-h	42,097 a-j
	Mycorrhiza	29,160 d-j	44,253 a-i	44,180 a-i	53,780 a-c	31,353 c-j
	Bacteria	31,497 c-j	30,613 c-j	44,873 a-h	60,527 a	45,603 a-h
	Myc+Bac	24,743 g-j	33,230 c-j	36,737 b-j	48,980 a-f	37,230 b-j
30 mM/lt NaCl	Control	30,693 c-j	20,933 ij	33,873 c-j	46,507 a-h	25,580 f-j
	Mycorrhiza	36,733 b-j	35,127 b-j	32,297 c-j	57,847 ab	37,123 b-j
	Bacteria	31,500 c-j	50,647 a-e	49,973 a-e	52,543 a-d	38,890 a-j
	Myc+Bac	23,103 h-j	25,980 f-j	30,203 c-j	46,737 a-h	28,747 e-j
60 mM/lt NaCl	Control	37,043 b-j	26,237 f-j	36,620 b-j	42,360 a-j	19,757 j
	Mycorrhiza	29,563 d-j	31,437 c-j	33,673 c-j	49,920 a-e	19,533 j
	Bacteria	32,057 c-j	30,117 d-j	24,793 g-j	48,327 a-g	35,513 b-j
	Myc+Bac	20,643 ij	35,900 b-j	28,903 d-j	51,730 a-e	33,273 c-j
Shoot dry weights (g)						
0 mM/lt NaCl	Control	1,817 kl	3,237 e-l	6,577 b-l	8,283 b-e	5,850 b-l
	Mycorrhiza	2,283 h-l	3,810 c-l	6,907 b-k	8,353 b-d	4,243 b-l
	Bacteria	2,793 g-l	4,263 b-l	5,540 b-l	5,860 b-l	6,920 b-k
	Myc+Bac	3,350 d-l	2,840 g-l	4,440 b-l	13,330 a	3,460 d-l
30 mM/lt NaCl	Control	2,947 g-l	3,557 d-l	6,300 b-l	5,763 b-l	4,613 b-l
	Mycorrhiza	2,470 h-l	2,300 h-l	3,810 c-l	8,847 bc	4,443 b-l
	Bacteria	3,207 e-l	2,730 g-l	7,063 b-j	7,693 b-g	6,520 b-l
	Myc+Bac	1,720 l	3,053 g-l	4,237 b-l	8,177 b-f	8,437 b-d
60 mM/lt NaCl	Control	2,800 g-l	2,717 g-l	4,767 b-l	6,570 b-l	4,623 b-l
	Mycorrhiza	2,077 j-l	3,923 c-l	5,983 b-l	7,257 b-i	3,437 d-l
	Bacteria	2,750 g-l	1,920 kl	7,337 b-h	7,343 b-h	3,140 f-l
	Myc+Bac	2,143 i-l	3,110 f-l	3,910 c-l	9,033 b	7,360 b-h
Root dry weights (g)						
0 mM/lt NaCl	Control	1,510 g	3,353 b-g	4,220 b-g	4,290 b-g	3,477 b-g
	Mycorrhiza	1,627 fg	5,683 ab	3,970 b-g	2,547 c-g	2,493 d-g
	Bacteria	2,070 d-g	4,647 a-e	3,983 b-g	2,853 b-g	4,283 b-g
	Myc+Bac	2,583 c-g	2,113 d-g	2,480 d-g	5,450 a-c	1,650 fg
30 mM/lt NaCl	Control	1,573 g	4,037 b-g	7,130 a	2,777 b-g	3,950 b-g
	Mycorrhiza	2,520 c-g	1,693 e-g	2,463 d-g	3,730 b-g	3,023 b-g
	Bacteria	2,897 b-g	2,983 b-g	3,927 b-g	3,730 b-g	2,930 b-g
	Myc+Bac	1,627 fg	2,823 b-g	3,263 b-g	4,077 b-g	3,667 b-g
60 mM/lt NaCl	Control	2,643 c-g	3,937 b-g	4,540 a-f	3,187 b-g	2,773 b-g
	Mycorrhiza	1,827 e-g	2,203 d-g	4,160 b-g	2,957 b-g	2,940 b-g
	Bacteria	2,007 d-g	2,513 c-g	4,250 b-g	3,117 b-g	1,917 e-g
	Myc+Bac	1,797 e-g	1,673 fg	2,547 c-g	4,880 a-d	3,013 b-g

