Hort. Environ. Biotechnol. 49(4):1-6. 2008.

Paclobutrazol Application Ameliorates the Negative Effect of Salt Stress on Reproductive Growth, Yield, and Fruit Quality of Strawberry Plants

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Abstract. Strawberry plants (*Fragaria* × *ananassa* Duch.) are very susceptible to salinity. In order to investigate the interaction effects of paclobutrazol (PP_{333}) and salinity on flowering, yield and fruit quality of strawberry plants, three levels of NaCl [0, 5, 10 mM] were incorporated into the nutrient solution and four levels of PP_{333} [0, 10, 20, 30 $mg \cdot L^{1}$] were sprayed. PP_{333} application caused reduction in inflorescence length, but increased inflorescence and flower number. Salinity reduced number of achenes per fruit. However 10 $mg \cdot L^{-1}$ of PP_{333} ameliorated the diverse effect of salinity at 10 mM NaCl salinity and increased significantly the number of achenes per fruit. Interaction of 20 and 30 $mg \cdot L^{-1}$ PP_{333} with 5 mM NaCl, showed increase in yield in comparison with 0 and 10 $mg \cdot L^{-1}$ PP_{333} at the same salinity level. Total soluble solids (TSS) were increased at 30 $mg \cdot L^{-1}$ PP_{333} in combination with 5 and 10 mM NaCl. also increased by this level of PP_{333}. The highest level of PP_{333} (30 $mg \cdot L^{-1}$) reduced titratable acidity (TA) on salt treated (5, 10 mM) plants. The ratio of TSS/TA tended to rise by application of PP_{333}. Salinity reduced vitamin C, but 20 $mg \cdot L^{-1}$ of PP_{333} significantly increased vitamin C in both levels of salinity (5, 10 mM). Therefore from the results of this study, PP_{333} application is recommended to increase reproductive growth, yield and improve fruit quality in saline condition.

Additional key words: Fragaria × ananassa Duch., Fruit Crop, Fruit Qualitative Properties, Growth Retardants, Productive Behaviour, Salt Stress

Introduction

Strawberry is an important crop which cultivation is expanding in open field and greenhouse in Iran. However saline soil and irrigation water threaten the production of this crop. Since salinity has a deliterious effect on fruit production at flowering, an important step in crop production, the strategies for finding a solution for this problem (Eshghi and Tafazoli, 2006) have been ongoing. Salinity is one of the most important factors limiting reproductive growth and reducing fruit yield and quality in several horticultural crops. Strawberry is quite sensitive to salinity (Awang et al., 1993) and decrease in strawberry fruit quality has been reported by Keutgen & Keutgen (2003) and Saied et al. (2005). Moderate salinity resulted in decrease of ascorbic acid and content of anthocyanins in strawberry (Keutgen and Pawelzik, 2007). The most common practice for reducing salinity is increasing leaching; however, this method is sometimes expensive and impractical. Few studies have focused on application of growth retardants to induce salinity tolerance. Uniconazole, a growth retardant from triazole compounds, application has been shown to decrease injury due to salt stress in *Vicia faba* (Bekheta Mohamed et al., 2006). PP_{333}, a gibberellin biosynthesis inhibitor, was known to promote salt stress tolerance in peach (*Prunus persica* L.)(Abou El-Khashab et al., 1997) while also to reducing symptoms and mortality due to salt stress in *Rhamnus alternus* seedlings (Banon et al., 2003). PP_{333} also modulated salt induced alterations in pigment content, proline level, lipid peroxidation, and antioxidant activity in the leaves of jujube seedlings (Fletcher et al., 2000). The persistence of PP_{333} residues in mango fruits was studied following its application at tree basin soil. Residues of PP_{333} were found in unripe mango fruits at levels below permissible level while generally not detected in fully mature mango fruits (Sharma and Awasthi, 2005). Additionally no toxicity endpoint was identified for an acute dietary assessment (Edwards, 2007). PP_{333} treatment reduced growth rate by 43% in strawberry petioles (Wiseman and Turnbull, 1999) and decreased shoot and root dry weight of strawberry plant (Deyton et al., 1991). Runner suppression and increase in branch crown formation after PP_{333} utilization has been reported in June-bearing strawberry (Archbold, 1989). The positive effect of PP_{333} on flowering of lychee has been reported by Menzel and Simpson (1990). These results imply that using growth regulators such as triazoles may be an effective way of improving plant stress tolerance. The aim of this study was

