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Influences of ascorbic acid and gibberellic acid in alleviating effects of salinity in *Petunia* under *in vitro*

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Abstract. Salinity is one of the abiotic stresses that limits the growth and productivity of many crops. A possible survival strategy for plant under saline conditions is to use compounds that could minimize the harmful effects of salt stress on the plant development. The objective of the presented study was to investigate the effect of exogenous ascorbic acid (ASA) with or without gibberellic acid (GA3) on key growth and biochemical parameters in two petunia cultivars 'Prism Rose' and 'Prism White' under saline (150 mM NaCl) and non-saline in vitro condition. Nodal cutting with an axillary buds were used as explants. Application of 1 mM ascorbic acid with or without 0.05 mM gibberellic acid into the MS medium stimulated the length of shoots and the number of new shoots of 'Prism Rose'; whereas, it decreased the root length and the number of roots of both 'Prism Rose' and 'Prism White' under non-saline condition. The addition of ascorbic acid with or without gibberellic acid into the MS medium under saline condition, increased the length of plants and the number of new shoots, but did not affect their root number and length. NaCl treatments increased the proline content and lipid peroxidation which was indicated by the accumulation of malondialdehyde (MDA). The study revealed a correlation between chlorophylls a and b content and the leaf pigmentation intensity – parameter a*. Addition of 1 mM ascorbic acid with 0.05 mM gibberellic acid into the MS medium plays a protective role in salinity tolerance by improving the shoot growth and the development as well as increasing the activities of the antioxidant enzymes and other antioxidant substances.

Keywords: AsA; GA3; Micropropagation; Petunia x atkinsiana D. Don; Salinity.

INTRODUCTION

Salt accumulation (Na^+ ions, in particular) is one of the main environmental factors limiting the soil fertility and the plant production (Azooz et al., 2013; Agami, 2014). The increase in osmotic pressure of plant induced by high saline concentration disturbs processes of nutrient uptake from the soil. The reaction of a plant to the salinity stress depends greatly on the genotype and the development stage of the plant. The complexity of interactions between stress factors and various molecular, biochemical and physiological reactions affects the plant growth and development (Hamdia & Shaddad, 2010; Khalid et al., 2015). The salinity stress has negative effects on fundamental metabolic processes occurring in a plant such as photosynthesis, lipid metabolism and protein synthesis (Agami, 2014). Large amounts of (Na^+ and Cl^- ions) NaCl salt can be accumulated in chloroplasts and cause destabilization of protein complexes and damage of photosynthetic pigments (Nandy et al., 2007). Furthermore, the negative effects of salinity stress on photosynthetic pigments could be due to the inhibition of chlorophyll biosynthesis or increase of its degradation by chlorophyllase, which is more active under salinity stress (Khan et al., 2011; Azooz et al., 2013). Therefore, the induction of salt tolerance in plants is important to sustain their economic yield (Agami, 2014). According to many authors (Tuna et al., 2008; Hamdia & Shaddad, 2010; Khan 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, indole-3-acetic acid or kinetin) as antioxidants mediated the salt tolerance as a selection factor and as a driving force for the improvement of resistance and the adaption to salt stress. Ascorbic acid (AsA) is an organic acid with antioxidant properties. The protective role of AsA in plant cells from the adverse effects of salinity stress was described by Younis et

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al. (2010) in *Vicia faba* seedlings, Bybordi (2012) in canola, Krupa-Małkiewicz et al. (2015) in tomato seedlings. For example, Bybordi (2012) reported that the application of ascorbic acid improved photosynthesis and germination of canola seed as well as mitigated the antioxidant enzyme activity. Thus, it could be hypothesized that exogenous AsA can scavenge the salt-induced reactive oxygen species, decrease activities of antioxidant enzymes, and thus enhance the ability of plant to resist salt stress.

