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EXOGENOUS APPLICATION OF NAPHTHALENE ACETIC ACID IMPROVES FRUIT SIZE AND QUALITY OF KINNOW MANDARIN (*Citrus reticulata*) THROUGH REGULATING FRUIT LOAD

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ABSTRACT

Citrus fruits are produced across the world because of their adaptability to different climatic conditions. Present study was executed to investigate the effect of naphthalene acetic acid (NAA) (0 mg.l^{-1}, 200 mg.l^{-1}, 300 mg.l^{-1}, 400 mg.l^{-1}, 500 mg.l^{-1}) on fruit thinning, fruit development (fruit size, weight), and physio-chemical attributes [juice percentage, total soluble solids (TSS), titrateable acidity (TA), ascorbic acid, total sugars, reducing sugars, and non-reducing sugars] of seedless Kinnow mandarin. This study was conducted on ten years old Kinnow (*Citrus reticulata* Blanco) plants grafted onto Rough lemon (*Citrus jambhiri* Lush.). Experiment was laid out according to randomized complete block design with three replicates per treatment. Foliar application of NAA was done on experimental plants after the June drop. NAA at 500 mg.l^{-1} caused heavy fruit thinning by reducing the crop load up to 55% whereas, 200 mg.l^{-1} NAA reduced the crop load up to 13%. As for as the fruit quality is concerned; NAA at the concentration of 200 mg.l^{-1} enhanced per fruit weight, juice percentage, and TSS compared with control. In conclusion, foliar application of NAA (200 mg.l^{-1}) after the June drop can be utilized as thinning agent for Kinnow mandarin to improve the fruit size and other fruit quality attributes.

Key words: *Citrus reticulata,* Fruit load, Fruit quality, Fruit thinning, NAA.

INTRODUCTION

Citrus fruits have a prominent position among the tree fruits and extensively grown in tropical and sub-tropical regions of the world (Khan *et al.,* 2016). These are preferred because of higher yield, fruit size, superior quality, aroma, and better adaptation to a range of soil and agro-climatic conditions (Ahmed *et al.,* 2006; Khan *et al.,* 2010). Citrus fruit are well known for their specific flavor and have nutritional properties. These are rich source of vitamins, sugars (Nawaz *et al.,* 2011), minerals, dietary fibers, and phyto-chemicals (Ahmed, 2006; Ahmed *et al.,* 2007). Pakistan ranks at 13th and 10th position in production and export, respectively, among the world's citrus producing countries. In Pakistan, citrus fruits are cultivated on an area of 193.4 thousand hectares with the annual production of 2.18 million tons; Punjab is the leading province with a share of 97.08%; and 70% of commercially grown citrus in Punjab is comprised of Kinnow mandarin (AMIS, 2014, 2017).

Fruit trees usually set more number of fruits than they can support leading to poor fruit quality. More number of fruits present on the plant results in smaller fruits and chances of alternate bearing, late frost susceptibility, insect pest attack, as well as branch breakage due to heavy crop load, collectively lead towards economic losses (Guardiola and Garcia-Luis, 2000; Davis *et al.,* 2004). In many plant species, plant growth regulators are used to modify plant growth, development and fruit quality (Saleem *et al.,* 2008; Yildirim *et al*., 2011; Khalid *et al.,* 2012). Fruit thinning is a management practice particularly used to enhance the size of remaining fruits on tree, since number of fruits becomes comparatively less and their competition for carbohydrates and nutrients is reduced. Citrus trees (Ponkan mandarin) that carried less fruits in previous year require heavy thinning in current year (Cruz and Moreira, 2012) to maintain plant health and to avoid alternate bearing. Plant growth regulators especially synthetic auxin such as NAA and 2,4-D are utilized as thinning agents in the form of foliar spray for many fruit crops particularly citrus species (oranges, mandarins, and grapefruit) to improve fruit size and fruit quality (El-Otmani *et al.,* 2000, 2004; Fishel, 2015). NAA application at the stage of early fruit development proves effective for fruit thinning and enhances the fruit size (Khan *et al.,* 2014), and diameter (mandarin and Washington naval orange) (Greenberg *et al.,* 2007; Amiri *et al.,* 2012), with reduced fruit loss because of fruit drop (Safaei-Nejad *et al.,* 2015). According to a report, higher concentration of NAA (400-500 mg.l^{-1}) caused more fruit thinning in citrus fruits, increased fruit yield, and improved fruit quality parameters (Barar *et al.,* 1992). Preharvest application of NAA improved fruit quality attributes by enhancing total soluble solids, juice percentage, reducing and non-reducing sugars compared with untreated Kinnow plants (Ahmad *et al.,* 2009).

