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RESEARCH PAPER

Influence of citrus rootstocks on growth performance and leaf mineral nutrition of 'Salustiana' sweet orange [*Citrus sinensis* (L). obsek]

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Key Message: The overall performance of 'Salustiana' scion cultivar budded on 'Rough lemon' rootstock was better in terms of morphological traits, photosynthesis rate, and nutrient uptake. Therefore, under the study area, 'Rough lemon' can be used as a potential rootstock for 'Salustiana' scion cultivar.

Abstract: Citrus fruits rank first among all fruit species and are widely grown across the globe in tropical and subtropical areas. The monopolized cultivation of 'Kinnow' mandarin in Punjab needs a substituted potential scion cultivar like 'Salustiana' for the diversification of the citrus industry of Pakistan. This current study was performed to examine the growth, vigour, photosynthesis, and nutrient utilization ability of 'Salustiana' orange (*Citrus sinensis* Osbeck L.) budded on one-year-old seedling of five rootstocks: 'Carrizo citrange' (*Poncirus trifoliata* (L) Raf × *Citrus sinensis* (L) Osbeck), 'Troyer citrange' (*Poncirus trifoliata* (L) Raf × *Citrus sinensis* (L) Osbeck), 'Trifoliate orange' (*Poncirus trifoliata* (L.), 'Rangpur lime' (*Citrus limonia* L. Osbeck) and 'Rough lemon' (*Citrus jambhiri* Lush.) rootstocks. The results suggest that all tested rootstocks exhibited distinct behaviour patterns concerning nutrient absorption from the soil. Regardless of scion growth, 'Salustiana' grafted on 'Rough lemon' rootstock had the most extended primary shoot length and had an increased trunk diameter of scion and vigorous root morphology as compared to the rest of selected rootstocks. 'Salustiana' plants on 'Rough lemon' rootstock had the highest mineral uptake efficiency in nitrogen (N), potassium (K), phosphorous (P), magnesium (Mg), calcium (Ca), zinc (Zn) and manganese (Mn). Rough lemon rootstock significantly showed better performance such as stronger root systems, higher mineral uptake capacity, and net accumulation of photosynthesis rate (Pn) with other selected rootstocks. The findings of this study will help horticultural breeders to choose the best compatible scion/rootstock combinations for 'Salustiana'.

Keywords: Citrus, Mineral nutrition, Rootstocks, Vegetative parameters, Vigour

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Introduction

Citrus fruits are cultivated worldwide due to its adaptability to diverse environmental conditions (Shireen et al., 2018). In Pakistan, citrus fruit trees cover an area of 199.9 thousand hectares, with an annual production of 2.132 million tons (Ghani et al., 2016). Citrus fruits are a valuable source of minerals, vitamins, carbohydrates, dietary fibres, and physico-chemical features (Prasad et al., 2015; Hayat et al., 2017). Citrus fruits are cultivated in all provinces of Pakistan. However, Punjab produces more than 95%, of the crop, and Kinnow accounts for 70% of citrus fruits (Khan et al., 2010). Kinnow mandarin is a leading cultivar in the area of Punjab, Pakistan, and the kinnow fruit has an excellent flavour, taste, physico-chemical, and nutritional characteristics along with unique medicinal and economic importance (Khan et al., 2016; Nawaz et al., 2019).

A single cultivar, Kinnow mandarin, has dominated Pakistan's citrus sector. The dominated cultivation of 'Kinnow' mandarin in Punjab needs a substituted potential scion cultivar like 'Salustiana' for the diversification of the citrus industry of Pakistan. Therefore attempts have been made to expand the citrus industry by promoting other suitable cultivars, including oranges, which seems to be the best choice on different rootstocks after kinnow mandarin. Fruit plants are commercially propagated through grafting, a technique that combines rootstocks with the scion of another, offering an excellent way to examine how these organs influence each other (Goncalves et al., 2019; Migicovsky et al., 2019). Rootstocks have played a vital role in the fruit industry, and its impact on morphological, physiological, anatomical and biochemical features may have contributed to distinguishing development and plant growth including, fruit quality and fruit maturation (Legua et al., 2014; Somkuwar et al., 2015; Tietel et al., 2020). The root system is fundamentally important because it anchors a plant to uptake nutrients and water from the soil substrate (Bellini et al., 2014). Root morphological traits differ among citrus cultivars, and differences in the root system architecture depend on the root area, root length, root diameter, lateral and feeder roots (Grace et al., 2012), and alterations in root characteristics have been documented to affect the accumulation of nutrients contents in leaves (Kumar et al., 2018).