During the action of stress factor toward a plant, a decline in the endogenous growth regulators concentration occurs very often (Tuna et al., 2008; Hamdia & Shaddad, 2010; Agami, 2014). Gibberellins (GAs) are phytohormones that regulate and influence various developmental processes, including seed germination, stem elongation, flowering, sex expression and fruit development (Li et al., 2010). According to Tuna et al. (2008) and Maggio et al. (2010), the decreased cytokinin and gibberellic acid (GA_3) and the increased abscisic acid (ABA) contents observed in plants are critical in stress adaptation and/or survival.

The cultivars of petunia used in this study are common flowering ornamental plants, largely produced for home-consumption and for gardening. Moreover, petunia is a model plant for studding plant development, due to various favourable biological features (Berenschot et al., 2008). The present paper reports the effects of exogenous application of 1 mM AsA with or without 0.05 mM GA_3 on morphological and some biochemical parameters of petunia (*Petunia x atkinsiana* D. Don) 'Prism Rose' and 'Prism White' shoots growth under saline (150 mM NaCl) and non-saline *in vitro* conditions.

MATERIAL AND METHODS

Plant material and growth conditions. The research material consisted of 15–20 mm stem nodes with an axillaries bud of *Petunia* x *atkinsiana* D. Don 'Prism Rose' and 'Prism White' obtained from sterile stabilized *in vitro* culture. The explants were transferred to MS medium according to Murashige & Skoog (1962) composition of vitamins, macro- and microelements. MS medium were supplemented with sodium chloride (0, 150 mM) either with or without 1 mM AsA or 0.05 mM GA_3, or 1 mM AsA + 0.05 mM GA_3. Each combination included 48 shoots (6 shoots per flask in eight replication). All cultures were incubated in growth room at a temperature of 24 ± 2 °C under 16 hours photoperiod with a photosynthetic photon flux density (PPFD) of 40 μmol/m^2/s^1 provided by Narva (Germany) emitting daylight cool white. All media contained 30 g/dm^3 sucrose (Chempur, Poland) and 100 mg/dm^3 myo-inositol (Duchefa, The Netherlands) and were solidified with 8 g/dm^3 agar (Biocorp, Poland), pH of the media was adjusted to 5.7. The media were heated and 30 ml were poured into 450 ml flask and next they were autoclaved at 121 °C (0.1 MPa) during the time required according to the volume of medium in the vessel. After the end of the experimental period (four weeks), explants were removed and washed with deionized distilled water, and the lengths of the shoots and roots were measured, and the number of new shoot and roots were counted. The plants were weighed for estimation of plant fresh mass and then dried at 70 °C for 48 hours. The dried plants were weighed to record a plant dry mass.

Biochemical analyses. The concentration of proline was determined according to the method of Bates et al. (1973). Content of the malondialdehyde (MDA) in plant tissue was determined by the method described by Sudhakar et al. (2001). The concentration of chlorophylls *a* and *b* were calculated according to Arnon et al. (1956) in modification to Lichtenthaler & Wellburn (1983) derived by Hendry & Grime (1993). Carotenoid content was determined according to Price & Henry (1991).

Leaf pigmentation intensity. The leaves (from the middle part of the shoot) pigments measurement was carried out using spectrophotometer CM-700d (Konica Minolta, Japan). Measurements were made in CIE L*a*b* system (Hunterlab, 2012). The 10° observer type and D65 illuminant was applied. Colour was measured in triplicates for each experimental combination.

Statistical analysis. Results obtained in *in vitro* cultures were statistically analysed 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 $\alpha < 0.05$. Proline, MDA, Chl *a*, Chl *b* and Car were measured in triplicates for each experimental combination. For comparison of colour parameter a* and Chl *a* or Chl *b* contents, coefficients of correlation were determined.

