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The Effect of Paclobutrazol on Fruit Yield, Leaf Mineral Elements and Proline Content of Strawberry Cv. Selva under Saline Condition

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Abstract: Since strawberry plants (*Fragaria×ananassa* Duch.) are susceptible to salinity, therefore a reduction of negative effects of salt stress is needed in strawberry production. To investigate the interaction effects of paclobutrazol (PP_{333}) and salinity on fruit yield, leaf mineral elements and free proline content, three levels of NaCl [0, 5, 10 mM] were incorporated into the nutrient solution and four levels of PP_{333} [0, 10, 20, 30 mg L^{-1}] were sprayed. Interaction of PP_{333} in 2 levels (20 and 30 mg L^{-1}) with 5 mM NaCl, showed increase in fruit yield. 10 mg L^{-1} of PP_{333} reduced proline content at 5 mM NaCl salinity. 20 mg L^{-1} of PP_{333} at 5 mM NaCl caused reduction in shoot sodium content. Similar result was also observed in the interaction of 20 and 30 mg L^{-1} of PP_{333} with 10 mM NaCl. PP_{333} application also reduced root sodium content. All levels of PP_{333} at 5 mM NaCl and 20 mg L^{-1} of PP_{333} at 10 mM NaCl, increased shoot potassium content. PP_{333} could overcome the increase in shoot and root chlorine content at 5 and 10 mM NaCl. PP_{333} application decreased the electrolyte leakage induced by salinity at 5 and 10 mM. From the above results, effective role of PP_{333} on fruit yield promoting and reducing damages of sodium and chlorine ions on strawberry plant has been proved.

Key words: Paclobutrazol · salinity · yield · mineral elements · proline content

INTRODUCTION

Strawberry is considered as a NaCl salinity sensitive plant. Elevated NaCl concentrations impair plants by osmotic and ion-specific effects. Salt tolerant plants differ from salt-sensitive ones mainly in having a low rate of sodium and chlorine transport to leaves and an enhanced ability to compartmentalize these ions in vacuoles [1]. Increase in shoot chlorine content has been reported in strawberry cvs. Elsanta and Korona and five citrus species when exposed to NaCl salinity [2, 3]. According to Ulrich *et al.* [4] for many strawberry cultivars, leaf chloride value higher than 0.5 % is associated with leaf necrosis and reduction in yield. Other studies on the effects of salt stress in strawberry cvs. Douglas and Toro showed that leaf scorch is basically due to increase in the foliar chlorine concentration [5]. Salt stress increased leaf proline content and reduced membrane stability index in pistacia rootstocks [6]. To overcome the devastating effects of salinity on strawberry plants, it is necessary to find an effective resolution. The most common practice is to increase leaching. However this method is sometimes expensive and impractical. Few studies have focused on application of growth regulators to induce tolerance. There is a report that paclobutrazol (PP_{333}), a gibberelline biosynthesis inhibitor, promoted salt stress avoidance in peach (*Prunus persica* L.) [7]. PP_{333} has also been shown to reduce the symptoms and mortality due to salt stress in *Rhamnus alternus* seedlings [8]. This growth regulator has also modulated salt induced altrations in leaf proline level of zuzuba seedlings [9]. Studies have also been undertaken to investigate the effect of paclobutrazol and uniconazole (Another Gibberelline biosynthesis inhibitor), on cold hardiness in *Actinidia arguta* during a dormancy cycle [10]. These results show that triazoles may be effective in improving plant stress tolerance. The aim of this study was to determine interaction effect of PP_{333} and salinity on strawberry cv. Selva.

MATERIALS AND METHODS

Cold stored rooted runner strawberry plants (*Fragaria×ananassa* Duch.) of 'Selva'cultivar were planted in 2 L containers filled with a mixture of perlite and

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peatmoss. Plants were fed continuously from the start of the experiment with nutrient solution (Melspray). Sodium Chloride (NaCl) was incorporated into the nutrient solution. Three levels of Nacl [0, 5, 10 mM] and four levels of PP_{333} [0, 10, 20, 30 mg L^{-1}] were imposed at the beggining of the experiment. A hand sprayer was used to apply PP_{333} to the point of runoff. The design of the experiment was a complete randomized with five replications and twelve treatments.

