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Original article

Influence of rootstocks on growth, yield, quality and physiological activity of 'Kinnow' mandarin grown in a semi-arid region

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Summary

Introduction – 'Kinnow' has revolutionised the citrus industry of India. It is commercially grown on *Jatti Khatti* rootstock in the arid and semi-arid regions. The monopolised cultivation of 'Kinnow' on *Jatti Khatti* needs a substituted rootstock for diverse agro-ecological regions. _Materials and methods_ – 'Kinnow' on seven rootstocks was evaluated to determine the effect of rootstocks on growth, yield, quality and physiological parameters. _Results and discussion_ – *Jatti Khatti* and rough lemon induced vigorous tree growth, while the citranges induced dwarfness. *Jatti Khatti*, Rangpur lime and sour orange excelled in quality attributes. *Karna Khatta* and rough lemon maintained higher relative water content (RWC). *Jatti Khatti* enhanced the photosynthetic rate (A), while stomatal conductance (gs) and transpiration rate (E) was higher on *Jatti Khatti* and rough lemon. Superoxide dismutase (SOD), peroxidise (POD), and glutathione reductase (GR) activities were higher on rough lemon, whereas catalase (CAT) activity and proline accumulation were higher on sour orange. The study clearly indicated that rootstocks have the differential ability to impart significant influence on tree morphology, fruit quality and physiological activity. _Conclusion_ – On the basis of plant vigour and yield, *Jatti Khatti* and rough lemon proved to be potential rootstocks for semi-arid region. Criteria such as enhanced photosynthetic rate, proline content, along with up-regulated antioxidant can be used as a reference for selecting appropriate rootstock representing different soil and climatic conditions.

Keywords
antioxidant enzymes, 'Kinnow', physiology, quality, rootstock, yield

Significance of this study

What is already known on this subject?

- 'Kinnow' mandarin, a tight skin hybrid, is a preferred fruit among consumers because of its attractive orange colour, high juice recovery and better fruit quality.
- *Jatti Khatti* is a commercially used rootstock for 'Kinnow' under semi-arid conditions.

What are the new findings?

- *Jatti Khatti* and rough lemon proved to be vigorous rootstocks with higher yield.
- Better quality fruits were induced by Rangpur lime and sour orange.
- Higher up-regulation of antioxidant enzymes suggest rough lemon to be suitable rootstock for arid and semi-arid regions and *Karna Khatta* for water-scarce areas because of its improved RWC and WUE*i*.

What is the expected impact on horticulture?

- This work would be useful to select responsive rootstock for diverse agro-climatic conditions, which could mitigate the adverse effect of abiotic stress and improve the productivity with better quality fruit.

Introduction

Citrus is an important fruit crop in India after banana and mango. Among citrus, 'Kinnow' mandarin, a hybrid between 'King' orange (*Citrus nobilis* Loureiro) and 'Willow' leaf mandarin (*Citrus deliciosa* Tenore) has revolutionised the citrus industry of India owing to its attractive orange-coloured fruits, high juice content, better yield and quality (Sharma *et al.*, 2016b). This beautiful mandarin in India is commercially grown on *Jatti Khatti* rootstock in the arid and semi-arid regions, which experiences constraining environmental conditions such as uneven rainfall, seasonal droughts, high temperature during summer (36–45 °C) and low temperature (6–11 °C) during winter. To achieve optimum productivity, excess irrigation is applied in certain productive pockets that may have significant impact on conditions affecting 'Kinnow' industry due to increased salinity.

To circumvent such crisis, appropriate rootstock-scion combinations are crucial because the physiology is affected by the rootstock. Strong relationships have been reported among the rootstocks on plant stature, fruit yield and the physiological activity of scion varieties in budded trees (Garcia-Sanches *et al.*, 2002; Jover *et al.*, 2012; Dubey and Sharma, 2016; Sau *et al.*, 2018). Moreover, scion behaviour depends in part on the rootstock and induces effects on leaf gas exchange (Syvertsen and Graham, 1985; Gonzalez-Mas *et al.*, 2009), hormonal signaling (Aloni *et al.*, 2010), *etc.* These relationships provide a basis for selecting appropriate rootstock under a given set of environmental conditions. Further, biotic and abiotic stresses lead to the over production of reactive oxygen species (ROS) which can have detrimental effect on normal metabolism through oxidative damage to lipids, proteins, carbohydrates and DNA, and can result in cell death

(Apel and Hirt, 2004; Uzun *et al.*, 2012). Therefore, strong antioxidant defense system is needed in plants to avoid cell damage due to excess generation of ROS. Similarly the capacity of plants to accumulate proline has been correlated with tolerance to several stresses (Krasensky and Jonak, 2012). Positive effects of rootstocks on altering various physiological processes have been reported in *Citrus* species such as grapefruit (Sharma *et al.*, 2015). Given that the rootstocks, unlike genotypes, may modify the growth, yield and physiological process in the scion cultivar, the present study was aimed to investigate the effect of different rootstocks on the growth, yield, fruit quality, and to ascertain the role of rootstocks in terms of leaf gas exchange parameters, antioxidant enzyme activities, proline accumulation, phenol content, *etc.*, in 'Kinnow' under semiarid conditions of North India.

