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Growth and physiological response of salt-sensitive and salt-tolerant rootstocks of citrus to paclobutrazol under salt stress

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ABSTRACT

The response of paclobutrazol was studied during 2006-07 on salt-sensitive 'Soh Sarkar' (*Citrus karna* Raf.) and salt-tolerant 'Rangpur lime' (*Citrus limonia* Osbeck) under salt stress. One-year-old seedlings of both rootstocks were grown in earthen pots of 10 inches size containing 5 kg soil. Paclobutrazol (0, 125 and 250 ppm) was applied 7 days prior to salinization is a 1 ½ litre solution/pot. Computed amount of NaCl (1 000 mg/kg soil) was added in solution form to get soil salinity level of 2 dS/m. Paclobutrazol mitigated the salinity stress effects in both the rootstocks. Application of 250 ppm paclobutrazol increased plant height (34.70%), leaves/plant (170.61%), root length (51.83%), shoot fresh weight (51.89%) and dry weight (44.88%), root fresh weight (14.42%) and dry weight (58.06%) under salt stress than salinized control without paclobutrazol application in salt-susceptible 'Soh Sarkar'. While in 'Rangpur lime', application of 125 ppm paclobutrazol increased maximum plant height (10.33%), root length (68.84%), shoot fresh weight (31.38%) and dry weight (41.25%), root fresh weight (95.40%) and dry weight (76.34%) under salt stress as compared to salinized seelings without paclobutrazol. Root to shoot ratio increased maximum in 'Soh Sarkar' (35.30%) and in 'Rangpur lime' (24.32%) with application of 125 ppm paclobutrazol under salt stress. Paclobutrazol-treated plants of the both rootstocks had less defoliation and toxicity symptoms appeared on fewer leaves. Relative water content, chlorophyll a and b, total chlorophyll and chlorophyll a to b ratio in both rootstocks increased by application of paclobutrazol. Membrane injury index reduced by 20.18% in Soh Sarkar and 51.83% in 'Rangpur lime' with application of 250 ppm paclobutrazol under salt stress than salinized seedlings without paclobutrazol treatment. The findings suggested that paclobutrazol promoted salt stress avoidance in both salt-sensitive and salt-tolerant rootstocks of citrus.

Key words: Citrus rootstock, Chlorophyll, Membrane injury index, Paclobutrazol, Root to shoot ratio, RWC, Salinity

Salinization is one of the most serious problems confronting sustainable agriculture in irrigated production system in semi-arid and arid regions (Ravindran *et al.* 2007). The most commonly used practice for alleviating salt stress is leaching, where large quantities of water are used to flush salts from the root zone. With diminishing supply of irrigation water, leaching may become an expensive or impractical option. Therefore, new approaches to agricultural production in salt-affected regions are needed.

Paclobutrazol is a growth retardant, which affects many plant species. Studies showed that paclobutrazol could inhibit endogenous gibberrelin biosynthesis, increase cytokinin content, cause transit rise in abscisic acid and decrease ethylene content. Earlier research indicated that paclobutrazol reduced the negative effect of salt stress on different crops, oleander (*Nerium oleander* L.) and datura (*Datura stramonium* L.) (Saeed 2005, Banon *et al*. 2005). In spite of well defined role of paclobutrazol in mitigating salinity induced effect in many crops, very little is known about the effect of paclobutrazol on amelioration of salinity induced effects in citrus. Citrus considered as salt-sensitive crop. The present investigation was carried out to examine the effect of paclobutrazol on salt-sensitive and salt-tolerant citrus rootstocks under salt stress.

MATERIALS AND METHODS

The experiment was performed with 1-year old seedlings of 2 citrus rootstocks, 'Soh Sarkar' (*Citrus karna* Raf.) and 'Rangpur lime' (*Citrus limonia* Osbeck) during 2006–07. All plants were grown from seeds in 10 inch earthen pots lined with polyethylene (200 gauze) and filled with 5 kg sandy loam (Typic Haplustep) with $pH_{1:2}$ 7.35, E $C_{1:2}$ 0.30 dS/m, CEC 10.65 cmol/kg and 0.43% organic carbon. Nucellar

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seedlings of both rootstocks were identified on the basis of leaf shape and size and vigour.

