International Journal of Plant Physiology and Biochemistry Vol. 3(3), pp. 43-49, March 2011
Available online at http://www.academicjournals.org/ijppb
ISSN-2141-2162 ©2011 Academic Journals

Full Length Research Paper

The effects of spermidine and putrescine polyamines on growth of pomegranate (*Punica granatum* L. cv 'Rabbab') in salinity circumstance

Elias Amri[1]*, Mohammad Mirzaei[2], Majid Moradi[3] and Kavoos Zare[4]

[1]Department of Horticulture, College of Agriculture, Shiraz University, Shiraz, Iran.
[2]Department of Horticulture, College of Agriculture, Jahrom Islamic Azad University, Jahrom, Iran.
[3]Department of Horticulture, College of Agriculture, Jiroft Islamic Azad University, Jiroft, Iran.
[4]Department of Agronomy, College of Agriculture, Arsenjan Islamic Azad University, Arsenjan, Iran.

Accepted 29 December,2011

Different foliar treatments of spermidine and putrescine polyamines were applied to investigate the responses of the commercial genotype of pomegranate, *P.* Rabbab, to salinity. Pomegranate cuttings were rooted and planted in the plastic bag that contained sand and perlite medium in a ratio of 1:1 and irrigated with complete Hoagland's solution immediately. Four salinity levels of irrigation water (0, 40, 80 and 120 mM NaCl) were used. At about 72 h after salinity treatments, foliar treatments of spermidine and putrescine (0, 1 and 2 mM) were used, while growth characteristics (that is, the length of the main stem, the length and number of internodes and the leaf surface) were measured during the experiment. At harvest, concentrations of Na, K and Cl in root, apical and basal leaves of two genotypes were separately determined 120 days after treatments. In Rabbab genotype, the increasing salinity proportional to NaCl concentration reduced the length of stem, the length and number of the internode and the leaf surface. There was an increase in the growth rate of salinity levels with an application of polyamines; although a decline in the growth rate occurred at salinity levels higher than 70 mM. With the increasing salinity level, the tissue concentration of Na and Cl increased, while the K/Na ratio decreased. No significant differences were observed among the two genotypes in Na, Cl and K concentrations of roots, apical and basal leaves. This result showed that the use of different degrees of exogenous polyamine can reduce the effects of stress on growth of pomegranate.

Key words: Pomegranate, polyamines, putrescine, salinity, spermidine.

INTRODUCTION

The pomegranate (*Punica granatum)*, which is an old fruit grown in the world originates from the Middle East and Orient, where it enjoys popularity to this day. In Iran, the pomegranate is commercially insignificant, probably due to the availability of a wide range of preferred alternative fruits. Rabbab is one of the varieties suitable for commercial production in the south of Iran. The pomegranate plant is very adaptable and will grow in regions ranging from temperate to subtropical. It is deciduous or semi-deciduous depending on its location. The best prospects for commercial fruit production exist in those parts of the region where the summer is warm to hot and where rainfall is minimal during late summer/autumn. However, water should be available for irrigation. Deep, loamy, well-drained soils are preferred but the pomegranate has some tolerance to less ideal drainage and mild alkaline conditions.

As a consequence, irrigation means have become a major factor for a successful plant production in dry climate zones. It is not only the absolute lack of water that is a problem, but also the poor quality of irrigation water, due to excessive ion concentrations. Today, more than one third of the irrigated land worldwide is affected by high salt concentrations in the soil solution (Postel, 1993). Among crop species, fruit trees are regarded as very sensitive to soil salinity. An electric conductivity (EC) of 4 mS cm^{-1} (corresponding to 40 mM NaCl or 0.27%

*Corresponding author. E-mail: elias.amri@gmail.com. Tel: +989171348761.

salt) of the saturation extract is regarded as critical in orchards. It is noteworthy that irrigation water for fruit trees should not exceed 2 mS cm^{-1}, due to the fact that pomegranate is moderately sensitive to salinity (salt effects at EC = 3 mS cm^{-1}).