***Corresponding author:**
Received ? ?, 200?; accepted ? ?, 200?.

to determine the effect of PP_{333} on reproductive growth, fruit quality, and yield of salt stressed strawberry plants in the hydroponic system.

Materials and Methods

Cold stored runner strawberry plants (*Fragaria* × *ananassa* Duch.) of 'Selva'cultivar were planted in 2 L containers filled with a 2:1 mixture of perlite and peat-moss. Plants were fed continuously from the start of the experiment with Melspray nutrient solution containing three levels of NaCl (0, 5, 10 mM). At the beginning of the experiment, four levels of PP_{333} (0, 10, 20, 30 $mg \cdot L^{-1}$) were sprayed to the shoot to run off. A factorial experiment with completely randomized design having twelve treatments and five replications was utilized.

Inflorescence length and number, and the number of flower and achenes per fruit were measured and counted once per weak along the flowering period. Mean fruit fresh weight was measured at harvest to determine yield.

Vitamin C was determined with oxidation/reduction procedure (Ting and Russeff, 1981). Ten ml of fruit juice with 10 ml of the metaphosphoric acid stabilizing solution was mixed. Duplicate 5 ml amounts of the mixture were titrated with indophenol solution until a pink color appears and persists for 10 seconds. Average of the two values was determined. Standard solution was prepared and F factor for mg of ascorbic acid equivalent to 1 ml of indophenol solution was recorded. Vitamin C expressed as mg vitamin C / 100 ml juice, was calculated as follows:

$$\text{Vitamin C} = \text{corrected volume (sample)} \times F \times 40$$
$$F = \text{mg ascorbic acid equal to 1 ml indophenol}$$

Data were analyzed using MSTATC software and means were compared by Duncan's multiple range test at the 5% significance level. Total soluble solid (TSS) was measured with a refractometer. Titratable acidicity (TA) was determined by titrating 10 ml of fruit juice with 0.2 N NaOH to pH 8.2, as indicated by phenolphthalein indicator. NaOH was added to the juice until dark orange color appeared and persisted for 20-30 seconds. The TA was expressed as percent titratable acidity using the following equation:

$$TA = (\text{ml NaOH} \times \text{N NaOH} \times \text{acid meq.factor /ml juice titrated}) \times 100$$

The taste quality of strawberries was indicated by the ratio of TSS and TA.

Results and Discussion

Reproductive growth

High salinity caused reduction in inflorescence length. Application of 20 and 30 $mg \cdot L^{-1}$ of PP_{333} showed the same results. Interaction of the highest level of PP_{333} and salinity significantly reduced inflorescence length. The effect of NaCl on inflorescence number was not significant; in contrast, PP_{333} application significantly increased the number of inflorescence. Interaction of 20 and 30 $mg \cdot L^{-1}$ PP_{333} with 5 mM NaCl and 10 $mg \cdot L^{-1}$ PP_{333} with 10 mM NaCl showed increase in florescence number (Table 1). Similarly it has been shown that PP_{333} (applied at 1 Kg ha^{-1}) increased inflorescence number of white clover (*Trifolium repens* L.) at peak flowering (Budhianto et al., 1995). As observed by Nazarpour (2005), at 12.5, 25 and 37.5 $mg \cdot L^{-1}$ of PP_{333}, there was no significant effect on inflorescence number, in strawberry cv. Camarosa, but another report indicated that treatment of strawberry cv. Nyoho with PP_{333} at the rate of 0.25 mg per pot, increased flower clusters (Fletcher et al., 2000). Five mM NaCl caused reduction in flower number. PP_{333} at 20 and 30 $mg \cdot L^{-1}$ significantly increased flower number. Interaction of 20 and 30 $mg \cdot L^{-1}$ of PP_{333} with 5 and 10 mM NaCl showed the same results. Maximum flower number was resulted in the interaction of 10 mM NaCl and 10 $mg \cdot L^{-1}$ of PP_{333} (Table 1).