Paclobutrazol (1.5 litre/pot) was applied at 125 ppm or 250 ppm 7 days before salinization. To get desired quantity of salts for 1 kg soil to develop 2 dS/m EC levels of the soil, a calibration curve was developed with stock solution of NaCl. This stock solution was then added in 10 g of soil of known electrical conductivity (0.30 dS/m) in different quantities (0.5 ml to 6 ml) and then distilled water was added in order to get 1: 2 soil: solution ratio for recording electrical conductivity of the samples after equilibration. A calibration curve was prepared and amount of salt required for 1 kg soil to get 2 dS/m EC level was calculated from the calibration curve (Dubey *et al*. 2006). Computed amount of salts (1 000 mg NaCl/kg soil) was dissolved in distilled water and one-and-half litre water containing 5 000 mg NaCl was poured into the pots. Treatments were started before new flush emergence so that the development of new flushes was under saline conditions. Thereafter, the plants were irrigated with tap water (0.25 dS/m) at regular interval to keep the soil moist. The electrical conductivity of the soil was tested at regular interval and the average final salinity levels recorded at the time of termination of the experiment were 2.23 dS/m (salinized pots of 'Soh Sarkar),' 2.24 dS/m (salinized pots of 'Soh sarkar' having paclobutrazol 125ppm), 2.26dS/m (salinized pots of 'Soh sarkar' having paclobutrazol 250 ppm), 2.17dS/m (salinized pots of 'Rangpur lime),' 2.24dS/m (salinized pots of 'Rangpur lime' having paclobutrazol 125ppm) and 2.19dS/m (salinized pots of 'Rangpur lime' having paclobutrazol 250 ppm).

The plants were uprooted after 90 days of salinization and washed with tap water and then 0.1N HCl, followed by 2 washings with de-ionized water. Shoots and roots fresh weight was recorded just after harvesting and dry weight after being placed in oven at 60±1°C until constant weight.

Relative water content (RWC) of leaf was determined according to Barrs and Weatherley (1962), by measuring fresh, saturated (6 hr in distilled water) and dry weight (70 °C until constant weigh) of leaf discs (8 mm). The RWC was then calculated as RWC (%) = [fresh weight – oven dry weight/ turgid weight-oven dry weight]×100. Membrane injury index was measured in leaf pieces (0.1g) taken from middle of leaf lamina and kept in 10 ml distilled water at 35 °C for 30 min. thereafter electrical conductivity (C1) was recorded using conductivity meter. These samples were then kept for 10 min. at 100 °C and electrical conductivity (C2) was recorded. Membrane injury index was then calculated by C1/C2. Chlorophyll content was estimated using the method given by Hiscox and Israelstom (1979).

The experiment was set up as factorial CRD including 2 rootstocks and 3 concentrations of paclobutrazol with five replications per treatment combination. Data were analyzed according to Gomez and Gomez (1986). The mean separation was performed using LSD test at 5% probability level.

RESULTS AND DISCUSSION

All growth variables increased with the application of paclobutrazol in both the rootstocks (Table 1) grown under NaCl stress. Application of paclobutrazol at 250 ppm was found effective in mitigating NaCl stress in salt-susceptible

Table 1 Growth of different rootstocks as affected by paclobutrazol under salt stress

