Research Article

Growth, Quality and Nutritional Profiling of Kinnow Mandarin Augmented through Rootstocks under Agro-Climatic Conditions of Sargodha, Pakistan

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Abstract | Citrus fruit production is getting much popularity throughout world, due to its high minerals and vitamin contents. The nutritional quality of citrus fruits primarily depends upon the selection of rootstock. Kinnow 'Mandarin' is a major citrus cultivar being cultivated in Pakistan. The current study aimed to investigate the nutritional status and growth and yield of Kinnow mandarin grafted on four overseas and two indigenous rootstocks in Pakistan. Experiments were conducted at experimental station of Citrus Research Institute, Sargodha from 2016 to 2020. Results revealed over all good adaptability, while no significant difference in juice contents was observed among all the tested cultivars. Physical quality of fruit (weight, firmness, size etc.,), and TSS were significantly affected by varying rootstocks. Cox mandarin was found as best cultivar as imparting maximum weight (180.7 g), size (74.10 mm), TSS (12.0 ^{0}B). Overall, cox mandarin budded with Kinnow performed well under local climatic conditions of Sargodha Pakistan.

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Keywords | Kinnow, Exotic rootstock, Canopy volume, Yield, Bio-chemical fruit quality

1. Introduction

Citriculture has great importance in the agriculture industry worldwide, occupying 1st position among the fruit crops in Pakistan (Shireen *et al*., 2018). Mandarin group is most widely cultivated and produced in Punjab, Pakistan, ultimately shares maximum foreign exchange (Khan *et al*., 2020a). Maximum citrus fruit (> 95%) is being produced by province of Punjab, and proportion of Kinnow (*Citrus reticulata blanco*) is more tha 70% of total production of citrus fruits (Niaz *et al*., 2004). Citrus fruits are much conspicuous and delicious with excellent nutritional value and organoleptic properties.

The growth and quality characteristics of citrus scion cultivars depend upon rootstocks. Properties of rootstocks vary with soils type, in scion tree vigour, fruit quality, yield and juice quality, tolerance to cold, drought resistance, root dispersion manner, mycorrhiza dependence (Hayat *et al*., 2021). These variations diversify the leaf mineral concentration, ultimately affecting the growth and productivity of citrus fruits escially influencing the vegetative growth (Basal, 2009). Rootstocks have been found to affect the leaf nutrient composition of scion cultivars (Jaskani *et al*., 2016; Hayat *et al*., 2019), especially potassium scion leaves (Sharples and Hilgeman, 1972) and variations in iron absorption (Pestana *et al*., 2005).

Moreover, Campeanu *et al*. (2009) suggested that scion cultivars should be considered for a better quality of mineral nutrients. A lot of researches have been conducted for effects of variation of rootstocks and their impacts of physiological, biochemical parameters of mandarin varieties, and found remarkable differences of results (Smith *et al*., 2004). Selection of rootstock or interstock has been found a crucial parameter, which remarkabley affects the canopy volume and other physiological parameters and production of various compounds i.e., chlorophyll (Richardson *et al*., 2003; Zhou *et al*., 2021). In addition, quality characteristics such as juice contents, total soluble solids and acid concentration of scion variety are greatly affected by the variety of rootstock. Uptake of nutrients and water from soil is directly affected by type of rootstocks. These effects have been widely studied around the globe under various environmental and climatic conditions significant results were found by varying rootstoks (Smith *et al*., 2004; Srivastav *et al*., 2005; Toplu *et al*., 2012; Khan *et al*., 2020b).

Many rootstocks are used globally due to their importance for fruit weight, size, juice and yield and in such way various rootstocks exhibited different results with different cultivars. Over all, sour orange is considered as efficient rootstock due to improved growth, yield, physical attricutes, biochemical attributes and production of quality fruits. Major benefit of using sour orange is its compatibility with major citrus varieties and compatibility towards climate chang and varying soil conditions (De Pasquale *et al*., 2006). In Pakistan, rough lemon and sour orange rootstocks are commercially used. Rough lemon is widely used in sandy, and clay soils of Punjab, whereas sour orange is commonly planted on the wet and caly soils of Khyber Pakhtunkhwa, Pakistan. In the Punjab province, commercial mandarin and sweet orange cultivars are generally budded or grafted on rough lemon (Jatti Khatti) rootstock. But a major problem with rough lemon is its high susceptibility to Phytophthora, infectious pododermatitis (foot rot) disease. This disorder reduces the fruit quality of Kinnow plants budded on rough lemon. Various studies have been conducted on the suitability of rootstock in advanced countries, i.e., China, Australia, Brazil, and USA (Davies and Albrigo, 1998; Kaplankiran *et al*., 2001; Bauer *et al*., 2005; Urbaneja-Bernat *et al*., 2020). Hayat *et al*., studied the characteristics of Feutrell's early budded with Troyer citrange and Cox mandarin, and concluded better fruit size than rough lemon without limiting any other physiological or biochemical character (Hayat *et al*., 2019).