The previous studies have shown that citrus rootstocks influence inversely on scion growth concerning environmental and soil conditions (Forner-Giner et al., 2014; Chahal & Gill, 2015). The selection of suitable graft combinations is essential for the production of fruits because of scion and rootstock relations effects physiology of each other (Sharma et al., 2015), minerals uptake (Toplu et al., 2012; Hayat et al., 2019), vigour and yield performance (Mallick et al., 2019; Martins et al., 2020). Previous work has been carried out to identify ideal rootstocks for successful worldwide citrus cultivars (Shafieizargar et al., 2012; Dubey & Sharma, 2016).

Rootstocks significantly affect the capacity of grafted plants to absorb nutrients and water, to synthesize hormones, and storage of photosynthates (Richardson et al., 2003). Rootstocks influence the nutrient accumulation of scion varieties, and perhaps the changes among scion/rootstock combinations have been related to mineral absorption ability of rootstocks due to their particular root system (Kumar et al., 2018; Sau et al., 2018). More than twenty horticultural characteristics are affected by the rootstocks, such as tree vigour, tolerance to temperature, disease resistance, and adoption to adverse soil conditions (Castle, 1995). Leaf mineral concentrations differed significantly among different rootstocks. Trees grafted with Volkamariana rootstock had a maximum level of N (2.23%), while minimum N concentration (1.14%) were recorded in Citrumelo 4475. Similarly, maximum P concentration (0.14%) was found in Yuma citrange, while minimum (0.08%) in Troyer citrange (Din et al., 2011).

Rootstocks impact on leaf photosynthetic efficiency could play an essential role in the behaviour of citrus plants in terms of vigour, fruit quality, and yield attributes (Richardson et al., 2003; Jover et al., 2012). Different rootstocks greatly influence mineral nutrients. Different scions absorb the variable amount of nutrients from the roots. Jahromi et al. (2012) documented that the Sour orange rootstock produced a higher amount of nitrogen (N) compared to those of the Mexican lime as well as Volkamer lemon rootstocks. Rootstock bark of Kinnow mandarin produced higher levels of nitrogen, particularly in comparison to scion bark, and the contrary pattern was observed in terms of potassium (Huchche, 1999). Moreover, Creste (1995) noticed the minimum nitrogen values with Satsuma on Sour orange and highest on Carrizo citrange rootstock. The mineral content of the scion varieties may differ, even when they are grown under the same agro-climatic environments.

Therefore, the evaluation of appropriate citrus rootstock is crucial because of the varying growth characteristics and concentrations of mineral elements (Khankahdani et al., 2019). Keeping in mind the above consequences, the present study aimed to understand the growth morphology, photosynthetic rate and nutrient accumulation pattern of five Citrus rootstocks (‘Carrizo citrange’, ‘Troyer citrange’, ‘Trifoliate orange’, ‘Rangpur lime’, and ‘Rough lemon’) budded with ‘Salustiana’ scion cultivar under Sargodha climatic conditions.

Materials and Methods

Experimental material and growth conditions

Citrus rootstocks trial was conducted in the greenhouse of Citrus Research Institute, Sargodha, Pakistan (Latitude 31° 46' N, longitude 72° 25' E) during March 2017. The seedlings were grown in 30-cm diameter plastic pots comprising a combination of garden soil: peat: sand = 3: 2: 1, v/v/v. The scions of ‘Salustiana’ were collected from 7-years old plants and T-budded on 1-year-old rootstocks viz., Rough lemon (*Citrus jambhiri* Lush.), Rangpur lime (*Citrus limonia* L. Osbeck), Trifoliate orange (*Poncirus trifoliata* (L.), Carrizo citrange (*Poncirus trifoliata* (L) Raf × *Citrus sinensis* (L) Osbeck), Troyer citrange (*Poncirus trifoliata* (L) Raf × *Citrus sinensis* (L) Osbeck) (Table 1). The plants were drip irrigated and exposed to conventional practices.