Mean fruit fresh weight was measured along the period of the experiment and expressed as fruit yield.

To determine proline content of the leaves, 0.5 g of plant material was homogenized in 10 ml of 3 % aqueous sulfosalcylic acid and the homogenate filtered through Whatman # 2 filter paper. Then 2 ml of filterate was reacted with 2 ml acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100°C and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance was read at 520 nm. The proline concentration was determined from standard curve and calculated as follows [11]:

$$\left[\frac{\frac{(\mu g \text{ proline/ml} \times \text{ml toluene})}{115.5 \ \mu g/\mu mole}}{\frac{(g \text{ sample})}{5}}\right] = \mu moles \text{ proline/g fresh weight}$$

For chemical analysis, ground samples were ashed at 550°C for 6 hours. The white ash was taken up to 50 ml with distilled water. Sodium and potassium were determined in these sample solutions and analyzed using a flame photometer. Chlorine was determined by titration with AgNo3 from the aqueous extract.

Electrolyte leakage was measured as an assessment of permeability. This procedure was based on Lutts *et al.* [12]. Electolyte leakage was measured using an Electrical Conductivity Meter (EC). Two mature leaves per plant were taken and cut into 1 cm segments. Leaf samples were then placed in individual vials containing 10 ml of distilled water, after three washes to remove surface contamination. These samples were incubated at room temperature on a shaker (100 rpm) for 24 h. Electrical Conductivity (EC) of bathing solution (EC1) was read after incubation. Samples were then placed in an autoclave at 120°C for 20 minute and the second reading (EC2) was determined after cooling solution to room temperature. The electrolyte leakage was calculated as EC1/EC2 and expressed as percent.

RESULTS AND DISCUSSION

Salinity caused reduction in fruit yield. The interaction of PP_{333} in 2 levels (20 and 30 mg L^{-1}) with 5 mM NaCl, showed increase in fruit yield, when compared with the treatment without PP_{333} (Table 1). Similar results were shown in strawberry and tomato fruit by increasing electrical conductivity of the nutrient solution [13, 14]. As observed by Chartzoulakis and Klapaki [15], total yield in pepper decreased with increasing salinity, however Mitchell *et al.* [16], reported that irrigation with saline water had no effect on tomato fruit yield. Christov *et al.* [17], reported that application of PP_{333} increased total yield in strawberry. A similar result was observed in peach tree cv. Redhaven [18].

Salinity caused increase in leaf proline content. The interaction of 10 mg L^{-1} PP_{333} and 5mM NaCl, reduced proline (Table 2). Kwan *et al.* [19] showed leaf proline accumulation in *Brassica rapa* L. at saline condition.

Increasing sodium content of shoot and root was observed by increasing concentration of salt. 20 mg L^{-1} of PP_{333} at 5 mM NaCl concentration, caused reduction in shoot sodium content, compared with similar treatment without PP_{333}. Similar result was also shown in the interaction of 20 and 30 mg L^{-1} of PP_{333} and 10 mM NaCl.

Table 1: The effect of NaCl and PP_{333} treatments on fruit yield (g)

	PP_{333} (mg L^{-1})			
Salinity (mM)	0	5	10	Mean
0	57.71de*	54.84e	44.47f	52.34C
10	166.1a	65.89d	58.49de	96.79A
20	119.30c	94.49c	67.33d	93.70AB
30	94.30c	85.08c	85.86c	88.42B
Mean	109.30A	75.08B	64.02C	

*Mean followed by the same letter (small letters for means and capital letters for means of rows and columns) are not significantly different at 5% level of probability using DMRT

Table 2: The effect of NaCl and PP_{333} treatments on leaf proline content (µmoles proline/g fresh weight)

