Journal of Plant Nutrition

1897

ISSN: 0190-4167 (Print) 1532-4087 (Online) Journal homepage: http://www.tandfonline.com/loi/lpla20

EFFECT OF SALINITY AND EXOGENOUSLY APPLIED POLYAMINES ON GROWTH AND ION RELATIONS IN SPINACH

Sawsan Suleiman , Clyde Wilson & Catherine M. Grieve

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF PLANT NUTRITION
Vol. 25, No. 12, pp. 2705–2717, 2002

EFFECT OF SALINITY AND EXOGENOUSLY APPLIED POLYAMINES ON GROWTH AND ION RELATIONS IN SPINACH

Sawsan Suleiman,[1] Clyde Wilson,[2,*] and Catherine M. Grieve[2]

[1]Tishreen University, Faculty of Agriculture, Lattakia, Syria
[2]George E. Brown, Jr., Salinity Laboratory, USDA-ARS, 450 West Big Springs Road, Riverside, CA 92507, USA

ABSTRACT

It has been suggested that polyamines are involved in many growth and development processes in plants and may serve as growth regulators. These nitrogenous compounds have been shown to be involved in cell division, nucleic acid and proteins synthesis, and normal senescence. It is thought by some that polyamines may be involved in the plant's mechanistic response to stress. More importantly, some reports indicate that exogenously applied polyamines can overcome the growth reduction brought about by salinity stress. In this report, growth studies were performed on to examine the effect of exogenously applied putrescine, spermidine, and spermine on the alleviation of salinity stress. *Spinacia oleracea*, L. cv. Space was chosen since much of our understanding of the sugar metabolism needed for growth has been derived from this plant. Polyamines were

*Corresponding author. E-mail: cwilson@ussl.ars.usda.gov

DOI: 10.1081/PLN-120015533
0190-4167 (Print); 1532-4087 (Online)

applied in a manner used in earlier reports, i.e., foliar spray. The effect of salinity and polyamines on leaf number, leaf area, fresh and dry weight was observed. Our analysis showed that putrescine and spermine had no significant effect on plant growth throughout the range of salinities studied (2 to 11.7 $dS\,m^{-1}$). Spermidine slightly decreased growth. Further studies on ion uptake indicated that none of the polyamines tested had a significant effect on plant ion content. While we failed to document any growth response to putrescine or spermine treatment, we did find a significant reduction of growth as salinity increased in the irrigation water. Further analysis of the ion data indicated that $K^+ : Na^+$ selectivity significantly increased with increasing salinity. This preferential increased influx of K^+ ions may be an important mechanism by which spinach maintains low Na^+ tissue levels relative to external concentrations. Additionally, $Ca^{2+} : Na^+$ selectivity as measured by the Gapon constant, K_g, greatly increased. Conversely, $Ca^{2+} : Mg^{2+}$ selectivity decreased resulting in an increase in Mg^{2+} tissue concentrations. Our results are discussed in terms of possible salt tolerance mechanisms in spinach as they relate to the preferential uptake of specific nutrient ions from saline irrigation waters.

INTRODUCTION

One consistent observation of nonhalophytes exposed to salinity is a reduction in shoot growth while root growth seems less affected.[1] At present, it is unclear what mechanism(s) are involved in this phenomenon. Munns and Termaat[1] have argued that the reduction in shoot growth is not related to limitations of any major soil or plant substrate such as water, nitrogen, photosynthate, or ATP. Instead, they suggested plant hormones (phytohormones) may be involved in the signaling process between root and shoot and are involved in regulating growth responses to salinity stress.

Several plant growth models exist that postulate a role for hormones in plant growth responses to salinity.[2] One model suggests that under saline conditions root-produced cytokinins may serve as a messenger from root to shoot in a manner similar to water stress.[3] In another proposal[4] abscisic acid, also produced in the root, acts as an alternative messenger, while Lerner et al.[5] postulated a role for both abscisic acid and cytokinins in adaptation to salinity stress. In another study involving sorghum, the data indicated that both hormones and plant nutrition could alleviate the effect of salinity stress.[6] In sorghum, it appears that increased mineral nutrition can overcome the growth reduction due

