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Effect of polyamines on physio-chemical and biochemical parameters of citrus rootstocks under NaCl stress

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ABSTRACT

The present study was carried out to understand the role of polyamines on physiological and biochemical changes in citrus rootstocks grown under NaCl stress. Citrus is highly salt sensitive crop and salinity is a major constraint hindering its successful production. In general, soil salinisation with NaCl resulted in lowered photosynthetic activity, growth as well as RWC but increased MII. However, application of polyamines in different concentrations reduced the negative effects of NaCl stress and improved the overall growth of the plants. In this experiment, citrus rootstocks, *viz.*, *Jatti khatti*, Rangpur lime, Attani-1 and *Soh-sarkar* were used. The maximum superoxidase dismutase (39.44 units 10 min^{-1} mg^{-1} of protein) and catalase (5.96 µmoles H_2O_2 reduced mg^{-1} protein^{-1}) activities were also noted in spermidine (0.75 mM) treated Rangpur lime rootstock under salt stress. The concentration of sodium and chloride in leaf tissue increased with NaCl treatment and maximum Na^+ (2.21%) and Cl- (3.18%) concentrations were observed in *Soh sarkar* rootstock. Putrescine (0.75 mM) in citrus rootstock *Jatti khatti* and *Soh sarkar* and spermidine (0.75 mM) in Rangpur lime and Attani-1 showed better effects in reducing the negative effects of NaCl stress. It was observed that polyamines could improve the salinity tolerance of citrus rootstocks by regulating absorption and accumulation of ions and improving antioxidant enzyme activities.

Key words: Biochemical changes, citrus rootstocks, polyamines, salinity.

INTRODUCTION

Citrus is classified as salt-sensitive fruit species and soil salinity is the most important factor responsible for poor productivity of citrus trees in traditional areas (Dubey *et al.*, 5). Salinization is one of the serious problems confronting sustainable agriculture in irrigated production systems in arid and semiarid regions (Marschner, 12). Salt induces various biochemical and physiological responses in plants and affects almost all metabolic processes. Many biochemical changes occurs when plants are subjected to salt stress like production of reactive oxygen species (ROS) can have detrimental effects on normal metabolism of plants through oxidative damage to lipids, proteins, and nucleic acids (Mittler, 13). Most halophytes react to environmental stresses with an effective ROS-scavenging system involving antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (O'Neil, 16). Therefore, technologies to improve antioxidant enzyme activities and to regulate translocation and accumulation of ions under salt stress conditions in citrus would be beneficial. Polyamines interact with negatively charged macro-molecules such as DNA, RNA, proteins, and phospholipids in such a way that they are involved in the regulation of the physical and chemical properties of membrane structure and functions of nucleic acids and modulation of enzyme activities. Polyamines accelerated the growth in stressed plants, inhibited Na^+ and Cl^- uptake, accelerated the accumulation of K^+, Ca^{++}, Mg^{++} and proline in the leaves, stabilized cell membrane, influenced protein and nucleic acid synthesis of salt-stressed plants and inhibited stress-induced senescence (Tang and Newton, 19). Despite the well defined roles of polyamines in upregulating the activity of antioxidant enzymes and regulating nutrient acquisition under saline conditions in many crops, very little is known about their interactive roles in improving salt tolerance in indigenous citrus rootstocks. Therefore, the present investigation was carried out on four commercial indigenous citrus rootstocks, to assess the impact of polyamines under NaCl stress.