Polyamines (PAs) are organic polycations found in all living organisms. In higher plants putrescine (Put), spermidine (Spd) and spermine (Spm) are the most abundant PAs and are involved in the various developmental processes (Tonon et al., 2004). For the fact that the levels of PAs increase during adaptation to stresses in a variety of plants, it is thought that they are also involved in these processes. However, the physiological role of stress-induced PA accumulation remains unknown. Polyamines titers, altered in different manners, are dependent on several factors, such as plant species, tolerance or sensitivity to stress and duration of stress. The exogenous addition of polyamines to stress-treated cells or tissues could lead to injury alleviation and growth promotion in most cases, although the effects varied between polyamines and among plant species (Shen et al., 2000; Bouchereau et al., 1999; Capell et al., 2004; Kauskabe et al., 2004). Plants under salinity stress are observed to be associated with a reduction in salt tolerance. So far, the physiological role of PAs in tolerance to environmental stress remains uncertain (Bais and Ravishankar, 2002; Capell et al., 2004; Amri and Shahsavar, 2010).

A salinity-induced increase in the endogenous polyamines has also been reported in various plants (Das et al., 1995; Chattopadhayay et al., 2002). It is well established that enhanced polyamine biosynthesis can protect plants from salinity by scavenging free radicals, stabilizing membrane and cellular structures, maintaining a cation–anion balance (Bouchereau et al., 1999), modulating ion channels (Lopatin et al., 1994) and energizing the cell through stimulation of ATP synthesis (Ioannidis et al., 2006). Numerous attempts have been made to improve the salinity tolerance of a variety of crops by traditional breeding programs, but commercial success has been limited thus far. In recent years, some methods, such as transgenic approaches and exogenous polyamine application, have been directed towards the agricultural use of polyamine ability for enhancing the salinity tolerance of plants. Alternatively, exogenous polyamine application is a convenient and effective approach for enhancing salinity tolerance of crops and eventually improving crop productivity under high salinity. Indeed, exogenous polyamine application has been successfully used for enhancing the salinity tolerance of plants (Chattopadhayay et al., 2002; Verma and Mishra, 2005). In the present study, pomegranate, which is an important horticultural crop, was selected as the test plant material because it is moderately sensitive to salinity. No major attempts have been made to improve salinity tolerance of pomegranate. Therefore, induction of salinity tolerance by the use of exogenous polyamines continues to be an objective of great interest. Several reports have shown that Spd plays an important role in chilling tolerance (Shen et al., 2000; Amri and Shahsavar, 2010) and the growth and development of young pomegranate plants (Fujihara and Yoneyama, 2001). The objective of the present study is to see how the exogenous Spd and Put affects polyamine metabolism and increases short-term salinity tolerance of pomegranate.

MATERIALS AND METHODS

The experiment was carried out during 2009 to 2010 in the greenhouse located in Fasa (South of Iran). Four salinity levels of irrigation water (0, 40, 80 and 120 mM NaCl) were used. At about 72 h, after salinity treatments, foliar treatments of spermidine and putrescine (0, 1 and 2 mM) were used and a non-ionic wetting agent (ricka 0.5 ml) was used in all treatments during 120 days.
Rabbab pomegrante genotyp of Iranian pomegrante were studied in this experiment and the cuttings of the pomegrantes were obtained from a pomegranate orchard located in Fasa. The cuttings were rooted and transplanted in a medium with 1:1 sand-perlite, while the plants were irrigated with the nutrient solution of complete strength Hogland every 5 days.

The length of the main stem, number and length of internodes and the surface of four leaves from the top of the plant were measured at the end of the experiment. At harvest, the leaves in the apical and basal section of the shoot, as well as the roots harvested separately, were washed thoroughly once with tap water and twice with deionized water, and were later ground and ashed at 550°C for 8 h. Consequently, the ash was dissolved in HCl (Chapman and Pratt, 1961). Concentrations of Na and K in the digest solutions were measured on a Flame photometer (Cottenie et al., 1982), while chloride concentration was determined by the coulometric-amperometric titration (Black et al., 1965).

The effects of treatments were evaluated using analysis of variance, while the means was compared by Duncan's Multiple Range Test (DMRT) at the 95% significance level, using SAS software (SAS Version 6, 4th Edition).