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

to salinity stress and, interestingly, a mixture of cytokinins and gibberellic acid can substitute for increased mineral nutrition. Current models of this stimulus-response coupling consist of a four-component system including (a) perception of the stimulus; (b) transduction of the signal; (c) alteration of gene expression; and finally (d) a physiological/morphological response.[7] The role of phytohormones in the salinity response in plants has been reviewed by Amzallag.[8]

A different class of chemicals, polyamines, has also been shown to influence plant response to a wide range of stresses, including salinity.[9,10] These nitrogenous compounds are ubiquitous in living cells in cellular concentration from 10 μM to mM[10] and may be involved in various physiological processes.[11,12] Some research indicates the intrinsically positive charge of these molecules at physiological cell pH allows them to associate to the negative charges of nucleic acids and phospholipids,[13,14] and thereby regulate plant response. Other researchers have shown that polyamines are involved in cell division, nucleic acids and proteins synthesis, senescence retardation.[15–17] It is possible that polyamines may, in fact, be a part of the plant's mechanistic response to salt, drought, and oxidative stresses.[17] For example, Ditomaso et al.[18] and Evans and Malberg[19] reported that putrescine accumulates under conditions of potassium deficiency, low pH and herbicide treatment. In the case of salinity stress, putrescine may be involved in nitrogen metabolism.[20]

Not only does the literature indicate that polyamines may be involved in the plant response to salinity stress, but also exogenously applied polyamines appear to overcome the reduced growth observed with salinity stress. In rice, Krishnamurthy[21] reported that foliar applied polyamines ameliorated the salt effect. Others have shown that exogenously applied polyamines are effective in the prevention of the appearance of senescence symptoms.[22]

Thus, inasmuch as polyamines may play a role in plant response to salinity, we investigated the effect of exogenous application of the three major polyamines, putrescine, spermidine, and spermine on the growth, development, and ion accumulation of salt-stressed spinach plants. We applied the polyamines to the leaves in a manner consistent with previous studies.[23,24] In contrast to other reports using polyamines, our foliar application of putrescine and spermine had no significant effect on any parameters of plant growth measured or foliar ion content. This was observed both in our nonsaline, control plants or throughout the range of salinities studied (2 to 11.7 $dS\,m^{-1}$). Spermidine slightly decreased growth. Seed pretreatment with polyamines showed the same lack of effect as those obtained by foliar treatment. However, in this case we did not observe a decrease in growth with spermidine. Further analysis of the salt-effect on spinach growth indicated that Na^+ levels increased in the leaf only 14-fold despite a 50-fold increase in the external Na^+ levels. Values for $K^+ : Na^+$ selectivity increased over 2-fold from 2 $dS\,m^{-1}$ to 5.4 $dS\,m^{-1}$. Additionally, we found an increase in $Ca^{2+} : Na^+$ selectivity as determined by the Gapon constant, K_g. Leaf

Mg^{2+} levels slightly increased in plants grown at the 11.7 dS m^{-1}. This may be related to a decrease in the $Ca^{2+}:Mg^{2+}$ selectivity.

MATERIALS AND METHODS

Seeds of spinach (*Spinacia oleracea* L., cv. Space) were planted in sand tanks in a greenhouse at Riverside, CA in three rows per tank. The plants were spaced 20 cm apart and 15 cm between rows. The seedlings were later thinned to four plants per row. The sand tanks ($1.2 \times 0.6 \times 0.5$ m deep) contained washed sand having an average bulk density of 1.2 Mg m^{-3} at saturation. The sand had an average volumetric water content of 0.34 m^3 m^{-3}. The plants were irrigated twice daily with modified Hoagland's solution consisting of (in mM): 2.5 Ca $(NO_3)_2$, 3.0 KNO_3, 0.17 KH_2PO_4, 1.5 $MgSO_4$, 0.05 Fe as sodium ferric diethylene-triamine pentaacetate (NaFe-EDTA), 0.023 H_3BO_3, 0.005 $MnSO_4$, 0.0004 $ZnSO_4$, 0.0002 $CuSO_4$, and 0.0001 H_3MoO_4 made up with City of Riverside, CA, municipal water. This solution served as the base nutrient solution. Irrigations were for 15 min duration, which allowed the sand to become completely saturated, after which the nutrient solution drained into 765 L reservoirs for reuse in the next irrigation. Water lost by evapotranspiration was replenished automatically each day to maintain constant electrical conductivity in the solutions. The solution pH was maintained around 6.5 by adding H_2SO_4 as required.