Table 1 Scion and rootstocks used in this study

Common name	Botanical name
Salustiana	*Citrus sinensis (L.)* Osbeck
Rough lemon	*Citrus jambhiri* Lush
Rangpur lime	*Citrus limonia* L. Osbeck
Trifoliate orange	*Poncirus trifoliata* L.
Carrizo citrange	*Poncirus trifoliata* (L) Raf × *Citrus sinensis* (L) Osbeck.
Troyer citrange	*Poncirus trifoliata* (L) Raf × *Citrus sinensis* (L) Osbeck.

Measurement of plant growth parameters

From March 2018 to November 2018, plant height (cm), node number, and internodal length (cm) were measured for each scion/rootstocks combination every month. The diameter of the scion stem (5 cm above from the graft union) was measured using Vernier caliper. The leaf area was measured using the LI-3100C Area Meter (LI-COR, Nebraska, USA). For the measurement of root–shoot ratio, healthy plants were removed from the pots and washed with clean water before the leaves in November of 2018.

Mineral analysis

The mature leaves were collected from all scion/rootstock combinations from the terminal position of the plant for mineral assessment during November 2018. Clean dried samples were crushed and digested in a combination of H_2SO_4–H_2O_2. Nitrogen (N) concentration was assessed using the Kjeldahl method (Nelson & Sommers, 1980). The concentration of other nutrients phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), iron (Fe) and zinc (Zn) were determined by using an inductively coupled plasma-mass spectrometer (ICP-MS) (Masson et al., 2010).

Photosynthetic measurements

A portable photosynthesis system was used for the measurement of net photosynthesis rate (Pn), intercellular CO_2 concentration (Ci), stomatal conductance (Gs), and transpiration (Tr), of the fully expanded leaves from 9:00 to 11:30, am during November 2018. All readings were undertaken based on the given environmental factors: leaf temperature 25 ± 2°C; relative humidity (RH) 65 ± 5%; photosynthetic photon flux 1200 µmol m^{-2} s^{-1} and external CO_2 concentration 400 µmol mol^{-1}.

Statistical analysis

The study was conducted in a completely randomized block design (RCBD) with five different rootstocks in three replications. The response of the rootstock to plant growth and mineral concentration was determined by the statistical software package (Statistix 8.1). The experimental data were analyzed by using analysis of variance techniques (ANOVA), and the means were compared using the least significant difference test 5% level of significance ($p < 0.05$) (Steel et al., 1997).

Results

Scion vegetative growth

Citrus rootstocks influenced the morphological traits of grafted trees. The morphological characteristics, including plant height, shoot length, and stem diameter of the scion on different rootstocks, differed significantly. 'Salustiana' scion cultivar budded onto 'Rough lemon' rootstock had the most extended primary shoot length (95.24 cm) and most vigorous growth. The weakest growth vigour and the lowest shoot length (72.67 cm) were obtained for 'Salustiana' budded onto 'Carrizo citrange' rootstock (Fig. 1). Eight months after grafting, there were substantial differences in the growth behaviors of the 'Salustiana' scion budded onto a range of size-controlling rootstocks (Table 2). Trees grown onto 'Rough lemon' rootstocks produced longer internodal length (2.24 cm), while the 'Carrizo citrange' rootstock resulted in the smallest internodal length (1.87 cm) (Table 2). The stem diameter of 'Salustiana' orange (13.68 mm) was considerably higher when grown onto the 'Rough lemon' rootstocks, especially in comparison to other selected rootstocks. Among the five scion/rootstock combinations, higher plant growth was encouraged by 'Rough lemon' rootstock, whereas 'Carrizo citrange' rootstock showed more dwarfing characteristics.