RESULTS AND DISCUSSION

Effect of AsA, GA_3 and NaCl on morphological traits. In the presented study, the addition of 1 mM AsA, 0.05 mM GA_3 or 1 mM AsA+0.05 mM GA_3 into MS medium under non-saline condition considerably reduced the length and the number of roots in both the *Petunia* cultivars, 'Prism Rose' and 'Prism White' (Table 1). The maximal shoot length (8.42 cm) and the number of new shoots (4.75) of 'Prism Rose' were obtained in plants grown in the MS medium supplemented with 0.05 mM GA_3 (Table 1). Furthermore, the addition of 0.05 mM GA_3 with 1 mM AsA to MS medium under non-saline condition increased the number of new shoots of 'Prism White' by 68.5% in comparison with plants growing in the control MS medium (Table 1). Compared to plants growing on MS medium without NaCl, AsA and GA_3, the shortest roots and the lowest number of roots were observed in petunia 'Prism Rose' grown in the MS medium supplemented with 1 mM AsA with 0.05 mM GA_3 (0.88 cm and 1.33, respectively) and in petunia 'Prism White' grown in the MS medium with the addition of 0.05 mM GA_3 (1.65 cm and 0.96, respectively). Increase in growth parameters of barley after the treatment of the seeds with AsA or proline (in the absence of the NaCl stress) was reported by Agami (2014). Also, Hussein & Alva (2014) indicated that application of 150 ppm AsA increased the height of millet as compared to other plants that were treated with tap water.

Table 1. Effects of 1 mM ASA and 0.05 mM GA3 on morphological traits of petunia 'Prism Rose' and 'Prism White' under non-saline and saline condition.

Trait	Salinity	Medium				
		MS	MS +1 mM ASA	MS + 0.05 mM GA_3	MS + 1 mM ASA + 0.05 mM GA_3	Mean
				'Prism Rose'		
Plant length (cm)	Control	6.31a	6.63a	8.42a	8.21a	**7.39A**
	150 mM NaCl	1.73b	2.23b	2.56b	2.81b	**2.33B**
	Mean	**4.02B**	**4.43B**	**5.49A**	**5.51A**	
No.of new shoot per explant	Control	2.46b	2.25b	4.75a	4.75a	**3.55B**
	150 mM NaCl	1.29b	1.38b	2.08b	1.83b	**1.64A**
	Mean	**1.87A**	**1.81A**	**3.41A**	**3.29A**	
Root length (cm)	Control	9.21a	5.30ab	2.98bc	8.88bc	**4.59A**
	150 mM NaCl	0c	0c	0c	0c	**0B**
	Mean	**4.60A**	**2.65AB**	**1.49AB**	**0.44B**	
No. of roots per explant	Control	7.92a	6.08ab	3.29abc	1.33bc	**4.65A**
	150 mM NaCl	0c	0c	0c	0c	**0B**
	Mean	**3.96A**	**3.04A**	**1.64A**	**0.66A**	
				'Prism White'		
Plant length (cm)	Control	9.10a	7.08ab	5.56b	7.79ab	**7.38A**
	150 mM NaCl	2.17c	2.04c	1.90c	2.56c	**2.16B**
	Mean	**5.63A**	**4.56A**	**3.73A**	**5.17A**	
No.of new shoot per explant	Control	2.67abc	1.92bc	3.38ab	4.50a	**3.11A**
	150 mM NaCl	1.67bc	1.42c	1.79bc	1.88b	**1.69B**
	Mean	**2.17A**	**1.67A**	**2.58A**	**3.19A**	
Root length (cm)	Control	12.92a	7.98b	1.65c	3.21c	**6.44A**
	150 mM NaCl	0.25c	0c	0c	0c	**0.06B**
	Mean	**6.58A**	**3.99AB**	**0.82B**	**1.60A**	
No. of roots	Control	12.00a	6.25b	0.96c	3.13bc	**5.58A**
	150 mM NaCl	0.25c	0c	0c	0c	**0.06B**
	Mean	**6.12A**	**3.12AB**	**0.48B**	**1.56B**	

Abbreviations: MS-Murashige and Skoog (1962) medium, ASA-ascorbic acid; GA3- gibberellic acid. Means in the same column followed by the same small letters are not significantly different among treatments within the same variety; the same capital letters indicate mean values for the main effects.