Achene number, yield, and vitamin C content

Salinity, PP_{333} and salinity × PP_{333} treatment effects on achene number, fruit yield and vitamin C were all significant. Salinity reduced number of achenes per fruit. However, PP_{333} treatment reversed this salinity effect, especially at 10 $mg \cdot L^{-1}$ along with 10 mM NaCl salinity, by significantly increasing the achene number (Table 2). The positive effect of PP_{333} on the formation of achenes of strawberry fruit was previously reported by Fletcher et al. (2000) as they observed from the greenhouse grown 'Shuksan' and 'Totem' cultivars. However, Nazarpour (2005) reported that 12.5, 25 and 37.5 $mg \cdot L^{-1}$ PP_{333} had no effect on the achene number of 'Camarosa' cultivar. Salinity reduced yield, however, at 10 $mg \cdot L^{-1}$ PP_{333} without any salinity, showed maximum yield. At 0, 10, 20 $mg \cdot L^{-1}$ PP_{333} in combination with salinity, a reduction in yield was observed but there was no difference between NaCl treatments at the highest level of PP_{333}. Interaction of 20 and 30 $mg \cdot L^{-1}$ of PP_{333} and 5 mM NaCl, showed increase in yield in comparison with 0 and 10 $mg \cdot L^{-1}$ of PP_{333} at the same salinity level. Also the effect of PP_{333} at 10 mM NaCl was much better than 0, 10 and 20 $mg \cdot L^{-1}$ PP_{333} (Table 2).

Table 1. The effect of NaCl and PP_{333} treatments on strawberry inflorescence length (cm), and the number of inflorescence and flower.

Treatment		Inflorescence length (cm)	Inflorescence number	Flower number
NaCl (nM)	PP_{333} ($mg \cdot L^{-1}$)			
0	0	14.22 bc*	5.2 c	16.20 d
	10	14.40 ab	6.2 abc	22.40 b
	20	11.20 d	6.6 abc	18.20 c
	30	11.20 d	7 a	18.20 c
5	0	15.64 a	5.2 c	13.20 e
	10	13.60 c	6.8 ab	9.60 g
	20	10.70 d	7 a	21.60 b
	30	10.90 d	7.6 a	21 b
10	0	15.0 ab	5.4 bc	11.20 f
	10	11.36 d	6.8 ab	24 a
	20	10.0 d	7.2 a	21.20 b
	30	8.52 e	6.2 abc	20.80 b
Means				
NaCl 0 mM		12.76 A	6.2 A	18.75 A
NaCl 5 mM		12.71 A	6.6 A	16.35 B
NaCl 10 mM		11.22 B	6.4 A	19.30 A
PP_{333} 0 mg L^{-1}		14.95 A	5.2 B	13.53 C
PP_{333} 10 mg L^{-1}		13.12 B	6.6 A	18.67 B
PP_{333} 20 mg L^{-1}		10.63 C	6.9 A	20.33 A
PP_{333} 30 mg L^{-1}		10.21 C	6.9 A	20 A
Significance				
NaCl		**	NS	**
PP_{333}		**	**	**
NaCl x PP_{333}		**	NS	**