MATERIALS AND METHODS

The pot experiment was conducted on *Jatti khatti* (*Citrus jambhiri*), *Soh sarkar* (*Citrus karna*), Rangpur lime (*Citrus limonia*) and Attani-1 (*Citrus rugulosa*) plants grown in polyhouse at a day temperature of

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25-33°C, night temperature of 15-20°C and relative humidity (RH) of 60-90%. Seeds were collected from the Citrus Germplasm block of the Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi. Seeds were thoroughly washed in running tap water immediately after extraction and sown in the nursery beds. Six-month-old nucellar plants were selected on the basis of uniformity, vigour, leaf size and shape. These plants were then transplanted in plastic pots (12 inch size) containing 7 kg of a mixture of garden soil and well-rotted farmyard manure at a ratio of 3:1. The soil having the EC of 0.37 dS m^{-1} was used as control. The treatment details were T_1: control (no salt + no polyamines); T_2: NaCl (100 mM) + Put. (0.5 mM); T_3: (NaCl 100 mM) + Put. (0.75 mM); T_4: NaCl (100 mM) + Spd (0.5 mM); T_5: NaCl (100 mM) + Spd. (0.75 mM); T_6: NaCl (100 mM) + no polyamines; T_7: Put. (0.75 mM) + Spd. (0.75 mM) + no salt. Membrane Injury Index (MII) was calculated as per the method suggested by Blum and Ebercon (3). The relative water content in recently matured leaves was determined following the method suggested by Brass and Wheatherly (4). Photosynthesis and stomatal conductance were measured by using an infrared gas analyzer (IRGA) (LI-6200, LI-COR Biosciences, Lincoln, NE, USA). The leaf chlorophyll contents were estimated using the method suggested by Hiscox and Israelstam (8). The activity of SOD in a leaf sample was determined by the method proposed by Fridovich (6). The method suggested by Luck (11) was followed to estimate the catalase activity in the plant sample. The activity of peroxidase in leaf samples was determined by the method proposed by Thomas *et al.* (20). Total leaf sodium and potassium contents in were determined using the diacid-digested plant sample using the method proposed by Jackson (9). Chloride content in the leaves was quantified by the mercury (II) thiocyanate method as suggested by Adriano and Doner (1). However, chloride extraction from the plant samples was done with 0.1 M sodium nitrate in 1:100 ratio (Gaines *et al.*, 7). The experiment was conducted in factorial randomized block design and two year pooled data were subjected to analyses using SPSS 11.0 (SPSS Inc., Chicago, IL, USA) for the calculation of F values. Significance of variance was estimated by applying the F test at the 5% level of significance.

RESULTS AND DISCUSSION

Results from this experiment revealed significant change in physiological and biochemical parameters of different citrus rootstocks grown under NaCl stress in response to polyamines treatment. It was noted that the photosynthetic rate was reduced significantly under salinized conditions. Polyamines Spd. and Put. had significant effects on the photosynthetic rate (Table 1) of citrus rootstocks. Seedlings treated with Put. and Spd. @ 0.75 mM had higher photosynthetic rate (7.82 μmol CO_2 m^{-2} s^{-1}) under non-saline conditions. In saline conditions, the photosynthetic rate was maximum (5.26 μmol CO_2 $m^{-2}s^{-1}$) in Put. (0.75 mM) treated citrus rootstocks. Among different rootstocks, maximum photosynthesis rate (6.28 μmol CO_2 m^{-2} s^{1}) was recorded in salt-treated Rangpur lime. Salinity causes a reduction in photosynthesis through its adverse impact on gas exchange parameters such as stomatal conductance and stomatal resistance. The findings of the present study are in agreement with the results on polyamines mediated stress tolerance observed by several authors (Zeid, 21; Tang and Newton, 19; Ndayiragije and Lutts, 14). Stomatal conductance was at a minimum in salinized plants and the effect of Spd. and Put. on stomatal conductance was significant (Table 1). The maximum stomatal conductance (0.18 mmol m^{-2} s^{-1}) under saline condition was found in with the application of Spd. (0.75 mM). Among different rootstocks, it was recorded maximum in Rangpur Lime (0.31 mmol m^{-2} s^{-1}), which was significantly higher over control. The transpiration rate was also affected due to salinity. Maximum transpiration rate (4.45 m mol H_2O m^{-2} s^{-1}) was recorded in Spd. (0.75 mM) treated citrus rootstocks under saline conditions which was significantly higher over complete saline conditions (2.75 mol H_2O $m^{-2}s^{-1}$). Our results suggest that the application of polyamines alone or in combination improved the photosynthetic rate, stomatal conductance and transpiration rate in different citrus rootstocks under saline conditions. This finding was in line with the earlier findings of Zekri (22) who reported a reduction in stomatal conductance under saline conditions in sour orange and Cleopatra mandarin. Sharma *et al.* (17) also reported that PBZ or Put. alone or in combination improved the photosynthetic rate and stomatal conductance in salt-susceptible *Karna khatta* rootstock under saline conditions.