Three salinity treatments were imposed when the first true leaves appeared by adding NaCl and $CaCl_2$ (1 : 1 by weight) to the nutrient solutions. The electrical conductivities (EC_i) of the irrigation water were increased to the desired level by incremental additions of the salts over 5 consecutive days to avoid osmotic shock to the seedlings. Final EC_i were 2.0, 5.4, 8.2, and 11.7 dS m^{-1}.

The experiment was a standard split-plot design with four salinity treatments, four polyamines treatments, and three replications. The salinity and polyamines treatment effects were analyzed using General Linear Model procedure in SAS.[25] Greenhouse temperature was controlled with a 28°C day/18°C night temperature.

The polyamines treatments were: control (0 mM polyamines), putrescine (5 mM), spermidine (5 mM), and spermine (5 mM) made up with deionized water containing 0.05% Tween 20 surfactant. The pH was adjusted to 6.0. Leaves of three plants in each sand tank were sprayed with one of the treatment solutions three times: (1) before adding salinity; (2) immediately after the imposition of the final salinity treatment level; and (3) seven days thereafter. Chemicals were purchased from Sigma Chemical Company (St. Louis, MO[1]).

[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

Shoot material was harvested 60 days after germination (53 days after initiation of salinization). Fresh weight and number of leaves were determined. Leaf area was measured using a LICOR LI-3000 leaf-area meter (Lincoln, NE). Shoots were washed in deionized water, dried at 65°C for 96 h, weighed, and ground to a fine powder. Sodium, K, Ca, and Mg were determined on nitric–perchloric acid digests of the tissues by inductively-coupled plasma optical emission spectroscopy (ICPOES). Chloride concentrations were determined on dilute nitric–acetic acid extracts by coulometric-amperometric titration.

The $K^+ : Na^+$ selectivity according to Pitman[26] where

$$S_{K,Na} = \left(\frac{\text{K content}}{\text{[K] medium}}\right) : \left(\frac{\text{Na content}}{\text{[Na] medium}}\right)$$

The Gapon selectivity constant relates the equivalent fractions of the exchange ions to the activities of the same ions in solution and is usually expressed for Ca−Na exchange.[27] The Gapon was calculated using:

$$K_g = \frac{E_{Ca} a_{Na}}{E_{Na} (a_{Ca}{}^{2+})^{0.5}}$$

As expressed above, E is the equivalent fraction of a given cation and a is the activity of the ion in solution.

RESULTS

Biomass Production

Data analysis showed that both salinity and polyamine effects were significant ($P \leq 0.05$). With respect to salinity, fresh weight decreased significantly with the 5.4 dS m^{-1} treatment [Fig. 1(A)]. Significant changes in dry weight were found beyond a salinity level of 5.4 dS m^{-1} [Fig. 1(B)]. As to polyamine treatments, we also found a significant effect. This was due to a small but significant decrease in growth in the spermidine treatment [Fig. 1(A)]. However, analysis of variance found no significant interaction between salinity treatments and polyamine treatments ($P > 0.05$).

These results are in contrast to other reports which showed that exogenously applied putrescine consistently and significantly increased biomass production of salt stressed rice,[28] mustard,[20] and broad bean.[23]