Addition of 150 mM NaCl solution to the MS medium had an inhibitory effect on the growth of plants in both the petunia cultivars. All plants growing in this medium had significantly lower plant length and smaller number of new shoots (Table 1). In addition, plants growing under saline conditions did not develop roots with an exception of petunia 'Prism White' grown in the MS medium supplemented with 150 mM NaCl. Addition of AsA along with GA_3 into the MS medium under saline conditions increased shoot length by 44.53% in 'Prism Rose' and 28.13% in 'Prism White' as well as the number of new shoots by 74.39% and 70.41%, respectively (Table 1).

Similarly, reduction in growth performance was found in broad bean plants in response to 150 mM NaCl (Azooz et al., 2013). However, Piwowarczyk et al. (2016) noted that the highest NaCl level (200 mM) significantly shortened both shoots and roots of grass pea compared to control conditions. Many studies have reported that vitamins, when used with optimal concentration, exhibited beneficial effect on the growth and yield of some crop plants grown under saline conditions (Khan et al., 2011; Azooz et al., 2013; Agami, 2014; Krupa-Małkiewicz et al., 2015). Treatment with AsA ameliorated the stress generated by NaCl and improved the plant growth and

Table 2. Effects of 1 mM ASA and 0.05 mM GA3 on fresh and dry mass of petunia 'Prism Rose' and 'Prism White' under non-saline and saline condition.

Trait	Salinity	Medium				
		MS	MS +1 mM ASA	MS + 0.05 mM GA_3	MS + 1 mM ASA + 0.05 mM GA_3	Mean
				'Prism Rose'		
Fresh mass per explant (g)	Control	1.56a	1.24abc	1.98a	1.34ab	**1.53A**
	150 mM NaCl	0.37c	0.17c	0.49bc	0.34c	**0.34B**
	Mean	**0.96A**	**0.71A**	**1.23A**	**0.84A**	
Dry mass per explant (g)	Control	0.09a	0.08a	0.11a	0.16a	**0.11A**
	150 mM NaCl	0.03a	0.02a	0.04a	0.03a	**0.03B**
	Mean	**0.06A**	**0.05A**	**0.07A**	**0.09A**	
				'Prism White'		
Fresh mass per explant (g)	Control	2.19a	1.29ab	1.61a	2.11a	**1.80A**
	150 mM NaCl	0.47b	0.48b	0.64b	0.63b	**0.55B**
	Mean	**1.33A**	**0.89A**	**1.13A**	**1.37A**	
Dry mass per explant (g)	Control	1.39a	1.22a	0.63bc	1.08ab	**1.08A**
	150 mM NaCl	0.34c	0.56bc	1.01abc	0.61bc	**0.63B**
	Mean	**0.87A**	**0.89A**	**0.82A**	**0.84A**	

Abbreviations: see Table 1

biochemical parameters in barley (Agami, 2014) and tomato (Krupa-Małkiewicz et al., 2015). According to Khan et al. (2011) and Agami (2014), AsA acts not only as an antioxidant, but also as a cofactor of many enzymatic reactions that allow the neutralization of negative effects of the salinity stress. Ascorbic acid causes an increase in IAA and GA_3 concentrations and stimulates the cellular divisions and/or their elongation and this, in turn, improves the plant growth (Khan 2011). Gonai et al. (2004) and Tuna et al. (2008) reported that the addition of GA_3 to the medium enhances the catabolism of ABA, the concentration of which is increased due to the environmental stress applied to a plant. Whereas, Iqbal & Ashraf (2013) suggested that the beneficial effects of GA_3 can be attributed to its effect on hormonal homeostasis and ionic uptake and partitioning (within shoots and roots) in the salt-stressed plants.