It was also observed that total chlorophyll contents were reduced due to salt stress. However, application of polyamines improved total chlorophyll content under salt stress. Maximum total chlorophyll content was recorded with the application of Put. (0.75 mM) + Spd. (0.75 mM) without any salt however under saline conditions, it was observed to be maximum (1.36 mg g^{-1} FW) with the application of Put. (0.75 mM), which was significantly higher than the chlorophyll contents under saline conditions (0.99 mg g^{-1} FW). A difference in reduction of chlorophyll under saline conditions in different citrus species was also reported by Nieves *et al.* (15). Zeid (21) had also reported improvement

Table 1. Effect of polyamines on photosynthesis rate, stomatal conductance and transpiration rate of citrus rootstocks under NaCl stress.

Treatment	Photosynthesis rate (μmol CO_2 m^{-2} s^{-1})				Mean	Stomatal conductance (mmol m^{-2} s^{-1})				Mean	Transpiration rate (m mol H_2O m^{-2} s^{-1})				Mean
	Jatti khatti	*Soh sarkar*	Attani-1	Rangpur lime		*Jatti khatti*	*Soh sarkar*	Attani-1	Rangpur lime		*Jatti khatti*	*Soh sarkar*	Attani-1	Rangpur lime	
T_1	6.34	6.80	8.04	8.23	7.35	0.13	0.15	0.37	0.46	0.28	4.14	5.04	7.16	7.35	5.92
T_2	5.39	4.45	5.34	5.53	5.18	0.08	0.09	0.16	0.25	0.14	2.98	3.88	4.21	4.31	3.84
T_3	5.88	4.94	5.02	5.21	5.26	0.08	0.10	0.15	0.24	0.14	3.06	3.96	4.12	4.41	3.89
T_4	4.74	4.31	5.52	6.02	5.15	0.07	0.08	0.22	0.31	0.17	2.13	3.03	5.19	5.09	3.86
T_5	4.03	4.09	6.19	6.28	5.15	0.06	0.09	0.23	0.32	0.18	2.94	3.84	5.83	5.17	4.45
T_6	3.51	3.17	3.36	3.56	3.40	0.04	0.06	0.09	0.12	0.08	2.17	3.07	2.84	2.93	2.75
T_7	6.55	6.61	8.96	9.16	7.82	0.13	0.15	0.38	0.47	0.29	4.50	5.40	7.75	7.94	6.40
Mean	5.21	4.91	6.06	6.29		0.08	0.10	0.23	0.31		3.13	4.03	5.30	5.31	
$CD_{0.05}$															
Treatment (T)			0.43					0.02					0.48		
Rootstock (R)			0.10					0.02					0.11		
T × R			0.53					0.03					0.51		

T_1: control (no salt + no polyamines); T_2: NaCl (100 mM) + Put. (0.5 mM); T_3: NaCl (100 mM) + Put. (0.75 mM); T_4: NaCl (100 mM) + Spd. (0.5 mM); T_5: NaCl (100 mM) + Spd. (0.75 mM); T_6: NaCl (100 mM) + no polyamines; T_7: Put. (0.75 mM) + Spd. (0.75 mM) + no salt.

in photosynthetic pigment by the application of Put. which was helpful in enhancement of total chlorophyll contents in tissues.