*Values within by the same letter are not significantly different at $P < 0.05$ by Duncan

and PAWELZİK (2009) reported that free amino acids and proline accumulated in salt conditions in cultivars, 'Elsanta' and 'Korona', but the latter revealed higher free amino acid content. However, higher accumulation of proline has been linked with better osmotic adjustment and, consequently, higher adaptation to salt conditions (SAIED et al., 2005). In strawberry cultivars, a dramatic accumulation of proline following salt stress was observed (KEUTGEN and PAWELZIK, 2009).

The effects of salt concentrations, applications, uprooting days, the interaction between salt and application, and the interaction between day, salt and application were determined to be statistically significant ($p > 0.05$) on anthocyanin level. It was observed that the anthocya-

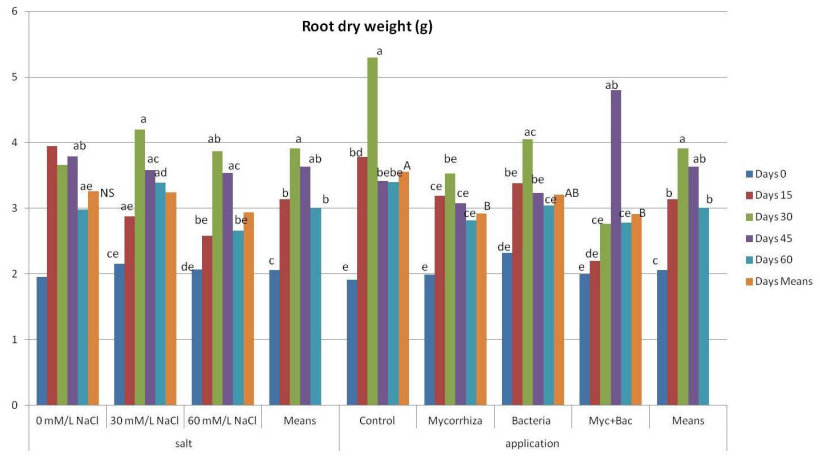

Fig. 3: Root dry weights of strawberry 'San Andreas' plants under salt (0, 30 and 60 mM/L NaCl) stress and applications (control, mycorrhiza, bacteria and myc+bac) for the periods of 0, 15, 30, 45 and 60 days.

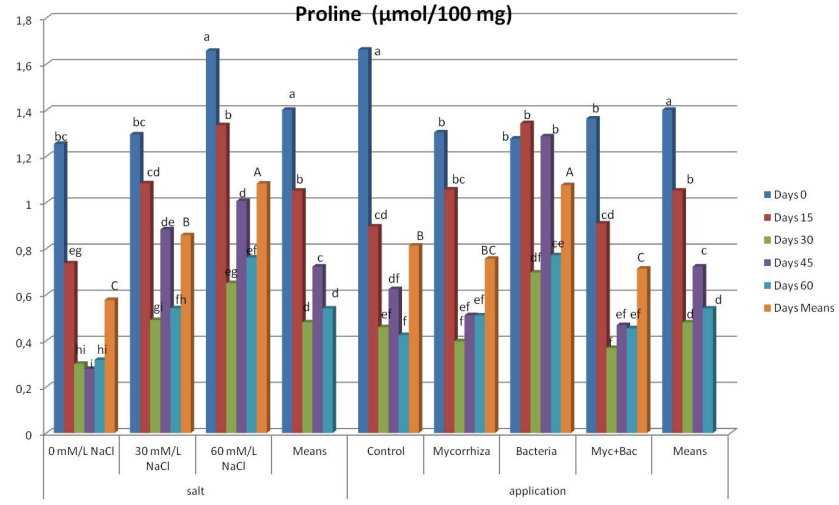

Fig. 4: Proline in the leaf tissues of strawberry cv. 'San Andreas' plants under salt (0, 30 and 60 mM/L NaCl) stress and applications (control, mycorrhiza, bacteria and myc+bac) for the periods of 0, 15, 30, 45 and 60 days.