Most of the citrus varieties are being budded with rough lemon rootstock in Punjab, Pakistan. In recent years, rough lemon is facing some challenges for growth and productivity. Major problem with rough lemon rootstock is increased vegetative growth, which cause the purity problems of fruits and decreased productivity. Plants budded/grafted with rough lemon rootstock resulted in dense plant growth, which makes it unsuitable for high density cropping system. Second major problem is the high incidence of citrus greening disease on citrus varieties budded/grafted with rough lemon. Because it is the oldest rootstock being used in Punjab, Pakistan and citrus greening has become more problematic from almost last two decades, which has increased acceptability of citrus greening by rough lemon rootstock. This has decreased the innate immunity of rough lemon against citrus greening, ultimately made it more susceptibile to citrus greening.

Hence, it is need of time to find a new substituted rootstock for monopolized cultivation of Kinnow, alreading being budded on rough lemon rootstock due to intensive change in climate, ultimately for improving yield and quality attributes, for increased productive life span of Kinnow mandarins in Punjab, Pakistan. Therefore, the current study was planned to investigate the compatibility of exotic rootstocks and their effects on growth yield and nutritional characteristics of Kinnow mandarin at experimental stations of citrus research institute (CRI), Sargodha.

2. Materials and Method

Procurement of raw material

Rough lemon mature fruits were collected in August from the germ plasm unit of CRI, Sargodha in 2010. Seeds of four exotic rootstocks (Cleopatra, Troyer citrange, Carrizo citrange, Cox mandarin) were imported from Australia under ASLP (Agriculture Sector linkage program) project for Pakistan and Australia. Seeds of two local rootstocks (rough lemon and sour orange) were extracted.

1.2 Experimental model

Treatment plan was T_1 (Cleopatra), T_2 (Troyer citrange), T_3 (Sour orange), T_4 (Cox mandarin), T_5 (Rough lemon) and T_6 (Carrizo citrange) budded with kinnow mandarin. T_5 (Rough lemon was considered as control). Extraction of seeds were done manually and shade dried seed were sown in trays in Screen house. Seedlings were transplanted in Polythene bags in July 2011. A-sexual propagation of plants was done using T-budding method in July 2012, and plantation was done at 15th Square of experimental station of CRI, Sargodha in August 2013. RCBD design was employed and standard agronomic practices as described in our manuscript Hayat *et al.* (2019) were carried on for appropriate growth and development of plants. As marketable fruit production of citrus plants normally takes at least 3–4 years, so data for physiological and biochemical parameters was collected constantly for four years i.e., from 2016 to 2020.

1.3 Measurement of yield and growth parameters

The initial reading of vegetative growth parameters including scion girth, stock girth, canopy volume, canopy spread etc., were measured using standard protocolos described by Bassal (2009). Plant height, spread of trees and canopy volumes were measured according standard methods described by (Albrigo, 1975). Plant height (meters) was measured from ground level to the tip of the plant canopy. Spread of trees (meters) was calculated by measuring maximum spread in north-south and east-west direction. The canopy volumes of selected plants were calculated using formula (Albrigo, 1975).

$$\text{PScv} = \frac{\pi D12}{4}\left[2\left(\frac{Ht-Hc}{3}\right)+\left(Hc-Hs\right)\right]$$ (Tumbo *et al.*, 2001)

PScv= canopy volume (m3); H_t= overall canopy height from ground level to point of maximum height of branches of canopy; D_1= canopy diameter parallel to the rows(m); H_c= Height from ground to maximum canopy of tree (m); Hs= Height from ground to canopy skirt (m).

1.4 Measurement of physical quality parameters

Physical parameters of fruits were calculated after harvesting during January of each year (2016-2020). Ten fruits were selected randomly from all four sides of the tree, i.e., North, South, East and West. The fruit weights (g) were measured by an electronic weighing balance. The fruit size and peel thickness (mm) were measured using a Vernier caliper (Mitutoyo, Kanagawa, Japan). Seed weight (g), pulp weight (g) and juice weight (g) were measured using an electronic weighing balance.

