PHYSIOLOGICAL RESPONSE AND QUALITY ATTRIBUTES OF PEACH FRUIT CV. FLORIDA KING AS AFFECTED BY DIFFERENT TREATMENTS OF CALCIUM CHLORIDE, PUTRESCINE AND SALICYLIC ACID

Irfan Ali, Nadeem Akhtar Abbasi* and Ishfaq Ahmad Hafiz

Department of Horticulture, PMAS-Arid Agriculture University Rawalpindi, Pakistan *Corresponding author's email: nadeemabbasi65@yahoo.com

Despite the ample increase in peach growing area, improvement in quality and average yield is still negligible. The influence of foliar application of calcium chloride (CaCl₂), putrescine (PUT) and salicylic acid (SA) on fruit yield and physicochemical characteristics of 'Florida King' peach cultivar was evaluated for two consecutive years (2011 and 2012). Results indicated that, application of CaCl₂ (1%), PUT (1, 2 and 3 mM) and SA (2 and 3 mM) increased fruit weight, fruit diameter, pulp: stone ratio and yield compared to control. In addition, fruit harvested from these growth elicitors treated trees did not show significant difference in total soluble solids and total sugar contents. All treatments resulted in higher fruit firmness, acidity and ascorbic acid contents than control. Higher calcium treatments proved toxic and significantly increased the calcium level in leaves and fruit. From present work it can be referred that spraying of CaCl₂, PUT and SA, significantly affected yield, and physico-chemical characteristics of peach cv. 'Florida King'. These growth elicitors are considered safe chemicals and can be applied to enhance production and improve fruit quality of peach without compromising the food safety standards.

Keywords: GRAS chemicals, physico-chemical characteristics, toxicity, safe food, fruit growth

INTRODUCTION

Peach (*Prunus persica* L.) is an important fruit crop of the world which was originated from China. It's delicious taste and unique flavour with high nutritional value have popularized it across the world. It is one of the important stone fruit of Pakistan having total cultivated area about 14700 ha with annual production of 55800 tones (FAO, 2010).

Over the last decade despite the ample increase in peach gro wing area, there has been a very small progress in average fruit yield. In recent years, the production and commercialization of stone fruits like peaches have increased briskly throughout the world including Pakistan, mainly because of introduction of improved, low-chilling cultivars and better handling of fruit. Northern areas and Baluchistan are the most prominent regions of Pakistan for production of peaches.

Various pre- and postharvest factors are associated with yield and quality of fruit including growth conditions, harvest maturity and post-harvest factors (Serrano *et al.*, 1996). Plenty of researchers have attempted to increase the productivity and quality of fruit crops. The use of growth elicitors and minerals in this respect had been reported on apricot, pear and apple (Nagwa *et al.*, 2007; Eissa *et al.*, 2008; Khalifa *et al.*, 2009; Ali *et al.*, 2010; Abd- El-Messeih *et al.*, 2010).

The effect of polyamines (PAs) in promoting fruit set and retention have been examined in apple, apricot, pear, litchi,

olives and mangoes (Ali *et al.*, 2010). Moreover, application of PAs had been an effective tool for improving embryo development in grapes. PAs have shown their functions for delaying maturity and ripening in apricot and grapes (Metha *et al.*, 2002; Ponce *et al.*, 2002) inhibiting abscission, reduced fruit drop, increased fruit firmness in peach, nectarine and pear (Bregoli *et al.*, 2002; Torrigiani *et al.*, 2004).

Pre and post-harvest application of calcium for improvement of quality and minimizing the disorders have been reported in various fruits like strawberry, peaches, nectarines and apples (Dunn and Able, 2006; Hernandez-Munoz et al., 2006). Similarly, use of salicylic acid (SA) was very effective in improving yield as well as physical and chemical characters in apple, kiwi, banana, jujube (Al-Obeed, 2012). Peach exhibits a double-sigmoidal growth curve like other stone fruits (Kushad, 1998). The initial increase in the fruit growth has been attributed to cell division, followed by cessation of mesocarp growth due to lignification of the endocarp (stone) tissue and is referred as pit hardening or lag phase stage which is second stage of fruit growth and development (Romani and Jennings, 1971; Westwood, 1978) while, third phase of fruit growth has been attributed to cell enlargement (Romani and Jennings, 1971). Flurkey and Jen (1978) reported an increase in total soluble solids and protein concentration during pit hardening, suggesting that pit hardening is an active growing period. The application of growth elicitors at these critical stages of fruit growth and development may prove more effective. But,

