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Original article

Alleviative effects of chitosan and ascorbic acid on *Petunia* × *atkinsiana* D. Don under salinity

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Summary

The genus *Petunia* of the family *Solanaceae* is one of the most valuable ornamental and floriculture crops. Therefore, it is crucial to determine the salt tolerance of this popular bedding plant during greenhouse production. The objective of this study was to evaluate the effect of 20 ppm chitosan and 1 mM ascorbic acid in response to 100 mM NaCl of petunia plants. The salinity of the irrigation water inhibits the elongation of shoot and root, affects the production of fresh and dry mass as well as the water contents of the studied species of *Petunia* compared to the control. Application of chitosan or ascorbic acid stimulated the shoot and root length as well as the fresh and dry masses of petunia under saline condition, but they did not cause any effect on the number of new shoots and roots. The induced salinity stress significantly decreased chlorophylls and carotenoid contents, while it increased the proline concentration. Whereas, no significant differences were found in MDA values after the application of chitosan or ascorbic acid under saline or non-saline condition. Irrigation of ascorbic acid under non-saline and saline condition increased chlorophylls significantly and decreased the proline content. The effects of chitosan or ascorbic acid in mitigating the adverse effect of salt stress pave the way to gain the insight into the potential role of these biologically active substances to improve the salt tolerance of petunia plants.

Keywords
ASA, chitosan, greenhouse, salt stress, *Solanaceae* spp.

Significance of this study

What is already known on this subject?

- Tolerance to the different abiotic stress and yield stability are complex genetic traits that are difficult to establish in many crops. Chitosan is a biopolymer with multiple applications in plant breeding and is completely safe for the environment. Ascorbic acid is a small molecule with antioxidant properties.

What are the new findings?

- It was observed that the application of 20 ppm of chitosan (8 kDa) as well as 1 mM of ascorbic acid under saline condition in *in vitro* culture could enhance the morphological and biochemical parameters in petunia plants.

What is the expected impact on horticulture?

- The research should encourage further investigations to protect petunia plants against soil salinity and improve their commercial production.

Introduction

Salinity is one of the major stress factors. It hampers the crop yield worldwide, because it reduces the germination percentage of seeds, early plant growth, and the development (Villarino and Mattson, 2011; Jabeen and Ahmad, 2013; Sen and Mandal, 2016; Moura et al., 2017; Salachna et al., 2017). The tolerance of crops to salinity is varying among species, plant genotypes, growth phase, types of salts, climatic condition, and the intensity of the stress (Niu and Cabrera, 2010). Salinity has a negative impact on the photosynthesis and the activity of antioxidant enzymes (Fercha et al., 2011; Jabeen and Ahmad, 2013). Moreover, salt stress can cause an imbalance in the uptake of mineral nutrients as well as phytotoxicity for excessive accumulation of Na^+ and Cl^- in the tissues (Fornes et al., 2007; Niu and Cabrera, 2010). The range of the adverse effect of salinity depends on the level of salt concentration and the time of exposure of the plant to this stress factor. Salt tolerance is often assessed based on the growth rate, flowering, and aesthetic value, or from the field reduction of specific plant parts such as seeds, roots, fruits, or leaves (Niu and Cabrera, 2010). The more the symptoms of the stress on the plant, the lower its tolerance to salinity will be. The salinity is caused by the inorganic chemicals and intensive fertilization practiced almost throughout the entire cultivation period, the accumulation of ballast compounds due to the use of low-grade fertilizers or the use of incorrect water for irrigation (Villarino and Mattson, 2011). Because of the high value of both ornamentals and floriculture species, it is crucial to determine the salt tolerance of popular bedding plants during greenhouse production.