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

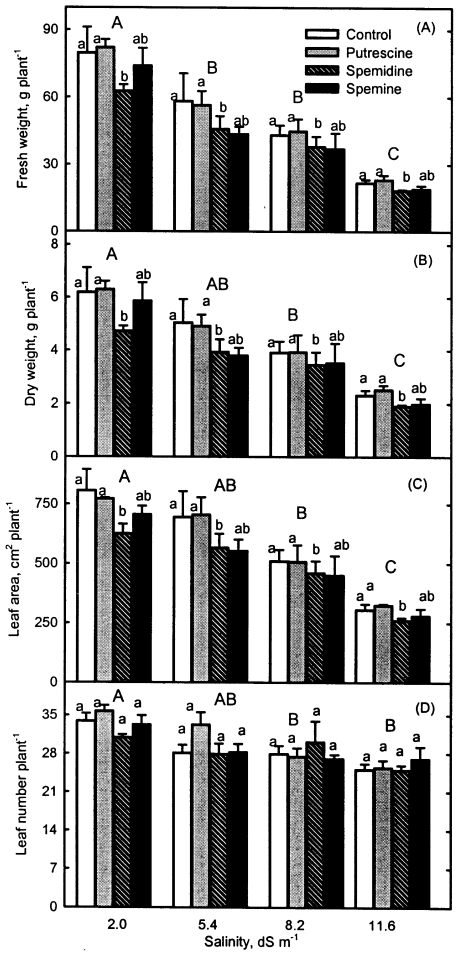

Figure 1. Effects of salinity and polyamine treatments on spinach fresh weight (A), dry weight (B), leaf area (C), and leaf number (D). Plants were harvested 60 days after germination (53 days after initiation of salinization). Sample size for each treatment is nine individual plants. Rectangle bars and stick bars are means $\pm$ 1 s.e. The experiment was a standard split-plot design. Salinity and polyamine treatment effects were analyzed using General Linear Model procedure in SAS[25] which shows that both salinity and polyamine effects are significant ($P \leq 0.05$). No significant interaction of salinity treatment with polyamine treatment was found ($P > 0.05$). Means were compared among the groups treated with different salinities and with polyamines using the multi-comparison method of Least Square Means. Significant differences at $P \leq 0.05$ are indicated by a different capital letter for salinity effect and by a different lowercase letter for polyamine effect. The same letter means no significant treatment difference ($P > 0.05$).

Leaf Area and Number of Leaves

Leaf area decreased markedly with increasing salinity [Fig. 1(C)]. Data analysis showed that treatment with polyamines had no effect on leaf area regardless of salinity level. Leaf area decreased in response to spermidine treatment at all salinity levels as compared to controls and plants treated with 5 mM putrescine. Spermidine tended to decrease leaf area under different salinity conditions but these effects did not differ significantly from growth-retarding effects of exogenously applied spermine.

The number of leaves decreased as the salinity levels increased [Fig. 1(D)]. However, exogenously applied polyamines did not have any significant effect on leaf number.

Ion Analysis

Polyamine treatments did not affect ion accumulation in spinach leaves (Table 1). Shoot Ca^{2+} increased significantly as salinity and substrate Ca^{2+} increased (Table 1). Ca^{2+} content in the plants was unaffected by polyamine treatment. Shoot Mg^{2+} increased at the highest salinity level, although substrate Mg^{2+} remained constant. In contrast, shoot Na^{+} increased significantly in the plants as Na^{+} in irrigation waters increased.

Shoot K^{+} decreased markedly as salinity increased. Shoot Cl^{-} increased significantly as the Cl^{-} concentration in the irrigation water increased. Its accumulation in plant tissues was less than that recorded for Na^{+}.

We considered the possibility that the polyamines in the foliar spray were unable to penetrate the spinach leaf cuticle. Thus, we pretreated the seeds by soaking them with polyamines. Four seed treatments and three time intervals of imbibition were imposed by soaking seeds for 6, 12, and 18 hours in: (1) deionized water only; (2) putrescine (1 mM); (3) spermidine (1 mM) and spermine (1 mM). Seeds were planted directly in the pre-salinized sand tanks used for experiment 1 (e.g., EC_i: 2.0, 5.4, 8.2, and 11.7 dS m^{-1}). As with the foliar treatment, we found no significant polyamine effects on the responses of spinach to salinity stress (data not shown).

Plant Ion Selectivity Under Salinity Stress

Since we did not find any significant effect of polyamine treatments on ion composition, we combined all the data for each salinity treatment to form a unique data set for each dS m^{-1} level to investigate the effect of salinity on ion uptake in spinach. Omielan et al.[29] suggested that a plant's ability to absorb K^{+} in environments containing high Na^{+} levels may be an important determinant of

Table 1. Effect of Salinity and Polyamine Treatments on Mineral Composition in Spinach Leaves