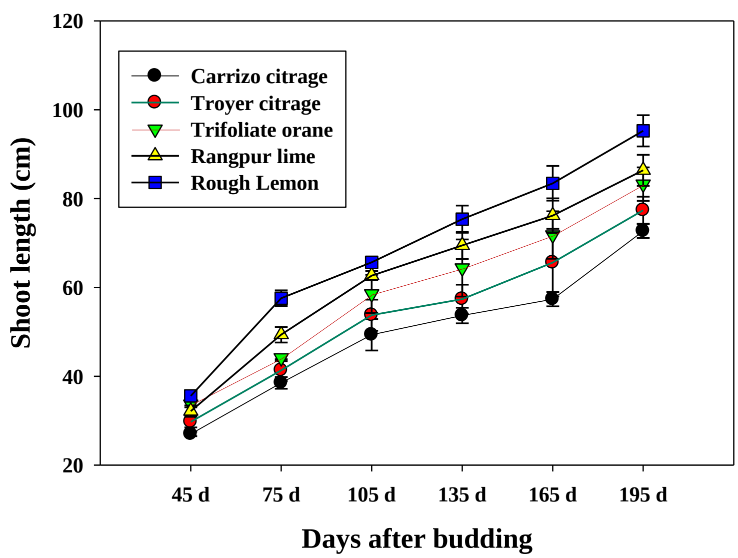

Fig. 1 Changes in primary shoot length in 'Salustiana' orange scion cultivar budded onto different rootstocks at different stages of growth and development

Table 2 Influence of different rootstocks on the growth parameters of 'Salustiana' orange scion cultivar

Rootstocks	Trunk diameter of the scion (mm)	Internodal length (cm)	Leaf area (cm^2)	Weight of overground part (g)	Weight of root (g)	Root-shoot ratio
Carrizo citrange	8.546^{e}	1.87^{d}	13.80^{e}	147.45^{e}	124.16^{e}	0.84^{a}
Troyer citrange	10.34^{d}	1.89^{cd}	14.63^{d}	170.53^{d}	134.42^{d}	0.78^{ab}
Trifoliate orange	11.30^{c}	1.91^{c}	14.75^{c}	189.13^{c}	143.32^{c}	0.75^{bc}
Rangpur lime	12.50^{b}	2.10^{b}	17.35^{b}	222.90^{b}	154.61^{b}	0.69^{cd}
Rough lemon	13. 68^{a}	2.24^{a}	18.28^{a}	242.90^{a}	162.98^{a}	0.67^{d}

The data are mean of five biological replicates. Different letters indicate significant difference at the P<0.05 LSD Comparisons Test

Leaf mineral content

'Salustiana' orange budded onto different rootstocks performed variably concerning the nutrient contents of the leaves (Table 3, 4**).** The results indicated that the maximum N content (3.55 %) was noticed in the 'Rough lemon' rootstock followed by 'Rangpur lime' (3.24 %), while the lower values of and 'Carrizo citrange' and 'Troyer citrange' (by 2.45 and 2. 66% respectively) were shown. Scion leaf on 'Rough lemon' rootstock had the highest P content (0.19 %) and the lowest P content (0.10 %) on 'Carrizo citrange' rootstock**.** In terms of leaf K content, 'Rough lemon' rootstock was also found higher (1.76 %), whereas 'Carrizo citrange' and 'Troyer citrange' rootstocks exhibited lower values (by 0.86 and 1.13% respectively). The maximum content of the leaf Ca was also recorded on 'Rough lemon' rootstock (3.45%), while the minimum Ca content was recorded in 'Rangpur lime' (1.78%) and 'Carrizo citrange' (1.53%) rootstocks respectively. The higher Mg content (0.90 %) was recorded on 'Rough lemon' rootstock whereas, 'Carrizo citrange' (0.63%) rootstock presented lower values. For the Na content, the result corresponding to 'Trifoliate orange' was higher but not statistically different from other rootstock treatments. Similarly, in the case of Fe content, there were no significant differences among rootstock treatments. The higher content of Zn (105.0 mg/kg), Mn (31.25 mg/kg), and Cu (26.95 mg/kg) was recorded with 'Rough lemon' rootstock, whereas the lower values were observed with 'Carrizo citrange' and 'Troyer citrange' rootstocks.