Tuna et al. (2008) found that the measurement of fresh and dry masses under salinity stress in shoots and roots of tested genotypes might be one of the assessment points of the salinity stress impact on the plants. Therefore, it could be assumed that the increased dry weight content in plants from stressed and control conditions could be associated with the increased tolerance to salt stress. In this study, the addition of 1 mM AsA with or without 0.05 mM GA_3 to the MS medium under non-saline condition had no significant effecton fresh and dry masses of both the petunia cultivars (Table 2). However, petunia plants growing under saline condition indicated a significant reduction of fresh and dry masses in comparison to plants grown on MS medium without NaCl, AsA and GA_3. The fresh masses of 'Prism Rose' and 'Prism White' were reduced by 76% and 79%, respectively, and the dry masses by 67% and 76%, respectively, in comparison to petunia plants grown in the MS medium without the addition of NaCl salt. However, the use of ascorbic and gibberellic acids combined under saline condition caused a reduction in the toxic effect of NaCl on the fresh and dry masses. In this case, the role of highest alleviation in the inhibition of the fresh and dry masses of the petunia plants was determined after the use of 0.05 mM GA_3.

A similar negative effect of NaCl salt on the reduction in fresh and dry masses was observed by Bybordi (2012) in canola. The inhibition in plant growth was significantly alleviated by ascorbic acid treatment. Tuna et al. (2008) reported that treatment with gibberellic acid was helpful in enhancing the growth of maize under saline conditions. Addition of 100 ppm GA_3 resulted in increase of dry mass of maize, which was close to the control group.

Effect of ASA, GA_3 and NaCl on proline and MDA contents. In the opinion of many authors (Demir & Kocaçalişkan, 2002; Bybordi, 2012; Krupa-Małkiewicz etal., 2015), elevated proline and MDA levels in plant tissues are quite good indicators of the negative effects of various stress factors of a plant. Excessive proline accumulation occurs as a strong reaction to the environmental stress, which

Table 3. Effects of 1 mM ASA and 0.05 mM GA_3 on proline, MDA, chlorophylls and carotenoid in shoots of petunia 'Prism Rose' and 'Prism White' under non-saline and saline stress.

Trait	Salinity	Medium				
		MS	MS + 1 mM ASA	MS + 0.05 mM GA_3	MS + 1 mM ASA + 0.05 mM GA_3	Mean
		'Prism Rose'				
Proline (μmol g^{-1} fm)	Control	1.69f	2.08f	1.59f	4.17e	**2.38B**
	150mM NaCl	35.69a	20.10c	34.82b	17.83d	**27.11A**
	Mean	**18.69A**	**11.09B**	**18.20A**	**11.0B**	
MDA (nmol g^{-1} fm)	Control	17.10d	10.81e	17.20d	21.39cd	**16.62B**
	150mM NaCl	29.51a	27.95a	25.42ab	23.27bc	**26.54A**
	Mean	**23.3A**	**19.37A**	**21.31A**	**22.33A**	
Chlorophyll *a* (μg g^{-1} fm)	Control	4.05a	3.37b	0.61d	1.20c	**2.30A**
	150mM NaCl	0.54d	0.66d	0.26c	0.62d	**0.52B**
	Mean	**2.29A**	**2.01A**	**0.43C**	**0.90B**	
Chlorophyll *b* (μg g^{-1} fm)	Control	1.44a	1.10b	0.19ab	0.42c	**0.79A**
	150mM NaCl	0.22de	0.28de	0.17e	0.32cd	**0.23B**
	Mean	**0.82A**	**0.70A**	**0.15B**	**0.36B**	
Carotenoid (μg g^{-1} fm)	Control	1.78a	1.50b	0.29d	0.58c	**1.04A**
	150mM NaCl	0.30d	0.36d	0.15e	0.36d	**0.30B**
	Mean	**1.04A**	**0.96A**	**0.22C**	**0.45B**	
		'Prism White'				
Proline (μmol g^{-1} fm)	Control	1.34e	1.65e	1.91e	2.15e	**1.76B**
	150mM NaCl	22.83b	8.42d	28.35a	11.32c	**17.73A**
	Mean	**12.08B**	**5.03D**	**15.13A**	**6.73C**	
MDA (nmol g^{-1} fm)	Control	9.62b	9.94b	9.78b	8.33b	**9.41B**
	150mM NaCl	17.57a	16.98a	16.98a	17.78a	**17.33A**
	Mean	**13.59A**	**13.46A**	**13.38A**	**13.05A**	
Chlorophyll *a* (μg g^{-1} fm)	Control	5.04a	4.74b	1.07d	2.14c	**3.24A**
	150mM NaCl	0.61e	0.30f	0.30f	0.49ef	**0.43B**
	Mean	**2.82A**	**2.51B**	**0.69D**	**1.30C**	
Chlorophyll *b* (μg g^{-1} fm)	Control	1.59a	1.51a	0.37c	0.65b	1.03**A**
	150mM NaCl	0.18d	0.06e	0.08e	0.18d	0.12**B**
	Mean	**0.87A**	**0.78B**	**0.22D**	**0.41C**	
Carotenoid (μg g^{-1} fm)	Control	1.53a	1.56a	0.50cd	0.89bc	1.11**A**
	150mM NaCl	0.32d	0.16d	0.15d	0.23d	0.21**B**
	Mean	**0.92A**	**0.85A**	**0.32B**	**0.55AB**	

