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Exogenous Application of 2,4-D, GA_3 and NAA at Flowering Improves Yield and Quality of Kinnow Mandarin (*Citrus reticulata* Blanco)

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ABSTRACT

This study was undertaken to assess the ability of plant growth regulators (PGRs) to improve fruit set percentage and quality, and to reduce the number of seeds in 'Kinnow' mandarin (*Citrus reticulata* Blanco). Various concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) (10, 20 and 30 mg/l), gibberellic acid (GA_3) (5, 10 and 20 mg/l) and 1-naphthalene acetic acid (NAA) (5, 10 and 20 mg/l), were applied at the full bloom stage on thirty 10-year-old 'Kinnow' mandarin plants grafted onto 'Rough lemon' (*Citrus jambheri* Lush) rootstock. A randomized complete block design was followed with three replications and a single tree was assumed to be an experimental unit. The overall objective of the experiment was to increase the productivity and quality of 'Kinnow' mandarin. Exogenous application of all the PGRs significantly increased fruit set percentage (from 43.89% with 10 mg/l 2,4-D to 82.77% with 10 mg/l NAA) leading to an increase in the total number of fruits/plant and fruit weight/plant (yield). Juice percentage, total soluble solids, acidity and vitamin C concentrations in fruit juice were also improved following the application of PGRs. PGRs at lower concentrations enhanced fruit set more efficiently than higher concentrations. The application of 20 mg/l GA_3 significantly reduced the number of seeds/fruit (15.82) compared to the control (19.63).

Keywords: citrus, foliar application, growth regulators, physiochemical properties, seedless

INTRODUCTION

Citrus fruits have special importance due to their distinct flavors and therapeutic value. Citrus fruits are an excellent source of vitamin C with fair amounts of vitamins A and B and minerals such as calcium, phosphorus and iron (Nawaz *et al.* 2008). The juice is refreshing, delicious and soothing. Besides their consumption as fresh fruit, a large number of products and byproducts are prepared and marketed at a premium price (Ahmed *et al.* 2007a, 2007b). Citrus fruits are ranked at the top in the world and are produced in more than 52 countries around the world. Brazil is the largest producer of citrus worldwide and produces 20.68 million tons (MT) of citrus fruits followed by China, USA, and Mexico with a production of 19.61, 10.01 and 6.85 MT, respectively (Anonymous 2007). Pakistan has a prominent position and is among the top 10 citrus-producing countries in the world. In Pakistan, citrus is cultivated over an area of 185,000 ha with an annual production of 2.2 million ton (Anonymous 2008). Among citrus, 'Kinnow' has gained special significance and has monopolized the citrus industry in Pakistan; it is commercially cultivated because of its better adaptation to soil and environmental conditions and it has not only excelled in area, production and quality relative to other mandarin cultivars but has also surpassed the sweet orange cultivars in the country (Khan *et al.* 2010). 'Kinnow' occupies about 70% of overall Pakistani citrus production. Even so, the average yield of citrus in Pakistan is just 9.076 tons/ha, while in many other citrus-growing countries it is much higher: Brazil (21.64 tones/ha), USA (25.98 tones/ha) and Turkey (26.73 tons/ha) (Nawaz *et al.* 2008).

There are other serious problems in Pakistani citrus orchards which are responsible for low yield, low fruit setting and excessive fruit drop, leading to losses for citrus farmers. Low fruit set and reduced quality of fruit is due to malnutrition, water stress, insect pest attack and most importantly, hormonal imbalance (Nawaz *et al.* 2008). The flowers and fruit of trees senesce when the concentration of auxin decreases and the concentration of abscissic acid (ABA) increases (Browning 1989; Marinho *et al.* 2005). Endogenous hormones and their balance play a modulating role in the mobilization of nutrients to the developing organs. The application of gibberellic acid (GA_3) increases fruit set (Albrigo and Sauco 2004) and reduces early fruit abscission in parthenocarpic citrus varieties (Fornes *et al.* 1992).