The maximum reduction in RWC occurred under salt-treated plants compared to non-salinized controls (Table 2). Interaction among salinity and polyamine showed that RWC was enhanced significantly in the non-saline and saline conditions with the application of polyamines. Under non-saline conditions along with Put. application, 26.11% increase in RWC was observed compared to saline conditions. The MII increased (63.56%) in salinised plants over non-salinised plants (Table 2). In the presence of NaCl, the maximum MII was recorded in salinised plants without Spd. or Put. application, whereas, the minimum (11.95) was measured in Put. (0.75 mM) and Spd. (0.75 mM) treated plants under non-saline condition. The results of this study demonstrate a marked reduction in relative water content and increase in membrane injury index under saline conditions. Furthermore, the application of Spd. or Put. alone or in combination improved RWC and significantly reduced MII in salinity conditions under salt stress. The findings of the present study agree with the results on polyamines mediated stress tolerance observed by Zeid (21).

Superoxide dismutase activity (SOD) was significantly enhanced under salinity (Table 3). Moreover, the application of polyamines (Spd. and Put.) increased SOD activity under both salinized and non-salinized conditions. Under salinized conditions, the SOD level was 20.53 mg^{-1} protein min^{-1}, which increased significantly to 33.67 mg^{-1} protein min^{-1} level by the treatment of Put. (0.75 mM) compared to control. Catalase activity in seedlings increased significantly under salt stress compared to that of non-salinised plants after the application of polyamines (Table 3). In the absence of NaCl stress, the application of Put. (0.75 mM) and Spd. (0.75 mM) both improved catalase activity. The maximum increase (30.53%) was observed with the application of Put. (0.75 mM). Maximum increase in SOD (39.44 units 10 min^{-1} mg^{-1} of protein) and catalase (5.96 µmoles H_2O_2 reduced mg^{-1} protein) activity were reported in Rangpur lime under saline conditions after application of spermidine (0.75 mM). Salinity increased peroxidase activity by 21.16% over non-saline conditions. Furthermore, the application of Spd. or Put. or combination of both increased peroxidase activity under both saline and non-saline conditions. Whereas, under salt stress, the maximum peroxidase activity (1.54 A_{436} unit min^{-1} µg g^{-1} of FLW) was noticed with the application of Spd. (0.75 mM). Among different rootstocks, *Jatti khatti* showed the maximum peroxidase activity (1.52 A_{436} unit min^{-1} µg $g^{-1}$of FLW). Salinity has been reported to increase

Table 2. Effect of polyamines on leaf total chlorophyll content, relative water content and membrane injury index of citrus rootstocks under NaCl stress.

Treatment	Total chlorophyll (mg g^{-1} FW)				Mean	RWC (%)				Mean	MII				Mean
	Jatti khatti	*Soh sarkar*	Attani-1	Rangpur lime		*Jatti khatti*	*Soh sarkar*	Attani-1	Rangpur lime		*Jatti khatti*	*Soh sarkar*	Attani-1	Rangpur lime	
T_1	1.53	2.65	1.67	1.770	1.91	92.41	93.79	95.10	92.93	93.56	11.36	13.7	11.4	12.7	12.29
T_2	1.29	1.41	1.22	1.417	1.33	78.24	79.62	77.12	73.95	77.23	25.7	27.5	29.5	30.8	28.38
T_3	1.39	1.51	1.17	1.367	1.36	80.31	81.69	73.19	71.02	76.55	25.0	25.8	31.1	31.4	28.33
T_4	1.26	1.38	1.26	1.457	1.34	77.31	78.69	77.70	75.54	77.31	26.3	28.1	27.3	27.6	27.33
T_5	1.12	1.24	1.35	1.543	1.31	75.92	77.30	79.05	77.88	77.54	26.8	29.6	26.3	28.3	27.75
T_6	0.87	0.99	1.06	1.041	0.99	69.07	70.45	69.08	67.91	69.13	31.3	33.1	34.1	36.4	33.73
T_7	1.52	2.79	1.82	1.917	2.01	92.60	93.98	95.26	93.09	93.73	11.6	13.4	10.2	10.6	11.95
Mean	1.28	1.71	1.36	1.50		80.84	82.22	80.93	78.90		24.01	24.46	24.27	25.40	
$CD_{0.05}$															
Treatment (T)			0.51					2.34					1.78		
Rootstock (R)			0.03					1.11					0.52		
T × R			0.64					2.52					2.10		

T_1: control (no salt + no polyamines); T_2: NaCl (100 mM) + Put. (0.5 mM); T_3: NaCl (100 mM) + Put. (0.75 mM); T_4: NaCl (100 mM) + Spd. (0.5 mM); T_5: NaCl (100 mM) + Spd. (0.75 mM); T_6: NaCl (100 mM) + no polyamines; T_7: Put. (0.75 mM) + Spd. (0.75 mM) + no salt.