Materials and methods

Experimental site

The experimental site (77°12'E; 28°40'N, 228 m a.s.l.) is characterized as semiarid and subtropical, with hot and dry summers and cold winters. The mean annual rainfall is 710 mm of which more than 75% is received during monsoon season (July to September). The soil type was sandy loam with bulk density 1.58 g cm^{-3}, pH of 7.4 and electrical conductivity [EC; 1:2 (W/V) in water] of 0.34 dS m^{-1} and organic carbon content of 0.39% (w/w) and a soil N, P and K concentration of 159.23, 536.1 and 314.78 kg ha^{-1}. The mean available Fe, Mn, Cu and Zn concentration in the soil was 9.35, 22.67, 7.52 and 4.83 mg kg^{-1} soil, respectively.

Planting material

'Kinnow' mandarin, budded onto seven rootstocks, *viz.*, rough lemon (*Citrus jambhiri* Lush.), *Karna Khatta* (*Citrus karna* Raf.), 'Carrizo' citrange (*Citrus sinensis* [L.] Osb. × *Poncirus trifoliata* [L.] Raf.), Rangpur lime (*Citrus limonia* [L.] Osb.), 'Troyer' citrange (*Citrus sinensis* [L.] Osb. × *Poncirus trifoliata* (L.) Raf.], *Jatti Khatti* (*Citrus jambhiri* Lush.) and sour orange (*Citrus aurantium* L.) were selected for the study.

The experimental block with the budded plants were established in September 2011 at a spacing of 6 × 6 m in a randomised block design with four replications (*i.e.*, four trees of each rootstock). The selected plants of different rootstock-scion combinations were investigated for growth, yield, fruit quality and physiological parameters during 2014–2015. Irrigation was applied through drip system having an EC of 1.3 dS m^{-1}. Each experimental tree received 100 g N, 50 g P and 100 g K per annum in the first year along with 25 kg farmyard manure (FYM) during the last week of February. From the fourth year onwards the doses were fixed at 600 g N, 400 g P and 600 g K along with 25 kg FYM $plant^{-1}$ and applied in two split doses, *i.e.*, February and September. Trees were not pruned and standard cultural practices were carried out uniformly.

Measurement of plant growth and yield

Plant growth in terms of plant height (m) and plant spread [N-S (*D1*)] and [E-W (*Dr*)] in the treatments were recorded two months after the emergence of spring flush in 2014–2015. Plant height was determined by measuring the distance from the ground to the top of the plant with the help of measuring scale. Plant spread [N-S (*D1*)] and [E-W (*Dr*)] was recorded with the help of a meter scale and canopy volume (*V*) was determined from individual measurements of tree height (*H*) and width in parallel (*D1*) and perpendicular (*Dr*) by the formula (Zekri, 2000):

$$V = (\pi/6) \times H \times D1 \times Dr$$

Fruits were harvested at maturity from each replication separately during 2014 and 2015. The fruit yield was calculated by multiplying the fruit weight (average of 20 fruits) to number of fruits $tree^{-1}$ and then the resultant was converted in kg $tree^{-1}$.

Fruit quality parameters

Random sample of 20 fruits from each replication of individual rootstocks which had the same color and almost the same diameter were selected, weighed, and their juice was extracted. Number of seeds per fruit were counted and the juice retrieved was weighed to calculate the juice percentage from the relationship between the weight of the extracted juice and the fruit sample weight. Total soluble solids (TSS in °Brix) were measured using an ATC-1E ATAGO hand-held refractometer from the translucent part of the juice. Total acidity (%) and ascorbic acid contents (mg 100 g^{-1}) were determined as per the guidelines of the Association of Official Analytical Chemists (AOAC, 2000).

Physiological parameters

Relative Water Content (RWC)

RWC was determined according to Barrs and Weatherley (1962). Five fully matured leaves were collected and washed immediately in distilled water and 8-mm discs (ten per leaf) were cut-out with a cork borer. Ten discs were selected and their individual fresh weight (FW) were measured. These were then floated in distilled water in closed Petri dishes for 4 h. The discs were then surface dried by placing them in between two sheets of Whatman No. 1 filter paper and the turgid weight (TW) of the discs ($n = 10$) was recorded. The leaf discs were then dried to a constant weight in a hot-air oven at (70 ± 1 °C for 2–3 days) and the dry weight (DW) of the leaf discs was recorded. The *RWC* was then calculated using the following formula:

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Leaf gas exchange

Photosynthetic rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*) and intercellular CO_2 concentration (*Ci*) of fully expanded leaves were recorded 60 days after the emergence of the rainy season (July) flush, *i.e.*, September. The gas exchange traits were measured between 10:00 and 11:30 am (IST) on clear day using an LCi-SD Ultra Compact Photosynthesis System (ADC Bio Scientific Ltd., Global House, Hoddesdon, U.K.). Four mature leaves $plant^{-1}$ (4^{th} leaf from tip of shoot) from exterior canopy position (one leaf each in North, South, East and West direction) and four plants per treatment were sampled for these measurements. The Intrinsic water use efficiency (WUE*i*) was calculated as the ratio of *A:gs* (During, 1994).