Abbreviations: see Table 1

results from its uncontrolled biosynthesis, limited oxidation, inhibition of its incorporation into proteins, and even the release from proteins due to proteolysis. Addition of 1 mM AsA with 0.05 mM GA_3 to the MS medium under non-saline condition significantly increased the proline concentration in petunia 'Prism Rose' by 147% and 'Prism White' by 60% in comparison with that of the control (Table 3). However, the addition of 150 mM NaCl to the MS medium enhanced the proline concentration in petunia 'Prism Rose' to above 2000% and 'Prism White' to above 1600% in comparison with the plants grown in the MS medium without NaCl (Table 3). Application of 1 mM AsA with or without 0.05 mM GA_3 to the MS medium alleviated the negative effect of NaCl. In case of 'Prism Rose' the highest decrease in the proline concentration (by 50%) under saline condition was observed after the addition of 1 mM AsA with 0.05 mM GA_3 to the MS medium, and in 'Prism White' (by 63%) after the addition

of 1 mM AsA to the MS medium, compared with that of plants treated with 150 mM NaCl. Similar rise in proline level was also found in canola (Bybordi, 2012). Proline was accumulated in the leaves of canola plants grown under salinity stress so that the proline concentration increased linearly with increasing salinity levels. In turn, Piwowarczyk et al. (2016) observed that the lower level of salt (50 mM NaCl) caused the greater accumulation of proline in grass pea 'Derek' roots. Results obtained in our study confirm the finding of Azooz et al. (2013), who reported that most vitamins tend to increase the proline content. They also showed that the application of AsA was effective in mitigating the adverse effect of stressed broad bean plants.

The addition of 1 mM AsA with 0.05 mM GA_3 to the MS medium increased the MDA content by 25% in petunia 'Prism Rose' shoots compared with that in the control grown under non-saline condition (Table 3). Under saline condition, MDA content decreased in response to AsA with or without GA_3 application in petunia 'Prism Rose' shoots, from 5 to 21%, compared to control. Otherwise in 'Prism White', the application of AsA with or without GA_3 had no influence on MDA contents under saline or non-saline condition. As shown in Table 3, the treatment of petunia 'Prism White' shoots with 150 mM of NaCl led to a higher content of MDA in the shoots over control. Similar rise in MDA level after the application of NaCl salt was also found in canola (Kattab, 2007; Dolatabadian et al., 2008), maize (Gunes et al., 2007) and tomato (Krupa-Małkiewicz et al., 2015). According to the abovementioned authors, the observed enhanced value for MDA content in plants raised on treatment with NaCl alone indicates that NaCl toxicity may be responsible for the increasing lipid peroxidation, which further leads to cell damage. However, the application of biologically active substances such as glutathione in canola (Kattab, 2007), salicylic acid in maize (Gunes et al., 2007) or AsA in tomato (Krupa-Małkiewicz et al., 2015) to non-stressed plants resulted in higher proline and MDA values than that of non-saline control plants. In contrast, Dolatabadian et al. (2008) showed that the application of AsA to non-stressed plants resulted in no significant differences in proline and MDA values, compared with control plants. Although the inhibitory effect of AsA and GA_3 on proline accumulation and lipid peroxidation in petunia plants has been demonstrated, but the actual mechanism is not yet clear. However, it can be assumed that AsA and/or GA_3 action is the result of many factors (such as nutrient homeostasis, enzymatic and hormonal activity), and some of them are out of control and difficult to predict.