RESULTS

Vegetative growth of seedlings declined under the influence of different levels of salinity. Also, with the increasing salinity levels, length of the main stem also significantly declined. Polyamines were able to use the growth rate of shoot to increase their salinity levels (Table 1). Putrescine polyamine when compared with spermidine, show that shoot growth could be significantly increased (Table 1). With the increasing concentration of polyamine, the protective role against them increased salinity (Table 1). Fresh weight and dry weight of seedlings were also affected by different levels of salinity. Increasing levels of salinity, fresh weight and dry weight were also significantly decreased (Table 1). However, the use of polyamine could cause dry and fresh weight increase in the salinity levels (Table 1). When putrescine was compared with spermidine, fresh weight and dry weight significantly increased (Table 1) and the

Table 1. Effect of salinity and spermidine treatment on shoot and root length [cm], shoot and root fresh masses (g) and shoot and root dry masses (g) of pomegranate seedlings.

Spermidine (mM)	Salinity level (mM)	Root dry mass (g)	Root fresh mass (g)	Root length (cm)	Shoot dry mass(g)	Shoot fresh mass(g)	Shoot length (cm)
0	0	7.2 c	13.8 c	10.6 d	14.6 bc	28.2 bc	36.8 bc
	40	5.2 ef	11.2 de	7.3 f	10.8 fg	24.6 cd	32.3 cd
	80	3.7 gh	9.4 fg	6.4 gh	8.3 hi	18.1 hi	30.2 d
	120	2.3 i	8 gh	5.1 hi	5.2 jk	17.2 i	24.1 fg
1	0	7.1 cd	13.2 cd	11.2 cd	13.1 cd	27.1 c	37.6 bc
	40	5.3 ef	12 d	7 ef	11.2 ef	25.3 de	31.2 cd
	80	4.2 fg	10.1 ef	6.1 gh	6.9 i	17.0 ij	27.1 e
	120	2.5 hi	8.3 hi	5.4 hi	5.3 jk	17.6 ij	25.6 ef
2	0	7.6 bc	14 b	13.1 b	15.6 b	28.9 b	38.5 b
	40	5.8 ef	12.1 d	6.5 g	12.4 de	26.3 d	34.1 c
	80	3.8 gh	8.9 gh	6.2 gh	7.8 i	17.9 hi	32.3 cd
	120	3.5 gh	8.6 h	6.1 fg	6.5 ij	17.8 hi	26.9 ef
Putrescine (mM)	**Salinity level (mM)**						
0	0	7.6 bc	13.2 cd	11.1 cd	13.2 cd	26.9 de	37.1 bc
	40	6.2 de	10 f	6.2 fg	11.6 ef	20.3 f	23.8 gh
	80	3.8 gh	8.1 hi	5.8 gh	8.1 hi	18.1 h	22.3 h
	120	2.1 i	7.6 h	5.3 hi	5.3 jk	15.6 ij	22.4 h
1	0	7.8 b	14.1 b	12.6 bc	15.3 b	29.1 ab	38.7 b
	40	6.1 de	12.6 cd	8.1 e	13.4 cd	26.1 cd	33.2 cd
	80	5.1 f	11.2 de	7.2 ef	9.1 gh	18.1 h	31.2 cd
	120	4 g	9 g	6.8 fg	8.6 hi	18.4 gh	24.5 f
2	0	8.2 a	15.1 a	13.2 a	16.1 a	31.5 a	42.5 a
	40	6.7 d	13.4 bc	7.4 bc	14.2 c	26.2 cd	34.6 c
	80	5.4 ef	9.7 fg	7.0 ef	9.8 fg	20.1 f	28.6 de
	120	4.3 fg	8.8 gh	7.1 ef	8.7 gh	19.4 fg	26.1 ef

For each plant part, the means in a column followed by the same letter are not significantly different at the 95% probability level (DMRT).

longitudinal growth of roots was influenced by different levels of salinity. Moreover, increasing thesalinity level significantly decreased the longitudinal growth of roots (Table 1). Thus, polyamine was able to use the longitudinal root growth in different levels of salinity increase

Table 2. Concentrations of Na, Cl and K in shoots and leaves of Rabbab pomegranate at different salinity levels and spermidine and putrescine polyamines.