Table 3 Effects of different rootstocks on the mineral contents of 'Salustiana' sweet orange scion cultivar

Rootstocks	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Carrizo citrange	2.45^{e}	0.10^{d}	0.86^{e}	1.53^{e}	0.63^{d}
Troyer citrange	2.66^{d}	0.14^{bc}	1.13^{d}	2.34^{c}	0.68^{c}
Trifoliate orange	2.86^{c}	0.17^{ab}	1.37^{b}	2.65^{b}	0.72^{c}
Rangpur lime	3.24^{b}	0.13^{cd}	1.24^{c}	1.78^{d}	0.84^{b}
Rough lemon	3.55^{a}	0.19^{a}	1.76^{a}	3.45^{a}	0.90^{a}

The data are the mean of three biological replicates. Different letters indicate "significantly different" at the P<0.05 LSD comparisons test

Table 4 Effects of different rootstocks on the mineral contents of 'Salustiana' sweet orange scion cultivar

Rootstocks	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Na (mg/kg)	Mn (mg/kg)
Carrizo citrange	84.31^{a}	22.84^{c}	72.12^{e}	0.05^{a}	18.07^{d}
Troyer citrange	52.58^{a}	23.30^{c}	79.48^{d}	0.071^{a}	27.44^{b}
Trifoliate orange	92.99^{a}	25.81^{b}	88.42^{c}	0.076^{a}	25.03^{c}
Rangpur lime	72.73^{a}	26.89^{a}	99.90^{b}	0.070^{a}	25.50^{c}
Rough lemon	99.33^{a}	26.95^{a}	105.0^{a}	0.073^{a}	31.25^{a}

The data are the mean of three biological replicates. Different letters indicate "significantly different" at the P<0.05 LSD comparisons test

Gas exchange measurements

The data regarding the gas exchange parameters of 'Salustiana' orange plants budded onto different rootstocks showed dissimilarly (Fig. 2). The maximum rate (11.037 $\mu molm^{-2}\ s^{-1}$) of photosynthesis (Pn) was noted in trees budded on 'Rough lemon' rootstock. On the other hand, the lowest rate (7.056 $\mu molm^{-2}\ s^{-1}$) of photosynthesis (Pn) was observed in the plants grown on the 'Carrizo citrange' rootstock. Plants budded with 'Rough lemon' rootstocks also had higher intercellular CO_2 concentration (496.53 $\mu molmol^{-1}$) and stomatal conductance (28.823 $\mu mol.m.^{-2}s^{-1}$) values compared with other treatment combinations. Transpiration rate (Tr) of 'Salustiana' orange was also considerably higher with 'Rough lemon' (0.63 mmol $m^{-2}\ s^{-1}$) followed by 'Rangpur Lime' rootstock, whereas the minimum values (0.29 mmol $m^{-2}\ s^{-1}$, 0.36 mmol $m^{-2}\ s^{-1}$ respectively) were presented by 'Carrizo citrange' and 'Troyer citrange' rootstocks.

Discussion

Rootstocks affect the growth characteristics of grafted fruit plants. Previously, the effect of rootstocks on growth morphology and productivity of certain fruit trees were investigated (Cantuarias-Aviles et al., 2010; Liu et al., 2017).

In the present study, we observed that the 'Salustiana' orange budded onto 'Carrizo citrange' rootstock prompted the small stature trees, although other rootstocks like, 'Rough lemon' and 'Rangpur lime' increased primary shoot length, scion trunk diameter, leaf area, and hence the entire plant growth. Compared with other rootstocks, plants budded on 'Rough lemon' rootstock were stronger/vigorous in morphological characteristics with higher yields (Singh et al., 2009), which is consistent with our findings. Similarly, Nasir et al. (2011) examined the influence of Kinnow mandarin budded on three different rootstocks. They reported that plants were grown on 'Rough lemon' rootstock, increased their growth concerning plant height, scion spread, and canopy size while plants grown on 'Rangpur lime' showed to be a dwarfing rootstock. The root is the central organ responsible for transporting water and nutrients from the soil medium to aerial parts; thus tree vigour, and yields in dense-planting are affected by root system architecture (Gregory et al., 2013). In this present study, we found that the root system of 'Rough lemon' was more extensive or stronger relative to all other rootstocks evaluated. The findings of this research are similar to those of previous studies, where the root system of 'Rough lemon' rootstock was also found stronger with higher morphological characteristics (Kumar et al., 2018). The alterations in root morphological traits of citrus rootstocks may be attributed to the genetic modifications among rootstocks (Eissenstat, 1991).