Table 4. Effects of 1 mM ASA and 0.05 mM GA_3 application on L*, a* and b* parameters of petunia 'Prism Rose' and 'Prism White' leaves under nonsaline and saline conditions.

Colour CIE L*a*b*	Salinity	Medium				Mean
		MS	MS + 1 mM ASA	MS + 0.05 mM GA_3	MS + 1 mM ASA + 0.05 mM GA_3	
			'Prism Rose"			
	Control	37.92c	64.19a	44.31c	65.42a	**52.96B**
L*	150mM NaCl	66.70a	51.69b	66.33a	66.70a	**62.86A**
	Mean	**52.31B**	**57.94B**	**55.32B**	**66.06A**	
	Control	11.33a	11.20a	-9.51cd	-8.68c	**1.09A**
a*	150mM NaCl	-10.12d	-12.64c	-4.83b	-9.53cd	**-9.28B**
	Mean	**0.60A**	**-0.71A**	**-7.1BC**	**-9.10C**	
	Control	20.80d	41.50b	30.38c	40.34b	**33.26B**
b*	150mM NaCl	40.40b	49.27a	33.03c	44.43ab	**41.78A**
	Mean	**30.60B**	**45.38A**	**31.70B**	**42.38A**	
			'Prism White'			
	Control	45.50cd	41.95d	67.61a	62.74ab	**54.45A**
L*	150mM NaCl	60.61b	50.36c	67.46a	50.30c	**57.18A**
	Mean	**53.05B**	**46.15C**	**67.53A**	**56.52B**	
	Control	-13.03d	-11.92cd	-6.88a	-11.48cd	**-10.83A**
a*	150mM NaCl	-8.53ab	-11.39ab	-10.17bc	-11.33cd	**-10.36A**
	Mean	**-10.78B**	**-11.65B**	**-8.52A**	**-11.40B**	
	Control	30.25e	25.33f	36.88d	42.03bc	**33.62B**
b*	150mM NaCl	46.07a	39.27a	46.02ab	38.30cd	**42.42A**
	Mean	**38.15A**	**32.29B**	**41.45A**	**40.16A**	

Abbreviations: see Table 1

Effect of ASA, GA_3 and NaCl on Chl a, b and Car contents. Measurement of photosynthetic (Chl *a*, Chl *b*) and nonphotosynthetic (Car) pigments under non-saline conditions in petunia 'Prism Rose' and 'Prism White' leaves showed that the application of 1 mM AsA with or without 0.05 mM GA_3 decreased Chl *a*, Chl *b* and Car contents, compared with those in the control (Table 3). Similarly, the high salinity caused a decrease in chlorophylls (Chl *a*, Chl *b*) and carotenoid contents in leaves of the tested petunia cultivars. Whereas, only plants of petunia 'Prism Rose' treated with 1 mM AsA with or without 0.05 mM GA_3 under saline conditions were less affected. A negative influence of NaCl on concentrations of chlorophylls in plants under salinity stress was observed in maize (Ali et al., 2007) canola (Bybordi, 2012) and barley (Agami, 2014). All the abovementioned authors suggested that a decrease in the content of photosynthetic pigments includes damage of photosynthetic apparatus. In the opinion of Bybordi (2012) it is associated with the high sensitivity of chlorophyll to the stress factors. Reduced chlorophyll concentrations due to the salinity stress were also observed in wheat (Ashraf et al., 2002) and tomato (Krupa-Małkiewicz et al., 2015).

