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Nutrient uptake, osmolytes and antioxidant potential of different citrus rootstocks subjected to acidic soils

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ABSTRACT

The present experiment was conducted during 2021–23 at ICAR-Indian Agricultural Research Institute, New Delhi to evaluate the response of five citrus rootstocks, viz. Rangpur lime (RL); Troyer citrange (TC); Cleopatra mandarin (CM); X-639 and *Jatti khatti* (JK) for leaf and root nutrient content including osmolytes and antioxidant potential in a soil *p*H range of 3.8–7.5. The experiment was initiated after the final establishment of plants in the pots in a factorial completely randomized block design (Factorial-CRBD) with four levels of soil *p*H and five different citrus rootstocks. Result indicated that RL recorded highest phosphorous content in leaf (0.43%) and root (0.36%) as well as total soluble sugars content (9.34 mg/g) in leaf. TC registered highest content of nitrogen (1.83%) and phosphorous (0.35%) in root; potassium (1.83 and 1.89%) and calcium (1.10 and 0.94%) in leaf and root, respectively, while significantly higher magnesium content (0.77%) and soluble protein (5.67 mg/g) were documented in the leaf as compared to other rootstocks. Maximum nitrogen content (3.10%), total phenols (6.76 GAE mg/g) and proline (40.27 µmol/g) were registered in leaves of CM whereas magnesium content in root (1.05%). The nitrogen content in leaves and roots of all the rootstocks as well as total soluble sugar tended to increase with decreasing levels of soil *p*H. Principal component analysis identified a distinct cluster of rootstocks comprising TC, X-639 and JK grouped together with notably high phenolic levels, while RL formed a separate cluster characterized by higher concentrations of total soluble sugar, proline and phosphorus. Thus, the present study demonstrated that citrus rootstocks prefer relatively lower soil *p*H for optimal growth. Among the investigated rootstocks, RL has demonstrated the ability to tolerate higher acidic soil conditions.

Keywords: Acidic soils, Antioxidants, Citrus, Macro nutrients, Osmolytes, Rootstocks

The citrus group comprising of different species is grown in more than 140 countries, with an annual production of 293.55 million tonnes (FAO 2024) in tropical, subtropical, and temperate parts of the world. In India, citriculture occupies an area of 1.10 million ha (15.64% of the total area under fruit cultivation) with a production of 14.25 million tonnes during 2023 (Anonymous 2023). Although the area under cultivation has increased by 29.55% since 2010–11, productivity has shown a declining trend because of various stresses (Anonymous 2023). Citrus species like sweet orange is reported to be severely affected by certain stresses under acidic soil condition rendering to economic loss to the growers (Li *et al.* 2020).

About 30% of the total global land area is covered by acidic soils (Hartemink and Barrow 2023). In India, acidic soils account for 92.80 million ha (Sarkar 2015) of which the pre-dominant acidic soil regions are north-eastern, eastern and southern peninsular regions of the country having a *p*H range of 4.5–5.5. Whereas, soils with *p*H values ranging between 5.6 and 6.5 are reported from Gujarat, Karnataka, Madhya Pradesh, Chhattisgarh, Uttar Pradesh, and Uttarakhand (Mandal *et al.* 2019). About 21 million ha of acidic soils are found in the Eastern Himalayan Region, comprising of eight states in the north-east India, acknowledged as a center of diversity for different citrus spp. Citrus cultivation had been encountering setbacks owing to the extensive use of seedlings for orchard establishment and the inadequate availability of rootstocks recommended for acidic soils. Citriculture in regions with acidic soils is often associated with high-quality fruit production worldwide. However, prolonged cultivation under acidic soil would invite several limitations in sustainable fruit production (Zhu *et al.* 2021). Therefore, crop husbandry in acidic soils will continue to be a significant concern for farmers and researchers in the near future (Zang *et al.* 2023).

Suitable citrus rootstocks capable of thriving across a broad spectrum of acidic soil conditions could represent a significant advancement for the sustainable citrus

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industry. There are numerous studies on abiotic stresses, including salinity, in citrus throughout the country as well as across the globe. However, studies with respect to citrus rootstocks in acidic soils are limited. The identification of rootstocks that exhibit optimal performance under a wider range of soil reactions would promote citriculture even in areas with acidic soils, thereby increasing production and productivity. Hence, the present investigation was taken up for nutrient acquisition, osmolytes and antioxidants potential to understand the performance of different citrus rootstocks under relatively low soil *p*H which could be used as a rootstock for propagation of commercial cultivars of sweet orange and other mandarin cultivars.