1.5 Determination of biochemical parameters

Biochemical parameters of fruits, *i.e.,* protein, fiber, ash, total soluble solids (TSS), acidity, vitamin C, pH, reducing sugars, non-reducing sugars, total sugars, α-carotene and β-carotene were calculated from the juice extracted.

Ash contents were measured by charring of pericarp followed by incineration in a muffle furnace at 550°C till constant weight (Ismail, 2017). A digital Refractometer was used to measure the TSS while percentage of acidity was calculated by titration, using sodium hydroxide (0.1 N) and phenolphthalein indicator. The juice pH will be measured by using a pH meter (Sugiura *et al.*, 1983). Vitamin C was measured by titration method and expressed as mg/100mL (Nasir, 2016).

Reducing sugars and total sugars were determined by the method described by Nasir (2016). For determination of reducing sugars, juice sample (10 mL) was titrated against standardized fehling solution (freshly prepared) and reducing sugars were calculated using following calculations:

$$\textit{Percent Reducing Sugars} = 6.25 \times (x \div y)$$

X= Standard sugar solution (mL) titrated against fehling solution (10 mL); Y= Sample solution (mL) used against fehling solution (10 mL).

Total sugars were calculated according to the procedure mentioned earlier by Nasir (2016) using formula:

Total Sugars (%) = 25 x (X / Z)

Where

X= volume (mL) of standard sugar used against 10 mL of Fehling solution; Z= volume (mL) of sample aliquot titrated against 10 mL of Fehling solution.

The Non-reducing sugars would be calculated according to the formula as given below:

Non-reducing sugars (%) = 0.95 x (Total sugar % – reducing sugars %)

1.6 Statistical analyses

Data was subjected to Statistix-10 Software, and mean values were analyzed and compared using LSD Test test at the 5% probability level (Steel and Torrie, 1984). Plot graphs were used to compare treatments and months using the same software.

2. Results and Discussion

Various rootstocks showed a significant effect on the growth and yield of Kinnow mandarin (Table 1). Maximum canopy volume in Kinnow mandarin trees was observed in trees budded on Cox mandarin (17.39 m^3) followed by Rough lemon rootstock (16.32 m^3) and Sour orange (15.73 m^3). Similarly, the highest plant height was observed in plants budded on Cox mandarin (3.10) followed by sour orange (3.09 m) and rough lemon (3.03 m) (Table 1).

Table 1: Performance of different rootstocks on growth and yield of Kinnow.

Treatments	Canopy (m^3)	Plant height (m)	Sci-on-stock ratio	Yield (Number of fruits)
Rough lemon	16.32ab	3.03a	0.88ab	376a
Troyer citrange	7.46d	2.48b	0.89a	129c
Carrizo citrange	13.13c	2.52b	0.89a	128c
Cleopatra	14.65bc	2.86ab	0.78b	242bc
Cox mandarin	17.39a	3.10a	0.89a	380a
Sour orange	15.73bc	3.09a	0.89a	306ab
HSD ($P \leq 0.05$)	2.57	0.409	0.1	127

NS represents not significant. Means within a column followed by the same letter are not significant at $P \leq 0.05$.

Physical fruit quality (fruit weight, firmness, peel thickness and fruit size) of Kinnow mandarin (Table 2) was significantly affected by different rootstocks. Maximum fruit weight was observed in cox mandarin (180.7 g) followed up by rough lemon (170 g) (Table 2). Maximum fruit firmness was recorded in Cox mandarin (Table 2). Peel thickness was highest in rough lemon (3.2 mm), sour orange (3.2 mm) and Cleopatra mandarin (3.06 mm) (Table 2). Similarly, maximum fruit size was recorded in Cox mandarin (74.10mm), rough lemon (72.13 mm), sour orange (72.13mm) and Cleopatra (71.93mm) (Table 2). Moreover, Any exotic rootstock did not significantly affect fruit juice (Table 2).

Table 2: Effect of different rootstocks on Physical fruit quality of Kinnow.