	PP_{333} (mg L^{-1})			
Salinity (mM)	0	5	10	Mean
0	0.168d*	0.629ab	0.724a	0.513B
10	0.271cd	0.499b	0.670a	0.480B
20	0.359c	0.695a	0.782a	0.612A
30	0.362c	0.691a	0.781a	0.611A
Mean	0.294C	0.628B	0.739A	

*Mean followed by the same letter (small letters for means and capital letters for means of rows and columns) are not significantly different at 5% level of probability using DMRT

Table 3: The effect of NaCl and PP_{333} treatments on shoot chlorine content (%)

	PP_{333} (mg L^{-1})			
Salinity (mM)	0	5	10	Mean
0	0.568cde*	0.801b	0.936a	0.768A
10	0.497def	0.603cd	0.641c	0.580B
20	0.595cd	0.489def	0.572cde	0.552BC
30	0.613c	0.450f	0.472ef	0.512C
Mean	0.568B	0.585B	0.655A	

*Mean followed by the same letter (small letters for means and capital letters for means of rows and columns) are not significantly different at 5% level of probability using DMRT

Table 4: The effect of NaCl and PP_{333} treatments on root chlorine content (%)

	PP_{333} (mg L^{-1})			
Salinity (mM)	0	5	10	Mean
0	1.531c*	0.795b	2.405a	1.910A
10	1.554c	1.595c	1.794b	1.648B
20	1.522c	1.603c	1.622c	1.582B
30	1.522c	1.575c	1.612c	1.570B
Mean	1.532C	1.642B	1.858A	

*Mean followed by the same letter (small letters for means and capital letters for means of rows and columns) are not significantly different at 5% level of probability using DMRT

Table 5: The effect of NaCl and PP_{333} treatments on electrolyte leakage (%)

	PP_{333} (mg L^{-1})			
Salinity (mM)	0	5	10	Mean
0	12.48d*	21.42b	25.63a	19.84A
10	12.75d	13.68d	17.92c	14.78B
20	12.57d	13.63d	14.30d	13.50BC
30	12.50d	12.98d	12.75d	12.75C
Mean	12.57C	15.43B	17.66A	

*Mean followed by the same letter (small letters for means and capital letters for means of rows and columns) are not significantly different at 5% level of probability using DMRT

In comparison with the treatment without PP_{333}, interaction of all concentrations of PP_{333} with 5 and 10 mM NaCl, decreased sodium content of the root (Fig. 1 and 2). 10 mM NaCl significantly reduced shoot potassium content. Interaction of PP_{333} in all concentrations with 5 mM NaCl and also 20 mg L^{-1} of PP_{333} with 10 mM NaCl, showed an increase in shoot potassium content (Fig. 3). Salinity treatments showed

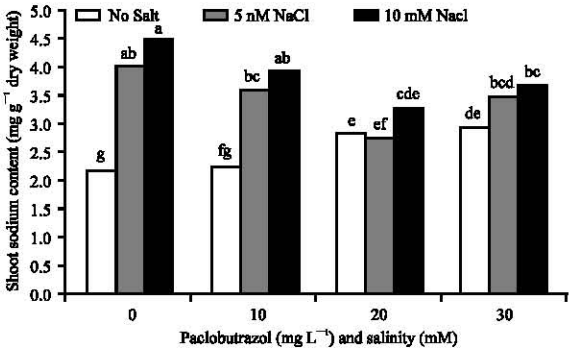

Fig. 1: The effect of NaCl and PP_{333} treatments on shoot sodium content (mg g^{-1} dry weight)

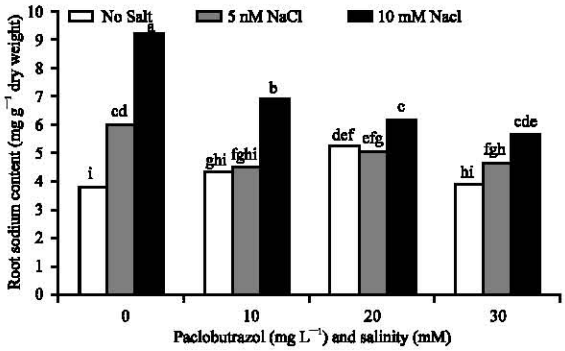

Fig. 2: The effect of NaCl and PP_{333} treatments on root sodium content (mg g^{-1} dry weight)