MATERIALS AND METHODS

Experimental site, planting material and setup: The present experiment was conducted during 2021–23 at ICAR-Indian Agricultural Research Institute, New Delhi (28.08°N and 77.12°E, 228.61 m amsl). Physiologically mature fruits of 5 citrus spp., viz. Rangpur lime (RL); Troyer citrange (TC); Cleopatra mandarin (CM); X-639 and *Jatti khatti* (JK) were collected from the rootstock germplasm repository to extract the seeds during 2021. The extracted seeds were treated with bavistin (soaking in solution @2 g/litre basis) before sowing on raised beds and the seedlings were maintained for three months. Thereafter the seedlings were sorted and subsequently transplanted to the secondary nursery to raise for another nine months before being transferred to the pots having dimension of 35 cm × 35 cm × 23 cm. The orchard soils collected from Dambuk (Lower Dibang Valley District) and Pasighat (East Siang District) of Arunachal Pradesh were grouped into *p*H values of 3.8, 4.6 and 5.35. The pots were filled with 10 kg each of these soils, while for control soil having near to neutral *p*H (7.5) was collected from ICAR-Indian Agricultural Research Institute, New Delhi. The initial organic carbon contents in the soils having *p*H 7.5, 5.35, 4.6 and 3.8 were recorded as 0.54, 0.75, 0.90 and 2.01%, respectively. Initial nitrogen contents were recorded as 351.23, 451.58, 426.50 and 413.95 kg/ha. The phosphorous content in these treatments were 79.74, 38.78, 2911 and 21.50 kg/ha, whereas potassium contents were estimated to be 192.86, 108.19, 98.34 and 82.10 kg/ha, respectively for the soils having *p*H as stated above.

The experiment was initiated after the final establishment of plants in the pots in a factorial completely randomized block design (Factorial-RCBD) with four levels of soil *p*H and five different citrus rootstocks. The potted plants were maintained in a shade-net house from February to September, 2023. During the monsoon season, all treatment combinations were exposed to uniform rainfall events, ensuring consistent conditions for all potted plants. The treatment combinations included four replications and two seedlings/replication. Regular irrigation @2 litre of water/pot was applied twice a week with tap water having *p*H of 6.8 and an EC of 0.29 dS/m throughout the experiment, ensuring that the soils were maintained at field capacity. At the termination of the experiment, 0 i.e. at 186 days, soil and plant samples were collected for analysis. The soil *p*H was determined again and the alterations were documented at varying levels i.e. 3.8, 4.6, 5.35 and 7.5 as 4.9, 5.44, 6.3 and 7.32, respectively.

Nutrient content in leaves and roots: At the termination of experiment, 25–30 mature leaves were collected from each of the replicated seedling rootstocks. The plants were then uprooted carefully ensuring minimum damage. Both the leaf and root samples were transported to the laboratory in an ice box and thoroughly washed in tap water followed by rinsing with 0.1 N HCl and distilled water. After drying in shade for about 6 h on blotting paper, the samples were properly labeled and kept in brown paper bags for drying in in a hot air oven at 65–70°C. The dried leaf and root samples were subsequently grounded and required quantity was taken for digestion in wet diacid using nitric acid (HNO_3) and perchloric acid ($HClO_4$). The digested materials were then diluted and filtered through Whatman No. 1 filter paper. The final volumes were made to 100 ml with double distilled water and were used for the estimation of mineral nutrients. The nitrogen content of the rootstocks was determined adopting the standard procedure (Jackson 1967), phosphorus content was estimated via the vanado-molybdo phosphoric acid yellow colour method as described by Jackson (1967) and the potassium content was estimated from the digested extract using a flame photometer as suggested by Piper (1966). The calcium and magnesium contents were determined via the EDTA titration method following the protocols described by Tucker and Kurtz (1961). The values were expressed as percentage.