Proper selection of tolerant genotypes from cultivated plants depends on the availability of a verified assessment method. The conventional plant breeding strategy for overcoming the negative effects of salt stress has still limited success (Rai et al., 2010). One of the possible strategies for plants at a specific level of salinity is the application of plant growth-regulating compounds that could alleviate the salt stress effect. The most characterized stress hormone is abscisic acid (ABA), which is involved in pivotal physiological responses required for salt stress adaptation, including ion and water homeostasis. In response to salt stress, other hormones, such as gibberellins (GA) must coordinately interact

with ABA and other stress metabolites (Maggio et al., 2010). According to many authors (Tuna et al., 2008; Fercha et al., 2011; Azooz et al., 2013; Krupa-Małkiewicz et al., 2015), the use of vitamins (such as ascorbic acid) or phytohormones (such as gibberellic acid or indole-3-acetic acid) as antioxidants has mediated the salt tolerance as well as a driving force for the improvement of resistance and the adaptation to salt stress. According to Agami (2014), ascorbic acid (ASA) is one of the commonly used effective compounds that were able to ameliorate the abiotic stresses. Ascorbic acid is a small, water-soluble molecule with antioxidant properties. Ascorbate acts as a primary substrate in the cyclic pathway for detoxification and neutralization of superoxide radicals and the singlet oxygen. ASA plays multiple roles in plant growth, such as cell division, cell wall expansion, and other developmental processes (Dolatabadian et al., 2008; Fercha et al., 2011; Bybordi, 2012; Saeidi-Sar et al., 2013; Krupa-Małkiewicz et al., 2015).

On the other hand, the role of chitosan under salinity stress is crucial in modulating physiological responses that will eventually lead to the adaptation to an unfavorable environment (Sen and Mandal, 2016). Chitosan is a biopolymer that is completely safe for the environment. This compound is a derivative of chitin and is characterized by biodegradability, bioactivity, and biocompatibility properties (Kowalski et al., 2006). According to many authors (Kowalski et al., 2006; Jabeen and Ahmad, 2013; Pongprayonn et al., 2013), chitosan may influence on the production of substances related to stress response, such as phytoalexins and chitinases. Chitosan treatments also influence the peroxidase activity, plant growth, and health in numerous crops.

Petunia × *atkinsiana* D. Don 'Prism White' belongs to *Solanaceae* family. It is commonly flowering ornamental plants, largely produced for home-consuming and for gardening (Fornes et al., 2007). Moreover, *Petunia hybrida* is often considered as a model plant for studding plant growth and development (Villarino and Mattson, 2011; Vandenbussche et al., 2016). Due to the important role of the *Solanaceae* family in agronomic and ornamental crops, holistic-scale approaches have been used to determine salt tolerance in this family. In this study, we would like to find out if the application of ascorbic acid and chitosan could alleviate the salt stress condition on growth and biochemical parameters of petunia plants. The ultimate purpose of our research is to provide an alternative treatment for preventing salt stress damage to petunia plants under greenhouse conditions.