Spermidine (mM)	Salinity level (mM)	Na (mg/kg)	Cl (mg/kg)	K (mg/kg)
0	0	0.98 ab	0.06 a	2.22 ef
	40	1.57 cd	0.94 c	2.32 de
	80	3.10 e	1.20 d	2.31 de
	120	4.20 gh	1.66 de	2.41 cd
1	0	1.20 bc	0.07 ab	2.13 fg
	40	1.39 c	0.95 c	2.65 bc
	80	2.80 de	1.33 de	2.54 c
	120	4.30 gh	1.52 de	2.34 de
2	0	0.88 a	0.04 a	2.56 bc
	40	0.96 ab	0.82 c	2.36 d
	80	2.60 d	1.33 de	2.33 de
	120	3.80 ef	1.28 de	2.26 e
Putrescine (mM)	**Salinity level (mM)**			
0	0	1.00 b	0.07 ab	2.14 fg
	40	1.43 bc	0.94 c	2.18 f
	80	3.10 e	1.35 de	2.06 g
	120	4.62 g	1.58 de	2.56 bc
1	0	1.10 bc	0.06 a	2.57 bc
	40	1.26 bc	0.91 b	2.96 a
	80	2.80 de	1.35 de	2.21 ef
	120	4.10 fg	1.33 de	2.86 ab
2	0	0.96 a b	0.04 a	2.75 b
	40	0.91 ab	0.85 b	2.65 bc
	80	2.40 d	1.22 d	2.33 de
	120	3.15 ef	1.12 cd	2.87 ab

For each plant part, the means in a column followed by the same letter are not significantly different at the 95% probability level (DMRT).

(Table 1). Putrescine, when compared with spermidine, shows that longitudinal root growth could be significantly increased (Table 1). Fresh weight and root dry weight was influenced by different levels of salinity. With the increasing salinity levels, fresh weight and root dry weight significantly decreased (Table 1). Likewise, the use of poly amine levels could increase the dry and fresh weight of root at different levels of salinity (Table 1). Putrescine when compared with spermidine was able to show that weight and root dry weight significantly increased (Table 1).

With an increase in salinity, the levels of sodium and chloride concentrations in the shoots of seedlings increased. This increase in salinity levels caused higher concentrations of toxic elements in the plant (Table 2). Use of putrescine and spermidine decreased the high concentrations of this element in the plant (Table 2). Potassium concentration, which was a factor that affected the resistance to stress, increased with an increase in salinity levels, but high levels of potassium salt concentration significantly decreased. However, the use of putrescine and spermidine increased the potassium concentrations in plant (Table 2).

Proline concentration, as a factor affecting the resistance to stress, increased with the increasing salinity levels, but high levels of salt significantly decreased the potassium concentration. Use of putrescine and spermidine increased the proline concentration in the plant's (Figure 1) internode length, which also affected different salinity levels. Internode length with increasing levels of salinity significantly decreased, and polyamine was able to use internode length at different levels of salinity increase. Putrescine, when compared with spermidine, was able to increase the internode length

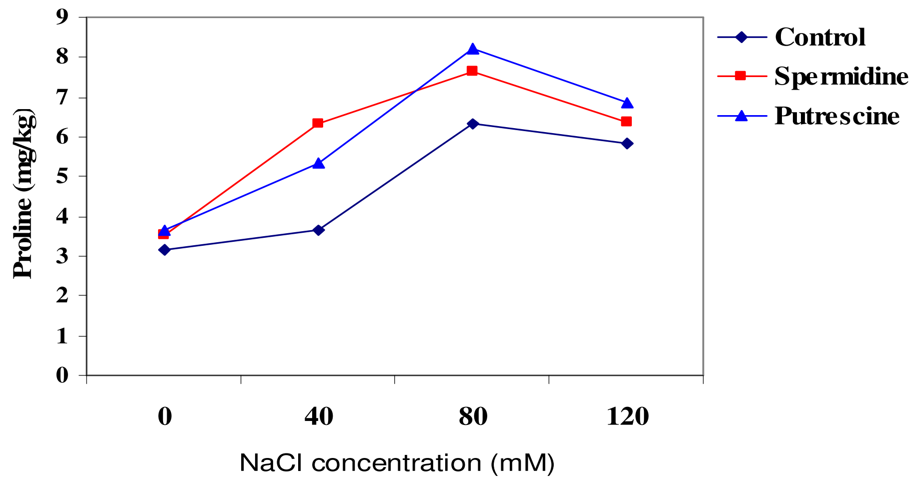

Figure 1. Effects of salinity and polyamines on proline accumulation in pomegranate leaves. Accumulation of proline was enhanced by salinity and polyamines in pomegranate leaves and more proline concentration was found in the leaves of pomegranate under salinity and polyamines. For each date, the means followed by the same letter are not significantly different at the 95% probability level (DMRT). However, the statistical analysis was shown only for the dates when significant differences were obtained.