Osmolytes and antioxidants: The biomolecules were extracted and estimated on fresh weight (FW) basis by collecting fresh recently mature leaves which were transported to the laboratory in an ice box. The quantification of total phenolic content in the leaf extracts was conducted following the Folin-Ciocalteu Reagent method as outlined by Malik and Singh (1980), wherein the total phenolic concentration was measured at 650 nm against the reagent used as a blank on a Perkin Elmer UV-visible spectrophotometer Lambda 365. For standard calibration, a plot was generated at 650 nm using known concentrations of gallic acid (GA). The concentration of phenols in the test samples was calculated from the calibration plot and expressed as milligrams of GA equivalent of phenol per gram of the sample. The proline content was assessed as per the protocol outlined by Bates *et al.* (1973), in which the absorbance was measured at 520 nm employing pure toluene as a blank. The total soluble protein content was determined following the Bradford method (Bradford 1976) for which the absorbance was measured at a wavelength of 595 nm with standard curve prepared using a bovine serum albumin stock solution. Total soluble sugars were estimated following the procedure described by Thimmaiah (2021) in which the absorbance was taken at 490 nm with 1% glucose taken for standard curve derivation.

Statistical analysis: The data were analyzed using SAS 9.3 version. The mean values were compared with the least significant difference (LSD) test ($P \leq 0.05$). R (programming language) software developed by R core and the R foundation was used to perform the principal component analysis (PCA).

RESULTS AND DISCUSSION

The soil *p*H significantly influenced soil biogeochemical processes, determines the solubility, mobility and bioavailability of elements thereby affecting plant growth and yield. The identification of appropriate rootstocks for acidic soils depends on the level of soil acidity, citrus genotypes being grown and the desired tree characteristics (Morales-Alfaro *et al.* 2021). In the present study, the soil *p*H was found to impact the nutrient and biomolecule contents in different citrus rootstocks raised in soils with varying *p*H values (Table 1).

Nutrient content in leaves and roots: The nitrogen content in leaves and roots was significantly influenced by soil *p*H and rootstock. Maximum leaf N content was documented in TC (3.64%) and RL (3.56%) at *p*H 4.6 and 3.8, respectively. Conversely, minimum values of N content were recorded in the leaves of TC (1.85%) and X-639 (1.79%) at 7.5 *p*H. In the roots, TC recorded maximum N content (2.16 and 2.13%) when they were raised under soil *p*H 5.35 and 3.80, respectively. Better uptake of nitroge in TC and RL may be attributed to better root system (data not presented) and N uptake in ionic form of nitrate is more under lower soil *p*H through energy dependent mechanism and transpirational stream as described by Davies and Albrigo (1994). Irrespective of soil *p*H, maximum P content in leaves (0.43%) and roots (0.36%) was registered in RL. Higher uptake and better accumulation of P in leaves and root suggest higher availability of P in soil *p*H between 6.0 and 7.0 due to high organic matter content in the experimental soil which solubilize fixed P in acid soils and hence not detrimental for plant growth. When the uptake of P is more than requirement for metabolism, it is accumulated in vacuoles creating phosphate homeostasis in the cytoplasm thereby avoids possible detrimental effects (Mimura and Reid 2024). Differential response of ion uptake by rootstocks as observed in our study have also been reported by Kumar *et al.* (2017) in Kinnow mandarin who reported better genotypic variations in root architecture resulting in difference in nutrient uptake.

In the present experiment, RL and TC raised in soil *p*H 5.35 recorded significantly maximum leaf K content (1.94 and 1.90%, respectively) and the differences were not significant. However, the K content in root was maximum in TC (1.98%) under *p*H 7.5 and X-639 (1.92%) under *p*H 4.6. Higher K content in RL at soil *p*H of 5.35 was due to mobile nature of K rendering its translocation from individual cell to the xylem and phloem consequently resulting in better stomatal function and photosynthesis. Our results of K content are in accordance with those of Alva *et al.* (2006) who studied on potassium management of citrus orchards in Sao Paulo, Brazil where *p*H of soil ranged between 4.8 and 5.5. Significantly maximum Ca content in the leaves (1.32%) was detected in X-639 under soil *p*H of 7.5, whereas in the roots was detected in X-639 and CM at the same *p*H.