Materials and methods

Experimental procedures

A greenhouse experiment was conducted at the West Pomeranian University of Technology Szczecin, Poland, located at the geographic coordinates of 53°26'84"N, 14°31'79"E. Plants of *Petunia* × *atkinsiana* D. Don 'Prism White' were derived from an *in vitro* culture. Explants were initially selected under the salinity condition in an *in vitro* culture (Krupa-Małkiewicz and Fornal, 2018) and then made adaptive to *in viv*o conditions in the second half of April 2016. The plantlets with well-developed shoots and roots were transferred into round black plastic pots of the diameter of 9 cm, filled with soil (deacidified peat + soil) at pH 5.5 and electrical conductivity (EC) under 1.5 mS cm^{-1} mixed with vermiculate in 1:1 ratio. The pots were placed on 60-cm high tables in the greenhouse at 20°C/15°C day/night temperature cycles and light intensity of 110 mol m^{-2} s^{-1} during the experimental period. The temperature inside the greenhouse was controlled by vents that opened automatically. After 10 days of the transplantation, the plants were irrigated with a solution of pure 99.9% sodium chloride (NaCl) (Chempur, Poland), ascorbic acid (ASA) (Stanlab, Poland), and chitosan (Center of Bioimmobilisation and Innovative Packaging Materials at West Pomeranian University of Technology, Szczecin, Poland). Chitosan was obtained according to Bartkowiak (2001). The petunia plants were irrigated in 100 mL per pot of the following solutions: 100 mM NaCl, 1 mM ascorbic acid and 20 ppm chitosan of molecular weight 8 kDa, with five different combinations (100 mM NaCl, 100 mM NaCl + 1 mM ASA, 100 mM NaCl + 20 ppm chitosan, 1 mM ASA, 20 ppm chitosan). Control plants were irrigated by 100 mL distilled water. Each treatment was repeated five times at weekly intervals. Before each treatment, the electrical conductivity (EC) of the soil was measured. The measurements were performed with electrical conductivity meter CC-411 (Elmetron, Poland), in aqueous extract (substrate:distilled water 1:2). The petunia plants were grown until six weeks. On the last day of the experiment, plants were removed from the pots, rinsed in tap water, and the morphological parameters were determined: shoot and root length (cm), number of new shoots per plant, number of roots. The plants were weighted for estimation of plant fresh mass (g) and then dried at 70°C for 48 h. The dried plants were weighted to record a plant dry mass (g).

Estimation of proline and MDA contents

The concentration of proline was determined according to the method of Bates et al. (1973). Leaf material (0.5 g) of young fully expanded leaves from each replicate were ground in 1.5 mL of 3% (w/v) aqueous sulfosalicylic acid. Proline was estimated by using ninhydrin reagent. The concentration of proline was calculated from a standard curve and expressed as μmol g^{-1} fresh mass. Content of the malondialdehyde (MDA) in plant tissue was determined by the method described by Sudhakar et al. (2001). The concentration of MDA was calculated from the absorbance at 600, 532 and 450 nm, while MDA contents were determined using the following Equation 1:

$$\text{MDA } (\mu\text{mol g}^{-1}\text{ fm}) = [6.45 \times (A_{532} - A_{600}) - 0.56\ A_{450}] \times V/fm \quad (1)$$

where: *V*, volume of the sample; *A*, absorbance; *fm*, fresh mass.

Determination of pigments

Leaves were sampled from the mid-section of each plant, in order to minimize age effect on the variability of results. Individual leaves were removed and then weighed to obtain fresh mass (fm). The levels of chlorophyll *a*, *b* and carotenoid (Car) were measured in 80% acetone extracts. Chlorophyll *a*, *b,* and carotenoid content were determined spectrophotometrically at 663, 645 and 440 nm. The concentration of Chl *a* and Chl *b* were calculated according to Arnon et al. (1956) with the modification of Lichtenthaler and Wellburn (1983) from Equations 2 and 3, respectively, derived by Hendry and Grime (1993).

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{ fm)} = [(12.7\ A_{663} - 2.69\ A_{645})/\ 1{,}000 \times fm] \times V \quad (2)$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{ fm)} = [(22.9\ A_{645} - 4.68\ A_{663})/\ 1{,}000 \times fm] \times V \quad (3)$$

Carotenoid content was determined by the Equation 4 of Price and Henry (1991).

$$\text{Carotenoid (mg g}^{-1}\text{ fm)} = [(A_{480} + 0.114\ A_{663}) - (0.638\ A_{663}) \times V/112.5 \times fm] \quad (4)$$

where: *V*, volume of the sample; *A*, absorbance; *fm*, fresh mass.

Statistical analysis

The experiment was designed as completely randomized with 80 plants, 20 plants per replication. The results were statistically evaluated using the Statistica v. 12 software. The significance of differences was determined by means of variance analysis (ANOVA) and Tukey's test, at the level of significance of $P \leq 0.05$. Proline, MDA, Chl *a*, Chl *b* and Car were measured in triplicates for each experimental combination.