there is no literature available on the effect of foliar sprays of the generally regarded as safe (GRAS) chemicals on these active growth phases on fruit quality. The present study was under taken to improve peach fruit quality and yield by foliar sprays of food grade chemicals like calcium chloride, putrescine and salicylic acid during cell division, pithardening and cell enlargement stage.

MATERIALS AND METHODS

The present study was conducted at farmer's orchard near Madrota, Tehsil Hazro, District Attock. (33° 7'S; 73° 6'E). Laboratory and cold storage facility of Department of Horticulture, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi (PMAS-AAUR), was utilized. A total of 33 peach trees (*Prunus persica* L.) cv. "Florida King" grafted on 'Peshawar Local' having eight years of age were selected for the study. The planting system used for the orchard is square system (6 m x 6 m). The trees were planted in East West direction. Trees of uniform size and free from pests and diseases were selected for the study. All the selected trees were given standard cultural practices.

Treatments of CaCl₂ (1, 2 and 3%), PUT (1, 2 and 3mM) and SA (1, 2 and 3mM) were applied at three different stages of fruit development, i.e. (i) cell division stage (20th of March), ii:) pit hardening or lag phase (7th of April) and iii:) cell enlargement stage (20th of April). Tween-20 (0.01%) was used as surfactant. Each tree was used as treatment unit in a block and the treatments was replicated thrice. Following parameters were recorded

Fruit growth curve: Fruit growth curve was noted by measuring the increase in the diameter of peach fruit with the help of vernier calipers. The data regarding fruit growth curve was taken on 3 days interval. The diameter was measured in mm.

Toxicity of the treatments: Toxicity symptoms (leaf and fruit burning, necrosis and chlorosis etc.) of the treatments were observed visually on the next day of spray each time.

Physical fruit quality characteristics: Sample of 10 mature fruit were taken for determination of the physical fruit quality characteristics like fruit weight (g.), fruit diameter (cm), pulp to stone ratio. Harvest dates and marketable yield (total weight of marketable fruit in Kg/ tree) were recorded at the time of harvest. Fruit firmness (N) was calculated by means of a digital penetrometer model BKD020 (WEL. New Zealand) equipped with an 8 mm plunger. Initial harvest dates were recorded at the day of first harvest. The fruit was harvested at three different occasions.

Fruit chemical characteristics: The fruits used to determine the fruit firmness were then cut into smaller pieces and juice was extracted for analysis of total soluble solids, total sugars and titratable acidity. Total soluble solids (TSS) were measured according to AOAC (1985) using hand refractometer at room temperature. Total sugars of juice

were estimated by the method described by Hortwitz (1960). To determine the titratable acidity, 10 mL extracted guava juice was mixed with 40 mL distilled water, and 2-3 drops of phenolphthalein were added in the juice. A 10 mL aliquot was taken in a titration flask and titrated against 0.1 N NaOH until permanent light pink color appears. Three consecutive readings were taken and percent acidity was calculated by using the following formula:

% Titratable Acidity =
$$\frac{\frac{N}{10} \text{ NaOH used} \times 0.0064}{\text{Volume of sample used}} \times 100$$

Ascorbic acid was determined according to the method described by Hans (1992). Three fruit slices from harvested fruit were taken for fruit tissue analysis followed by dry ashing procedure (Chapman and Pratt, 1961) and grinding subsequently. Calcium contents were determined as described by Berry and Johnson (1966).

RESULTS

Growth curve: In order to characterize and identify the developmental stages of peach fruit (cv. Florida King), growth data were collected during two different years (2011) and 2012) by measuring fruit size. A typical growth profile with data collected during the two years for each developmental stage, are shown in Figure 1. 'Florida King' peaches exhibited a double-sigmoidal growth curve, one of the characteristics of stone peaches. The initial increase in the fruit growth (14 to 30 day after full bloom, i.e. 20th March to 7th April) has been attributed to cell division, while third phase of fruit growth (46 days after full bloom i.e. from 18th April) has been attributed to cell enlargement. Cessation of mesocarp and seed growth at 30 days after full bloom is due to lignification of the endocarp (stone) tissue and is referred as pit hardening or lag phase stage which is second stage of fruit growth and development.