The use of plant growth regulators (PGRs) has become an important component of agrotechnical procedures. Synthetic auxins have generally been used as an antagonist to reduce abscission of mature fruit with all citrus species but mainly oranges, mandarins and grapefruits (Hield *et al.* 1964). PGRs play a paramount role in citrus biology and can affect several processes connected with flowering, fruit setting and fruit development. Saleem *et al.* (2008) reported that final fruit set was significantly affected by GA_3 treatments individually as well as in a mixture with 2,4-dichlorophenoxy acetic acid (2,4-D), with maximum fruit set of 32.32% in 'Blood Red' sweet orange trees treated with 45 mg/l GA_3 compared with the control. Fruit yield, in terms of number of fruit/tree as well as kg/tree was significantly affected by the treatments compared with the control. Similarly, preharvest application of GA_3 can effectively be used to control fruit drop in sweet orange cv. 'Salustiana' in which fruit drop percentage was reduced to the lowest val-

Received: 14 April, 2011. Accepted: 13 August, 2011.

Original Research Paper

ues (1.70 and 2.28) after the first application of GA_3 (100 mg/l), and after the second application of GA_3 (50 mg/l) compared to control where fruit drop percentage was 4.50 and 3.85%, respectively (Ibrahim *et al.* 2011).

Although a lot of work has been done to increase fruit set and improve yield and quality in sweet oranges (*Citrus sinensis* Osbeck), there is no precise recommendation for the control of fruit drop in 'Kinnow' mandarin. Moreover, fruit drop of 'Valencia' orange in Florida was not reduced by the application of PGRs, stressing the importance of climatic conditions on the effectiveness of PGR treatments (Greenberg *et al.* 1975). Thus there was a need to test the efficacy of PGRs to increase fruit set, reduce the number of seeds/fruit, improve fruit quality and yield under agro-environmental conditions of Punjab, Pakistan.

MATERIALS AND METHODS

Chemicals and reagents

PGRs, namely 2,4-D, GA_3 and 1-naphthaleneacetic acid (NAA) were purchased from BDH Chemicals, Dorset, UK while sodium hydroxide (NaOH), ethanol, Tween-20 and phenolphthalein of reagent grade were purchased from Sigma-Aldrich, St. Louis, MI, USA.

The research work was conducted at the fruit experimental orchard, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. Thirty 'Kinnow' mandarin plants grafted onto 'Rough lemon' rootstock of uniform size and age (10 years) were selected for this study. Foliar application of PGRs were made at different concentrations [2,4-D (T_2 = 10 mg/l, T_3 = 20 mg/l, T_4 = 30 mg/l); GA_3 (T_5 = 5 mg/l, T_6 = 10 mg/l, T_7 = 20 mg/l) and NAA (T_8 = 5 mg/l, T_9 = 10 mg/l, T_{10} = 20 mg/l)] during the 2nd week of March at full bloom (3.5 l/tree) to check their effect on fruit set and physiochemical properties of the fruit. T_1 was the control, i.e. no PGRs applied. The experiment consisted of 10 treatments (including the control) with three replications; a single tree was considered to be an experimental unit.

Fruit set percentage was recorded from four pre-tagged branches 2.5 cm in diameter, one from each side of the tree in the 2nd week of April. Fruit set percentage was determined by using the following formula (Moeen-Ud-Din *et al.* 2001):

$$\text{Fruit set \%age} = \frac{\text{Total fruit set}}{\text{Total bloom}} x\, 100$$

Yield/tree was recorded by weighing and counting the total number of fruits/tree at harvest time. Fruit size was measured by randomly measuring the diameter of 10 fruits/tree with the help of a digital vernier caliper. To determine the number of seeds/fruit, 10 fruits were removed from each tree of each treatment; fruit peels were removed manually and the seeds were extracted, counted and the average number of seeds/fruit was calculated.

