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RESEARCH PAPER

Influence of rootstocks on plant growth and internal quality of sweet orange (*citrus sinensis*) cv. Musambi

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Key Message: Various rootstocks to enhance vegetative growth and quality characteristics of sweat orange Musambi were tested in agro-climatic conditions of Punjab. Troyer, a prominent rootstock of California being highly polyembryonic and hardy and manifested the best results among five contender treatments.

Abstract: Musambi fruit is famous for its distinguished characters for taste and unique flavor in soil and climate of Punjab, Pakistan. The productivity of musambi is low which is greatly influenced by rootstock. Therefore, the experimental evaluation of rootstocks including exotic and local (rough lemon rootstock) was done at Citrus Research Institute Sargodha. A study trial was initiated using RCBD design with three replications per treatment. Musambi was budded on rootstocks (five treatments including T_o Rough lemon, T_1 Troyer citrange T_2 Benton, T_3 Cox mandarin, T_4 Carrizo citrange) in the year 2011. The fruit samples were collected for quality and quantity parameters. The analyzed results showed significant compatibility success. Overall, the maximum plant height (1.70 m) was attained in T_0 (Rough lemon) with maximum canopy volume (4.79 m^3). Maximum fruit weight (184 g), fruit size (73.2 mm), and almost equal juice percentage (46%)) were obtained on T_1 Troyer citrange as compared to rough lemon and other rootstocks. The determined characters in Troyer citrange citrus rootstock were significant, and can boost multifaceted attributes in growing conditions of suitable agroclimate like Sargodha, Punjab. In future, the productive life span of grafted plants in orchards could be achieved with the enhanced quality attributes for orange cultivar; Musambi.

Keywords: Compatibility, Musambi, Plant growth, Quality, Troyer citrange

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Introduction

The productivity of citrus is greatly influenced by selected cultivars (Nawaz et al., 2012). The quality citrus can be grown in tropical and subtropical environments under sandy loam soil texture and favourable growing conditions (Shah, 2004). The selection of rootstocks ensured variety performance to tolerate the abiotic impact with successive quality improvemnets i.e. yield and fruit quality (Martinez-Cuenca et al., 2016). Citrus is extensively grown due to valueable major antioxidant (ascorbic acid or vitamins C) (Sikarwar & Tomar, 2018). Citrus comprises of major commercial varieties groups including Sweet oranges, Mandarin, Grape fruit, Lemon and Lime which are being grown commercially in Punjab (95%). Global production of oranges during year 2019-20 is forecast to fall 5.8 million metric tonnes from the previous year to 47.5 million metric tonnes due to unfavorable weather conditions. Brazil the leading producer of the world is also expecting 22 percent fall to 15.1 million metric tonnes due to weather related problems (United States Department of Agriculture [USDA], (2020). Its attractable color and pleasant flavor, proceeed for juice contents (Toplu et al., 2008). Oranges are enriched with minerals like potassium, manganese, phosphorus, iron and copper so can be recommended for hemoglobin deficient bodies and for hemoglobin production (Czech et al., 2020). Musambi is a very popular orange variety due to its sweet taste which is driven by Kinnow mandarin. Sweet orange contributes about 60% in world's citrus production (Khan et al., 2014).

In Pakistan, sweet orange is considered as a high valued commercial crop from the month of September to December earlier to mandarins. Among four provinces, Khyber Pakhtunkhwa is a hub of oranges and its major orange producing districts include Malakand, Swat, Nowshera, Lower Dir, Dera Ismail Khan, Mardan and Haripur. In Punjab, plantation of Musambi orange has been declined due to its short bearing or low productive life span. Despite of plant germination issue due to polyembryony in 18th century, major citrus trees were grown from seedlings for commercial purpose. Moreover, it was the era for outbreak of phytophthora foot rot in oranges which sghifted to citrus industry towards rootstock multiplication (sour orange; *Citrus aurantium* L.) for grafting of desired variety. After that in 20th century, the most devastating problem of citrus, Tristeza virus to sweet oranges grafted on sour orange rootstock drive the industry to utilize the alternate rootstocks. The quality and superior nature of rootstock characters include (i) shorter duration of vegetative growth and standard trees' strength (ii) must enhance the qaualitative and quantitative characters of selected grafted variety in term of higher yield, desireable fruit quality and suitable commercial fruit size (iii) increment of resistance in citrus associated pathogens and diseases likewise phytophthora rots, root weevils, burrowing nematodes, Tristeza virus and (iv) enhance trees tolerance in grown habitat to abiotic stresses such as salinity in soil, drought resistance, flooding of water, cold tolerance and high soil pH. Consequently, the presence of all desired characters of rootsctock is still lacking. Various factors are responsible for the production of quality fruit like nutrient management, quality of nursery plants, rootstock onto which scion is grafted and appropriate cultural practices. Rootstock type has an important role in growth, development and production of citrus crop. Citrus rootstock differ in compatibility to soil type, manner of root dispersion, affiliation to mycorrhiza (Nawaz et al., 2012).