Treatment	Height (cm)	Leaves/ plant	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Rootstock							
'Soh Sarkar'	63.27	102.67	21.54	30.35	17.46	14.74	8.43
'Rangpur lime'	56.24	117.42	24.97	31.07	11.97	12.98	5.54
Rootstock, CD (P = 0.05)	5.65	5.55	1.93	2.47	1.27	1.27	1.84
Paclobutrazol							
Saline without PBZ	54.83	70.13	18.88	27.05	12.74	12.13	5.37
Saline+125 ppm PBZ	57.35	94.63	25.61	30.05	16.70	13.95	7.78
Saline+250ppm PBZ	67.09	165.38	25.29	34.58	14.70	15.51	7.89
Paclobutrazol, CD (P = 0.05)	6.80	6.79	2.37	3.03	1.56	1.55	2.26
Rootstock×Paclobutrazol							
'*Soh Sarkar'*							
Saline	56.25	65.5	18.33	26.40	16.78	13.10	6.63
Saline+125 ppm PBZ	55.78	65.25	18.48	24.55	16.40	12.15	8.33
Saline+250 ppm PBZ	75.77	177.25	27.83	40.10	19.20	18.98	10.48
'*Rangpur lime'*							
Saline	53.40	74.75	19.43	27.70	8.70	11.15	4.10
Saline+125ppm PBZ	58.92	124.00	32.73	36.45	17.00	15.75	7.23
Saline+250ppm PBZ	56.40	153.50	22.75	29.05	10.20	12.03	5.30
Rootstock×Paclobutrazol, CD (P = 0.05)	13.18	18.65	3.35	4.28	2.21	2.20	3.20

PBZ, paclobutrazol

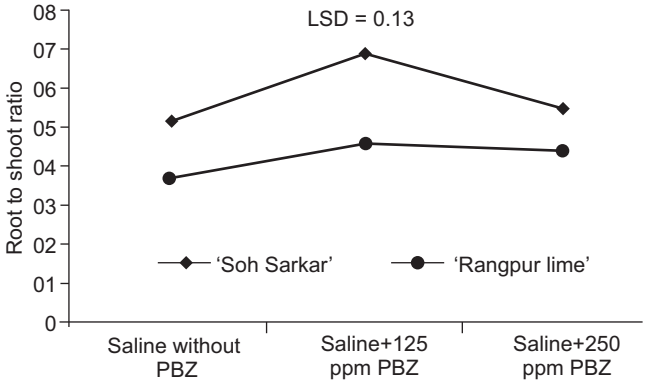


Fig 1 Effect of paclobutrazol concentration (ppm) on root to shoot ratio of 'Soh Sarkar' and 'Rangpur lime' under salt stress.

'Soh Sarkar', whereas 125 ppm paclobutrazol improved stress tolerance in salt-tolerant 'Rangpur lime'. Application of paclobutrazol at 250 ppm to 'Soh sarkar' resulted in the highest plant and number of leaves, root length, shoot fresh and dry weight. Whereas, application of 125 ppm paclobutrazol in 'Rangpur lime' resulted in maximum plant height, root length, shoot fresh and dry weight, root fresh and dry weight.

Root to shoot ratio was increased with application of paclobutrazol and maximum increase (35.30%) in root / shoot ratio was recorded in 'Soh Sarkar' and 'Rangpur lime'. Maximum increase (24.32%) was measured with the application of 125ppm paclobutrazol (Fig 1). It was reported that paclobutrazol significantly decreased plant height resulting in decrease in fresh and dry weight of shoot (Hasihashemi and Kiarostami 2007). However, in our study, plant height, fresh and dry weight of shoot and roots increased with application of paclobutrazol in the both rootstocks. It might be due to the fact that application of paclobutrazol reduced the harmful effect of salinity in both the rootstocks and increased number of leaves and height comparing to salt-stressed plants without paclobutrazol treatment, where merisatmatic tip died due to salt injury.

Membrane injury index reduced with the increased concentration of paclobutrazol and lowest in 'Soh Sarkar' (0.253) and in 'Rangpur lime' (0.131) was recorded on application of 250 ppm paclobutrazol as compared to 0.317 and 0.272 in salinized plants without paclobutrazol treatment in both rootstocks, respectively (Fig 2). Reduction in membrane injury index was more pronounced in salt- tolerant rootstock than salt-sensitive rootstock.