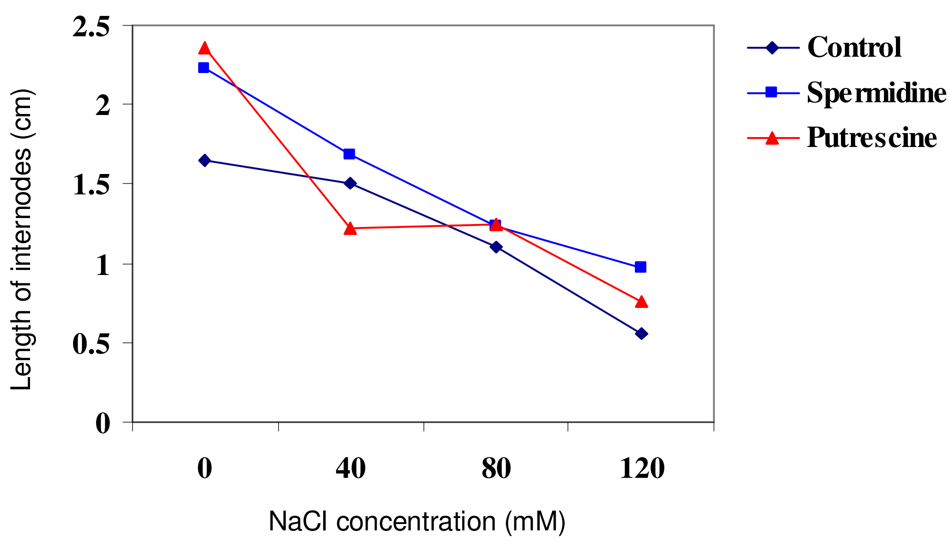

Figure 2. Effects of salinity and polyamines on length of internodes in Pomegranate. For each date, the means followed by the same letter are not significantly different at the 95% probability level (DMRT). The statistical analysis was shown only for the dates when significant differences were obtained.

significantly (Figure 2). However, the increasing salinity level of the internode number was reduced significantly, in that internode number also affected salinity levels.

As a consequence, polyamine was able to use them in the internode number of different levels to increase salinity. Putrescine, when compared with spermidine, could increase the internode number significantly (Figure 3). Likewise, the leaf seedlings under the influence of different levels of salinity increased as well. However, the increasing salinity level leaf area also decreased significantly and was able to use polyamine levels in leaves of different levels to increase salinity (Figure 4). Putrescine compared with spermidine, the leaf area could significantly increase (Figure 4). With the increasing

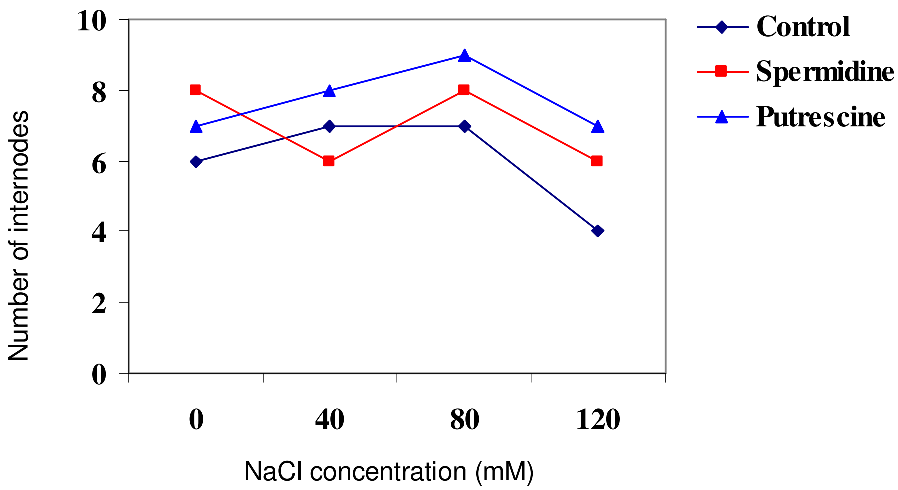

Figure 3. Effects of salinity and polyamines on number of internodes in Pomegranate. For each date, the means followed by the same letter are not significantly different at the 95% probability level (DMRT). The statistical analysis was shown only for the dates when significant differences were obtained.