Table 3. Effect polyamines on leaf superoxide dismutase, catalase and peroxidase activities in citrus rootstocks under NaCl stress.

Treatment	SOD (mg^{-1} protein min^{-1})				Mean	CAT (µmoles H_2O_2 hydrolyzed min^{-1} mg^{-1} $protein^{-1}$)				Mean	POD (A_{436} unit min^{-1} µg g^{-1} FLW)				Mean
	Jatti khatti	*Soh sarkar*	Attani-1	Rangpur lime		*Jatti khatti*	*Soh sarkar*	Attani-1	Rangpur lime		*Jatti khatti*	*Soh sarkar*	Attani-1	Rangpur lime	
T_1	16.82	14.94	13.39	14.62	14.94	1.03	4.16	3.91	4.14	3.31	1.26	1.243	0.93	0.87	1.08
T_2	22.69	25.81	21.81	33.04	25.84	4.33	8.07	4.48	4.71	5.40	1.53	1.513	1.41	1.45	1.48
T_3	33.53	37.75	29.08	34.31	33.67	5.19	8.93	4.13	4.36	5.65	1.64	1.623	1.39	1.43	1.52
T_4	28.62	29.74	31.64	37.83	31.96	4.13	5.21	6.19	5.42	5.24	1.37	1.353	1.56	1.60	1.47
T_5	24.06	27.18	33.25	39.44	30.98	3.16	5.35	6.73	5.96	5.30	1.43	1.413	1.63	1.67	1.54
T_6	17.16	17.28	23.24	24.43	20.53	1.92	4.19	4.42	3.95	3.62	1.47	1.353	1.31	1.35	1.37
T_7	19.17	16.29	17.18	15.37	17.00	2.03	5.74	4.73	4.26	4.19	1.91	1.493	1.47	1.51	1.60
Mean	23.15	24.14	24.23	28.43		3.11	5.95	4.94	4.69		1.52	1.43	1.39	1.41	
$CD_{0.05}$															
Treatment (T)			1.16					0.23					0.16		
Rootstock (R)			0.23					0.21					0.11		
T × R			1.48					0.30					0.22		

T_1: control (no salt + no polyamines); T_2: NaCl (100 mM) + Put. (0.5 mM); T_3: NaCl (100 mM) + Put. (0.75 mM); T_4: NaCl (100 mM) + Spd. (0.5 mM); T_5: NaCl (100 mM) + Spd. (0.75 mM); T_6: NaCl (100 mM) + no polyamines; T_7: Put. (0.75 mM) + Spd. (0.75 mM) + no salt.

SOD activity in citrus (Almansa *et al.*, 2). Application of polyamines enhanced the activities of SOD, CAT and POD under saline conditions. The results of the present investigation suggested that the application of spermidine or putrescine alone or in combination improved antioxidant systems in plant to repair the damage caused by ROS.