Proline

Proline content was estimated using a rapid colorimetric method as suggested by Bates *et al.* (1973). A fresh leaf sample (0.5 g) was homogenized in 5 mL of 3% sulpho-salicylic acid and centrifuged at 7826 ×*g* for 10 min at 4 °C (model-HERMLE Z 323K). The reaction mixture contained 1 mL of supernatant, 5 mL each of acid ninhydrin reagent and glacial acetic acid and heated at 100 °C. After 1 h, 4 mL of toluene was added, vortexed and absorbance of the chromophore-containing toluene layer was read on UV-vis double beam PC 8 scanning auto cell spectrophotometer (UVD 3200, Labomed, Inc., USA) at 520 nm.

Total phenols

Total phenols were determined by the method described by Malik and Singh (1980). A fresh leaf sample of 0.5 g was ground with a pestle and mortar in 10-time volume of 80% ethanol and diluted extracts of different concentrations were taken in test tubes and total volume was made to 3 mL with double distilled water. Then 0.5 mL of Folin-Ciocalteu reagent (1:1 with water) and 2 mL of 20% Na_2CO_3 were added to each tube and heated to dryness on water bath. A blue colour was developed in each tube due to the reduction of phenols by phosphomolybdic acid present in Folin-Ciocalteu reagent. Absorbance was measured on a spectrophotometer (UVD 3200, Labomed, Inc., USA) at 650 nm. The concentration of total phenols was calculated using a standard curve and expressed as mg catechol equivalent of phenol per gram of sample.

Polyphenol oxidase

The polyphenol oxidase activity in leaf samples was determined by the method suggested by Lerner *et al.* (1971) with slight modifications. PPO enzyme was extracted at 4 °C by macerating one gram of chopped sample in pestle and mortar with 5 mL of 100 mM phosphate buffer (pH 7.3) containing sodium ascorbate. Thereafter, the extract was filtered through four layers of gauze and treated for 20 min with 1.5% Triton X-100 solution prepared in 100 mM phosphate buffer (pH 7.3). Final volume of extract was made with 100 mM phosphate buffer of pH 7.3 containing 10.0 mM sodium ascorbate. It was then centrifuged at 15,000 ×*g* for 1 h at 4 °C. The aliquot so obtained was used as an enzyme source.

Both catecholase and cresolase activities were measured on a spectrophotometer (UVD 3200, Labomed, Inc., USA) at 400 nm according to the procedure described by Sa *et al.* (1988). Catecholase activity was measured using 30 mM of 4-methyl catechol (4MC) as substrate, made up in 10 mM sodium acetate buffer (pH 4.5). Three mL of 100 mM phosphate buffer (pH 7.3) was added to 1 mL crude enzyme extract and 1 mL of substrate was added at zero time. Cresolase activity was measured in the same way, except that 4-methyl phenol (p-ceresol) was used as substrate, made up in 10 mM phosphate buffer (pH 7.0). The enzyme activity was represented as ΔA_{400} g^{-1} $min^{-1} \times 10^{-3}$.

Antioxidant enzymes

Preparation of enzyme extract

Fresh leaf samples were collected from each treatment in ice box to prevent the proteolytic activity. One g of cleaned sample was homogenized in pre-chilled mortar and pestle at 4 °C in 5 mL of chilled phosphate buffer (50 mM; pH 7.0). The homogenate was centrifuged at 15,000 ×*g* for 20 min at 4 °C (Model-HERMLE Z 323K). The supernatant was filtrated through two layers of muslin cloth and stored at -20 °C to be used as extract for the estimation of the following antioxidant enzymes.

Superoxide dismutase (SOD)

The SOD activity (EC 1.15.1.1) was measured by its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) using the method proposed by Dhindsa *et al.* (1982). The 3 mL reaction mixture contained 0.2 mL of 0.2 M methionine, 0.1 mL of 3 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mL of 1.5 M Na_2CO_3, 0.1 mL of 2.2 mM NBT, 0.1 mL of 2 μM riboflavin, 0.1 mL of enzyme extract and phosphate buffer (1.5 mL of 100 mM; pH 7.8). Riboflavin was added last and the tubes were shaken well and placed 30 cm below a light bank consisting of two 15 W fluorescent lamps for 15 min. The absorbance by the reaction mixture was read on spectrophotometer (UVD 3200, Labomed, Inc., USA) at 560 nm. One unit of SOD activity was defined as the amount of enzyme extract which caused 50% reduction in the absorbance compared to the tubes lacking enzymes. Finally, the SOD was quantified on the basis of total soluble protein content of the sample and expressed as U min^{-1} mg^{-1} protein.

Catalase (CAT)

The activity of CAT enzyme (EC 1.11.1.6) was based on the absorbance of hydrogen peroxide (H_2O_2) at 240 nm as described by Aebi (1984). A 3-mL reaction mixture was prepared by mixing 1.5 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.5 ml of 75 mM H_2O_2, 5 μL of enzyme extract and water to make-up the volume. The absorbance was recorded for 1 min in the spectrophotometer and CAT activity was expressed as concentration of H_2O_2 reduced min^{-1} mg^{-1} protein.

Peroxidase (POD)

The activity of POD (EC 1.11.1.7) was determined as increase in optical density due to the oxidation of guaiacol to tetra-guaiacol (Castillo *et al.*, 1984). The absorbance of reaction mixture (3 mL) containing 1 mL of 100 mM phosphate buffer, 0.5 mL of 96 mM guaiacol, 0.5 mL of 12 mM H_2O_2, 25 μL of enzyme extract and water was recorded on spectrophotometer (UVD 3200, Labomed, Inc., USA) at 470 nm wave length. Activity was calculated by using extinction coefficient of tetra-guaiacol (ε) 26.6 mM^{-1} cm^{-1} and expressed as μ moles of tetra-guaiacol formed min^{-1} mg^{-1} protein.