RWC increased with the increasing concentration of paclobutrazol in both the rootstocks (Fig 2). The difference between treatments was significant in increasing (7.38% and 4.18%) RWC in both 'Soh Sarkar' and 'Rangpur lime', respectively with application of 250 ppm paclobutrazol. In general, salinity reduced relative water content but maintained turgor potential in paclobutrazol-treated plants in both rootstocks as a consequence of osmotic adjustment.

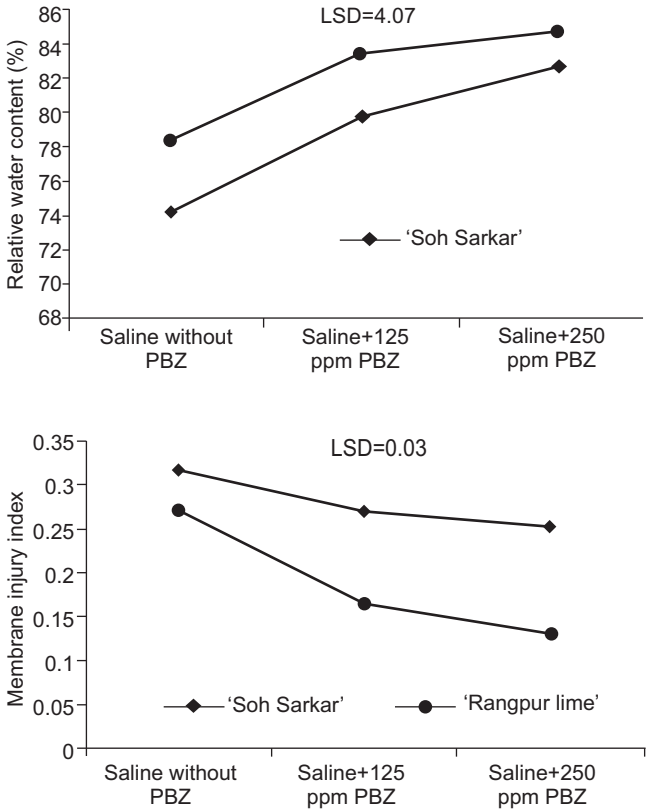


Fig 2 Effect of paclobutrazol concentration (ppm) on membrane injury index and relative water content (%) in 'Soh Sarkar' and 'Rangpur lime' under salt stress

paclobutrazol promoted a process of osmotic adjustment probably through the accumulation of organic compounds (Banon *et al.* 2005). The negative effect of salt stress on RWC in salt-sensitive rootstock 'Soh Sarkar' was more than salt-tolerant rootstock 'Rangpur lime' because average value of reduction (6.88%) in salinized plants of salt-sensitive rootstock was more than salinized plants of salt tolerant rootstock (4.01%) as compared to the value of 250 ppm paclobutrazol-treated plants. It showed that higher-concentration of paclobutrazol was more effective in reducing the NaCl stress in salt-sensitive 'Soh Sarkar'. The reduction in shoot and root biomass under salt stress could be due to reduction of availability of water or accumulation of toxic ions in the plant. A significant negative correlation was reported between cellular injury and RWC in salt-stressed wheat (Farooq and Azam 2006) and in barley (Kocheva *et al*. 2004). In general, NaCl slows down water uptake by plants, thereby decreasing the RWC, followed by reduction in fresh weight in both rootstocks, especially, salt-sensitive 'Soh Sarkar'. It is hypothesized that the fresh weight of shoot and roots increased in paclobutrazol-treated plants because cellular water content was enhanced. Therefore, it would be predicted that paclobutrazol could induced adaptive mechanism in citrus under salt stress. Higher concentration