In reclaimed soils, the growth and fruiting of citrus are highly affected by environmental condition especially soil factor, which negatively affected the growth of sour orange rootstock (Ahmed et al., 2007). The availability of wide range of rootstocks in the era is much important. The rootstock capability is measured by its tolerance rate to the prevailing condition of grown environment, agro-climatic condition and disease resistance with proper quality prodcution (Sau et al., 2018). Rootstocks have a superior capability which enhance minerals & nutrients utilization, bunchy root spreading that lead to supporting of grafted cultivars and ultimately boosting the vegetative and quality aspects of fruit (Bassal, 2009). Rootstock showed the resistance by avioding the detrimental influence on growth and intervent several biotic and abiotic stresses near rootzone (Webster, 1995). Therefore, positive contigent influence to translocate the water and assimilates between the scion-rootstock along with promoting and inhibiting circulation of endogenous hormones (Ahmad et al., 2007). Large fruit size with high auxin levels resulted from auxin accumulation with compatible rootstock (Liu et al., 2015).

As rootstock can influence twenty variables of citrus, so evaluation of the best rootstock for commercial fruit production is a dire need of the time (Shafqat et al., 2014). Size of tree, precocity, quality fruit production and time of maturity is significantly affected by the relationship between canopy and root volume of plant (Gimeno et al., 2015). Leaf chlorophyll contents (Garcia-Sanchez et al., 2002) and mineral nutrients like N, P, K of citrus leaves are greatly influenced by rootstocks (Toplu et al., 2008). Maximum yield of Kinnow mandarin as grafted on Brazillian Sour orange was comparable with other rootstocks (Ahmed et al., 2007). A remarkable difference was noticed in fruit size of various citrus scions grafted onto different rootstocks (Shafqat et al., 2014; Kumar, 2015). Rough lemon mostly used as a rootstock in Punjab has advantages of being compatible with most of the commercial citrus varieties resulting in quality produce with better yields. However, rough lemon rootstock susceptiblity enhanced a quick decline. So, to overcome this major consequence, the selection of suitable and compatible rootstock is utmost demand for citrus blooming industry for better yield and quality of sweet orange cv. Musambi. Due to irregular and poor growth (Nel & Bennie, 1984), expanded growth in South Punjab districts; Layyah, Bhakkar, Dera Ghazi Khan and Vehari are encountering the production with lower quality and quantity. Therefore, this study was designed to observe and evaluate better rootstock performance for growth and productivity.

Materials and Methods

Plant material and growing conditions

The rootstock nursery of rough lemon and four others namely Troyer, Cox mandarin, Benton and Carrizo Citrange (imported from Australia) were grown in citrus foundation block at Citrus Research Institute, Sargodha, Pakistan in August, 2009 (Fig. 1). The grown seedlings were transplanted in green house in September 2010. The budding of Musambi scion employing T-Budding was done in October, 2011. Proper fertilization and management practices were apllied to the experimental field.

Data collection for vegetative growth and fruit yield

The vegetative data was recorded from 2014 to 2018 consecutively for five years, while fruit quality and yield data was collected during 2017-2018 for two years. The success of compatibility was calculated by measuring scion and stock girth. For estimating the dwarfism, height of the plants was measured in meters from the ground level to the tip of plant by measuring rod. Canopy height, canopy diameter, maximum height of canopy and height of canopy from ground (skirt) was measured to calculate the canopy volume indicating the growth potential of plants. The canopy volume of the trial plants were computed using formula (Albrigo, 1975).

$$PScv = \frac{\pi D12}{4}\left[2\left\langle\frac{Ht-Hc}{3}\right\rangle + \langle Hc - Hs\rangle\right]$$

Where

PScv = Canopy volume (m^3); Ht= Overall canopy height above ground level; D1 = Canopy diameter parallel to the row; Hc = Height to the point of maximum canopy diameter; Hs = Height from ground to canopy skirt

At the time of optimum maturity, fruit was harvested from the plants of all budded plants at all rootstocks. For yield, total fruits were counted and weighed (kg).