Glutathione reductase (GR)

This enzyme (EC1.8.1.7) was assayed according to Smith *et al.* (1988). Each 3.0 mL reaction mixture contained 1.0 mL of 0.2 M potassium phosphate buffer, pH 7.5 containing 1.0 mM EDTA, 0.5 mL of 3.0 mM DTNB (5, 5 dithio-bis-2-nitrobenzoic acid) in 10 mM potassium phosphate buffer, pH 7.5, 0.1 mL of 2.0 mM NADPH, 0.1 mL of crude leaf enzyme extract, and distilled water to a final volume of 3.0 mL. The reaction was initiated by adding 0.1 mL of 2.0 GSSG (oxidised glutathione) and the increase in absorbance at 412 nm was recorded at 25 °C over a period of 10 min on a UV-VIS double beam PC 8 scanning auto cell spectrophotometer (UVD 3200, Labomed, Inc., USA).

Statistical analysis

The data were analysed using statistical analysis system software (9.3 SAS Institute, Inc., USA) to calculate *F* values followed by Tukey's honest test. Values of $P \leq 0.05$ were considered as significant.

Results and discussion

Growth and yield

Growth behaviour of 'Kinnow' scion in terms of plant height and canopy volume was significantly influenced by the rootstocks ($P \leq 0.05$). Trees budded on *Jatti Khatti* rootstock had highest plant height (2.96 m) which was statisti-

Table 1. Influence of rootstocks on plant growth, fruit yield and quality of 'Kinnow' mandarin (mean data of 2014 and 2015).

Rootstocks	Plant height (m)	Canopy volume (m^3)	Yield (kg $plant^{-1}$)	Fruit weight (g)	Number of seeds $fruit^{-1}$	Juice (%)	TSS (°Brix)	Acidity (%)	Ascorbic acid (mg 100 g^{-1})
Rough lemon	2.73^{ba}	9.10^{b}	49.69^{b}	258.12^{a}	24.25^{cb}	46.20^{cb}	10.88^{d}	0.97^{dce}	43.65^{cb}
Karna Khatta	2.51^{bc}	7.21^{c}	26.95^{de}	221.42^{c}	18.75^{d}	42.32^{cd}	11.77^{cb}	1.09^{bc}	46.20^{a}
Carrizo citrange	1.90^{d}	4.07^{ed}	19.85^{e}	182.55^{e}	26.25^{b}	39.71^{d}	11.20^{cd}	1.21^{ba}	36.18^{d}
Rangpur lime	2.24^{c}	5.16^{d}	39.18^{c}	228.77^{b}	19.75^{cd}	47.65^{b}	12.03^{b}	1.05^{dc}	42.41^{c}
Troyer citrange	1.77^{d}	3.70^{e}	23.57^{de}	195.92^{d}	26.75^{b}	42.80^{cd}	11.25^{cd}	1.25^{a}	34.52^{e}
Jatti Khatti	2.96^{a}	11.53^{a}	59.20^{a}	231.04^{b}	31.75^{a}	58.10^{a}	12.96^{a}	0.94^{de}	45.73^{a}
Sour orange	2.35^{c}	9.04^{b}	33.25^{dc}	202.36^{d}	26.75^{b}	48.43^{b}	11.78^{cb}	0.88^{e}	44.07^{b}
LSD ($P \leq 0.05$)	0.32	1.42	8.63	6.95	4.82	4.10	0.65	0.13	1.32

cally at par with rough lemon (2.73 m) followed by sour orange (2.30 m), whereas lowest plant height was measured on 'Troyer' citrange (1.77 m) which did not differ significantly with 'Carrizo' citrange (1.90 m). Canopy volume exhibited similar trend with highest values measured on *Jatti Khatti* (11.53 m^3) followed by rough lemon (9.10 m^3), whereas the lowest canopy volume was measured on 'Troyer' citrange (3.70 m^3) followed by 'Carrizo' citrange which has not differed significantly with trees on Rangpur lime (Table 1). Rootstocks also significantly influenced fruit yield of 'Kinnow' (kg $tree^{-1}$). Mean values (2014–2015) illustrated significantly higher yield from trees on *Jatti Khatti* (59.20 kg) followed by trees on rough lemon (49.69 kg) (Table 1). Fruit yield on the other rootstock-scion combinations did not exhibit any significant variation, except the citrange rootstocks which had significantly the poorest yield.