Salinity ($dS\,m^{-1}$)	Polyamine	Ca	Mg	Na	K	Cl
		$mmol\,kg^{-1}$				
2	Control	176.3d	620.7b	19.9d	3,515a	189d
	Putrescine	185.0d	632.7b	18.6d	3,543a	181d
	Spermidine	177.0d	640.3b	17.0d	3,423a	180d
	Spermine	186.7d	624.7b	17.7d	3,409a	180d
5.4	Control	265.0c	632.7ab	106.8c	2,749b	568c
	Putrescine	252.0c	631.3ab	90.5c	2,849b	559c
	Spermidine	252.3c	642.3ab	102.1c	2,904b	561c
	Spermine	249.0c	667.0ab	99.6c	2,895b	560c
8.2	Control	324.7b	688.7ab	168.0b	2,745b	905b
	Putrescine	345.3b	675.0ab	211.0b	2,732b	938b
	Spermidine	304.7b	667.0ab	169.3b	2,819b	875b
	Spermine	308.3b	680.7ab	156.0b	2,688b	868b
11.7	Control	478.7a	704.3a	263.7a	2,372c	1,490a
	Putrescine	482.7a	702.3a	266.0a	2,373c	1,444a
	Spermidine	450.7a	694.7a	245.3a	2,341c	1,478a
	Spermine	477.7a	717.3a	269.0a	2,383c	1,486a

Sample size for each treatment is nine individual plants. Values are means of three replications. Control indicates that polyamines were not applied. The experiment has a standard split-plot design. The salinity and polyamine treatment effects were analyzed using General Linear Model procedure in SAS.[25] Significant salinity effect was found ($P \leq 0.05$). No significant polyamine effect and no significant interaction of salinity treatment with polyamine treatment were found for all the means ($P > 0.05$). Means were also compared among the groups treated with different salinities and polyamines using the multi-comparison method of Least Square Means. Significant treatment differences at $P \leq 0.05$ are indicated by a different letter within a column for each leaf mineral concentration. The same letter indicates no significant difference ($P > 0.05$).

salt tolerance. With this in mind, we calculated $K^+ : Na^+$ selectivity according to Pitman.[26] Spinach displayed a relatively low $K^+ : Na^+$ selectivity at $2\,dS\,m^{-1}$ (Table 2). Selectivity increased sharply upon the addition of salts increasing from 95.6 at $2\,dS\,m^{-1}$ to 216.6 at $5.4\,dS\,m^{-1}$. Beyond that point, we found no significant change up to the highest salinity tested ($11.7\,dS\,m^{-1}$).

In order to determine the effects of salinity on the plant's ability to accumulate Ca^{2+}, we utilized the approach of Suarez and Grieve[30] who used ion-exchange theory to investigate Ca^{2+} and Na^+ uptake. The Gapon selectivity constant relates the equivalent fractions of the exchange ions to the activities of the same ions in solution and is usually expressed for Ca–Na exchange.[27] Thus,

Table 2. Effect of Salinity on Selectivity Coefficients ($S_{K,Na}$) and Gapon Constant (K_g) in Spinach

Salinity ($dS\,m^{-1}$)	$K^+ : Na^+$ ($S_{K,Na}$)	$Ca^{2+} : Na^+$ (Kg)	$Ca^{2+} : Mg^{2+}$ (Kg)
2.0	95.6b	0.74b	0.45a
5.4	216.6a	1.76a	0.35b
8.2	209.0a	1.38a	0.27c
11.7	222.9a	1.92a	0.29c

Data were analyzed as described in Table 1. Significant differences at $P \leq 0.05$ are indicated by a different letter within a column for each leaf mineral concentration. Means within a column followed by the same letter are not significantly different based on a Fisher's protected LSD test at the 5% level of probability.

we expressed the Gapon constant as explained in the Materials and Method section. The data in Table 2 show that $Ca^{2+} : Na^+$ selectivity in spinach increased the 2.0 $dS\,m^{-1}$ control from K_g of 0.74 to 1.76 at 5.4 $dS\,m^{-1}$. After that point K_g did not change significantly with increasing salinity. These findings indicate that spinach has a decreased ability to accumulate Ca^{2+} with increasing salinity.

The same approach was used to investigate the effect of increasing salinity on $Ca^{2+} : Mg^+$ selectivity. Interestingly, the K_g for $Ca^{2+} : Mg^+$ selectivity significantly decreased as salinity increased indicating an increasing preference for Mg^+ over Ca^{2+} (Table 2). This result was supported by the finding that chlorophyll levels did not change with salinity (data not shown).