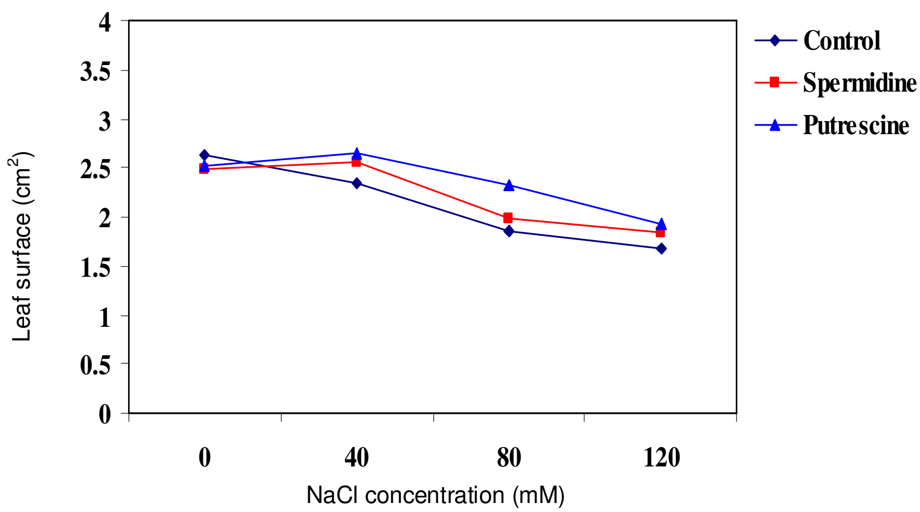

Figure 4. Effects of salinity and polyamines on the leaf surface in Pomegranate. For each date, the means followed by the same letter are not significantly different at the 95% probability level (DMRT). However, statistical analysis was shown only for the dates when significant differences were obtained.

concentrations of polyamine, the protective role against them was to increase salinity.

DISCUSSION AND CONCLUSION

Due to the bad effects of salinity on plant growth, the increasing salinity levels, as well as plant growth, decreased. Salinity levels induced a reduction both in length and number of internodes (Figures 2 and 3), which resulted in a reduction of growth. Salinity also decreased the leaf area (Figure 4) and reduced the rate of photosynthesis, thereby making plants to have smaller sizes. Using poly-amine due to the increased length of internode (Figure 2), the number of internode (Figure 3) and leaf (Figure 4) increased the growth of seedlings. Accordingly, bad effects of salinity on the vegetative growth significantly reduced.

As a result of plant stress on proline and potassium, concentrations in the plant increased, but with the increasing levels of salinity and potassium, concen-tration of proline reduced again. Using polyamine, due to increased concentrations of potassium in plant proline, could increase plant resistance to salinity levels.

Fundamentally, sodium and Cl ions concentrations in root and leaves did not differ among pomegranate genotypes, whereas on the other hand, pomegranate genotypes varied in response to the relative high Na and Cl concentrations in their tissues. This is in accordance with the different results (Levy et al., 1999) reported earlier that the threshold of toxic ions were not the same for different genotypes of plants.

Polyamines are involved in plant defense to environmental stresses (Bouchereau et al., 1999). In general, plant species and cultivars with high stress tolerance are endowed with a great capacity to enhance polyamine biosynthesis in response to environmental stresses including salinity. This suggested that the initiation of polyamine accumulation requires an osmotic signal (Imai et al., 2004). Also, it suggests that an osmotic, rather than ionic effect is the main signal triggering the polyamine response under salinity. High salinity levels disturbed polyamine homeostasis in pomegranate roots. It may act as a protection for the plasma membrane against stress damage by maintaining membrane integrity (Roy et al., 2005), thereby preventing superoxide-generating NADPH oxidases activation (Shen et al., 2000) or inhibiting protease and RNase activity (Bais et al., 2002).