Sodium content in leaves was affected significantly by salinity. In the absence of NaCl, the minimum Na^+ accumulation was recorded with the treatment of Put. (0.75 mM) and SPd. (0.75 mM). However, in salinised plants, the application of 0.75 mM Put. was more effective in reducing Na^+ accumulation in leaf tissues (Table 4). Among different rootstocks, minimum accumulation of Na^+ was noticed in *Jatti khatti* rootstock. Cl^- content in the leaves of salinised seedlings was increased significantly compared to that of control (Table 4). Application of Spd. or Put. alone or in combination had a significant effect on Cl^- accumulation (Table 4). Under salinized condition, the application of either 0.75 mM Put. or 0.75 mM Spd. reduced the leaf Cl^- concentration by 17.53% compared to that of controls. It was also noted that all the treatments reduced Cl^- accumulation in leaf tissues compared to the salinized control (Table 4). Minimum accumulation of Cl^- was noticed in Rangpur lime rootstock. Leaf K^+ content was higher in Spd. and Put. treated plants (Table 4). Interaction among salinity and polyamines showed that the leaf K^+ content was higher when the plants were sprayed with 0.75 mM Spd. However, Rangpur lime had maximum accumulation of K^+ among different rootstocks. One of the primary responses of plant to salinity is an influx of sodium and chloride concentration in plant tissues. The results from present study clearly showed that NaCl treatment resulted in an increase in sodium and chloride concentrations in leaf tissues, which was significantly reduced by application of polyamines. Our results are consistent with the findings that suggested role of exogenously applied polyamines in ion homeostasis regulation and absorption and translocation of toxic ions in cucumber (Suping *et al.*, 18), and PBZ in strawberry (Jamalian *et al.*, 10).

In conclusion, citrus rootstocks *Jatti khatti*, *Soh sarkar*, Rangpur lime and Attani-1 behaved differently under salt treated conditions after the application of polyamines, which played vital role in alleviating the NaCl stress. The response of salt susceptible citrus rootstock *Jatti khatti* and *Soh sarkar* was more for putrescine (0.75 mM), however, salt tolerant Rangpur lime and Attani-1 showed more response towards Spd. (0.75 mM) in reducing the negative effects of NaCl stress.

Table 4. Effect of polyamines on leaf sodium, chloride and potassium content in leaf tissues of citrus rootstocks under NaCl stress.

Treatment	NA (%)				Mean	Cl (%)				Mean	K (%)				Mean
	Jatti khatti	*Soh sarkar*	Attani-1	Rangpur lime		*Jatti khatti*	*Soh sarkar*	Attani-1	Rangpur lime		*Jatti khatti*	*Soh sarkar*	Attani-1	Rangpur lime	
T_1	0.57	0.66	0.43	0.45	0.53	0.83	0.77	0.76	0.71	0.77	2.27	2.35	3.03	3.11	2.49
T_2	0.81	1.19	1.86	1.88	1.44	2.44	2.53	2.12	2.07	2.29	2.03	2.03	1.66	1.74	1.87
T_3	0.89	1.31	1.48	1.50	1.30	1.98	2.37	2.26	2.21	2.21	2.17	2.15	1.41	1.49	1.81
T_4	1.57	1.66	1.39	1.41	1.51	2.66	2.97	1.98	1.93	2.42	1.67	1.95	1.62	1.70	1.74
T_5	0.92	1.37	1.64	1.66	1.40	2.78	2.85	2.07	2.02	2.40	1.43	1.71	1.87	1.95	1.74
T_6	2.12	2.21	2.03	2.05	2.10	2.79	3.18	2.53	2.68	2.80	1.15	1.43	1.12	1.20	1.23
T_7	0.63	0.66	0.38	0.40	0.51	0.78	0.68	0.67	0.62	0.69	2.28	2.56	3.16	3.24	2.81
Mean	1.07	1.30	1.32	1.34		2.04	2.19	1.77	1.75		1.86	2.05	2.00	2.08	
$CD_{0.05}$															
Treatment (T)			0.14					0.48					1.10		
Rootstock (R)			0.11					0.20					0.37		
T × R			0.26					0.53					1.17		

T_1: control (no salt + no polyamines); T_2: NaCl (100 mM) + Put. (0.5 mM); T_3: NaCl (100 mM) + Put. (0.75 mM); T_4: NaCl (100 mM) + Spd. (0.5 mM); T_5: NaCl (100 mM) + Spd. (0.75 mM); T_6: NaCl (100 mM) + no polyamines; T_7: Put. (0.75 mM) + Spd. (0.75 mM) + no salt.

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