The variable effect of rootstocks on growth parameters of four year old 'Kinnow' plants, exhibited superiority of *Jatti Khatti* and rough lemon (both *Citrus jambhiri*) in terms of plant height and canopy volume. Better growth of 'Kinnow' on these rootstocks is supported by our published data on root morphology, which reported higher root projected, surface area and root volume in both the rootstocks (Kumar *et al*., 2018). Efficient root system of these rootstocks must have resulted in better water uptake, mobilisation of nutrients and its ultimate effect on plant growth. The superiority of rough lemon and *Jatti Khatti* as vigorous rootstock for different *Citrus* species have been reported earlier by several workers (Castle, 1987; Sharma *et al*., 2016a; Sau *et al*., 2018). Restricted vegetative growth of 'Kinnow' on both the citrange rootstocks may be related to reduced physiological activity and high phenol content as observed in our study. Phenol influence the synthesis, level and polarity of IAA transport and cell wall permeability (Lockard and Schneider, 1981). The dwarfing effect of 'Kinnow' and lemon 'Kagki Kalan' on 'Troyer' citrange have also been reported by Goswami *et al.* (2001) and Dubey and Sharma (2016). *Jatti Khatti* and rough lemon excelled over other rootstocks by having highest yield kg $tree^{-1}$, while the yield was lowest on the citrange rootstocks. Variation in yield might be due to differences in nature, plant growth and physiology of rootstocks. Furthermore, higher canopy volume and enhanced physiological activity in *Jatti Khatti* and rough lemon must have resulted in more photo assimilates supporting more fruits and higher yield realization as also observed in the present study. Difference in yield due to rootstock influence in citrus had also been reported earlier by several researchers (Ahmed *et al*., 2006; Sau *et al*., 2018).

Fruit quality parameters

Significant variation with different rootstock-scion combinations was observed for fruit quality attributes. Weight of 'Kinnow' fruits from trees on rough lemon were significantly higher followed by *Jatti Khatti* which has not differed significantly with trees on Rangpur lime. Fruits with minimum weight (182.55 g) were recorded in trees on 'Carrizo' citrange. Although the fruit weight was significantly higher from trees on rough lemon, the juice recovery percentage in the fruits from trees on *Jatti Khatti* was significantly higher (58.10%) followed by Rangpur lime (47.65%) which had statistical parity with sour orange and rough lemon. 'Kinnow'-'Carrizo' citrange combination had the lowest juice recovery (39.71%) which was non-significant with trees on 'Troyer' citrange (42.32%) and *Karna Khatta* (42.80%). Number of seeds/fruit also varied significantly due to rootstocks and least number of seeds (19.0 seeds $fruit^{-1}$) were recorded in fruits from trees on *Karna Khatta* and Rangpur lime while the most seeded fruits in rootstock-scion combination were recorded from trees on *Jatti Khatti* (32 seeds $fruit^{-1}$) followed by sour orange (27 seeds $fruit^{-1}$) which did not differ significantly with trees on 'Troyer' citrange rootstock (Table 1).

Jatti Khatti followed by Rangpur lime which had statistical parity with sour orange and *Karna Khatta* were identified with high TSS in 'Kinnow' fruits than other rootstocks and rough lemon which culminated into low TSS. More acidic fruits were from trees on 'Troyer' citrange, which showed statistical similarity with trees on 'Carrizo' citrange. The differences in the result of acidity on other rootstocks were statistically insignificant, except fruits from trees on sour orange and *Jatti Khatti*, which gave less acidic fruits with statistical parity. 'Kinnow' fruits from trees on *Karna Khatta* and *Jatti Khatti* had a significantly higher ascorbic acid, whereas the lowest quantity of ascorbic acid (34.52 mg 100 g^{-1}) was recorded in 'Kinnow'/'Troyer' citrange combinations (Table 1).

In our study, except the citrange rootstocks, all other rootstocks produced medium to large sized fruits being biggest fruits on rough lemon. Although, rough lemon excelled in fruit weight, the fruit quality traits, *viz*., juice recovery percentage, TSS and ascorbic acid were induced more from the trees on *Jatti Khatti* thus confirming the positive effect of *Jatti Khatti* on the quality attributes of 'Kinnow'. *Karna Khatta* and Rangpur lime proved to be the superior rootstocks for producing fruits with lower seeds which otherwise is a major problem in 'Kinnow'. Such rootstock mediated changes on quality attributes of citrus have been reported by earlier workers (Castle, 1983; Yomento *et al*., 2005; Zekri, 2011; Dubey and Sharma, 2016, Sau *et al*., 2018).

Physiological parameters

All the physiological parameters (RWC, *A*, *Ci*, *gs*, *E*, and WUE*i*) in 'Kinnow' leaves were significantly influenced by rootstocks. The maximum value for RWC in scion leaf was observed on *Karna Khatta* (89.47%), which was statistically non-significant with trees on rough lemon (88.61%), whereas the minimum value was observed on 'Carrizo' (81.14%) which did not differ significantly with 'Troyer' (82.84%) rootstocks (Figure 1). The photosynthetic rate (*A*) and internal cellular CO_2 (*Ci*) was significantly higher on *Jatti Khatti* (6.9; 251.63 μmol m^{-2} s^{-1}) followed by trees on rough lemon (5.81; 218.75 μmol m^{-2} s^{-1}) which did not differ significantly from sour orange. The minimum *A* and *Ci* was observed in scion leaves of 'Kinnow' trees budded on 'Troyer' rootstock (3.16; 163.38 μmol m^{-2} s^{-1}) (Figure 1B-C). The stomatal conductance (*gs*) (Figure 1D) and transpiration rate (*E*) (Figure 1E) followed a pattern similar to *A* and *Ci* thus recording higher *gs* and *E* values ion *Jatti Khatti* (0.095; 4.02 mmol m^{-2} s^{-1}) and rough lemon without any significant difference. A relatively low *gs* and *E* was noticed on 'Troyer' rootstock (0.037; 2.01 mol m^{-2} s^{-1}). The highest WUE*i* (96.64 μmol m^{-1} H_2O m^{-2} s^{-1}) was recorded in 'Kinnow' leaves on 'Troyer' rootstock with similar statistical values in 'Kinnow' trees on Rangpur lime and *Karna Khatta* (Figure 1F).