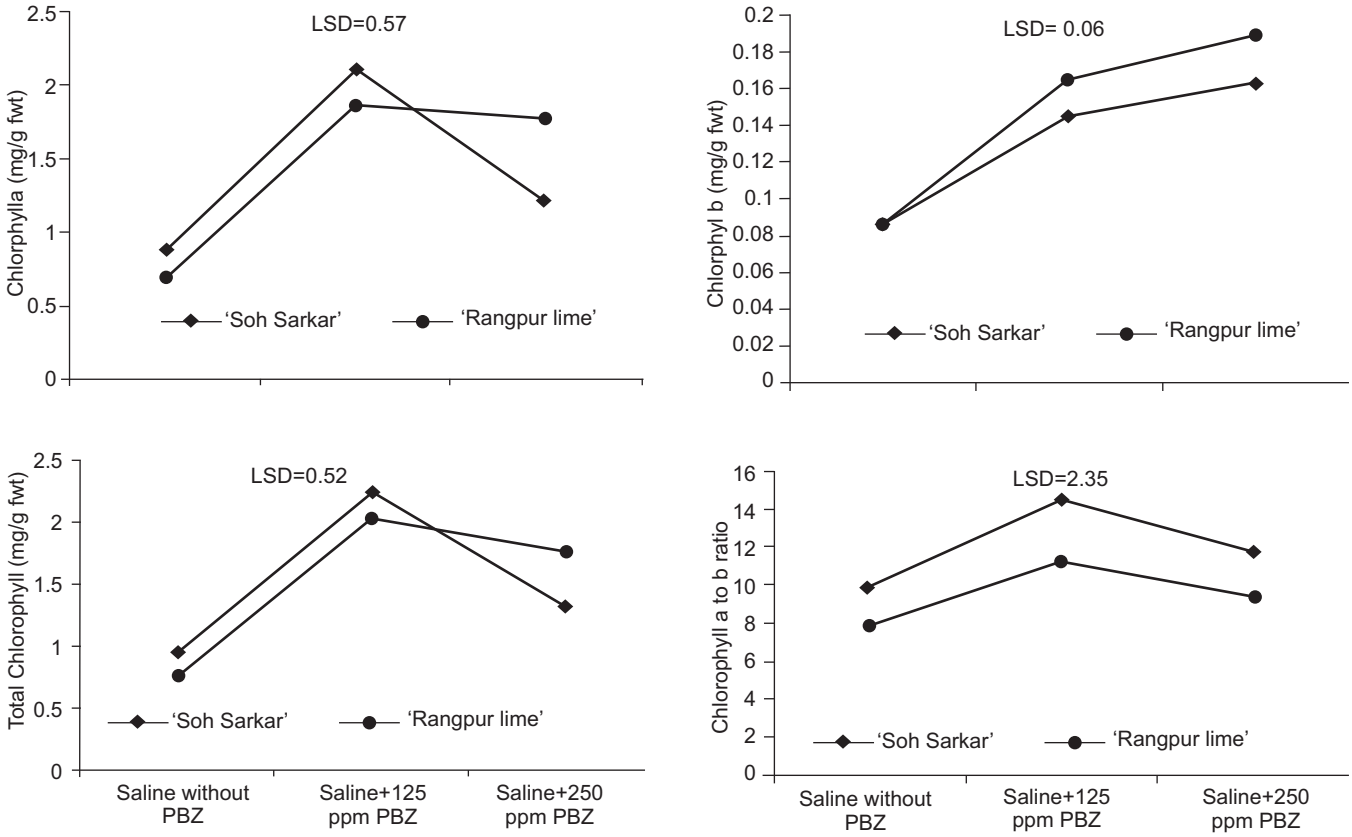


Fig 3 Effect of paclobutrazol concentration (ppm) on contents of chlorophyll a, b and total chlorophyll and chlorophyll a: b ratio in 'Soh Sarkar' and 'Rangpur lime' under salt stress

of paclobutrazol (250 ppm) was required in salt-sensitive rootstock to osmoregulate the turgor and increase the root and shoot fresh weight. However, in salt-tolerant rootstock 125 ppm paclobutrazol was optimum to maintain turgor.