Results and discussion

Effects of NaCl, ASA and chitosan and on the electric conductivity of the substrate and morphological traits of petunia

During the control treatment, EC remained relatively stable throughout the experiment, and it increased across the weekly periods for the added salt (Figure 1). The application of ascorbic acid and chitosan reduced the EC level in the soil. The reduced EC level of soil leachates from ASA or chitosan plants might be related to the induction of antioxidant responses that protect the plant from oxidative damage. Conversely, pH remained stable across the treatment period and averaged 6.0 (data not shown).

The influence of stress on plant growth is often determined on the basis of the shoot and root measurements. There are doubts whether roots or shoots are more sensitive to salinity. The statistical analyzes of growth parameters (Figure 2, Table 1) in this study, revealed a significant effect of NaCl salt on the shoot and root length and the number of roots. But, it was observed that neither NaCl and ascorbic acid nor chitosan has influenced the number of new shoot per plant.

Irrigated by NaCl salt caused the reduction of shoot and root length by 48% and 33%, respectively, compared with control. In addition, the number of the petunia root was significantly decreased under stress condition (Table 1). Irrigation by ascorbic acid in non-stressed petunia plants decreased the shoot length and the number of roots by 19% and 39%, respectively, compared with control. However, application of chitosan to non-stressed plants was not associated with significant differences, with the exception of the number of roots. Watering the petunia plants with a solution of ascorbic acid or chitosan under stress conditions did not significantly affect the length of the roots and their number. Addition of ascorbic acid or chitosan under saline condition increased the shoot length by 34% and 46%, respectively, compared with that of plants treated with 100 mM NaCl.

Similar reduction in growth performance under salinity stress condition was obtained by Kattab (2007) in canola, Beltagi (2008) in chick pea, Villarino and Mattson (2011) in fourteen floriculture species, Jabeen and Ahmad (2013) in safflower and sunflower, Azooz et al. (2013) in broad bean, Arun et al. (2016) in petunia, Moura et al. (2017) in Passiflora. According to many authors (Beltagi, 2008; Azooz et al., 2013; Mahdavi and Rahimi, 2013), the reduction in growth and development of the plant under high salinity level could be attributed to the toxic effect of NaCl salt stress. In addition, salt stress could induce physiological drought by which plants would not be able to absorb water due to increased ionic concentration in their cells. The result is the deformation of macromolecules by disrupting their shell or bound water (Beltagi, 2008). As reported by Jabeen and Ahmad (2013) and Azooz et al. (2013), chitosan or vitamin treatments, when used in optimal concentration, had stimulation effects on growth parameters under saline stress conditions. Jabeen and Ahmad (2013) reported that irrigation by chitosan reduced detrimental effects of salt stress in both safflower and sunflower. Similar results were found by Mahdavia and Rahimi (2013), who reported that ajowan seeds treated with chitosan showed higher growth than the control under salinity stress. The oligosaccharides act on plants as phytohormone-like compounds in the regulation processes of morphogenesis, growth, and development. According to Guan et al. (2009), chitosan has the ability to increase the water and essential nutrient uptake through adjusting the cell osmotic pressure. On the other hand, beneficial effect of the exogenous application of ascorbic acid in partially mitigating the effects of salt stress in chick pea was described by Beltagi (2008), in durum wheat by Fercha et al. (2011), and in pepper by Mohammed (2013). Such increase of plant growth may be due to ascorbic acid that acts as an antioxi-