In a budded tree, scion behaviour depends on the type of rootstock which affects many scion properties such as RWC, chlorophyll fractions, photosynthetic capacity and the allocation of carbohydrates from source leaves to reproductive or

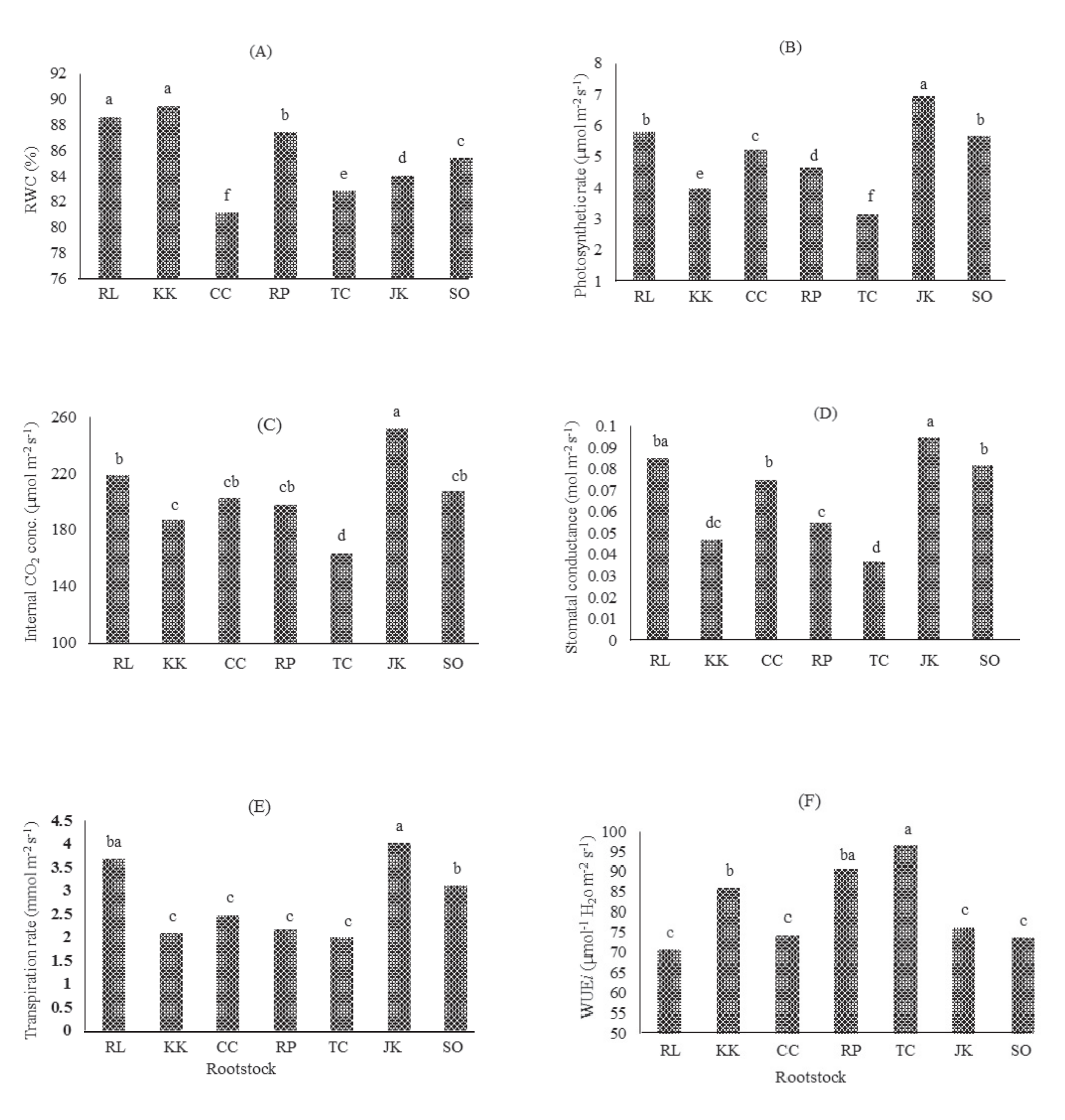

FIGURE 1. Effect of different rootstocks on: (A) Relative water content; (B) Photosynthetic rate; (C) Internal CO_2 concentration; (D) Stomatal conductance; (E) Transpiration rate; and (F) Intrinsic water use efficiency. Rootstocks: RL: Rough lemon, KK: *Karna Khatta*, CC: 'Carrizo' citrange, RP: Rangpur lime, TC: 'Troyer' citrange, JK: *Jatti Khatti*, SO: Sour orange.

Table 2. Influence of rootstocks on the leaf proline, phenol content and polyphenol oxidase activities in 'Kinnow' (FW: fresh weight).