* 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

NS, *,** Nonsignificant or significant at P = 0.05 or 0.01, respectively

Similarly it has been reported that raised salinity (8 ds m^{-1}) had negative impact on strawberry (cv. Elsanta) fresh fruit yield (Ondrasek et al., 2006). The yield of tomato fruit was also decreased after saline water irrigation at 6 ds m^{-1} (Mizrahi et al., 1988). Similar results were shown in the greenhouse tomato by increasing plants when they were exposed to the nutrient solution of 2.3-5.1 ms cm^{-1} salt (Dorai et al., 2001). Chartzoulakis and Klapaki (2000) reported that total yield decreased significantly with increasing salinity in two pepper cultivars, but Mitchell et al. (1991) reported that irrigation with saline water had no effect on tomato fruit yield. Kirschbaum (1998) indicated that application of 50-400 $mg \cdot L^{-1}$ PP_{333} increased total fruit yield in strawberry cv. Sweet Charlie. A similar result was observed in young pear trees (Jaumien et al., 1986).

Vitamin C content did not differ significantly among salinity treatments when no PP_{333} was applied and the same results were observed when 10 and 30 $mg \cdot L^{-1}$ PP_{333} were applied. 20 $mg \cdot L^{-1}$ PP_{333} significantly increased vitamin C at 5 and 10 mM NaCl salinity (Table 2). Keutgen and Keutgen (2003) reported that the concentration of vitamin C was not significantly affected by NaCl salinity (40 and 80 meq L^{-1}) in both 'Elsanta' and 'Korona' strawberry cultivars.

Fruit TSS, TA, and TSS/TA

Salinity treatments had no significant effect on TSS, TA and TSS/TA. The same result was observed for interaction effect of salinity and PP_{333} for TSS (Table 3). PP_{333} at 30 $mg \cdot L^{-1}$ caused an increase in TSS. Other concentrations of PP_{333} were not significantly different from each other, but they were raised in comparison with control (Table 3). It has been reported that fruits from a processing tomato cultivar exposed to various degrees of salinity had higher

Table 2. The effect of NaCl and PP_{333} treatments on strawberry fruit achene number, yield (g) and vitamin C (mg/100 ml juice).

Treatment		Achene number	Fruit yield	Fruit vitamin C
NaCl (nM)	PP_{333} ($mg\cdot L^{-1}$)			
0	0	270.2 b*	57.71 de	26.25 i
	10	309.2 a	166.1 a	50.25 bcd
	20	231.8 c	119.3 b	75 a
	30	222.4 cd	94.3 c	43 def
5	0	141 g	54.84 e	33 ghi
	10	164.4 fg	65.89 d	49.25 bcde
	20	190.4 def	94.49 c	58 b
	30	89.80 h	85.08 c	39.75 efg
10	0	99.80 h	44.47 f	29.25 hi
	10	208.8 cde	58.49 de	46 cdef
	20	164.6 fg	67.33 d	53.25 bc
	30	172.4 efg	85.86 c	37 fgh
Means				
NaCl 0 mM		258.4 A	109.3 A	48.63 A
NaCl 5 mM		164.4 B	75.08 B	45 AB
NaCl 10 mM		161.4 B	64.02 C	41.38 B
PP_{333} 0 mg L^{-1}		170.3 C	52.34 C	29.50 D
PP_{333} 10 mg L^{-1}		227.5 A	96.79 A	48.50 B
PP_{333} 20 mg L^{-1}		195.6 B	93.70 AB	62.08 A
PP_{333} 30 mg L^{-1}		161.5 C	88.42 B	39.92 C
Significance				
NaCl		**	**	*
PP_{333}		**	**	**
NaCl x PP_{333}		**	**	**

* 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.
$^{NS, *, **}$ Nonsignificant or significant at P = 0.05 or 0.01, respectively.