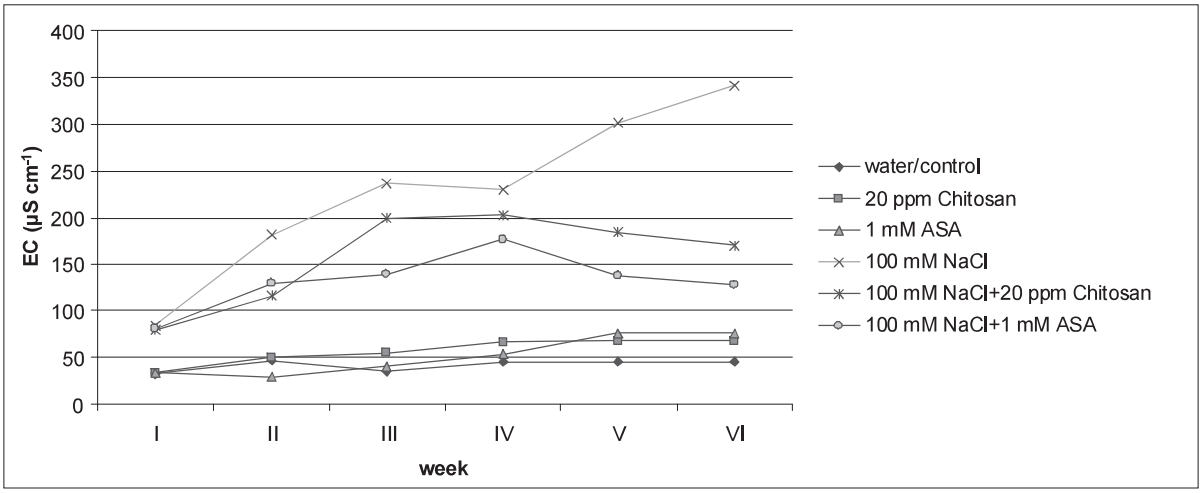


Figure 1. Effect of a different combination of treatment on EC of the medium in every week of experiment.

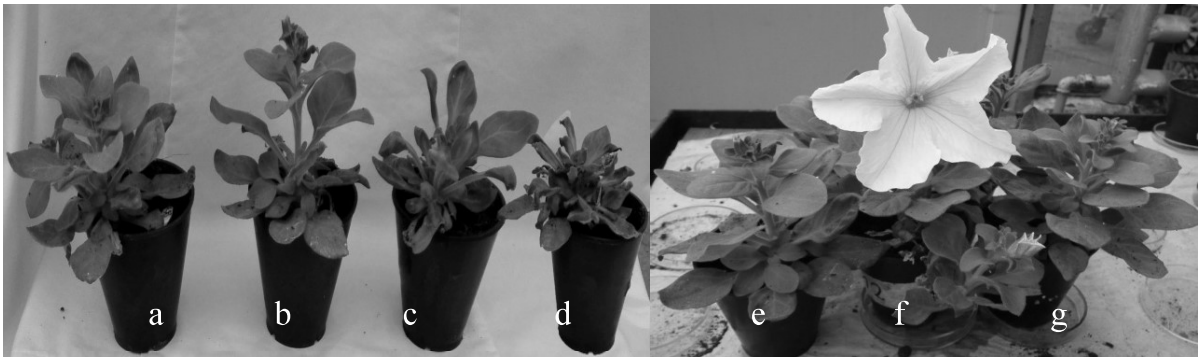


Figure 2. The effect of 20 ppm Chitosan (b), 20 ppm Chitosan + 100 mM NaCl (c), 100 mM NaCl (d), 1 mM ascorbic acid (f), 1 mM ascorbic acid + 100 mM NaCl (g) on growth of petunia plants compared with control (a, e).

Table 1. Effect of chitosan, ascorbic acid and salt treatment on morphological traits of petunia 'Prism White'.

Treatment	Morphological trait						
	Shoot length (cm)	No. of new shoots per plant	Root length (cm)	No. of roots	Total fresh mass (g)	Total dry mass (g)	Plan water contents (%)
0 mM NaCl (control)	27.91a*	2.4a	18.42a	51.67a	11.58a	6.20a	71.32a
20 ppm Chitosan	23.8ab	1.5a	15.75ab	30.67b	10.76a	3.56b	66.91ab
1 mM ASA	22.6b	2.0a	15.17ab	31.50b	7.21b	2.61bc	64.47b
100 mM NaCl	14.5c	1.6a	12.08b	13.0c	4.08c	1.17c	46.45c
20 ppm Chitosan + 100 mM NaCl	21.16b	2.0a	13.42ab	17.67c	6.95bc	2.39bc	65.61ab
1 mM ASA + 100 mM NaCl	19.43b	1.8a	13.17ab	17.0c	5.01bc	1.78bc	63.80b
$LSD_{\alpha\ 0,05}$	5.01	1.07	5.29	11.78	3.08	2.16	7.21