Rootstocks	Proline	Phenol	Catecholase	Cresolase
	(μg g^{-1} FW)	(mg g^{-1} FW)	(ΔA_{400} g^{-1} min^{-1} 10^{-3})	
Rough lemon	232.00^{c}	53.33^{d}	3.12^{d}	3.23^{d}
Karna Khatta	144.00^{d}	44.99^{g}	4.11^{c}	3.39^{c}
Carrizo citrange	41.40^{f}	64.78^{b}	2.53^{e}	2.43^{d}
Rangpur lime	324.00^{b}	46.61^{f}	4.66^{b}	3.98^{b}
Troyer citrange	146.80^{d}	84.81^{a}	2.22^{f}	2.40^{e}
Jatti Khatti	133.80^{e}	56.54^{c}	7.34^{a}	4.68^{a}
Sour orange	354.00^{a}	53.64^{e}	2.10^{f}	2.40^{d}
LSD ($P \leq 0.05$)	2.95	0.81	0.12	0.12

vegetative sinks (Garcia-Sanches *et al*., 2002). In the present study, higher RWC in leaves of 'Kinnow' on *Karna Khatta* and rough lemon reflects the improved hydraulic conductivity of these rootstocks, osmoregulation, and their ability to sustain growth under water stress conditions. The higher values for RWC in citrus were also observed by Maotani and Machida (1980) and grapefruit 'Redblush' grafted on *Karna Khatta* rootstock (Sharma *et al*., 2015). Reduction in RWC in 'Kinnow' leaves on 'Carrizo' and 'Troyer' might be associated with poor root system and hydraulic conductivity of these rootstocks.

Significantly higher physiological (*A*, *gs*, *E* and *Ci*) activity in 'Kinnow' plants on *Jatti Khatti,* rough lemon and sour orange rootstocks indicates the well acclimatized character of 'Kinnow' mandarin on these rootstocks under semiarid conditions. The optimum air temperature during autumn (30–35 °C) season might have resulted in better partitioning of assimilates, favouring higher physiological activity. Higher physiological activity of 'Kinnow' on *Jatti Khatti* and rough lemon under semiarid conditions have also been reported by Panigrahi *et al.* (2014). Lower physiological activity of 'Kinnow' on 'Troyer' citrange rootstocks may be due to reduced density of foliar tissue (data not shown) that might have led to a decrease in the fractional volume of intercellular spaces and stomatal conductance ultimately causing photo-inhibition of photosynthesis due to decreased carboxylation efficiency, as found in other citrus cultivars (Hu *et al*., 2007; Kumar *et al*., 2017).

The higher WUE*i* in 'Kinnow' leaves on 'Troyer', Rangpur lime and *Karna Khatta* rootstocks may be attributed to a greater decrease in *gs* and *E* value. Decreased *gs* and *E* values reflect the poor sensitivity of the scion on these rootstocks to soil water stress to produce higher WUE*i* in leaf compared to other rootstocks. Similar findings were also reported by Vu and Yelenosky (1988) in 'Valencia' orange and Ribeiro *et al.* (2009) in 'Satsuma' mandarin.

Leaf proline, phenol and polyphenol oxidase activities were significantly influenced by the rootstocks (Table 2). In our study proline concentration was found maximum in 'Kinnow' leaves on sour orange (354.0 µg g^{-1} FW) followed by Rangpur lime (324.0 µg g^{-1} FW) and rough lemon (232.0 µg g^{-1} FW) while minimum accumulation was recorded on Carrizo (41.4 µg g^{-1} FW) and *Jatti Khatti* (133.8 µg g^{-1} FW) thus exhibiting a decrease of 2.64- and 8.55-fold over the sour orange rootstock. Rootstock also imparted a significant influence on the accumulation of phenol content. Plants budded onto 'Troyer' rootstock accumulated higher phenol (84.81 mg g^{-1} FW) followed by 'Carrizo' (64.78 mg g^{-1} FW) and *Jatti Khatti* (56.54 mg g^{-1} FW), thus accumulating 1.88-, 1.43- and 1.25-fold more phenol in their leaves compared to *Karna Khatta* which accumulated minimum phenol (44.99 mg g^{-1} FW). Polyphenol oxidase activity expressed as (ΔA_{400} g^{-1} min^{-1} 10^{-3}) varied significantly exhibiting higher catecholase (7.34) and cresolase activity (4.68) in scion leaves on *Jatti Khatti* followed by Rangpur lime and *Karna Khatta*. Similar trend was recorded with respect to the minimum values exhibiting lower catecholase (2.40) and cresolase (2.22) activity on 'Troyer'.

Higher proline concentration in leaves of 'Kinnow' on sour orange, Rangpur lime and rough lemon may be attributed to the genetic character of these rootstocks to accumulate higher proline as they confer tolerance to abiotic stress such as drought and salinity. Accumulation of free proline in citrus and other plant species subjected to environmental stresses have also been reported by other workers (Balal *et al*., 2012). Minimum proline concentration in leaves of 'Kinnow' on 'Carrizo' citrange rootstock, which is a drought- and salt-sensitive rootstock, might be due to reduced leaf RWC and poor osmotic adjustment due to higher accumulation of chloride in the leaves because 'Carrizo' is considered to be a chloride includer (Brumos *et al*., 2010). Reduction in proline level under salinity in 'Carrizo' seedlings have also been reported (Perez-Perez *et al*., 2007). Higher phenol accumulation in leaf tissue of 'Kinnow' on 'Troyer' and 'Carrizo' suggest an activation of defence reaction to evaluate the stress mechanism in these scion-stock combinations under reduced leaf RWC and antioxidant enzymes. Similar findings were also reported in apple (Bolat *et al*., 2014).