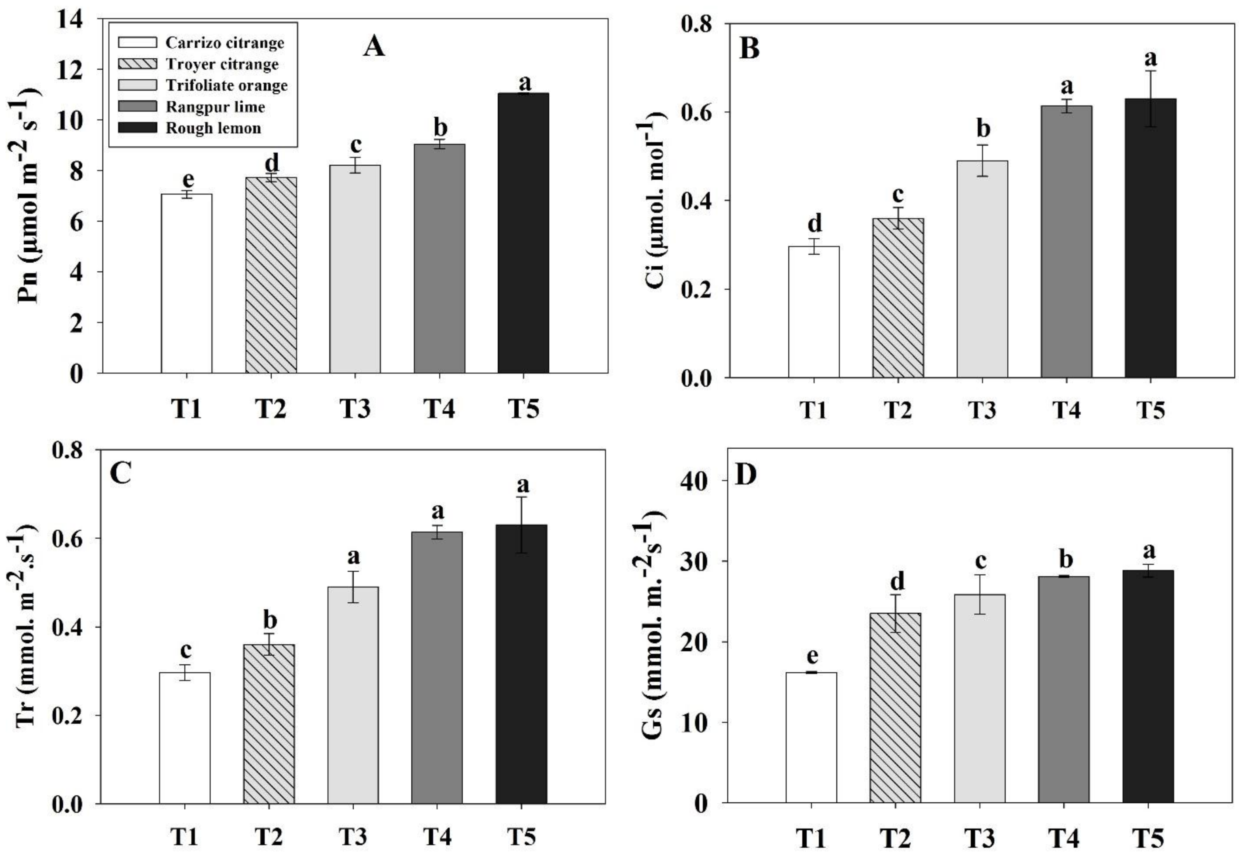

Fig. 2 The Influence of rootstocks on ret photosynthesis (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), transpiration (Tr) of 'Salustiana' orange citrus leaves. Error bars show the standard error of three biological replicates. Different letters indicate significant differences by LSD (P ≤ 0.05).