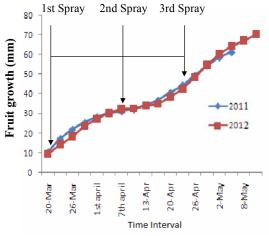


Figure 1. Fruit growth curve (after fruit set till harvest)

Toxicity of the treatments: All the pre-harvest sprays were well tolerated except 2% and 3% CaCl₂ resulted in noticeable leaf and fruit burn which was higher at 3% CaCl₂ than at 2% CaCl₂ (Figure 2 and 3). The leaf burn appeared as brown necrotic tissue and could encompass the entire leaf. The phyto-toxicity of the calcium sprays was more prominent on the young developing leaves and fruit compared to the older ones.



Figure 2. Toxicity on fruit and leaves caused by 2 % CaCl₂ foliar sprays



Figure 3. Toxicity on fruits and leaves caused by 3 % CaCl₂ foliar sprays

Fruit physical characteristics at harvest: Fruit weight, diameter and pulp to stone ratio were increased significantly in treated fruits. A significant increase in fruit weight was noted in both the years by all treatments compared to untreated fruit. SA 1 mM had non-significant effect on these attributes. Moreover, data of both the years showed a significant increase in fruit diameter by all the sprayed chemicals compared to control (untreated). Maximum fruit weight and diameter were obtained by spraying 1% CaCl₂ and 2 mM PUT in both years (Table 1). Furthermore, higher pulp to stone ratio was observed by the treatments containing PUT @ 2 mM and CaCl₂ @ 1% followed by PUT 3mM and SA 3 mM treatments.

Fruit firmness values (Table 1 and Table 2) also significantly increased during both seasons compared to control. However, sprays of PUT at 2 mM showed the highest firmness values (109.07 N). In contrast to this, the lowest firmness in fruits was observed in control with minimum values as 75.33 N whereas, in the second experimental season all treatments were at par in maintaining the firmness values compared to control.

Marketable yield and harvest dates: The effect of all the treatments on yield of 'Florida King' peach trees has been shown in Table 2. It is clear from the data that most of the treatments treatments effectively increased fruit yield except calcium treatments which caused toxicity while SA 1 mM reduced the yield non-significantly compared to untreated trees. Maximum fruit yield was achieved by spraying putrescine at 2 mM and 1% CaCl₂ in both the seasons. Lowest yield in the trees receiving higher doses of CaCl₂ was because of the toxic effect of CaCl₂ on leaves and fruits that caused excessive fruit drop before harvest and leaving many fruits defected at the time of harvest as noted in the present study.

Table 1. Effects of different treatments of calcium chloride, putrescine and salicylic acid applied at different stages of fruit growth and development on peach fruit physical characteristics in the year 2011 and 2012

Treatments		Weight (g)	Fruit Diameter (mm)			tone Ratio	Firmness (N)	
	2011	2012	2011	2012	2011	2012	2011	2012
Control	123.33C	106.67 B	57.113CD	54.419 C	9.291 C	8.917D	75.33 E	97.44B
CaCl ₂ 1%	148.33AB	120.56 A	65.890A	59.249 A	13.914A	13.083A	96.50 BC	105.05AB
CaCl ₂ 2%	123.89C	108.89AB	57.110CD	55.087 BC	9.821C	11.689BC	85.10 CDE	98.21AB
CaCl ₂ 3%	125.00 C	117.22 AB	56.443D	55.203 BC	9.750 C	10.667 C	93.60 BCD	98.21AB
PUT 1 mM	136.78BC	110.56AB	61.780B	55.284BC	9.791C	10.900 C	87.70CD	104.62AB
PUT 2 mM	153.00A	121.11 A	66.000 A	57.977AB	13.668A	12.944AB	109.07A	112.82A
PUT 3 mM	137.55BC	115.33AB	59.220 CD	55.866 BC	12.001B	12.500AB	101.60AB	110.26AB
SA 1 mM	123.33C	108.33AB	58.663BCD	54.557C	9.788C	10.450 C	83.00DE	100.00AB
SA 2 mM	123.22C	112.22 AB	58.667BCD	55.849BC	11.942B	11.083C	85.47CDE	101.28AB
SA 3 mM	132.56C	112.11 AB	60.333 BC	55.846 BC	12.139B	12.592AB	94.67BCD	107.69AB
LSD	15.065	12.870	3.8418	3.0182	1.3010	1.2941	12.063	15.134