DISCUSSION

In the present study, we examined whether or not polyamines could influence the growth of spinach grown under saline conditions. However, in contrast with reports on other species, analysis of our growth data showed that polyamines treatment did not ameliorate salinity stress in spinach. Growth of spinach was not influenced by putrescine or spermine, either applied via a foliar spray or by presoaking the seeds (data not shown). This insensitivity to these polyamines extended across the entire range of salinity tested (2 to 11.7 $dS\,m^{-1}$). However, we did note a small, but significant, decrease in growth with spermidine treatment as compared to putrescine treatment. Apparently, a plant may respond to one polyamine in a different manner than another. Delgado et al.[31] found that while spermine and spermidine increased the growth rate in chickpea at moderate

concentrations, at high level of spermine growth rate was greatly reduced. Thus, it is possible that the response appears to be concentration dependent. These investigators also reported changes in ion content with polyamines treatment. We did not find any significant changes in ion concentrations in the shoot (Table 1).

It is unclear as to the reason we were not able to achieve similar results for spinach to those reported for other plants. In our case, we applied the polyamines using a foliar spray in a manner previously reported in the literature[23] at three different times (1) before adding salinity; (2) immediately after the imposition of salinity; and (3) seven days thereafter. We considered the possibility that the polyamines were not able to penetrate the cuticle of the leaves. Earlier results with polyamines were based on studies where polyamines were applied by presoaking the seeds.[26] Thus, we also tried presoaking the seed (data not shown). In either approach, we were unable to find any effect of polyamines treatment.

The absence of a polyamine effect on spinach contrasts with results obtained by Parakash and Prathapasenan[28] who showed that putrescine reduced Na^+ and Cl^- accumulation, and stimulated K^+ levels in leaves and stems of rice. Krishnamurthy[21] showed that exogenous supply of putrescine inhibited Na and Cl uptake, and accelerated the accumulation of K^+, Ca^{2+}, and Mg^{2+} in salt-tolerant rice.

While we failed to document any changes in spinach growth with respect to polyamines, we did document a highly significant salt effect. Both Maas[32] and Francois and Maas[33] describe spinach as "moderately salt sensitive" based on shoot fresh weight. Our results are consistent with this classification. However, we should point out that this assessment is based on salinization early in the spinach life cycle. We salinized seven days after germination. Spinach seems to display more tolerance if salinized later in its life cycle.[34]

It is generally accepted that the Ca^{2+}-nutritional status of plant is important in maintaining selectivity, and hence, the integrity of cellular membranes, and that the ionic composition of irrigation waters may influence Ca^{2+}. In our spinach leaves, Ca^{2+} levels increased with increasing salinity due, in part, to the larger amounts of Ca^{2+} in the external media. However, the $Ca^{2+}:Na^+$ selectivity as represented by the Gapon constant (K_g) increased significantly from 2 to 5.4 dS^{-1} and then did not change. The ability to increase Ca^{2+} in the presence of increasing external Na^+ levels may be an important adaptive trait.[29,35]

It is also worth noting that leaf Mg^{2+} levels increased 11% at the highest salinity level imposed despite the fact that external Mg^{2+} levels remained constant. This is a result of an increasing preference for Mg^{2+} over Ca^{2+} (Table 2). The stimulation of Mg^{2+} content in spinach plant at high salinity is unusual. It's possible that spinach plants developed certain mechanism to accumulate high levels of Mg^{2+} in their tissues. This response may be important in maintaining photosynthesis under stress as chlorophyll levels were not affected by increasing salinity levels or polyamines treatment (data not shown).

In conclusion, it does not appear from our data that the polyamines, putrescine, spermidine, spermine can ameliorate the effect of salinity stress on growth in spinach. However, salinity stress significantly influenced plant growth and leaf area. As evidenced by our ion data, spinach excludes Na^+ by increasing $K^+ : Na^+$ selectivity. However, as Na^+ levels increased, spinach also displayed a enhanced ability to take up Ca^{2+}. This may be an important component of spinach's salt sensitivity. Future work should focus on the interaction between Na^+ concentrations in the substrate and Ca^{2+} uptake.