In Pakistan, Kinnow is the leading citrus fruit, marketed locally and also exported to other countries. The fruit with better size get premium price in local and international market, while small sized fruits fetch a nominal price in the market and in most of the cases utilized for processing purposes only. Although it has been reported that application of plant growth regulators particularly synthetic auxin can enhance fruit size and quality by reducing fruit drop of some citrus fruits (Sweet oranges and Kinnow mandarin) (Nawaz *et al.,* 2008; Saleem *et al*., 2008; Khalid *et al*., 2012; Khan *et al.,* 2014), but comparatively limited information regarding the use of NAA as a fruit thinning agent in Kinnow mandarin is available. Additionally, fruit thinning in Ponkan mandarin is comparatively difficult compared with other citrus species (Cruz and Moreira, 2012). Present research was aimed to investigate whether the effect of NAA is universal for other citrus cultivars/species such as Kinnow mandarin or only confined to some particular species. An experiment was planned to study the effect of NAA application on fruit thinning and quantitative and qualitative attributes such as fruit size, single fruit weight, juice percentage, total soluble solids (TSS), acidity, vitamin C, and sugars of Kinnow fruits.

MATERIALS AND METHODS

The study was carried out on ten year old seedless Kinnow mandarin (*Citrus reticulata* Blanco) plants grafted on Rough lemon (*Citrus jambhiri* Lush) at experimental area square number 32, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan (latitude 31°4181′ N, longitude 73°0776′ E, and altitude of 175m above the sea level). Experiment was laid down in randomized complete block design with three replicates and a single plant was taken as treatment unit. Fifteen uniform sized plants were selected and were treated with different concentration of NAA (0, 200, 300, 400, and 500 mg.l^{-1}) after June drop period on July 20, 2014. Aqueous solution for all treatments was prepared and applied to plants in the form of foliar spray. While, control trees were sprayed with tap water only. Uniform mature fruits were selected for further physical and biochemical analysis.

The numbers of dropped fruits before and after the spray were counted and fruit drop percentage was calculated by using the following formula

$$\text{Fruit drop percentage} = \frac{\text{Total dropped fruits after spray}}{\text{Total number of fruits}} \times 100$$

To measure the fruit size, twenty fruits per plant were used at the time of harvest, and fruit size was measured with digital Vernier caliper (KINCROME, part no. 2313, Victoria, Australia). Average fruit weight was calculated by weighing 10 fruits harvested from each experimental plant. The juice of the 10 harvested fruit was extracted by juice squeezer and weighed by using a digital electric balance. The average juice percentage was calculated by using the following formula

$$\text{Juice percentage} = \frac{\text{Juice weight per fruit}}{\text{Average fruit weight}} \times 100$$

Total soluble solids were measured with digital refractometer (ATAGO, RX5000, USA) by placing 1-2 drops of juice on the prism of refractometer. Acidity of juice was determined by taking 10 ml of juice from each sample and diluted with distilled water in a 100 ml beaker; 2-3 drops of phenolphthalein were added as an indicator. The samples were titrated against N/10 NaOH (Hortwitz, 1960), and the results were expressed as percent citric acid.

$$\text{Titrateable acidity} = \frac{0.1\ \text{N NaOH used} \times 0.0064}{\text{Volume of juice used}} \times 100$$

Vitamin C in juice was measured according to the method described by Ruck (1961). Sugars (reducing sugars, non-reducing sugar, and total sugars) in the juice were estimated by using method described by Hortwitz (1960). Briefly, 10 ml of juice of each sample was taken in 250 ml volumetric flask having 100 ml distill water, 25 ml lead acetate solution (25%), and 10 ml potassium oxalate (20%) solution and the volume was made up to 250 ml with distill water and filtered. The filtrate was used for the measurement of sugars.

Statistical Analysis: The experiment was designed according to randomized complete block design. Treatments response on fruit quality was determined by statistical analysis of data with software Statistix 8.1. The data was analyzed by using analysis of variance techniques (ANOVA) and the means were compared by using least significant difference test at $P \leq 0.05$ (Steel *et al*., 1997).