The root is the central organ responsible for transporting water and nutrients from the soil medium to aerial parts; thus tree vigour, and yields in dense-planting are affected by root system architecture (Gregory et al., 2013). In this present study, we found that the root system of 'Rough lemon' was more extensive or stronger relative to all other rootstocks evaluated. The findings of this research are similar to those of previous studies, where the root system of 'Rough lemon' rootstock was also found stronger with higher morphological characteristics (Kumar et al., 2018). The alterations in root morphological traits of citrus rootstocks may be attributed to the genetic modifications among rootstocks (Eissenstat, 1991).

Several studies suggested that rootstocks greatly influence leaf mineral nutrients in citrus trees (Ahmed et al., 2007; Toplu et al., 2008). In the present study, 'Salustiana' scions budded onto ‘Rough lemon’ rootstock was found to be more efficient in the absorption of some mineral nutrients (N, P, K, Ca, Mg, Cu, Zn, and Mn) than ‘Rangpur lime’, ‘Trifoliate orange’, ‘Troyer citrange’ and ‘Carrizo citrange’ rootstocks. Aguirre et al. (2001) stated that low vigour rootstock could be one of the possible reasons for mineral deficiencies in apple leaves, and these deficit symptoms in ‘Golden Delicious’ were mentioned by Amiri et al. (2008). Several scientists have shown that trees grafted on taller/stronger rootstocks have greater magnesium (Mg) and potassium (K) concentrations than size-controlling rootstocks. The nutrients have been found to accumulate at different rates in the scion due to different rootstocks, i.e., in peach, apple (Tsipouridis & Thomidis, 2005, Amiri et al., 2014). Similarly, Smith (1975) found that rootstocks significantly affect the vigour and yield of grafted plants through a differential ratio of absorption and translocation of mineral elements from the soil substrate that ultimately affect growth behavior.

Higher mineral uptake capacity was linked with the more vigorous root system (total root length, number of tips, and forks) that can directly affect nutrient uptake efficiencies (Kumar et al., 2018; Hayat et al., 2019). The higher content of minerals in leaf might have resulted from higher root absorption and faster transportation from the root system to the aerial part (Fallahi et al., 1984). Such distinctions could also be demonstrated by the genetic effect contributing to different nutrient absorption ability (Kucukyumuk & Erdal, 2011). Additional explanations for these variations in mineral absorption may be the diversified capacity of hydraulic conductance in different rootstocks, which correlated positively with growth vigour and accumulation of nutrients (Cohen & Naor, 2002).
Photosynthetic productivity has been generally known as a main physiological parameter to assess the plant growth vigour, and, ultimately, biomass and yields (Bosa et al., 2016). In the current research, plants grafted onto ‘Carrizo citrange’ rootstock presented lower photosynthetic rates compared with other rootstock treatments. This could be explained by that low vigour rootstocks reduce the capacity of water transport, affecting the level of stomatal opening and absorption of light and CO_2. Consequently, the vigour of trees is restricted, and the photosynthetic efficiency declines (Zhao et al., 2016).

Conclusion

In summary, our findings suggested that citrus rootstocks had a substantial impact on the morphological, biochemical, and physiological responses in ‘Salustiana’ orange. Regardless of scion vigour, ‘Salustiana’ scion grafted onto ‘Rough lemon’, rootstock had the most extended primary shoot length and scion diameter and the higher net photosynthesis rate (P_n) compared with other rootstocks. The overall performance of ‘Rough lemon’ rootstock was better in mineral nutrient uptake due to the stronger root system as compared to different rootstocks used in this study. Therefore, under the study area, ‘Rough lemon’ can be used as a potential rootstock for ‘Salustiana’ orange.

Author Contribution Statement: Muhammad Asim and Tehseen Ashraf conceived and designed the research project. Muhammad Nawaz Khan conducted a research experiment. Muhammad Asim and Faisal Hayat wrote the first draft of the manuscript. Sumeera Asghar contributed in statistical analysis for the evaluation of the best rootstocks, i.e. technical assistance during data analysis. Shahid Iqbal and Faisal Hayat edited the manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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