REFERENCES

1. Munns, R.; Termaat, A. Whole-Plant Responses to Salinity. Aust. J. Plant Physiol. **1986**, *13*, 143–160.
2. Poljakoff-Mayber, A.; Lerner, H.R. Plants in Saline Environments. In *Handbook of Plant and Crop Stress*, 2nd Ed., Revised and Expanded; Pessarakli, M., Ed.; Marcel Dekker, Inc.: New York, 1999; 125–152.
3. Bradford, K.J.; Hsiao, T.C. Physiological Responses to Moderate Water Stress. In *Physiological Plant Ecology. II. Water Relations and Carbohydrate Assimilation*; Lange, O.L., Nobel, P.S., Osmond, C.B., Zeigler, H., Eds.; Encycl. Plant Physiol. New Series, Springer-Verlag, Berlin, 1982; Vol. 12B, 263–324.
4. Hartung, W.; Peuke, A.D.; Davies, W.J. Abscisic Acid: A Hormonal Long-Distance Stress Signal in Plants Under Drought and Salt Stress. In *Handbook of Plant and Crop Stress*, 2nd Ed., Revised and Expanded; Pessarakli, M., Ed.; Marcel Dekker, Inc.: New York, 1999; 731–747.
5. Lerner, H.R.; Amzallag, G.N.; Friedman, Y.; Goloubinoff, P. The Response of Plants to Salinity: From Turgor Adjustments to Genome Modification. Israel J. Plant Sci. **1994**, *42*, 285–300.
6. Amzallag, G.N.; Lerner, H.R.; Poljakoff-Mayber, A. Interaction Between Mineral Nutrients, Cytokinin and Gibberellic Acid During Growth of Sorghum at High NaCl Salinity. J. Exp. Bot. **1992**, *43*, 81–87.
7. Naqvi, S.S.M. Plant Hormones and Stress Phenomena. In *Handbook of Plant and Crop Stress*, 2nd Ed., Revised and Expanded; Pessarakli, M., Ed.; Marcel Dekker, Inc.: New York, 1999; 709–730.
8. Amzallag, G.N. Role of Phytohormones in Adaptation of Sorghum to Salinity. In *Linnean Society Symposium Series, No. 13*: Desertified Grasslands: Their Biology and Management; International Symposium, London, England, UK, February 27–March 31, 1991; Chapman, G.P., Ed.; Academic Press: London, UK, 1992; 315–319.
9. Galston, A.W. Polyamines as Modulators of Plant Development. Bio. Sci. **1983**, *38*, 382–388.

10. Galston, A.W.; Kaur-Sawhney, R. Polyamines in Plant Physiology. Plant Physiol. **1990**, *94*, 406–410.
11. Smith, T.A. Polyamines. Annu. Rev. Plant Physiol. **1985**, *36*, 117–143.
12. Smith, T.A. Plant Polyamines Metabolism and Function. In *Polyamines and Ethylene: Biochemistry, Physiology and Interaction*; Flores, H.E., Arecteca, R.N., Shannon, J.C., Eds.; American Society of Plant Physiologists: Rockville, MD, 1990; Vol. 5, 1–23.
13. Cohen, A.S. *Introduction to the Polyamines*; Prentice-Hall; Englewood Cliff, NJ, 1971.
14. Robert, D.R.; Dumbroff, E.B.; Thompson, J.E. Exogenous Polyamines Alter Membrane Fluidity in Bean Leaves: A Basis for Potential Misinterpretation of Their True Physiological Role. Planta **1986**, *167*, 395–401.
15. Slocum, R.D.; Kaur-Sawhney, R.; Galston, A.W. The Physiology and Biochemistry of Polyamines in Plants. Arch. Biochem. Biophys. **1984**, *235*, 283–303.
16. Tabor, C.W.; Tabor, H. Polyamines. Annu. Rev. Biochem. **1984**, *53*, 749–790.
17. Flores, H.E. Change in Polyamine Metabolism in Response to Abiotic Stress. In *Biochemistry and Physiology of Polyamines in Plants*; Slocum, R., Flores, H.E., Eds.; CRC Press: Boca Raton, FL, 1991; 213–228.
18. Ditomaso, J.M.; Rost, T.L.; Ashton, F.M. Herbicide-Induced Diamine Accumulation in Pea Roots: The Effect of Napropamide on Polyamine Levels. Plant Cell Physiol, **1988**, *29*, 1367–1372.
19. Evans, P.T; Malberg, R.L. Do Polyamines Have Role in Plant Development? Annu. Rev. Plant Physiol. Mol. Biol. **1989**, *40*, 235–269.
20. Mishra, S.N.; Sharma, I. Putrescine as a Growth Inducer and a Source of Nitrogen for Mustard Seedlings Under Sodium Chloride Salinity. Ind. J of Exp. Biol. **1994**, *32*, 916–918.
21. Krishnamurthy, R. Amelioration of Salinity Effect in Salt Tolerant Rice (*Oryza sativa* L.) by Foliar Application of Putrescine. Plant Cell Physiol. **1991**, *32*, 699–704.
22. Galston, A.W.; Kaur-Sawhney, R. Polyamines and Senescence in Plants. In *Plant Senescence: Its Biochemistry and Physiology*; Thompson, W.W., Nothnagel, E.A., Huffaker, R.C., Eds.; American Society of Plant Physiologists: Rockville, MD, 1987; 167–181.
23. Ashour, N; Thalooth, A.T. Effect of Putrescine on Growth and Photosynthetic Pigment of Broad Bean Plants Grown Under Chloride Salinization Conditions. Biochem. Physiol. Pflanzen (BPP), Bd. **1971**, *162 S*, 203–208.
24. Feray, A.; Hourmant, A.; Beraud, J.; Brun, A.; Cann-Moisan, C.; Caroff, J.; Penot, M. Influence of Polyamines on the Long Distance Transport of Potassium Rubidium-86) in Potato Cuttings (Solanum tuberosum cv. Sirtema): Comparative Study with Some Phytohormones. J. Exp. Bot. **1992**, *43*, 403–408.