RESULTS

Fruit Thinning (%): Fruit thinning is considered an important practice to reduce crop load, and improve fruit size and fruit quality; as fruit size is inversely proportional to the number of fruits. The results for fruit thinning show that NAA application at early fruit developmental stage (after June drop) induced the fruit thinning. The increased concentration of NAA enhanced the fruit thinning intensity. NAA at 200 mg.l^{-1} resulted in

13% thinning of fruit, while 300 $mg.l^{-1}$, 400 $mg.l^{-1}$, 500 $mg.l^{-1}$ NAA induced fruit thinning up to 41%, 53%, and 55% respectively (Table 1).

Physical characteristics: Fruit size has paramount importance from marketing point of view. Results regarding fruit size revealed that maximum fruit size of 92.37 mm was recorded in case of 400 $mg.l^{-1}$ NAA followed by 200 $mg.l^{-1}$ NAA, 300 $mg.l^{-1}$ NAA, and 500 $mg.l^{-1}$ NAA with an average fruit diameter of 88.75 mm, 84.92 mm and 83.47 mm respectively (Table 1). Whereas, minimum fruit size of 73.20 mm was found in control.

Data regarding weight $fruit^{-1}$ showed that it was significantly affected by NAA treatments compared with control as maximum fruit weight (177.46 g) was found in plants treated with 200 $mg.l^{-1}$ NAA followed by 300 $mg.l^{-1}$ NAA (160.97 g) (Table 1). No significant difference for fruit weight was observed for 400 $mg.l^{-1}$ NAA (154.64 g) and 500 $mg.l^{-1}$ NAA (151.26 g) compared with control plants (148.13 g). NAA at 200 $mg.l^{-1}$ improved fruit weight by 20% compared with non-treated fruits (control).

The results regarding juice percentage depicted significant differences among various treatments. Maximum juice (54%) was found in the fruits treated with 200 $mg.l^{-1}$ NAA followed by 300 $mg.l^{-1}$ NAA (53%). The fruits subjected to 400 $mg.l^{-1}$ NAA and 500 $mg.l^{-1}$ NAA also performed well compared with control having juice content of 50% and 51%, respectively. Whereas, minimum juice content (46%) were found in untreated fruits.

Bio-Chemical characteristics: Total soluble solids (TSS) are considered the indirect measurement of sugar content; and fruits with high TSS are better. In citrus fruits, sugars contribute for 75-85% of the total soluble solids. In this study maximum total soluble solids (10.90°) were recorded for the fruits treated with 200 $mg.l^{-1}$ NAA followed by 300 $mg.l^{-1}$ NAA (10.84°), 400 $mg.l^{-1}$ NAA (10.82°), and 500 $mg.l^{-1}$ NAA (10.57°) respectively, whereas lowest (8.29°) TSS was observed in non-treated fruits (control) as shown in Table 2. Data pertaining acidity and ascorbic acid content showed non-significant differences among the different NAA treatments (Table 2). While, data regarding total sugars shows that maximum total sugar content (7.05%) were found at 400 $mg.l^{-1}$ NAA, followed by 500 $mg.l^{-1}$ NAA with total sugars of 6.77% (Table 2). The least amount of total sugars (6.40%) was recorded in 200 $mg.l^{-1}$ NAA. Maximum reducing sugar percentage (3.65%) was attained by fruits treated with 500 $mg.l^{-1}$ NAA followed by 300 $mg.l^{-1}$ NAA (3.64%) and both treatments were at par with each other. Control and 200 $mg.l^{-1}$ NAA attained reducing sugar percentage of 3.21% and 3.32% respectively. For non-reducing sugars, maximum percentage (4.12%) was acquired by fruits treated with 400 $mg.l^{-1}$ NAA, whereas, 200 $mg.l^{-1}$ NAA, 500 $mg.l^{-1}$ NAA and control fruits performed well with the percentage of 3.08%, 3.12% and 3.32%, respectively.

Table 1. Effect of NAA on fruit thinning and physical characteristics of Kinnow mandarin fruits.

TREATMENTS	Fruit Thinning (%)	Fruit Size (mm)	Single Fruit Weight (g)	Juice Percentage
Control	5 c	73.20 c	148.13 b	46 c
200 $mg.l^{-1}$ NAA	13 c	88.75 ab	177.46 a	54 a
300 $mg.l^{-1}$ NAA	41 b	84.92 b	160.97 ab	53 a
400 $mg.l^{-1}$ NAA	53 a	92.37 a	154.64 b	50 ab
500 $mg.l^{-1}$ NAA	55 a	83.47 b	151.26 b	51 ab

Means in column followed by similar letters are non-significant at $P \leq 0.05$.

Table 2. Effect of NAA on chemical characteristics of Kinnow mandarin fruits.