On the other hand, exogenous spermidine application alleviated growth inhibition of *Brassica juncea* (Verma and Mishra, 2005) and improved grain yield of rice plants under salinity (Velikova et al., 1998). These results indicate that the accumulation of free spermidine may be detrimental for growth and development of pomegranate plants. These conjugated forms of polyamines could be a valuable source of the free polyamines under conditions that demand their presence in active forms (Tonon et al., 2007; Amri and Shahsavar, 2010). Also, they have the potential to act as free radical scavengers (Bouchereau et al., 1999). In the present study, among the enhanced polyamines, the insoluble bound spermidine content was most different between the pomegranate under either salinity conditions or spermidine application conditions.

Therefore, it seems that the bound spermidine is most closely involved in the salinity tolerance of pomegranate. These polyamines may serve as membrane surface stabilizers through interaction with phospholipids or other negatively charged groups of membrane (Wan et al., 2006; Amri and Shahsavar, 2010). Photosynthesis is one of the most important processes inhibited under salinity. In recent years, there have been several reports establishing that polyamines, especially the thylakoid-bound polyamines, participate in the regulation of the structure and function of the photosynthetic apparatus under environmental conditions, like UV-B radiation (Wne et al., 2006), low temperature (Urano et al., 2003) and salinity (Imai et al., 2004).

Oxidative damage in plants is caused by polyamine acting as direct free radical scavengers or binding to the antioxidant enzyme molecules to scavenge as free radicals (Bais et al., 2002). Higher endogenous levels of polyamines, particularly spermidine are positively correlated with greater increase in the antioxidant. This suggests that spermidine accumulation was able to increase the metabolism and growth effect on the plant, through osmotic effects.

Plants, which gathered compatible metabolisms under salinity stress conditions, such as proline, seem to be visitors of such substances that must play the role of plant protection against stress. It was seen in the seedlings of this experiment that plants respond to salinity stress action under proline accumulation.

Basically, the increasing salinity levels intervals increased damage to the cell membrane, while the percent damage to the plants under salinity conditions, with increasing irrigation intervals, significantly increased when compared to control plants, which indicated that the cell strength has less salinity conditions.

REFERENCES

Amri E, Shahsavar A (2010). Response of lime seedlings (*Citrus aurantifolia* L.) to exogenous spermidine treatments under drought stress. AJBAS, 4(9): 4483-4489.

Bais HP, Ravishankar GA (2002). Role of polyamines in the ontogeny of plants and their biotechnological applications, Plant Cell Tiss. Organ Cult., 69: 1-34.

Capell T, Bassie L, Christou T (2004). Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. USA. Proc. Natl. Acad. Sci., 101: 9909-9914.

Imai R, Ali A, Pramanik HR, Nakaminami K, Sentoku N, Kato H (2004). A distinctive class of spermidine synthase is involved in chilling response in rice. J. Plant Physiol., 161: 883-886.

Kauskabe YL, Nada S, Hara S (2004). Overexpression of Spermidine Synthase Enhances Tolerance to Multiple Environmental Stresses and Up-Regulates the Expression of Various Stress-Regulated Genes in Transgenic Arabidopsis thaliana, Plant Cell Physiol., 45: 712-722.

Levy Y, Lifshitz J, De Malach Y, David Y (1999). The response of several citrus genotypes to high-salinity irrigation water. Hort. Sci., 34: 878-88

Tonon G, Kevers C, Faivre-Rampant O, Grazianil M, Gaspar T (2004). Effect of NaCl and mannitol iso-osmotic stresses on prolin and free-polyamine levels in embryogenic *Fraxinus angustifolia* callus. J. Plant Physiol., 161: 701-708.

Urano KY, Yoshiba T, Nanjo Y, Igarashi M, Seki F, Sekiguchi K, Yamaguchi-Shinozaki K (2003). Characterization of *Arabidopsis* genes involved in biosynthesis of polyamines in abiotic stress responses and developmental stages. Plant Cell Environ., 26: 1917-1926.

Velikova VB, Yordanov IT, Georgieva KM, Tsonev TD, Goltsev V (1998). Effects of exogenous polyamines applied separately and in combination with simulated acid rain on functional activity of photosynthetic apparatus. J. Plant Physiol., 153: 299-307.

Verma S, Mishra SN (2005). Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. J. Plant Physiol., 162: 669-677.

Wan X, Shi G, Xu Q, Hu J (2006). Exogenous polyamines enhance copper tolerance of Nymphoides peltatum. J. Plant Physiol., doi:10.1016/j.jplph.2006.06.003, pp.1234-1240.