25. SAS Institute. *SAS/STAT*® Software: Changes and Enhancements Through Release 6.12; SAS Institute: Cary, NC, 1998.
26. Pitman, M.G. Ion Uptake by Plant Roots. In *Encyclopedia of Plant Physiology. Transport in Plants II. Part B. Tissues and Organs*; Lüttge, U.; Pitman, M.G., Eds.; Springer-Verlag, Berlin, 1976; Vol. 2, 95–128.
27. Sposito, G. *The Thermodynamics of Soil Solutions*; Oxford University Press: Oxford, UK, 1981.
28. Parakash, L.; Prathapasenan, G. Effect of NaCl Salinity and Putrescine on Shoot Growth, Tissue Ion Concentration and Yield of Rice (*Oryza sativa* L. var. GR-3). J. Agron. Crop Sci. **1988**, *160*, 325–334.
29. Omielan, J.A.; Epstein E.; Dvorak, J. Salt Tolerance and Ionic Relations of Wheat as Affected by Individual Chromosomes of Salt-Tolerant *Lophopyrum elongatum.* Genome **1991**, *34*, 961–974.
30. Suarez, D.L.; Grieve, C.M. Predicting Cation Ratios in Corn from Saline Solution Composition. J. Exp. Bot. **1988**, *39*, 605–612.
31. Delgado, M.M.; Sanchez-Calle, I.M.; Diaz-Miguel, M.; Matilla, A.J. Effects of Polyamines on the Ion Content and Solutes Excretion of Isolated Embryonic Axes and the Germination Rate of Seeds of Chickpea. J. Plant Nutr. **1989**, *12*, 509–522.
32. Maas, E.V. Salt Tolerance of Plants. Appl. Agric. Res. **1986**, *1*, 12–24.
33. Francois, L.E.; Maas, E.V. Crop Response and Management of Salt-Affected Soils. In *Handbook of Plant and Crop Stress*; Pessarakli, M., Ed.; Marcel Dekker, Inc.: New York, 1999; 169–201.
34. Shannon, M.C.; Grieve, C.M.; Lesch, S.M.; Draper, J.H. Analysis of Salt Tolerance in Nine Leafy Vegetables Irrigated with Saline Drainage Water. J. Amer. Soc. Hort. Sci. **2000**, *125*, 658–664.
35. Wilson, C.; Lesch, S.M.; Grieve, C.M. Growth Stage Modulates Salinity Tolerance of New Zealand Spinach (*Tetragonia tetragonioides*, Pall.) and Red Orach (*Atriplex hortensis* L.). Ann. Bot. **1999**, *85*, 501–509.