Treatments	Fruit weight (g)	Firm-ness (N)	Peel thickness (mm)	Fruit size (cm^2)	Fruit Juice (%)
Rough lemon	170 a	1.76 b	3.2 a	72.13 a	40.53
Troyer citrange	160 ab	1.76 b	2.7 bc	67.23 ab	37.9
Carrizo citrange	109 d	1.66 b	2.46 c	58.50 c	40.4
Cleopatra	153 bc	2.10 a	3.06 a	71.93 a	40.23
Cox mandarin	180.7 a	2.13 a	2.76 b	74.10 a	43.23
Sour orange	170.7 a	1.76 b	3.2 a	72.13 a	42.6
HSD ($P \leq 0.05$)	27.47	0.15	0.3	6.5	NS

NS represents not significant. Means within a column followed by the same letter are not significant at $P \leq 0.05$.

The protein content, fiber content and ash content of Kinnow mandarin were not affected by different exotic rootstocks (Table 3). The results showed that different rootstocks do not affect protein content, fiber content and ash content (Table 3). The TSS and acidity of 'Kinnow' juice were significantly affected using different rootstocks (Table 4). Maximum TSS was found with Cox mandarin (12.00 °Brix) and Carrizo citrange (11.8°Brix) (Table 4).

Table 3: Effect of different rootstocks on Bio-chemical fruit quality of Kinnow.

Treatments	Protein (%)	Fiber (%)	Ash (%)
Rough lemon	0.86	1.2	1.83
Troyer citrange	0.9	1.2	1.86
Carrizo citrange	0.9	1.23	1.86
Cleopatra	0.83	1.2	1.86
Cox mandarin	0.86	1.56	1.86
Sour orange	0.86	1.2	1.83
HSD ($P \leq 0.05$)	NS	NS	NS

NS represents not significant. Means within a column followed by the same letter are not significant at $P \leq 0.05$.

Vitamin C in "Kinnow" mandarin was not significantly affected by any rootstock (Table 4). Thus, showing that vitamin C was not affected by any rootstock. The pH of Kinnow mandarin juice was significantly affected by different rootstocks. It was observed that maximum pH in Kinnow was found with sour orange, and minimum pH was observed in cox mandarin rootstock (Table 4).

Table 4: Effect of different rootstocks on Bio-chemical fruit quality of Kinnow.

Treatments	TSS (°Brix)	Acidity (%)	Vitamin C (mg/100 mL)	pH
Rough lemon	9.8 c	0.7 d	34.96	3.93b
Troyer citrange	11.03 ab	0.8 c	35.70	3.73bc
Carrizo citrange	11.8 a	1.0 a	33.06	3.53 cd
Cleopatra	10.73 abc	0.81 c	34.30	3.7 c
Cox mandarin	12.00 a	0.64 e	35.83	3.46 d
Sour orange	10.46 bc	0.91 b	33.93	4.33 a
HSD (P≤0.05)	1.08	0.05	NS	0.2

NS represents not significant. Means within a column followed by the same letter are not significant at $P \leq 0.05$.

Different rootstocks significantly affect reducing sugars, non-reducing sugars and total sugars of Kinnow mandarin (Table 5). Maximum reducing sugars was observed in sour orange, *i.e.*, 3.80%, while minimum reducing sugars was found with rough lemon 3.33% (Table 5). The highest non-reducing sugars (8.63%) were calculated with Troyer citrange (Table 5). Similarly, maximum total sugars were found in Kinnow mandarin budded on Troyer citrange (12.33%) and cox mandarin (12.23%) (Table 5). Al-Hosani *et al.* (2011) observed highest sugars in Hamlin orange on plants grafted on sour orange while low sugars were found on acid lime rootstocks and found similar results. β-carotene and α-carotene of Kinnow mandarin juice were not significantly altered with any rootstock (Table 5). Thus, different rootstocks have a non-significant effect on carotene content of Kinnow.

Grafting is commercially practiced for variety development in horticultural crops around the globe (Hayat *et al.*, 2021). Important thing to consider for grafting is selection of appropriate rootstock, as rootstock is major regulator for scion phenotyping and other characteristics of final fruit including fruit size, weight, productivity, yield, quality attributes, stress tolerance and response towards climate change (Liu *et al.*, 2017; Hayat *et al.*, 2022). Previously, the effect of rootstocks on growth morphology and productivity of certain fruit trees were investigated (Cantuarias-Aviles *et al.*, 2010; Khan *et al.*, 2020b).