Peel thickness for each fruit was measured in mm with a digital vernier caliper for the 10 selected fruits from each treatment and then average peel thickness was calculated. The juice of 10 harvested fruit was extracted with the help of manually operated juice squeezer and weighed; average juice weight was calculated separately for each treatment and juice percentage was obtained from the following formula (Nawaz *et al.* 2008):

$$\text{Juice \%age} = \frac{\text{Juice weight per fruit}}{\text{Average fruit weight}} x\, 100$$

Total soluble solids (TSS) were measured by an automatic digital refractometer (RX 5000, ATAGO, Tokyo, Japan) by placing 1-2 drops of juice on the prism of the refractometer while the acidity of juice was determined by diluting 10 ml of juice from each sample with distilled water in a 100 ml beaker; 2-3 drops of phenolphthalein were added for assessing the end point. The samples were titrated against a 0.1N solution of NaOH (Hortwitz 1960). The results were expressed as percent citric acid (Ahmed *et al.* 2006):

$$\text{Acidity \%age} = \frac{\text{N/10 NaOH used} \times 0.0064}{\text{Volume or weight of sample used}} x\, 100$$

Vitamin C (ascorbic acid) in juice was estimated according to the method described by Ruck (1961): 10 ml juice was placed into a 250-ml conical flask and the volume was made up to the mark using 0.4% oxalic acid solution. Filtered aliquot (5 ml) was poured into a flask and titrated against 2,6-dichlorophenoindophenol dye until a light pink colour formed which persisted for 10-15 s. Vitamin C content was calculated as:

$$\text{Vitamin C (mg ascorbic acid/100 ml juice)} = \frac{1 \times R1 \times V}{R \times W \times V1} x\, 100$$

where

R1 = ml dye used in titration of aliquot;

R = ml dye used in titration of 1 ml of standard ascorbic acid solution prepared by adding 1 ml of 0.1 % ascorbic acid + 1.5 ml of 0.4% oxalic acid;

V1 = volume of juice used (ml);

V = volume of aliquot made by addition of 0.4% oxalic acid;

W = volume of aliquot used for titration (ml).

The PGR solutions for spraying were prepared by dissolving each PGR (2,4-D, GA_3 and NAA) in 5 ml of 70% ethanol and then the required solution was prepared by adding distilled water with 0.1% Tween-20 as the surfactant (Khan *et al.* 2006). Recommended doses of nitrogen (N), phosphorous (P) and potash (NPK) (1000, 500 and 500 g, respectively) fertilizer and 50 kg of farm yard manure (FYM)/plant were supplied to experimental plants (Malik 1994). N was applied in two split doses, first in February in combination with P and K (500g each) while the second application was made in the last week of August (500 g N) and 50 kg of FYM was applied to each plant in December.

The experiment was executed according to a randomized complete block design (RCBD) and the means of data collected were separated by one-way analysis of variance (ANOVA). Significant differences between treatment means were compared by the least significant difference (LSD) test at P = 0.05 (Steel *et al.* 1996).

RESULTS AND DISCUSSION

There is limited literature regarding the importance of citrus cultivars and PGRs for desert citrus production. PGRs in citriculture are known to have a profound impact on tree vigour, health, yield and quality of fruit. The results obtained in this study for various parameters are explained next.

Fruit set (%age)

Fruit set is the critical phase in the transformation of a flower to a fruit to obtain good yield and to increase a grower's returns (Lovatt 1999). Fruit set was counted on marked branches following bloom and petal fall. There were significant differences in fruit set percentage among the various PGR treatments. All PGRs (2,4-D, GA_3 and NAA) significantly increased fruit set compared to the control. Maximum fruit set (82.77%) was observed in T_5 (5 mg/l GA_3) followed by T_6 (10 mg/l GA_3), T_7 (20 mg/l GA_3) and T_2 (10 mg/l 2,4-D) with fruit set being 77.80, 76.63 and 63.35%, respectively. The lowest fruit set (43.89%) was observed in T_1 (control). 2,4-D and NAA at high concentrations increased fruit set less efficiently, i.e., T_{10} (20 mg/l NAA) and T_4 (30 mg/l 2,4-D) compared to lower concentrations, i.e., T_2 (10 mg/l 2,4-D) and T_8 (5 mg/l NAA). Our results regarding fruit set were in agreement with those of Huang and Huang (2005), who concluded that a foliar spray of GA_3 at 50 mg/l on ¾ flower fall stage of citrus resulted in good protection of fruitlets and increased fruit set and yield by 100% compared to the control in 'Nanfengmiju' mandarin. Similarly, Agusti *et al.* (1982) found that a single spray of GA_3 (5 mg/l) at the petal fall stage increased fruit set significantly (29%) in 'Navelate' sweet orange. The application 2,4-D (100 mg/l) and GA_3 (100 mg/l) to fruiting branches increased fruit set by 17.3 and 21.4%, respectively in ginkgo (*Ginkgo biloba*) trees (Zhao *et al.* 2005). The results of our study indicate that 5 mg/l GA_3 can be used to enhance fruit set in 'Kinnow' mandarin.