Physico-chemical characteristics

The selected mature fruits were harvested in the month of January every year for consective five years. After washing and drying, fruit samples were divided into three replicates for physical and biochemical analysis. A representative of 20 mature fruit samples from a rootstock was selected. Fruit diameter (mm) of selected mature fruits was assessed at mature/ripened phase with digital vernier caliper. Weight (g) of the selected fruit (20 fruits per replication) was taken with the digital electronic balance and their average was computed for further analysis. The peel thickness (mm) was recorded in average for desired replication with digital Vernier caliper by separating whiter albedo layer from the peel. All collected fruit samples were analyzed to asses the physico-chemical characters. Average data of peel weight in term of percentage was further measured with this formula:

$$\text{Peel weight (\%)} = \frac{\text{Average peel weight}}{\text{Average fruit weight}} \times 100$$

After peeling the whole fruit, juice was squeezed and rag material was collected and weighed. The average data of rag weight was determined in percentage with the given formula:

$$\text{Rag weight (\%)} = \frac{\text{Average rag weight}}{\text{Average fruit weight}} \times 100$$

Fruit juice was squeezed by using electronic squeezer. Pulp and seeds of each fruit were separated by sieving the juice, then average juice percentage per fruit was recorded as described by Nasir et al. (2016). The juice weight percentage of samples was determined with this formula:

$$\text{Juice weight (\%)} = \frac{\text{Average juice weight}}{\text{Average fruit weight}} \times 100$$

Total soluble solids (^{o}Brix) of juice were determined by the use of digital ATC Refractometer (automatic temperature compensation, corrected at 20 ºC of HANNA Japan). Acidity was assessed as percentage of citric acid in juice through titrations. Titratable acid (TA) percentage (citric acid g per 100 ml) was measured by titrating 10 ml of juice against 0.1 N NaOH solutions to the persistent pink color end point achieved at pH 8.1. All measurements were made in triplicate to record the averages as described by Miri et al. (2018); Nawaz et al. (2019). Percentage of acidity was calculated as following;

$$\text{Acidity (\%)} = \frac{\text{NaOH used} \times 0.0064}{\text{Sample weight}} \times 100$$

TSS/acid ratio representing maturity index was calculated as following;

$$\text{TSS/Acid ratio} = \frac{\text{Average TSS}}{\text{Average acidity}}$$

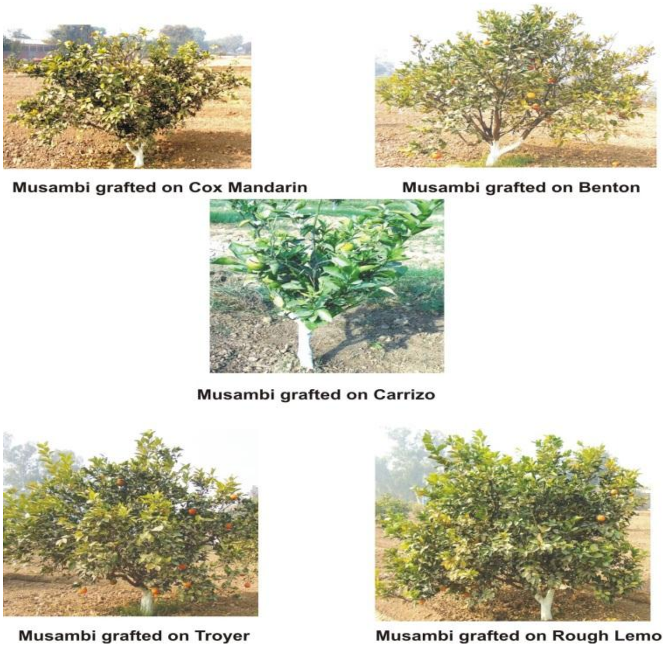

Fig. 1 Pictorial description of grafted plants on different rootstocks

Statistical analysis

The budded seedlings were arranged in a randomized complete block design (RCBD), with three replication and one plant per treatment. Analysis of Variance was conducted by using statistix 8.1 and means were compared by LSD test at $P \leq 0.05$ (Steel & Torrie, 1960).