nin content increased in line with the increasing salt concentration. Anthocyanin content increased in plants subjected to bacterial application. On the other hand, the 60th day uprooting was determined to be the uprooting with the highest content of anthocyanin (Fig. 5). In terms of salt × application interaction, it was determined that the anthocyanin content was high at 30 mM/L NaCl concentration with control and bacteria applications and at 60 mM/L NaCl concentration with control, mycorrhiza and bacteria applications. Anthocyanin content was determined to be rather low at both salt concentrations with the application of mycorrhiza and bacteria (Tab. 2). Concerning the interaction between uprooting days, salt concentrations and implemented applications, high levels of anthocyanin were determined in plants subjected to bacteria application made on the plants uprooted on the 60th day with 30 mM/L NaCl concentration, and on those that were subjected to control, mycorrhiza and bacteria applications made on the plants uprooted on the 60th day with 60 mM/L NaCl concentration (Tab. 4). KEUTGEN and PAWELZIK (2009) reported that salt stress increased the contents of anthocyanins in fruits of both cvs and the highest increase of 94 % occurred in cv. 'Elsanta' at 40 mmol NaCl/l.

Membrane permeability is an important indicator that points out to membrane stability. A low level of membrane permeability is important for the robustness of the membrane. In general terms, the salt applied on the plants causes an increase in membrane permeability thus disrupts membrane stability and becomes a significant factor damaging the plant. In studies conducted on strawberry, it was determined that application of salt increases membrane permeability (KAYA et al., 2003). In addition, subjecting plants under salt stress to bacteria application caused a decrease in membrane permeability and thus helped in the maintenance of membrane stability. In our study, the effects of salt concentrations, applications, uprooting days, the interaction between salt and application, and the interaction between day, salt and application were determined to be statistically significant ($p < 0.05$) on membrane permeability. Membrane permeability increased at applications with 30 mM/L and 60 mM/L NaCl concentrations. Among the applications, it was determined that the membrane permeability of the control application increased and in other mycorrhiza, bacteria and myc+bac applications it was lower. It was observed that membrane permeability increased in the uprootings of the 15th, 45th and 60th days (Fig. 6). In salt × application interaction, membrane permeability was determined to be high in plants subjected to control application at 30 mM/L and 60 mM/L NaCl concentrations. The lowest interaction value was measured with bacteria and myc+bac applications at 0 mM/L NaCl concentration (Tab. 2). Concerning the interaction between uprooting days, salt concentrations and applications, it was determined that the control application

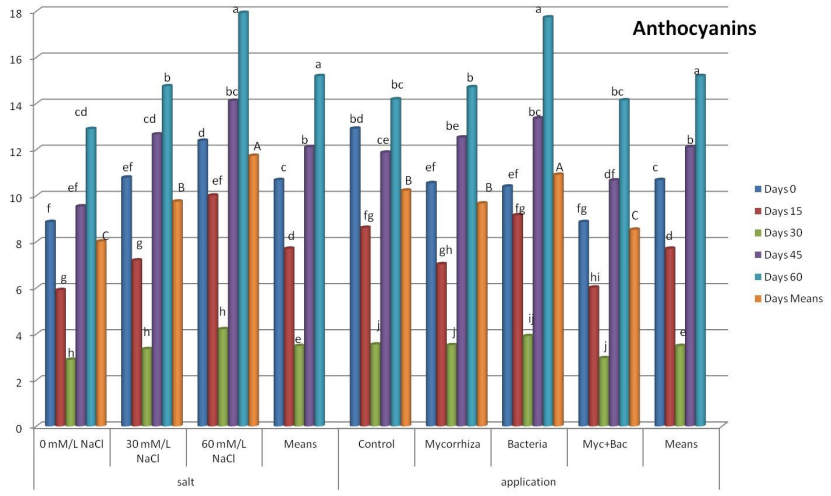

Fig. 5: Anthocyanins in the leaf tissues of strawberry cv. 'San Andreas' plants under salt (0, 30 and 60 mM/L NaCl) stress and applications (control, mycorrhiza, bacteria and myc+bac) for the periods of 0, 15, 30, 45 and 60 days.