Yield

Fruit yield is of prime concern to orchardists. They grow plants for better yield and good quality fruit production (Ahmed *et al.* 2006). Yield is measured in terms of number of fruits/plant and fruit weight/plant; the results regarding both of these parameters are described next.

1. Number of fruits/plant

There were significant differences among various treatments. All PGR treatments increased the total number of fruits/plant (**Table 1**). The highest number of fruits/plant (682.33) was observed in T_5 (5 mg/l GA_3) followed by T_2 (10 mg/l 2,4-D) and T_8 (5 mg/l NAA) with 659.66 and 647.66 fruits/plant, respectively; fewest fruits/plant (422.00) was found in T_1 (control). All three PGRs, at lower concentrations, increased yield but at higher concentrations fewer fruits/plant were observed in most treatments. Since 2,4-D and NAA (auxin) have a dual function, i.e., when they are used at lower concentrations they act as growth promoters while at higher concentrations they act as growth inhibitors, they sometimes adversely affect the vegetative and reproductive behaviour of citrus (Saleem *et al.* 2008). This might explain why fewer fruits/plant formed at higher concentrations of GA_3 by promoting vegetative growth at the expense of reproductive growth (fruits). The intensity of leaves on a flush was significantly affected by PGR treatments compared to the control in 'Blood Red' sweet orange. A maximum number of leaves (13.76) formed on trees treated with 30 mg/l of a mixture of GA_3 + 2,4-D each, while fewest leaves formed in control trees having 4.11 leaves/flush; similarly, shoot length and maximum shoot length (7.39 cm) also significantly increased in trees treated with GA_3 + 2,4-D but the minimum size of a flush (2.30 cm) was recorded in control trees (Saleem *et al.* 2008).

2. Fruit weight/plant (kg)

Maximum fruit weight/plant (96.14 kg) was observed in T_5 (5 mg/l GA_3) closely followed by T_2 (10 mg/l 2,4-D) and T_7 (20 mg/l GA_3) with fruit weight/plant being 90.14 and 89.17 kg, respectively. Minimum fruit weight/plant (52.49 kg) was observed in T_1 (control). In this case it is clear from the data that higher yield was observed at higher concentrations of PGRs, except for GA_3 (**Table 1**); at higher concentrations of GA_3 fewer fruits/plant were observed while fruit size was greater at higher concentrations; consequently, greater fruit weight/plant was observed. T_5 (5 mg/l GA_3) and T_2 (10 mg/l 2, 4-D) were the best treatments. The results for yield were similar to those of Agusti *et al.* (1982), who reported that the application of 5 mg/l of GA_3 on 'Navelate' sweet orange at the end of petal fall stage significantly increased yield up to 89 kg/tree compared to untreated tress (80 kg/tree). Huang and Huang (2005) reported that spraying GA_3 (50 mg/l) on citrus achieved good results by protecting fruitlets and increasing yield in 'Nanfengmiju' mandarin. Similarly, Saleem *et al.* (2008) observed that application of 45 mg/l GA_3 to 15-years-old 'Blood Red' sweet orange plants at the full bloom stage increased yield (71 kg/tree) more than the control (48 kg/tree).

Fruit size (mm)

The size of the fruit is important, not only because it is a component of productive yield, but also because it determines the acceptance of fruit by the consumer. The importance of fruit size as a parameter of quality of citrus fruits has increased markedly in recent years. The consumer's preference for large fruit causes huge differences in price between large and small fruit to the point that income from smaller fruit is often less than the picking and handling costs (Guardiola and García-Luis 2000).