*Means followed by the same letter are not significantly different ($P \leq 0.05$) determined by Tukey's test.

dant and therefore has an effect as plant growth regulators (Mohammed, 2013). According to Wassel et al. (2007), the positive effect of the application of exogenous ascorbic acid on plant growth may be due to its auxinic action as well as its improved role in many metabolic and physiological processes and enhancing the synthesis of carbohydrates.

Applied 100 mM NaCl salt solution decreased fresh and dry mass by 65% and 81%, respectively, as well as plant water contents by 35%, in reference to petunia plants grown under non-saline condition (Table 1). The effects of ascorbic acid or chitosan treatment on fresh and dry mass as well as plant water contents showed that petunia plants under non-saline condition exhibited a significant reduction ($p \leq 0.05$) in their water status in response to control plants. Besides non-stressed plant, there were no significant differences in the fresh mass between the chitosan and control treatments. However, the use of ascorbic acid or chitosan alleviated the toxic effect of NaCl on the fresh and dry masses. Reduction in plant water content under salinity stress may be attributed to decreased water uptake due to low substrate water potential, or to injury of the root system (Piwowarczyk et al., 2016).

According to Munns (2005), the evaluation of plant dry mass should be one of the assessment points of the salinity stress impact on the plants. In many cases, salinity stress decreased the fresh and dry mass as well as the plant water content in the plants. Therefore, according to Piwowarczyk et al. (2016), improving the tolerance to salinity stress by new research methods should be associated with the increased or unchanged dry weight content in plant from stressed and unstressed conditions. Similar reduction in fresh and dry mass as well as plant water contents was found in some other plants under saline condition (Beltagi, 2008; Fercha et al., 2011; Jabeen and Ahmad, 2013; Azooz et al., 2013; Mahdavi and Rahimi, 2013; Moura et al., 2017). According to Mahdavi and Rahimi (2013), the interaction between the salinity and 0.2% chitosan treatment in ajowan plants significantly affected the dry weight of the shoot and the relative water content. However, Beltagi (2008) observed that the addition of 4 mM ascorbic acid significantly increased chick pea stem dry mass at low level of NaCl (20 mM). In addition, chick pea stem fresh mass, and the root dry mass were increased at 40 mM NaCl salt. In summary, Zeng et al. (2012) described an excellent property of chitosan that helps forming films on the seed surface, which consequently can promote the seed germination under stress condition. While according to Fercha et al. (2011) and Azooz et al. (2013), ascorbic acid induces the improvement of membrane integrity, which may have contributed in reducing the ion leakage and water loss in plant cell.

Effects of NaCl, ASA and chitosan on chlorophylls and carotenoid contents

The application of 100 mM NaCl salt significantly decreased the concentration of chlorophylls (Chl *a* and Chl *b* by 13% and 37%, respectively), and carotenoid (by 41%), in comparison with the control (Table 2). Concentrations of chlorophylls (Chl *a* and Chl *b*) of petunia significantly increased (by 22% and 70%, respectively) after application of 1 mM ascorbic acid in non-saline conditions as compared to their control (Table 2). These parameters in petunia plants were not affected by 20 ppm chitosan under unstressed conditions. In addition, treatment with ascorbic acid or chitosan did not influence the carotenoid concentration under non-saline condition.

Table 2. Effect of chitosan, ascorbic acid and salt treatment on chlorophylls *a* and *b*, and carotenoid content in petunia 'Prism White' leaves.