Compared to plants grown under salt stress without paclobutrazol treatment, the leaves of paclobutrazol-treated plants were greener and there was an increase in chlorophyll 'a' 'b', total chlorophyll levels and chlorophyll a to b ratio (Fig 3) in both rootstocks. Chlorophyll a (174.18%) and chlorophyll b (119.76%) were more in leaves of salt-stressed plants of 'Rangpur lime' treated with 250ppm paclobutrazol. The total chlorophyll content was found maximum in salt-stressed plants of both rootstocks when treated with 125ppm paclobutrazol but per cent increase was higher in 'Rangpur lime' (164.57%) as compared to 136.84% increase in 'Soh Sarkar'. Results showed that application of 125ppm paclobutrazol increased chlorophyll a/b ratio in both rootstocks and the maximum increase was noticed in leaves of salt-stressed plants of salt-sensitive 'Soh Sarkar' by application of 125ppm paclobutrazol. Increase in chlorophyll fractions might be due to stimulation of chlorophyll synthesis in paclobutrazol-treated plants is mediated through an effect on cytokinins. It was in agreement with findings of Saeed (2005).

Findings of present investigation suggest that paclobutrazol could mitigate the harmful effect of salinity stress in citrus. Marked differences exist between the salt-tolerant 'Rangpur lime' and salt-sensitive 'Soh Sarkar' with regard to response to paclobutrazol treatments. Paclobutrazol was found more effective in protecting shoot of salt-sensitive rootstock, whereas its effect was more pronounced on roots of salt-tolerant rootstock as indicated by fresh weight data. Under salt stress, paclobutrazol improved RWC more effectively in 'Soh Sarkar' than 'Rangpur lime'. Salinity reduced relative water content but maintained turgor potential in paclobutrazol treated plants of both rootstocks as a consequence of osmotic adjustment. Effect of paclobutrazol on chlorophyll content in both rootstocks suggested that paclobutrazol had a role in enhancing the chlorophyll. Paclobutrazol treatment increased more chlorophyll a and b in salt-tolerant rootstock. The positive effect of paclobutrazol on various parameters, such as relative water content, membrane injury, chlorophyll content suggests that paclobutrazol is effective in mitigating salt stress effects in both rootstocks. Paclobutrazol has potential to protect citrus plants gown in saline area or irrigated with saline water. Our results suggests that 250ppm paclobutrazol is more effective in salt-sensitive rootstock 'Soh Sarkar' while 125ppm paclobutrazol is more effective in salt tolerant 'Rangpur lime' under moderate salinity stress (2dS/m).

REFERENCES

Banon S, Fernadez J A, Ochao J and Sanchez-Blanco M J. 2005. Paclobutrazol as an aid to reduce some effect of salt stress in oleander seedlings. *European Journal of Horticultural Science* **70**: 43–9.

Dubey A K, Srivastav, Manish, Singh, Room, Pandey R N and Deshmukh P S 2006. Response of mango (*Mangifera indica* L.) genotypes to graded levels of salt stress. *The Indian Journal Agricultural Sciences* **76**: 670–72.

Farooq S and Azam F. 2006. The use of cell membrane stability (CMS) technique to screen for salt-tolerant wheat varieties. *Journal of Plant Physiology* **163**: 629–39.

Gomez KA and Gomez AA. 1986. *Statistical Procedure for Agricultural Research,* 2nd edn. John Willey and Sons, New York.

Hasihashemis and Kiarostami K. 2007. Effect of paclobutrazol and salt stress on growth and ionic content in two cultivars of wheat. *Pakistan Journal of Biological Sciences* **10**: 41–8.

Kocheva K, Lambrev P, Giorgiev G, Goltsev V and Karavaliev M. 2004. Evaluation of chlorophyll fluorescence and membrane injury in the leaves of barley cultivars under osmotic stress. *Bioelectrochemistry* **63**: 121–4.

Ravindran K C, Venkatesa K, Balakrishnan V, Chellappan KP and Balasubramanian T. 2007. Restoration of saline land by halophytes for Indian Soils. *Soil Biology and Biochemistry* **39**: 2661–4.

Saeed WT. 2005. Pomegranate cultivars as affected by paclobutrazol, salt stress and change in finger prints. *Bulletin,* Faculty of Agriculture, Cairo University **56**: 581–615.