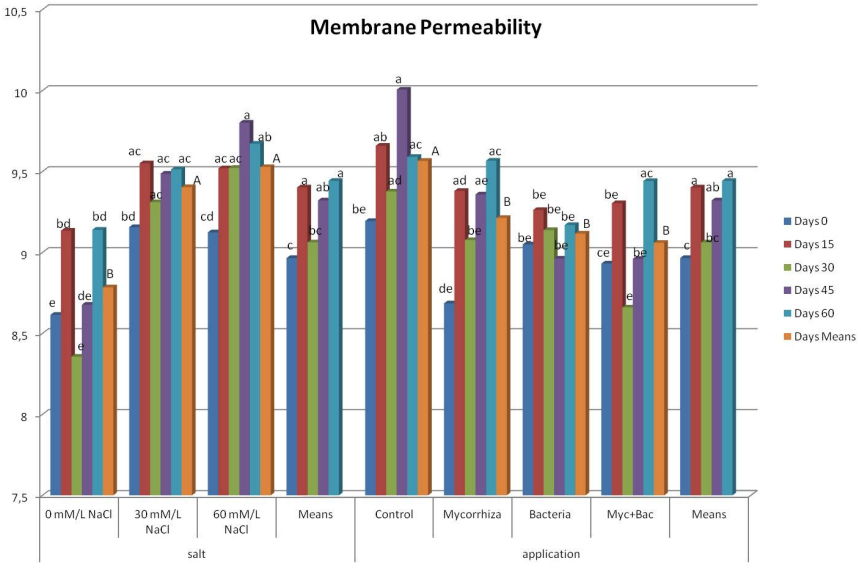

Fig. 6: Membrane permeability in the leaf tissues of strawberry cv. 'San Andreas' plants under salt (0, 30 and 60 mM/L NaCl) stress and applications (control, mycorrhiza, bacteria and myc+bac) for the periods of 0, 15, 30, 45 and 60 days.

was statistically different ($p < 0.05$) at 60 mM/L NaCl concentration on the plants uprooted on the 45th day (Tab. 4).

Conclusions

Our study demonstrates that under saline conditions, proline, anthocyanins and membrane permeability increase in strawberry leaves, while LSA decreases. The mycorrhiza and bacteria applications carried out during planting caused an increase in the leaf area of plants under salt stress. Bacteria application was determined to be the prominent application that helps the plant to tolerate the negative effects of salt stress by increasing proline and anthocyanin levels.

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Tab. 4: Proline, anthocyanins and membrane permeability in the leaf tissues of strawberry cv. 'San Andreas' plants under salt (0, 30 and 60 mM/L NaCl) stress and applications (control, mycorrhiza, bacteria and myc+bac) for the periods of 0, 15, 30, 45 and 60 days.