Primary objective of conducting current study was to investigate the compatibility of exotic rootstocks with Kinnow mandarin and their effects on physical and biochemical/nutritional parameters under agroclimatic conditions of Sargodha, Pakistan. Scion stock ratio clearly shows no issue of scion stock incompatibility (Table 1), which is in line with Hayat *et al.* (2019) working on Feutrell's budded with different exotic rootstock under the agro-climatic condition of Sargodha. 'Kinnow' mandarin budded on Cox mandarin (380) showed a significantly higher yield than other rootstocks. Whereas, Rough lemon (376 fruits/tree) and sour orange (306 fruits/tree) showed the highest yield among different rootstocks. Results were in contradiction with the study of Fallah and Rodeny (1992). They observed that rough lemon is low yielder than citrange while experimenting on Fair Child mandarin as in the current study, rough lemon showed more yield than citrange in the case of Kinnow mandarin.

In the present study, Cox mandarin rootstock performed better compatibility with Kinnow mandarin thus resulting in higher yield than other varieties. But all other rootstocks were compatible with rough lemon. The current study results are in line

Table 5: Effect of different rootstocks on Bio-chemical fruit quality of Kinnow.

Treatments	Reducing sugars (%)	Non reducing sugars (%)	Total sugars (%)	B-Carotene ↿ mg/100g	Anti-Oxidant (%DPPH)
Rough lemon	3.33 b	8.36 abc	12.00 ab	61.66	72.73
Troyer citrange	3.40 ab	8.63 a	12.33 a	59.63	72.83
Carrizo citrange	3.36 ab	8.46 ab	12.06 ab	62.26	73.33
Cleopatra	3.63 ab	7.63 c	11.53 b	61.16	72.53
Cox mandarin	3.66 ab	8.30 abc	12.23 a	61.56	72.63
Sour orange	3.80 a	7.83 bc	11.83 ab	61.16	73.40
HSD (P≤0.05)	0.45	0.75	0.66	NS	NS

NS represents not significant. Means within a column followed by the same letter are not significant at $P \leq 0.05$.

with Georgiou (2000), who found that Sour orange, Rough lemon, Troyer, and Carrizo are compatible with mandarins. Vitamin C in "Kinnow" mandarin was not significantly affected by any rootstock (Table 4). Thus, showing that vitamin C was not affected by any rootstock. The results are in disagreement with the findings of Khalifa and Hamdy (2015) who found increase in vitamin C of mandarin after budding on Valencia late rootstock.

The increase in TSS was further supported by Khalifa and Hamdy (2015) findings, who found an increase in TSS trees budded on sour orange. Similarly, Nasir *et al*. (2014) found a significant difference in Kinnow TSS when budded on different rootstocks. The highest acidity (%) was found in Carrizo citrange, i.e., 1%, whereas minimum acidity was recorded in Cox mandarin rootstock (0.64%) (Table 4). The results are in contrast with the findings of Al-Hosani *et al*. (2011), who found the highest acidity in trees budded on acid lime rootstock. In contrast, lowest acidity was observed in Cox mandarin. Hifny *et al*. (2013) and Khalifa and Hamdy (2015) observed highest acidity on fruit trees budded on sour orange.

Conclusions and Recommendations

Current study was aimed to investigate the compatibility of exotic rootstocks grafted / budded with Kinnow mandarin and their physiological and biochemical parameters. Project concludes that the agro-climatic conditions of Sargodha, Pakistan welcomes the cultivation of exotic rootstocks. Significant difference in juice contents was not observed other parameters exhibited remarkable differences. It may be concluded that better vegetative and reproductive could be attained in Kinnow budded with Cox mandarin without imparting any remarkable effects of growth, yield, physiological, biochemical and nutritional profile. Physico-chemical and proximate analysis reveals that Cox mandarin has excelled under trial rootstocks. Cox mandarin has found potential candidate for replacement of rough lemon, which is facing serious problems in Punjab, Pakistan including increased vegetative growth and susceptibility towards citrus greening disease. It is pertinent to mention, though Cox mandarin has not been studied for resistance against for biotic and abiotic factors, but after comprehensive studies of bearing length of plants and other parameters, Cox mandarin can be future widely cultivated rootstock.

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Novelty Statement

Five exotic rootstocks (Cleopatra, Troyer citrange, Sour orange, Cox mandarin and Carrizo citrange) were studied for their physiological and nutritional profiles upon budding with Kinnow mandarin. Finding a new rootstock for Kinnow mandarin is becoming vital due to various problems associated with rough lemon including increased vegetative growth and higher adaptability of microbial infections (previously utilizing rootstock).

Author's Contribution

All the Authors have equal contribution.

Conflict of insterest

The authors have declared no conflict of interest.

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