Average of 3 replicates; Mean separation within rows using LSD Test (5% level); Means within a column having same letters are statistically non- significant using Least Significant Difference Test

Table 2. Effect of different treatments of calcium chloride, putrescine and salicylic acid applied at different stages of fruit growth and development on harvesting dates, yield, leaf calcium contents, fruit peel calcium contents and fruit pulp calcium contents at harvest for both the years.

Treatments	Harvesting		Yield (Kg/tree)		Leaf calcium		Fruit peel calcium		Fruit pulp calcium	
	dates				contents (%)		contents (%)		contents (%)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Control	Zero	zero	49.083C	43.206BC	0.39C	0.37C	0.056C	0.058CDE	0.021C	0.023C
CaCl ₂ 1%	Zero	zero	61.447AB	50.889AB	0.52B	0.48B	0.068 A	0.069B	0.025B	0.028B
CaCl ₂ 2%	Zero	zero	32.003D	30.483D	0.56B	0.59A	0.069 A	0.071AB	0.027AB	0.029AB
CaCl ₂ 3%	Zero	zero	27.157D	28.550D	0.64A	0.61A	0.070 A	0.072A	0.028A	0.031A
PUT 1 mM	Zero	zero	54.552BC	46.290ABC	0.39C	0.36C	0.056 C	0.056EF	0.022C	0.024C
PUT 2 mM	Zero	zero	62.688A	51.444A	0.40C	0.39C	$0.059~\mathrm{B}$	0.058CD	0.021C	0.024C
PUT 3 mM	Zero	zero	52.316C	45.769ABC	0.40C	0.34C	0.059 B	0.058C	0.022C	0.024C
SA 1 mM	Zero	zero	47.377C	42.761C	0.39C	0.34C	0.055 C	0.056 DEF	0.022C	0.024C
SA 2 mM	+2	+1	47.400C	44.330ABC	0.39C	0.34C	0.056 C	0.055BF	0.022C	0.025C
SA 3 mM	+2	+2	52.071C	44.112ABC	0.40C	0.39C	0.057 C	0.056B-F	0.022C	0.025C
LSD			7.819	5.198	0.050	0.065	0.002	0.002	0.002	0.002

(+, days after, -, days before the control harvest date); Average of 3 replicates; Means within a column having same letters are statistically non -significant using Least Significant Difference Test

Data in Table 2 showed that the spray treatments of $CaCl_2$ and putrescine did not affect the fruit maturity on the tree, since control, $CaCl_2$ and PUT treated fruits were harvested on the same date according to their external appearance. Higher doses of SA delayed fruit colour change thus; harvest date was delayed two to three days (Table 2).

Effect on chemical characteristics of fruit: Data in Table 3 reveals that soluble solid contents and total sugars were not affected significantly by different treatments. Concerning total acidity (%), data shows that it was significantly increased by spraying different chemicals compared to untreated trees. The lowest total acidity of fruit juice was observed in control fruit while highest acidity value was recorded in the fruits treated with 2, 3 mM PUT, 1% CaCl₂

and 3 mM SA which are statistically at par with each other during both the seasons.

Ascorbic acid contents of fruit were also affected significantly by different treatments. The effect of PUT was more visible than all the other treatments in increasing ascorbic acid. PUT 2 mM, 3 mM and 1% CaCl₂ were the best treatments on increasing the ascorbic acid contents of fruit followed by the higher doses of salicylic acid. Lowest ascorbic acid contents were noticed in the fruits which were not sprayed by any chemical (Table 3).