values for TSS (Mizrahi and Pasternak, 1985). Nazarpour (2005) indicated that PP_{333} application at 12 $mg\cdot L^{-1}$ increased TSS in 'Camarosa' cultivar of strawberry. The same response was observed in greenhouse tomato (Dorai et al., 2001), but D'Anna et al. (2003) reported that sugar content was scarcely influenced in strawberry by salinity treatments (1.5-4.5 ms cm^{-1}). Decrease in TSS with 30 and 60 mM NaCl salinity was reported about strawberry cultivars 'Korona' and 'Elsanta', respectively (Saied et al., 2005). The highest level of PP_{333} (30 $mg\cdot L^{-1}$) significantly showed reducing effect at saline and non saline conditions (Table 3). A higher value for TA has been reported in a processing tomato cultivar at saline condition (Mizrahi and Pasternak, 1985). As indicated by Keutgen and Keutgen (2003), TA was decreased by salinity (80 meq L^{-1} of NaCl in 'Elsanta' cultivar of strawberry. Same results on greenhouse tomato were obtained by Dorai et al. (2001). Higher fruit acid concentration was resulted from irrigation with saline water (8.1 ds m^{-1}) in tomato (Mitchell et al., 1991). The taste quality of strawberries depends strongly on the ratio of TSS per TA. This ratio tended to rise by application of 30 $mg\cdot L^{-1}$ PP_{333} at saline and non saline conditions (Table 3). Increasing fruit quality by salinity as indicated by TSS/TA ratio has been reported by Awang et al. (1993) but Saied et al. (2005) indicated that this ratio decreased significantly with increasing salinity (60 mM NaCl) in both cultivars 'Elsanta' and 'Korona' of strawberry. Teferi Belayneh (2005) observed that TSS/TA ratio increased by PP_{333} application in mango fruits.

Conclusion

From the above results, it can be concluded that PP_{333} application improves reproductive growth. Interaction of 20 and 30 $mg\cdot L^{-1}$ PP_{333} with 5 mM NaCl and 10 $mg\cdot L^{-1}$ PP_{333} with 10 mM NaCl showed increase in florescence

Table 3. The effect of NaCl and PP_{333} treatments on strawberry fruit TSS (%), TA (%) and TSS/TA.

Treatment		TSS	TA	TSS/TA
NaCl (nM)	PP_{333} ($mg\cdot L^{-1}$)			
0	0	7 f*	0.34 c	20.10 c
	10	8.12 def	0.46 b	17.38 cd
	20	9 bcd	0.54 a	16.69 cd
	30	10.63 a	0.35 c	30.41 a
5	0	7.62 ef	0.44 b	17.28 cd
	10	8.27 de	0.45 b	18.65 cd
	20	8.62 cde	0.54 a	15.91 d
	30	9.87 ab	0.35 c	28.23 ab
10	0	7.87 def	0.46 b	17.01 cd
	10	7.87 def	0.47 b	16.53 cd
	20	8.12 def	0.44 b	18.34 cd
	30	9.5 bc	0.36 c	26.39 b
Means				
NaCl 0 mM		8.68 A	0.42 A	21.14 A
NaCl 5 mM		8.62 A	0.44 A	20.02 A
NaCl 10 mM		8.34 A	0.43 A	19.56 A
PP_{333} 0 mg L^{-1}		7.5 C	0.41 C	18.13 B
PP_{333} 10 mg L^{-1}		8.12 B	0.46 B	17.52 B
PP_{333} 20 mg L^{-1}		8.58 B	0.5 A	16.98 B
PP_{333} 30 mg L^{-1}		10 A	0.35 D	28.34 A
Significance				
NaCl		NS	NS	NS
PP_{333}		**	**	**
NaCl x PP_{333}		NS	**	**

* 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.
NS, *,** Nonsignificant or significant at P = 0.05 or 0.01, respectively.

number. Application of 20 $mg\cdot L^{-1}$ PP_{333} increased vitamin C at 5 and 10 mM NaCl salinity. TSS/TA tended to rise by using 30 $mg\cdot L^{-1}$ PP_{333} at saline and non saline conditions. This experiment also showed that salt stress reduced yield, but 20 $mg\cdot L^{-1}$ PP_{333} could overcome the negative effect of 5 mM NaCl. Moreover the distinct effect of PP_{333} on yield promoting seems to be a result of the growth inhibiting effect. Its role as anti-gibberellin is considered sufficient for the induction of higher yield and for improvement of the quality.

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