Results and Discussion

Scion/stock ratio

Statistical analysis showed that scion/stock ratio was significantly affected by different rootstocks. On analysis, vegetative growth by each rootstock varied significantly as shown in Table 1. The maximum positive scion stock relationship was observed in Carrizo citrange and mimimum relationship was observed in Cox mandarin.. The average data of scion and stock ratio showed that Carrizo, Rough lemon and Troyer have good compatibility consecutively. Cox mandarin had least compatibility during the period of study. Suppressed scion growth was observed in case of Cox Mandarin and Benton. This might be due to the fact that rootstock may have strong bud union configuration with scion (Patil, 2017).

Plant height (m)

The maximum plant height was observed in Cox mandarin and minimum height was observed in Carrizo citrange. The plant height ranged between 1.00 to 1.80 m in all observed grafted plants**.** Plant height of sweet orange cv. Musambi showed significant difference (Table 1). Maximum plant height in 'Musambi' was found in Cox mandarin rootstock which was significantly at par with rough lemon. The increase in plant height may be due to better compatibility between scion and rootstock (Singh et al., 2019). Furthermore, rootstock affected the tree size, as it directly suppresses the plants to translocate nutrients and water from the soil. This devastating behavior of translocation significantly alter the pattern of canopy development and various plant characters such as photosynthetic rate (Iglesias et al., 2003).

Canopy volume (m^3)

Canopy volume is a vital parameter to gauge the vegetative growth of the plants. Effect of different rootstocks on canopy volume of sweet orange cv. Musambi showed a significant change (Table 1). The maximum canopy volume was observed in Rough lemon, while minimum canopy volume was observed in Caarizo citrange rootstock. Maximum canopy volume was attained in Musambi budded with Rough lemon followed by Troyer and Cox mandarin (Table 1). All mentioned three rootstocks performed well. While minimum canopy volume was observed in plants budded on Benton and Carrizo Citrange rootstocks. Dwarfing character of canopy was also observed in citrus Carrizo citrange rootstock (Vidalakis et al., 2007).

Assessment of fruit physical quality

Fruit weight, size and juice weight (%) showed significant difference among treatments (Table 2.). Data regarding influence of rootstock on fruit weight reveals that Musambi budded on Troyer produced the heaviest fruit followed by Rough lemon. The lowest fruit weight was recorded in Carrizo Citrange. Similarly, maximum fruit size in term of diameter was registered in Troyer budded with Musambi (Table 2). The highest juice percentage was recorded in fruits of Musambi budded on Rough lemon followed by Troyer. Juice percentage in fruits from both rootstocks was statistically at par between them and significantly different from the rest of the rootstocks (Table 2). Peel thickness, peel weight, seed weight and rag weight were not significantly affected by rootstock (Table 4). Rootstock especially in case of citrus has many scion interactive effects on tree growth, yield and fruit quality (Castle et al., 2011). Treeby et al. (2007) concluded that rootstock genotype controls the external fruit quality of citrus fruit as well as involved in accumulation and transportation of photosynthates. Furthermore, rootstocks also influences internal factors such as juice contents and colour (Fellers, 1985). Thus, citrus rootstocks play vital role in improving the fruit quality (Castle, 1995). The results were correlated with the findings of Sonkar et al. (2002) who reported the maximum juice contents and fruit weight in Valencia late when budded on rough lemon rootstock. Similarly Mosambi, Valencial late and Blood red produced more juice contents when budded on Rough Lemon rootstock (Parameshwar et al., 2018). Thus, the rootstock had a significant effect on external condition (fruit size, shape, rind thickness, colour and juice quality) of scion fruits (Castle, 1995; Treeby et al., 2007).