Treatment	Chlorophyll *a* (µg g^{-1} fm)	Chlorophyll *b* (µg g^{-1} fm)	Carotenoid (µg g^{-1} fm)
0 mM NaCl (control)	44.60b*	10.81b	20.21a
20 ppm Chitosan	46.80b	11.14b	20.93a
1 mM ASA	54.57a	18.33a	22.13a
100 mM NaCl	38.88c	6.80d	11.92c
20 ppm Chitosan + 100 mM NaCl	39.20c	6.84d	13.80bc
1 mM ASA + 100 mM NaCl	40.08c	9.22c	15.41b
$LSD_{\alpha\ 0.05}$	2.67	1.40	2.21

*Means followed by the same letter are not significantly different ($P \leq 0.05$) determined by Tukey's test.

The negative effects of salinity stress on photosynthetic (Chl *a* and Chl *b*) pigments and carotenoid was observed by Niu et al. (2007) in *Lantana montevidensis* (Spreng.) Briq., Beltagi (2008) in chick pea, Azooz et al. (2013) in broad bean, Krupa-Małkiewicz et al. (2015) in tomato, Arun et al. (2016) in petunia, Salachna et al. (2017) in curly kale. According to the mentioned authors a large amount of salt affects the photosynthetic apparatus, negatively, and exerts the inhibition of chlorophyll biosynthesis or increase of its degradation by chlorophyllase, which is more active under salinity stress. Moreover, the degradation of chlorophyll can increase the amount of reactive oxygen species (ROS), which are highly reactive, and may cause cellular damage. According to Dzung et al. (2011), chitosan oligomer of 60 ppm was optimal to the increased content of chlorophylls of coffee seedlings in greenhouse and field experiment. The same results were also shown on other plants. Dzung (2005) reported that 2 kDa chitosan oligomer at a concentration of 30 ppm increased the content of chlorophylls of soybean and peanut by 17.9% and 23%, respectively. However, Limpanavech et al. (2008) showed that chitosan with a molecular weight of 45 kDa significantly increased the chloroplast diameter of *Dendrobium* orchid at the concentration of 10–50 ppm in young leaves and at 50 ppm in old leaves. In this study, the application of chitosan with the molecular weight of 8 kDa at the concentration of 20 ppm did not influence on chlorophylls and carotenoid contents under non-saline or saline conditions.

The inhibitory effect of salinity stress on photosynthetic pigments was partially or completely ameliorated as a result of ascorbic acid treatments (Beltagi, 2008; Dolatabadian et al., 2008; Bybordi, 2012; Azooz et al., 2013; Krupa-Małkiewicz et al., 2015). According to the abovementioned authors, ascorbic acid acts as a detoxifier and neutralizer of superoxide radicals and other singlet oxygen species. Moreover, ascorbic acid prevents the activation of free radicals, which can enhance the content of chlorophylls.

Effects of NaCl, ASA and chitosan on proline and MDA contents

The survival of plants growing under environmental stress conditions depends on the mechanism of osmotic adjustment. Such a mechanism might be manifested by the accumulation of inorganic ions and/or organic compounds (Piwowarczyk et al., 2016). According to many authors (Dolatabadian et al., 2008; Bybordi, 2012; Jabeen and Ahmad, 2013; Azooz et al., 2013; Krupa-Małkiewicz et al., 2015), proline accumulation is one of the most frequently reported modifications induced by environmental stresses. Plants under stress condition accumulated an excessive amount of proline, which results from its uncontrolled biosynthesis, limited oxidation, inhibition or its incorporation into proteins, and even the release of proteins due to proteolysis (Azooz et al., 2013). In the presented study, proline accumulation in petunia plants increased by 48%, after application of 100 mM NaCl in comparison with the plants grown under non-saline condition. While, the treatment of 1 mM ascorbic acid or 20 ppm chitosan under non-saline condition did not influence the proline contents in petunia 'Prism White' plants, relative to the control when no ascorbic acid or no chitosan were applied (Table 3). Application of 1 mM ascorbic acid or 20 ppm chitosan in this experiment alleviated the negative effect of NaCl. The highest decrease in the proline concentration (by 13%) under saline condition was observed after irrigated in ASA, compared to the plants treated with 100 mM NaCl.