The exogenous application of PGRs significantly increased the fruit size of treated plants compared to the control (**Table 1**). However, 2,4-D and GA_3 increased fruit size more. The fruit with greatest diameter (73.63 mm) was found in T_6 (10 mg/l GA_3) followed by T_3 (20 mg/l 2,4-D; 72.57 mm); smallest fruit (64.02 mm diameter) was found in T_1 (control). All three PGRs increased fruit size more than the control, with the best PGR treatments being 10 mg/l GA_3 (T_6) and 20 mg/l 2,4-D (T_3). Our results agree with those of Thomas and Lovatt (2004), who reported that foliar application of 15 mg/l 3,5,6-trichloro-pirydiloxyacetic acid (3,5,6-TPA) increased fruit size most in 'Clementine' mandarin and ultimately increased yield due to larger fruits. The net increase in yield/acre averaged over the 3-year experiment for the 3,5,6-TPA-treated trees, compared to the control trees, was: 610.00 kg of jumbo (fruit 63.51-69.85 mm in diameter); 1289.56 kg of large (57.16-63.50 mm); and 1165.73 kg of medium (50.81-57.15 mm) size fruit. No other treatment increased the yield of large size fruit compared to the control. Similarly, Zhang and Whiting (2011) noted that 200 mg/l GA_3 applied at 9 days after full bloom improved final fruit ('Bing' sweet cherry under USA field conditions) weight by 15%, and 56% of the fruit from this treatment had fruit weight/fruit > 9 g; to the weight of untreated limbs only increased 15%.

Percentage juice

Juice is an extremely important parameter for industrial processing, also related to size, which, in turn, although determined by the genetic characteristics of a cultivar, is affected by cultural practices such as the application of PGRs (Nawaz *et al.* 2008). Significant differences in juice percentage, calculated on the basis of juice weight, were observed among the various treatments (**Table 1**). The application of PGRs significantly increased the juice percentage, maximum (53.29%) in T_6 (10 mg/l GA_3) followed by T_7 (20 mg/l GA_3) and T_4 (30 mg/l 2,4-D) with 51.18 and 50.76%, respectively; the lowest juice percentage (42.56%) was found in T_1 (control). The juice percentage could be increased by as much as 10% simply by the application of PGRs. An increase in juice percentage was observed following the application of PGRs, which affect many physiological and biochemical processes within plants. Davies *et al.* (1997) also concluded that the application of GA_3 (18 g ai (active ingredient)/acre) had the potential to improve processing juice extraction weight by 3.2 to 9.4% depending on the cultivar and harvesting time.

Peel thickness (mm)

Peel thickness is an important parameter of fruit quality. There were no significant effects of PGRs on peel thickness (**Table 1**) although maximum peel thickness (3.48 mm) was observed following the application of GA_3 at 20 mg/l (T_7), least in T_2 (10 mg/l 2,4-D). Agusti *et al.* (1994) reported that the application of 50 mg/l 2,4-DP (2,4-dichlorophenoxypropionic acid) applied at the end of physiological drop on 'Owari Satsuma' mandarin (*Citrus unshiu* Marc.) increased fruit size and juice percentage significantly while no significant effect on peel thickness was observed as the peel thickness of treated fruit was 3.74 mm compared to the control (3.66 mm); however, a denser and firmer peel was produced. Similarly, Nawaz (2007) reported that preharvest application of PGRs [2,4-D (10, 20, 30 mg/l), GA_3 (10, 50, 100 mg/l) and NAA (10,15, 20 mg/l) at the preharvest fruit drop stage had no significant effect on peel thickness in 'Kinnow' mandarin.

Number of seeds/fruit

Seed dispersal in fleshy fruits like citrus is an unwanted characteristic. 'Kinnow' fruit should be seedless – or at least with less seed/fruit – because seeded fruits are more inconvenient to eat and add bitterness during processing, thereby satisfying the demands of customers and the processing industry (Ahmed 2005). The data regarding plump seed

Table 1 Effect of growth regulators on physical characteristics of 'Kinnow' mandarin.