Biochemical fruit quality

The results of this study depicted that numerous rootstocks (Rough lemon, Troyer citrange, Benton, Cox mandarin & Carrizo citrange) have non-significant influence on bio-chemical properties of sweet orange cv. Musambi cultivar (Table 3). Our findings correlate with the study of Gorinstein et al. (2001) where no variation was observed in biochemical quality of citrus varieties. Citrus fruit have higher amount of qualitative soluble dietry fiber due to the high valued antioxidant properties in kinnow. Moreover, rootstocks can affect the internal fruit quality including juice soluble solids, acid concentration and their ratio (Fellers, 1990). The rootstock comparatively improves the tolerance against biotic and abiotic factors which ultimately boost the plant growth under varying environment (Martinez-Cuenca et al., 2016). The rootstock alters the fruit quality factors ranged between 5 to more than 30% which ultimately lowers the yield (Castle, 1995). Keeping in view the importance of rootstock, budded fruit of rough lemon are considerably large, less soluble solids and acid amount and thicker peeled (Castle, 1993). Furthermore, no significant change in biochemical fruit quality was observed (Table 2). These findings correlate with a previous research study by Castle (1995) who suggested that fruit quality is an inherent scion cultivar trait which can not be changed without genetic manipulation. Therefore, stage of fruit maturity has a significant effect on physical and chemical composition of citrus (Riaz et al., 2015).

Taste of extracted fruit juice is correlated with maximum TSS contents. The major components include more than 80% of fructose, glucose and sucrose, 10% of organic acid (mainly citric acid), 1% nitrogen compound and the rest 9% include vitamins, minerals with other soluble substances (El-Otmani & Coggins, 1991). Therefore, in earlier studies, use of citrus rootstock modifies TSS contents (Fadel et al., 2018; Sau et al., 2018) and presence of internal biochemical factors may also affect the perception of acidity (Zhou et al., 2018). Our results are in disagreement with the findings of Treeby et al. (2007) whose study reported a significant change in TSS and acidity of Bellamy navel orange by grafting on suitable rootstock of Troyer and Carrizo citrange, Cleopatra mandarin, sweet orange and trifoliate orange. In another study, high TSS in Bellamy navel orange was found in Trifoliate orange and Carrizo citrange rootstock, while Carrizo citrange has minimum titratable acidity (TA) (El-Otmani & Coggins, 1991).

Tree fruit yield

Fruit yield of sweet orange cv. Musambi when budded on different rootstocks i.e. Rough lemon, Troyer citrange, Benton, Cox mandarin and Carrizo citrange showed the highly significant difference in a number of fruits per tree (Fig. 2). Troyer citrange excelled with respect to tree fruit yield (60 number of fruits per tree) followed by rough lemon (47 fruits per tree) as compared to all other rootstock contestant. The increase in yield can be justified with the fact that both scion and stock showed better compatibility and interaction. Castle (1995) found that when Valencia late was budded on different rootstocks (Carrizo citrange, Cleopatra mandarin, Rough lemon, Sour orange, Swingle citrumelo and Trifoliate orange), a significant difference in yield was found and maximum number of fruits was recoded in Rough lemon followed by Carrizo Citrange. Similarly, Treeby et al. (2007) observed that fruit numbers were significantly higher for trees on Troyer citrange and Trifoliate orange compared to Cleopatra mandarin and sweet orange. Singh et al. (2019) observed a significant increase in yield of Valencia late when budded on rough lemon rootstock. Ananthakrishnan et al. (2006) reported higher production of sweet orange on rough lemon than that of sour orange rootstock. Bajwa et al. (1972) also reported similar findings of the highest mean fruit yield per tree (249.9 fruits per tree) and 201.3 per tree was recorded in Nucellar and Sathgudi, respectively. Ahmed et al. (2007) indicated highly significant difference among rootstocks, and Brazillian sour orange excelled overall other rootstocks, Citrumello 4475, Citrumello 1452, Volkamariana, Yuma citrange, Rough lemon, Mithi, Troyer citrange, Carrizo citrange and Brazillian sour orange.

Conclusion

The selection of rootstock under the changing climate scenario has representative influcene on production and quality. Our findings showed that rootstock genotype do not alter the TSS, acidity and TSS: TA ratio of Musambi. As far as the selection criteria is concerned, Troyer citrange rootstock is better to enhance crop productivity in Musambi as compared to other utilized rootstock. In future, it is important to improve rootstock characters in accordance with soil and climatic response with the enhanced productivity.