The results obtained in this study confirmed earlier findings (Fercha et al., 2011; Azooz et al., 2013; Krupa-Małkiewicz et al., 2015). In view of the effect of ascorbic acid on proline content, ASA probably improved the growth of stressed plants. Fercha et al. (2011) reported that the application of ascorbic acid was effective in mitigating the adverse effect of salt stress on plant growth of durum wheat due to enhanced proline accumulation. However, Jabeen and Ahmad (2013) observed that proline content in stressed seedlings of safflower and sunflower decreased at low concentration of chitosan (0.25%), while increased levels of proline were observed at high chitosan concentration (0.75%).

Leaf (MDA) malondialdehyde accumulation, the product of lipid peroxidation, is a prominent indicator of membrane impairment in plants exposed to saline regimes (Jabeen and Ahmad, 2013). Malondialdehyde accumulation of petunia plants was increased by 49% after application of 100 mM NaCl compared with the control (when no salt was applied).

Table 3. Effect of chitosan, ascorbic acid and salt treatment on proline and malondialdehyde (MDA) content in petunia 'Prism White' leaves.

Treatment	Proline (µmol g^{-1} fm)	MDA (nmol g^{-1} fm)
0 mM NaCl (control)	4.45c*	7.09b
20 ppm Chitosan	4.83c	7.17b
1 mM ASA	5.38c	7.57b
100 mM NaCl	6.59a	10.56a
20 ppm Chitosan + 100 mM NaCl	6.04b	7.81b
1 mM ASA + 100 mM NaCl	5.71bc	7.65b
$LSD_{\alpha\ 0.05}$	0.44	0.58

*Means followed by the same letter are not significantly different ($P \leq 0.05$) determined by Tukey's test.

However, after application of 1 mM ascorbic acid or 20 ppm chitosan under non-saline and saline condition the accumulation of MDA was compared with the control (Table 3).

Jabeen and Ahmad (2013) showed that the application of chitosan under unstressed condition significantly decreased MDA content compared to the control. Moreover, the decline of the MDA level at low concentrations of chitosan (0.25%), whether or not seedlings were stressed, suggested that seed priming with chitosan reduced lipid peroxidation. This is due, that chitosan could retard lipid oxidation by chelating metal ions or by combining with lipids. Chitosan could alleviate membrane lipid peroxidation and ultra-structure phytotoxicities and protect cells from stress (Jabeen and Ahmed, 2013). Similar to the obtained results, Dolatabadian et al. (2008) showed that the application of ASA to non-stressed plants resulted in no significant differences in MDA content compared with the control. Rise in MDA level after the application of NaCl alone was also found in alfalfa (Hu et al., 2006), canola (Dolatabadian et al., 2008), and tomato (Krupa-Małkiewicz et al., 2015). According to the above-mentioned authors, the accumulation of MDA under stress condition may be related to the higher activities of antioxidative enzymes. Salinity may cause oxidative damage of the membrane system of plants, which follows from an increase in ROS (reactive oxygen form).

Conclusion

Considering the results, it seems that the application of ascorbic acid as well as chitosan under saline condition could enhance the shoot and root length of petunia 'Prism White'. The biologically active substances used in this study can ameliorate the harmful effect of salinity on biochemical parameters, such as proline, chlorophylls and carotenoid contents in petunia plants. The increase of tolerance to salinity stress by chitosan may be due to its influence on the relative water content, and by ascorbic acid may be due to the enhanced antioxidant capacity, and may also be through the restoration of the hormone balance, which have to be verified in the future study.

Thus, the findings of this study suggested that treatment with exogenous ASA or chitosan could protect petunia plants against soil salinity and improve their commercial production.

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