When calcium contents of fruit and leaves were determined, calcium treated fruits had significantly higher concentrations of these cations compared with other treatments (Table 2). All the other treatments had no effect on the calcium

Table 3. Effect of different treatments of calcium chloride putrescine and salicylic acid applied at different stages of fruit growth and development on some chemical characteristics of peach fruit at harvest in the year 2011 and 2012

Treatments	TSS (^o Brix)		Total Sugars (%)		TA (%)		AA (mg/100g)	
	2011	2012	2011	2012	2011	2012	2011	2012
Control	9.323A	9.167A	4.51A	4.649A	0.93F	1.088C	4.33D	4.900C
CaCl ₂ 1%	9.323A	9.300A	4.64A	4.727A	1.14ABC	1.165AB	5.60AB	6.450A
CaCl ₂ 2%	9.374A	9.333A	4.53A	4.664 A	1.02CDEF	1.102BC	4.83CD	5.400BC
CaCl ₂ 3%	9.706A	9.267A	4.53A	4.746A	1.01DEF	1.108BC	4.83CD	6.033AB
PUT 1 mM	9.349A	9.233A	4.53A	4.775A	1.10A-E	1.108BC	4.96BCD	5.700ABC
PUT 2 mM	9.528A	9.333A	4.69A	4.770A	1.22A	1.199A	6.16A	6.383A
PUT 3 mM	9.451A	9.400A	4.60A	4.669A	1.15AB	1.134ABC	5.96A	6.100AB
SA 1 mM	9.451A	9.033A	4.50A	4.620A	0.98 EF	1.101 BC	4.76CD	5.333BC
SA 2 mM	9.400A	9.167A	4.64A	4.785A	1.09B-E	1.123BC	5.00BCD	5.650ABC
SA 3 mM	9.400A	9.367A	4.60A	4.752A	1.11A - D	1.145ABC	5.20BC	6.083AB
LSD	0.509	1.100	0.101	0.202	0.12	0.070	0.68	0.890

TA = Titratable acidity, AA (Ascorbic acid); Average of 3 replicates; Means within a column having same letters are statistically non-significant using Least Significant Difference Test.

contents. Thus foliar application of CaCl₂ was effective in inducing calcium assimilation.

DISCUSSION

The double sigmoid growth curve of peach fruit was observed in this study. The main objective was to find out the exact dates in local conditions so that the spray time can be determined. The study showed that cell division starts after fruit set and continues till 31st day after fruit set (7th April), pit hardening starts after 31st day after fruit set (7th April) and continues till 46th day after full bloom (20th April) while pit hardening stage started after 46th day of full bloom and continued till harvest. In the present study the higher concentrations of CaCl₂ proved to be toxic for leaves and fruits. CaCl₂ molecule contains 66% chloride. CaCl₂ is dissociated into free ions of calcium (Ca) and chloride (Cl) in solution form. Sodium (Na) ions that are present in fruits, leaves, irrigation water and soil residues, form sodium chloride by combining with these chloride (Cl) ions. This build up of the salt dry out leaf and fruit tissues causing sunburn and tissue deterioration. The other reason of the toxicity may be related to the change in chemistry of CaCl₂ when it yield ions in water, even in little quantity that may be present on fruit and leaves from respiration, condensation and transpiration. Free hydrogen (H⁺) form hydrochloric acid after reacting with chloride (Cl) ions, which may reduce fruit skin brightness and burn lenticels (Techflo, 2013). Similar toxicity from preharvest calcium (Ca) application has also been reported on apple (Lidster et al., 1977).

In the present study higher doses of SA delayed the initial harvest dates of the fruit. SA has been reported to delay harvest maturity in dates and jujuba when applied before harvest (Al-Obeed, 2010; Kassem *et al.*, 2011). The delaying effect of SA might be because of its inhibitory effect on ethylene biosynthesis

Foliar application of growth elicitors i.e., CaCl₂, PUT and SA significantly improved fruit physical characteristics like fruit weight, size and pulp to stone ratio. This may result in greater price in the market, since large size fruits are more appreciated in the market. Previous studies also reported that CaCl₂ and PUT improved the physical characteristics of the stone fruits (Serrano *et al.*, 2004; Ali *et al.*, 2010; El-Alakmy, 2012). The improvement in fruit physical properties as a result of treatments especially PUT might be due to their influence in enlarging the cell size and enhancing the strength of carbohydrate sink, thus increasing fruit size and weight. In this connection, it has been reported that PAs are essential for cell growth and differentiation and their intracellular concentration increase during periods of rapid cell proliferation (Valero *et al.*, 2002).