The higher catecholase and cresolase activity in 'Kinnow' trees on *Jatti Khatti* and 'Troyer' citrange suggests their protective role in overcoming biotic stress and as a defensive component against soil borne pathogen. In 'Troyer', higher catecholase and cresolase activity might have been contributed from *Poncirus trifoliata* which is one of the parents in 'Troyer' citrange, and is well known for its tolerance to biotic stresses*.* Variation in PPO activity in scion cultivars of mango have also been reported by Dayal *et al*. (2016).

Antioxidant enzymes

In our study, significant variations in antioxidant enzymes were observed in leaves of 'Kinnow' on different rootstocks (Table 3). The antioxidant enzymes SOD (60.49 U min^{-1} mg^{-1} protein) and POD (31.20 µmol Tetra-guaiacol formed min^{-1} mg^{-1} protein) were most active in in 'Kinnow' leaves on rough lemon followed by *Karna Khatta* and *Jatti Khatti* rootstocks respectively. Minimum activity of these enzymes were recorded in leaves budded on sour orange, thus exhibiting a reduction of 6.44% and 38.65% over the mean maximum value recorded in rough lemon. The catalase activity was,

TABLE 3. Influence of rootstocks on the leaf anti-oxidant enzyme activities in 'Kinnow'.

Rootstocks	SOD (unit min^{-1} mg^{-1} protein)	Catalase (μmol H_2O_2 hydrolyzed min^{-1} mg^{-1} protein)	Peroxidase (μmol Tetra-guaiacol formed min^{-1} mg^{-1} protein)	GR (ΔA_{412} min^{-1} mg^{-1} protein)
Rough lemon	60.49^{a}	9.68^{b}	31.20^{a}	0.490^{a}
Karna Khatta	59.97^{b}	7.16^{d}	21.73^{c}	0.197^{e}
Carrizo citrange	57.53^{d}	7.24^{d}	21.44^{c}	0.114^{g}
Rangpur lime	57.24^{d}	7.82^{c}	24.02^{b}	0.295^{c}
Troyer citrange	57.94^{c}	7.62^{c}	20.96^{c}	0.142^{f}
Jatti Khatti	58.06^{c}	9.65^{b}	24.41^{b}	0.320^{b}
Sour orange	56.59^{e}	11.19^{a}	19.14^{d}	0.278^{d}
LSD ($P \leq 0.05$)	0.36	0.31	1.09	0.005

however, significantly enhanced on this scion-rootstock combination (11.19 μmol H_2O_2 hydrolyzed mg^{-1} protein min^{-1}) followed by scion leaf on rough lemon (9.68 μmol H_2O_2 hydrolyzed mg^{-1} protein min^{-1}) and was statistically similar to *Jatti Khatti*. Catalase activity was recorded minimum on *Karna Khatta* (7.16 μmol H_2O_2 hydrolyzed mg^{-1} protein min^{-1}) with parallel values on 'Carrizo'. As compared to the mean maximum value recorded on sour orange a reduction of 36.01% and 35.29% was registered on these rootstocks. Maximum glutathione reductase (GR) activity (0.490 ΔA_{412} min^{-1} mg^{-1} protein) was recorded in leaves of 'Kinnow' on rough lemon while minimum (0.118 ΔA_{412} min^{-1} mg^{-1} protein) was observed on 'Carrizo' rootstock which was 4.29-fold lower than on rough lemon.

Variations in antioxidant enzymes in leaves of 'Kinnow' on different rootstocks, suggest the differential ability of the rootstocks to scavenge Reactive Oxygen Species (ROS). Up-regulated SOD, POD and GR activity in 'Kinnow' on rough lemon and *Jatti Khatti* suggests that the trees on these rootstocks were more potential in removing O_2 by catalyzing its dismutation and thus have the ability to overcome stress and can be very well adapted under arid and semiarid conditions. SOD catalyses the dismutation of the O_2^- free radical to O_2 and H_2O_2. Reducing H_2O_2 to non-toxic levels is accompanied by CAT and POD by converting H_2O_2 to water and oxygen (Apel and Hirt, 2004). Induced CAT accumulation in leaves on sour orange rootstock also suggests that this scion-stock combination has the ability to overcome stress by scavenging higher H_2O_2 and preventing any potential damage to leaf tissue. Variation in anti-oxidant enzymes caused by rootstocks has also been reported for different citrus species (Santini *et al.*, 2012; Sharma *et al.*, 2016a).

Conclusion

Jatti Khatti and rough lemon induced larger tree size and higher yield. Fruits of high internal quality were induced from trees on *Jatti Khatti*, Rangpur lime and sour orange. On the basis of physiological activity (*A*, *gs*, *E* and *Ci*) although *Jatti Khatti* shows meritorious characteristics for its commercial cultivation in semi-arid regions under normal soil moisture conditions, higher up-regulation of the antioxidant enzymes in the leaves of 'Kinnow' on rough lemon clearly reflects the ability of this rootstock to scavenge ROS and to protect 'Kinnow' trees from environmental stress under both arid and semi-arid conditions. Improved RWC and WUE*i* on *Karna Khatta* suggest that this rootstock can sustain drought and can be a better rootstock for water-scarce areas. The up-regulated catalase and proline activity in sour orange indicated its utility as a rootstock for moderate drought-prone areas.

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