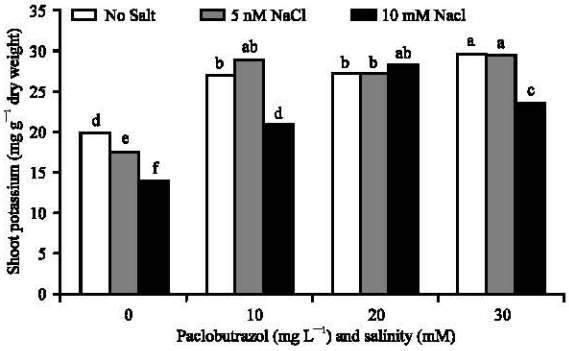

Fig. 3: The effect of NaCl and PP_{333} treatments on shoot potassium content (mg g^{-1} dry weight)

reduction in root potassium but PP_{333} application did not affect the reduction trend (Fig. 4). Cusido *et al.* [20], reported that increase in leaf sodium content, causes reduction in leaf potassium content of *Nicotina rustio*. Salinity increased root sodium content in two pepper cultivars but leaf sodium content remained unchanged [15]. A reduction trend in leaf potassium content of peach trees was shown by use of PP_{333} [21].

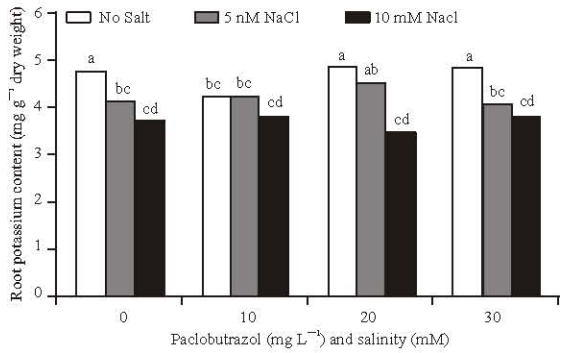

Fig. 4: The effect of NaCl and PP_{333} treatments on root potassium content (mg g^{-1} dry weight)

The results reported here showed a rise in shoot and root chlorine content at 10 mM NaCl, however PP_{333} application could overcome this increase at 5 and 10 mM NaCl salinity (Table 3 and 4). It has been shown that toxicity symptoms of salt stressed strawberry plants cvs. Douglas and Toro is caused by leaf chlorine accumulation, not sodium [5].

Salt treatments increased electrolyte leakage and membrane permeability but interaction of PP_{333} in all levels with 5 and 10 mM NaCl salinity caused reduction in electrolyte leakage (Table 5). Kaya *et al.* [22], have reported an increased membrane permeability with addition of 35 mM NaCl in strawberry cv. Oso Grande.

CONCLUSION

From the above results it can be concluded that PP_{333} application in both salinity levels (5 and 10 mM), increased fruit yield. This effect may be due to changes in the assimilate partitioning from leaves to roots or increase in mineral elements and soluble proteins in the leaves. Similar postulation has been previously reported by Rai and Brist [23]. In saline condition PP_{333} reduced sodium and chlorine absorption and provided an appropriate ion distribution among organs in peach trees, therefore it has caused an increase in fruit yield [24]. Proline overproduction may result from a decreased activity of the enzyme involved in the degradation of proline [25]. Sometimes it may be due to changes in metabolic pathway of glutamic acid to proline [26]. Potassium deficiency in saline condition may also cause proline overproduction [20]. After PP_{333} application in *Rhamnus alternus* plants, salt accumulation in leaching water was observed and indicates that PP_{333} inhibited sodium absorption by plant [8]. PP_{333} reduced root chlorine content at high salinity level. It has been reported that in strawberry cv. 'Korona' NaCl tolerance was due to a more effective limitation of Cl^- transport into leaves [27]. The distinct effect of PP_{333} on yield promoting and reducing negative effects of harmful ions in plants may be usefull in saline condition.

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