Effect of ASA, GA_3 and NaCl on L*a*b* parameters. In the present study the addition of 1mM AsA into the MS medium under saline conditions had positive effects on the intensity of the green colour of leaves of both 'Prism Rose' and 'Prism White' cultivars determined by the parameter a* (Table 4).

The a* value, providing information of the position in the colour gamut between green and red, measured on the leaf surface ranged from 11.20 ('Prism Rose') to -11.92 ('Prism White'). The colour of the leaf surface is defined by the b* parameter, indicating the location on the axis between yellow and blue colours. Under stress conditions (NaCl), leaves of both petunia cultivars were dark yellow compared with that of the control, which was indicated by the parameter b*, ranged from 40.40 ('Prism Rose') to 46.07 ('Prism White'). The obtained results are in agreement with Ochmian et al. (2013). They showed that the content of chlorophylls is highly correlated with the green colour index-parameter a*, which is responsible for the green colour. According to Ochmian et al. (2013), the amount of chlorophyll in leaves is connected with more nutrients in the medium. This is in agreement with the results obtained in our study, where it was observed that the salt stress reduced Chl *a* and Chl *b* content in leaves of petunia 'Prism Rose' and 'Prism White'. Moreover, Table 5 shows significant correlation between the Chl *a*, Chl *b* and intensity of green colour of leaves indicated by parameter a*.

Table 5. Correlation coefficients (R) between Chl a, Chl b and colour parameter a* of petunia 'Prism Rose' and 'Prism White'.

Chlorophyll		
	Chl a	**Chl** b
Colour parameter		
	'Prism Rose'	
a*	0.445*	0.448*
	'Prism White'	
a*	0.613*	0.637*

* significant at $P \leq 0.05$

Contents of chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) are expressed as μg g^{-1} fresh mass

Parameter L* is related to the physiological attributes of visual response (Wrolstad et al., 2005). Hue describes the visible colour. Index of L* is usually useful for tracking colour changes. In the present study, values of variable L* ranged from 37.92 ('Prism Rose') to 45.50 ('Prism White') under non-saline conditions, which indicated that leaves of 'Prism Rose' were darker since the variable L* varies from 0 that represent black to 100 that represent white (Table 4).

Under non-saline conditions leaves of both the tested petunias grown in the MS medium enriched with 1 mM AsA with or without 0.05 mM GA_3 were characterized by a lighter colour, for which the parameter L* increased from 17% to 72.5% for 'Prism Rose' and from 38% to 48.5% for 'Prism White'. However, the addition of 1 mM AsA with 0.05 mM GA_3 into the MS medium supplemented with 150 mM NaCl had no significant influence on the parameter L*. Similar results were obtained by Krupa-Małkiewicz et al. (2017) for petunia explants. They observed that by increasing the NaCl concentration to 150 mM in the medium, the value of L* parameter was higher in comparison to the control by 13.5% and 17.6%. Moreover, all petunia plants tested from this medium were characterized by lighter leaf coloration in comparison to the control.

The finding of our study can be summarized as follows. Salinity stress had a negative influence on the apical growth of plants as well as the development, and biochemical parameters of both petunia cultivars grown under *in vitro* conditions. However, plant growth reduced by the salinity stress was different in the two *Petunia* cultivars. Exogenous application of AsA and GA_3, as antioxidant compounds, increased the shoot length and the number of new shoots under saline and non-saline conditions and decreased proline and MDA values under saline condition of petunia 'Prism Rose'. Better growth under saline condition was observed in the *Petunia×atkinsiana* D. Don 'Prism White' cultivar. Only these explants developed roots and had longer shoots with darker leaves under saline condition, compared to 'Prism Rose' explants.

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