Salt	Applications	0	15	30	45	60
Proline (µmol/100 mg)						
0 mM/lt NaCl	Control	1,536 b-e	0,746 m-t	0,315 w-z	0,157 z	0,156 z
	Mycorrhiza	1,209 f-i	0,725 n-t	0,188 yz	0,323 v-z	0,345 u-z
	Bacteria	1,105 h-k	0,775 l-s	0,538 r-x	0,276 x-z	0,442 t-z
	Myc+Bac	1,165 g-j	0,696 n-t	0,160 z	0,355 u-z	0,327 v-z
30 mM/lt NaCl	Control	1,534 b-e	0,897 j-p	0,462 t-z	0,842 k-r	0,439 t-z
	Mycorrhiza	1,252 e-h	1,024 h-m	0,523 s-x	0,563 r-x	0,515 s-x
	Bacteria	1,232 f-h	1,442 d-g	0,634 o-v	1,636 b-d	0,748 m-t
	Myc+Bac	1,159 g-j	0,969 h-n	0,341 u-z	0,492 s-y	0,462 t-z
60 mM/lt NaCl	Control	1,920 a	1,045 h-l	0,598 p-w	0,873 j-q	0,680 n-t
	Mycorrhiza	1,450 d-g	1,418 d-g	0,484 s-y	0,646 o-u	0,668 n-t
	Bacteria	1,494 c-f	1,814 ab	0,915 i-o	1,951 a	1,124 h-k
	Myc+Bac	1,768 a-c	1,062 j-l	0,605 p-w	0,555 r-x	0,573 q-x
Anthocyanins						
0 mM/lt NaCl	Control	11,470 e-k	7,643 o-u	3,557 y- β	8,627 l-s	10,523 g-m
	Mycorrhiza	7,457 o-v	4,970 v-β	2,487 β	9,970 i-o	13,100 c-g
	Bacteria	8,627 l-s	5,753 t-y	2,873 z β	11,003 e-m	15,417 bc
	Myc+Bac	7,890 n-t	5,257 u-z	2,633 β	8,547 l-s	12,537 d-h
30 mM/lt NaCl	Control	12,657 d-h	8,437 m-s	3,217 y- β	12,587 d-h	13,167 c-f
	Mycorrhiza	10,720 f-m	7,147 p-v	3,577 y- β	13,427 c-e	13,397 c-e
	Bacteria	10,457 h-n	6,970 q-w	3,487 y- β	13,530 c-e	17,827 a
	Myc+Bac	9,327 k-q	6,217 s-x	3,110 z β	11,097 e-l	14,607 cd
60 mM/lt NaCl	Control	14,610 cd	9,743 j-p	3,870 x- β	14,400 cd	18,860 a
	Mycorrhiza	13,460 c-e	8,970 k-r	4,487 w- β	14,183 cd	17,630 ab
	Bacteria	12,110 d-j	14,740 cd	5,370 t-z	15,533 bc	19,963 a
	Myc+Bac	9,360 k-q	6,577 r-w	3,120 z β	12,347 d-i	15,277 bc
Membrane permeability						
0 mM/lt NaCl	Control	8,918 b-g	9,265 b-f	9,150 b-f	9,378 b-f	9,375 b-f
	Mycorrhiza	7,913 hi	9,113 b-g	8,633 d-h	9,222 b-f	9,548 b-d
	Bacteria	8,808 c-h	9,070 b-g	8,396 f-h	7,964 hi	8,455 e-h
	Myc+Bac	8,819 c-h	9,088 b-g	7,244 i	8,146 gh	9,184 b-f
30 mM/lt NaCl	Control	9,369 b-f	9,813 bc	9,421 b-e	9,626 b-d	9,584 b-d
	Mycorrhiza	9,081 b-g	9,679 bc	9,114 b-g	9,397 b-f	9,476 b-e
	Bacteria	9,202 b-f	9,436 b-e	9,575 b-d	9,467 b-e	9,509 b-d
	Myc+Bac	8,971 b-g	9,272 b-f	9,129 b-g	9,455 b-e	9,484 b-d
60 mM/lt NaCl	Control	9,295 b-f	9,898 b	9,556 b-d	11,012 a	9,811 bc
	Mycorrhiza	9,062 b-g	9,347 b-f	9,481 b-d	9,457 b-e	9,674 bc
	Bacteria	9,136 b-f	9,280 b-f	9,442 b-e	9,456 b-e	9,544 b-d
	Myc+Bac	9,004 b-g	9,551 b-d	9,608 b-d	9,279 b-f	9,655 b-d

*Values within by the same letter are not significantly different at $P < 0.05$ by Duncan

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