TREATMENTS	TSS (°Brix)	TA (%)	Ascorbic acid (mg $100g^{-1}$)	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)
Control	8.29 b	1.21 a	53.75 a	6.53 b	3.21 bc	3.32 b
200 $mg.l^{-1}$ NAA	10.90 a	1.19 a	47.30 a	6.40 b	3.32 abc	3.08 b
300 $mg.l^{-1}$ NAA	10.84 a	1.14 a	45.15 a	6.45 b	3.64 a	2.81 b
400 $mg.l^{-1}$ NAA	10.57 a	1.16 a	45.16 a	7.05 a	2.93 c	4.12 a
500 $mg.l^{-1}$ NAA	10.82 a	1.15 a	53.75 a	6.77 ab	3.65 a	3.12 b

Means in column followed by similar letters are non-significant at $P \leq 0.05$. TSS (total soluble sugars), TA (titrate able acidity).

DISCUSSION

Chemical thinning in fruits is considered an important approach to regulate crop load. The developing fruits have competition for carbohydrates and nutrients. Chemical compounds such as synthetic auxins are used to reduce number of fruits per tree (Hardy and Falivene, 2008). In this study, it was observed that application of NAA at early fruit developmental stage (after June drop) induced fruit drop and caused fruit thinning. It is well established facts that in case of fruit drop, the development of subsequent fruits becomes better, leading towards enhanced sizing potential as well as quality of fruit. It has been reported that application of NAA during fruit growth and development increases rate of respiration and abscission induced by ethylene production in immature fruits five times greater that of control (McArtney, 2002) that significantly reduces crop load up to 30-48% (Maria *et al.*, 2013).

Fruit size and weight are considered important quality parameters for commercial and consumer's point of view (Nawaz *et al.,* 2008; Khan *et al.,* 2010). NAA treated fruits attained better per fruit weight and size compared with non treated fruits. This might be due to the reason that too much fruits on tree compete with each other for carbohydrates and all fruit remains comparatively smaller. This competition is reduced after the fruit thinning as some of the fruits (15-50%) drop while the remaining fruits get more carbohydrates. NAA application (400 $mg.l^{-1}$) showed better fruit size by increasing more cells per fruit after cell division period while at higher concentration (500 $mg.l^{-1}$) the fruit size was comparatively decreased because of abnormal physiological activities and adverse effects of NAA (Guardiola and Garcia-Luis, 2000).

Juice percentage is an important qualitative parameter from industrial point of view and it is affected by the application of plant growth regulators. Atawia and El-Desouky (1997) reported that pre-harvest growth regulator application remarkably increased relative juice content in citrus species. In this study, NAA treatment at fruit development stage (after June drop) significantly increased fruit juice percentage compared with non-treated fruits. TSS is measurement of sugars content, as in citrus fruits sugars constitute 85% of total soluble solids (Nawaz *et al*., 2011). Results regarding TSS showed that it was improved by foliar application of NAA compared with control. However, a non-significant difference of TSS was observed between the different concentrations of NAA (200-500 $mg.l^{-1}$). TSS in various citrus fruit cultivars was improved by the application of NAA (Nawaz *et al.,* 2008; Taghipur *et al.* 2011; Safaei-Nejad *et al.*, 2015). Acidity in citrus fruits is one of the quality traits. In mandarins, fruit quality is considered better if acidity is low (Nawaz *et al.,* 2008). In our study, non-significant differences were observed. However according to some previous reports, the application of plant growth regulators particularly auxin reduces the fruit acidity in different citrus species (Ahmad *et al.*, 2009; Yildrem *et al*., 2011). Sugars are the source of energy therefore, considered as imperative parameter for quality determination. Plant growth regulator application contributes to an increase in sugar content of citrus fruits. In this study, maximum sugar contents were observed at higher NAA concentrations (400 $mg.l^{-1}$ and 500 $mg.l^{-1}$). Normally, the sugar contents increases with the advancement of maturity and auxin acts as bio-regulators that delays maturity and increases sugars content of citrus fruits (Saleem *et al*., 2008). Moreover, the application of plant growth regulators modify the fruit shape, improve fruit size, and enhance carbohydrates availability leading towards increased hexose and sucrose levels within the fruits (El-Otmani *et al.*, 2004).

The fruit size and quality is an important parameter for commercial and consumer's point of view. This study shows that exogenous application of NAA can regulate crop load by thinning effect, and can improve the fruit size and other quality parameters such as per fruit weight and juice percentage of Kinnow mandarin.

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