Treatments	Fruit set (%)	Number of fruits/plant	Fruit weight/ plant (kg)	Fruit size (mm)	Juice (%)	Peel thickness (mm)	Number of seeds/fruit
T_1 (control)	43.89 e	422.00 c	52.49 d	64.02 c	42.56 c	3.15	19.63 a
T_2 (10 mg/l 2,4-D)	63.35 bc	659.66 ab	90.96 b	68.90 b	50.09 ab	3.00	18.60 ab
T_3 (20 mg/l 2,4-D)	55.17 cd	644.66 ab	88.63 ab	72.57 ab	48.70 b	3.06	19.40 a
T_4 (30 mg/l 2,4-D)	51.23 cd	616.00 ab	89.13 ab	72.16 ab	50.76 ab	3.11	18.20 abc
T_5 (5 mg/l GA_3)	82.77 a	682.33 a	96.14 a	72.36 ab	49.59 ab	3.21	16.65 bc
T_6 (10 mg/l GA_3)	77.80 ab	631.33 ab	85.92 ab	73.63 a	53.29 a	3.31	16.18 cd
T_7 (20 mg/l GA_3)	76.63 ab	624.00 ab	89.17 ab	70.36 ab	51.18 ab	3.48	15.82 d
T_8 (5 mg/l NAA)	60.33 bc	647.66 ab	86.50 ab	69.90 ab	49.49 ab	3.34	19.40 a
T_9 (10 mg/l NAA)	53.04 cd	642.66 ab	87.46 ab	69.44 ab	50.73 ab	3.26	18.87 a
T_{10} (20 mg/l NAA)	52.79 cd	610.33 ab	79.64 c	69.23 ab	49.04 b	3.30	19.22 a

Means in a column followed by similar letters are not significantly different at $P = 0.05$ (LSD).

(seeds with developed cotyledons and embryos) count/fruit showed significant differences between various treatments. The maximum number of seeds (19.63) was observed in T_1 (control), closely followed by T_8 (5 mg/l NAA), T_3 (20 mg/l 2,4-D) and T_{10} (20 mg/l NAA) with the number of seeds being 19.40, 19.40 and 19.22, respectively; the minimum number of seeds (15.82) was recorded in T_7 (20 mg/l GA_3). The treatments clearly formed three groups (**Table 1**): auxins (T_2 (10 mg/l 2,4-D), T_3 (20 mg/l 2,4-D), T_4 (30 mg/l 2,4-D), T_8 (5 mg/l NAA), T_9 (10 mg/l NAA), T_{10} (20 mg/l NAA)) formed the 1st group with a high number of seeds; GA_3 (T_5 (5 mg/l GA_3) and T_6 (10 mg/l GA_3)) formed the 2nd group with relatively fewer seeds; T_7 (20 mg/l GA_3) formed the 3rd group with the fewest seeds/fruit. GA_3 reduced the number of seeds compared to the control and other treatments. The reduction in the number of seeds/fruit may be due to the stimulatory effect of GA_3 on parthenocarpic fruit development (Zhang 2003). It can be inferred from the data that PGRs can reduce the number of seeds in 'Kinnow' mandarin, which is highly prized but contains many seeds which is discouraged by the citrus juice industry/processors. However, to reduce the number of seed, further detailed studies are required to standardize the concentration and time of application with special reference to our local agro-ecological conditions.

Chemical characteristics

1. Total soluble solids (TSS)

TSS is an important measure of the sugar content of fruits as sugars constitute approximately 85% of the soluble solids in citrus fruits (sweet oranges and mandarins) (Wardowski *et al.* 1979). Maximum TSS (11.73%) accumulated in T_9 (10 mg/l NAA) followed by T_{10} (20 mg/l NAA) and T_4 (30 mg/l 2, 4-D) i.e., 11.38 and 11.37%, respectively; minimum TSS (9.67%) was observed in T_1 (control). All the three PGRs increased the TSS compared to the control. It can also be inferred from the data that auxin (2,4-D and NAA) increased TSS more efficiently than GA_3. Ingle *et al.* (2001) reported that foliar sprays of 10 mg/l 2,4-D, 30 mg/l NAA and 25 mg/l GA_3 in February, August and September significantly increased fruit quality parameters such as juice percentage, TSS, ascorbic acid content, acidity and peel thickness, similar to our findings. Similarly, Nawaz *et al.* (2008) reported a significant increase in TSS compared to the control in 'Kinnow' mandarin following the foliar application of various concentrations of 2,4-D at the preharvest stage; 30 mg/l 2,4-D increased TSS to 12.03% compared to the contol which had a TSS of 10.43%. However, Berhow (2000) found that early foliar spray treatments of 100 mg/l GA_3 on grapefruit (*Citrus paradisii* Mcaf.) significantly lowered the concentration of the bitter flavonoid naringin in grapefruit fruit tissues and finally resulted in larger mature fruit, which yielded juice with the same TSS value as juice obtained from control fruits.