The general positive effects observed during this study on the fruit firmness of peach as a result of applying PUT and CaCl₂ could be attributed to the characteristic of these generally regarded as safe (GRAS) chemicals to inhibit the activities of fruit softening enzymes like PG and PE and also because of creating rigidification by making cross links with pectic substances as reported in plums and peaches (Khan et al., 2007; Manganaris et al., 2005). Similar effects of CaCl₂ and PUT on improvement of fruit firmness at the time of harvest were already reported in other fruits like apricot and apple (Ali et al., 2010; Asgharzade et al., 2012). Application of CaCl₂ and PUT at three stages of fruit growth increased the yield of peach crop possibly due to the role of these chemicals in improving the development of flowers and fruits (Galston et al., 1997). However, PAs are also known as polycationic nitrogenous and antisenecence compounds (Aziz et al., 2001). It is very likely that these bio-regulators play a vital role in fruit growth and development especially at cell division stage, consequently affecting size and weight of fruit, ultimately the yield of peach crop. While the lowest yield in 2% and 3% CaCl₂ is due to the toxic effects of the treatments which led to excessive fruit drop.

The treatments had no effect on soluble solid and total sugar contents at harvest, while the sprays of these chemicals were effective in maintaining the higher level of titratable acidity and ascorbic acid. A reduction in sugar and TSS as a result of PUT applications has been reported in apple (Costa et al., 1986). While, a lower concentration (0.01 mM) of PUT applied to litchi increased acidity and reduced total sugars and sugar acid ratio (Mitra and Sanyal, 1990). The present study shows that the putrescine treatments had no significant effect on sugar contents of peach that might be because putrescine behaves differently in different crops. Severe increase in respiration rate of untreated fruits (ethylene production and fruit senescence) appears to provoke organic acids consumption leading to reduction of titratable acidity of the fruits more compared to the treated fruit. Higher acid contents in putrescine treated fruit may be due to slowing down the fruit respiration rate by hindering the ethylene production. Increased ascorbic acid content in fruit treated with a PUT may be ascribed to the suppression of ascorbate oxidase activity as a result of increased levels of endogenous polyamines in fruit pulp. The effects of PAs on activities of ascorbate oxidase in peach are yet to be investigated. Higher endogenous concentration of PUT in bell pepper compared to tomato fruit has been associated with higher ascorbic acid level (Yahia et al., 2001).

The increase in calcium contents in peach and nectarine flesh could be responsible for the higher pulp firmness observed at harvest than control. A significant role has been proposed for calcium in conferring mechanical strength on the cell wall, as a result of its binding to pectin to form calcium pectate, which increases the rigidity of the middle lamella of the cell wall (Conway *et al.*, 1997). Accordingly, multiple Ca²⁺ sprays on peach and nectarine cultivars proved to be effective in increasing fruit calcium content (Johnson

et al., 1998), similar results were also reported by Serrano et al. (2004). In contrast to this, for other cultivars grown in the western USA, similar treatments did not increase the endogenous calcium content in the flesh (Crisosto et al., 2000). Therefore differences in calcium uptake by the fruits in those reports suggest that calcium mobility, which usually is extremely low (Poovaiah et al., 1988) may also be limited by growth conditions and cultivar type.

Conclusion: In general, the results showed positive influence of all sprayed agro-chemicals on the quality of peach fruit. Ca, PUT and SA sprays had better effect than untreated trees. Ca and PUT sprays were more effective than SA. Application of Ca and PUT improved fruit physical and chemical characteristics such as fruit size, fruit weight, yield, fruit firmness, titrable acidity and vitamin C content. Moreover, Ca sprays were effective in enhancing calcium contents of leaves and fruit. Finally, the application of GRAS chemicals can be easily used to improve yield and quality of peach fruit.

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