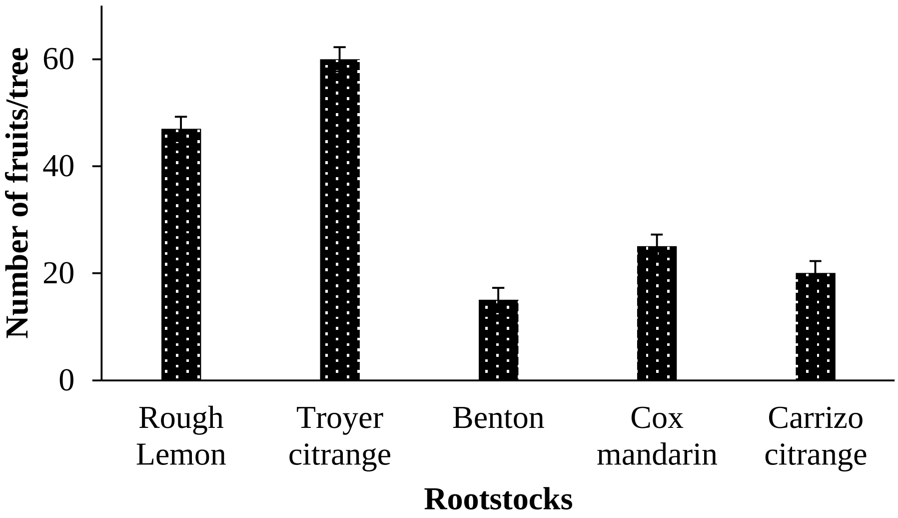

Fig. 2 Effect of various rootstocks on yield (fruits per tree) of sweet orange (*Citrus sinensis*) cv. Musambi. Vertical bars represent the standard errors (± SE) of the means. n = 3 replicates.

Table 1 Influence of rootstocks on scion stock ratio, plant height and canopy volume of sweet orange (*Citrus sinensis*)

Treatments	Scion stock ratio	Plant height (m)	Canopy volume (m^3)
Rough lemon	0.94^{ab}	1.70^{b}	4.79^{a}
Troyer citrange	0.92^{b}	1.57^{c}	2.78^{b}
Benton	0.88^{b}	1.20^{d}	0.49^{d}
Cox mandarin	0.79^{c}	1.80^{a}	2.45^{c}
Carrizo citrange	1.00^{a}	1.00^{e}	0.09^{e}
LSD ($P \leq 0.05$)	0.02	0.24	1.37

Means given in column with different letters showed significant difference at $P \leq 0.05$

Table 2 Influence of rootstocks on fruit weight, fruit size and peel thickness of sweet orange (*Citrus sinensis*)

Treatments	Fruit weight (g)	Fruit size (mm)	Juice weight (%)
Rough lemon	175^{b}	70.0^{a}	47^{a}
Troyer citrange	184^{a}	73.2^{a}	46^{a}
Benton	161^{c}	69.0^{a}	39^{b}
Cox mandarin	131.5^{d}	64.0^{b}	40^{b}
Carrizo citrange	90^{e}	58.3^{b}	41^{b}
LSD ($P \leq 0.05$)	11.32	3.52	11.32

Means given in column with different letters showed significant difference at $P \leq 0.05$; NS means are non-significant

Table 3 Influence of rootstocks on biochemical characteristics of sweet orange (*Citrus sinensis*)

Treatments	TSS (%)	Acidity (%)	TSS/Acid (ratio)
Rough lemon	9.2	0.64	14.37
Troyer citrange	10.2	0.65	15.69
Benton	10	0.75	13.33
Cox mandarin	9.7	0.93	10.43
Carrizo citrange	10.8	0.82	13.17
LSD ($P \leq 0.05$)	NS	NS	NS

NS shows non-significant values

Table 4 Influence of rootstocks on fruit weight, fruit size and peel thickness of sweet orange (*Citrus sinensis*)

Treatments	Peel thickness (mm)	Peel weight (%)	Seed weight (%)	Rag weight (%)
Rough lemon	2.58	22	1	30
Troyer citrange	3.23	24	1	29
Benton	3.86	45	2	45
Cox mandarin	3.39	24	2	34
Carrizo citrange	3.58	31	1	27
LSD ($P \leq 0.05$)	NS	NS	NS	NS

Means given in column with different letters showed significant difference at $P \leq 0.05$; NS means are non-significant

Author Contribution Statement: The authors Abdul Aziz, Akbar Hayat & Ehsan-Ul-Haque developed the manuscript concept and designed the experiment. Muhammad Nawaz Khan, Ahmed Raza, Faheem Khadeja help the author for conducting the research and performing the field as well as laboratory analysis and writing of the research paper. Maqsood Ahmed deeply review the whole manuscript on given concept.

Conflict of Interest: Authors of the research article declare no conflict of interest.

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