Table 2 Effect of growth regulators on chemical characteristics of 'Kinnow' mandarin.

Treatments	Total soluble solids (TSS)	Acidity (%)	Vitamin C (mg/100 g)
T_1 (control)	9.67 b	1.10 a	35.84 d
T_2 (10 mg/l 2,4-D)	11.34 a	1.01 ab	39.32 bcd
T_3 (20 mg/l 2,4-D)	10.97 a	0.91 b	43.28 abc
T_4 (30 mg/l 2,4-D)	11.37 a	0.91 b	44.44 a
T_5 (5 mg/l GA_3)	10.95 a	0.91 b	39.10 bcd
T_6 (10 mg/l GA_3)	11.13 a	0.94 b	38.73 cd
T_7 (20 mg/l GA_3)	10.93 a	0.97 b	41.91 abc
T_8 (5 mg/l NAA)	11.30 a	0.90 b	41.02 abc
T_9 (10 mg/l NAA)	11.73 a	0.94 b	45.11 a
T_{10} (20 mg/l NAA)	11.38 a	0.78 c	43.80 ab

Means in a column followed by similar letters are not significantly different at $P = 0.05$ (LSD).

2. Percentage acidity

Fruits sprayed with PGRs showed a significant decrease in acidity. Highest acidity (1.10%) was recorded for T_1 (control) followed by T_2 (10 mg/l 2,4-D) i.e., 1.01% whereas, minimum acidity (0.78%) was recorded in T_{10} (20 mg/l NAA). For all other treatments it ranged between 0.97 and 0.90%. The application of PGRs reduced the acidity of fruit which is a desirable character for superior fruit quality. Fruit harvest in 'Kinnow' is delayed in Pakistan due to high acidity of juice which imparts a bitter taste. Harvesting can be hastened by at least 15 days by using 15 mg/l NAA at the preharvest stage (Nawaz *et al.* 2008).

Vitamin C

Vitamin C (ascorbic acid) is essential for life and is a powerful water-soluble antioxidant, it must be ingested for survival as it prevents humans from many serious diseases like atherosclerosis and cancer by scavenging reactive oxygen species (ROS) and protecting tissues from ROS-induced oxidative damage (Parke 1999; Padayatty *et al.* 2003). The concentration of vitamin C content in fruits varies for various citrus species and is affected by environmental factors, the time of fruit harvest, plant vigour, age of the plant and by the application of PGRs (Ingle *et al.* 2001). Vitamin C content was significantly increased by various concentrations of PGRs, maximum (45.11 mg/100 ml) in T_9 (10 mg/l NAA) and T_4 (30 mg/l 2,4-D). Minimum ascorbic acid content (35.84 mg/100 ml) was observed in the control. The application of auxins (2,4-D and NAA) was better than the use of GA_3 in improving the vitamin C content of 'Kinnow' mandarin (**Table 2**).

CONCLUSION

PGRs are versatile compounds. Exogenous application of PGRs (2,4-D, GA_3, NAA) in this study significantly increased 'Kinnow' mandarin fruit set percentage and increase the average fruit yield/plant. Fruit quality (juice percentage, TSS, acidity and fruit size) was also improved by the application of PGRs which, at lower concentrations, enhanced fruit parameters more efficiently than higher concentrations. Of particular interest, GA_3 significantly reduced the number of seeds/fruit compared to the control. However, more detailed studies are required to check the efficacy of GA_3 in producing seedless/ or less seeded fruits in 'Kinnow' and other citrus cultivars of commercial importance.

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