The maximum Ca content in the X-639 and CM at soil *p*H of 7.5 is associated with their tolerant adaptive to high salinity, high *p*H, and cancerous soils (Davies and Albrigo 1994). CM is also described as an efficient absorber of cations like Ca, Mg, and K (Tripathi 2024). Similar were the observation of Shankar *et al.* (2023) in CM and X-639 at varying levels of water salinity stress. In the present study, the maximum Mg in leaves (0.77%) was recorded in JK at a soil *p*H of 7.5, whereas in roots it was recorded in CM (1.14%) at soil *p*H of 4.6. Irrespective of rootstocks, Ca content in leaves and roots as well as Mg content in leaves exhibited decreasing trend with lowering in soil *p*H. However, the Mg content in roots increased with lowering in soil *p*H. This may be associated with highly soluble nature of magnesium minerals when soil *p*H is below 7.5 (Sureshkumar and Sandeep 2015). The underlying physiological processes responsible for the observed patterns of calcium and magnesium uptake and translocation within plants grown in low *p*H soils need to be fully understood.

Osmolytes and antioxidants: Abiotic stress conditions induce, the accumulation of soluble proteins and carbohydrates in plants. Sugars are vital osmolytes having significant roles in the defensive mechanism of plants (Ikram *et al.* 2022). In the present study, varying soil *p*H impacted the accumulation of total soluble protein and sugars in the leaves of the rootstocks (Fig. 1). Irrespective of varying soil *p*H, the maximum total soluble protein content was recorded in TC (5.67 mg/g FW), followed by JK and RL. Higher total soluble protein in the leaves of these rootstocks suggests its potential to adjust in a broader range of soil acidity for mitigating stress like situation. Similar were the observations of Hussain *et al.* (2018) in a drought stress studies on six citrus rootstocks. They reported higher accumulation of total soluble protein in leaves and roots of Carrizo citrange compared to the other rootstocks. However, there are no reports on the differential accumulation of total soluble protein under acidic stress.

Sugars constitute the primary substrates that provide structural material and act as signal molecules interacting with hormonal pathways to regulate the intrinsic plant immune system (Morkunas and Ratajczak 2014). Interesting results were obtained under this study with respect to accumulation of total sugar in the different rootstock. Irrespective of rootstocks, the maximum accumulation of total soluble sugars was recorded at soil *p*H of 3.8 (8.62 mg/g FW) as compared to those in soil *p*H 4.6 (8.04 mg/g FW), 5.35 (6.93 mg/g FW), and 7.5 (6.85 mg/g FW). Under varying soil *p*H (3.8–7.5), the maximum total soluble sugar content registered in RL (9.34 mg/g FW) could be attributed to maximum total dry weight and low chlorophyll degradation. The minimum content of soluble sugars in leaves of TC (5.78 mg/g FW) corresponded to significantly maximum Mg content (0.77%) as higher Mg concentration in leaves enhances transport of photoassimilates from leaves

Table 1 Effects of different soil *p*H on the nutrient contents (%) in 5 citrus rootstocks

Treatment	Leaf					Root				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
Soil *p*H (S)										
7.5	2.21^{d}	0.37^{b}	1.74^{a}	1.17^{a}	0.69^{a}	1.35^{c}	0.29^{d}	1.64^{b}	0.92^{a}	0.75^{c}
5.35	2.99^{c}	0.40^{a}	1.60^{b}	1.05^{b}	0.70^{a}	1.53^{b}	0.34^{b}	1.60^{b}	0.87^{b}	0.76^{b}
4.6	3.15^{b}	0.35^{c}	1.77^{a}	0.94^{c}	0.69^{a}	1.53^{b}	0.35^{a}	1.80^{a}	0.76^{c}	0.83^{a}
3.8	3.24^{a}	0.34^{d}	1.76^{a}	0.88^{d}	0.64^{b}	1.64^{a}	0.30^{c}	1.61^{b}	0.68^{d}	0.83^{a}
Rootstock (R)										
RL	2.95^{c}	0.43^{a}	1.80^{b}	0.87^{e}	0.72^{b}	1.51^{c}	0.36^{a}	1.45^{e}	0.71^{d}	0.58^{e}
TC	3.03^{b}	0.35^{c}	1.83^{a}	1.10^{a}	0.77^{a}	1.83^{a}	0.35^{a}	1.89^{a}	0.94^{a}	0.81^{c}
CM	3.10^{a}	0.35^{c}	1.70^{c}	0.98^{d}	0.60^{c}	1.57^{b}	0.30^{b}	1.77^{b}	0.84^{b}	1.05^{a}
X-639	2.80^{d}	0.38^{b}	1.67^{d}	1.02^{c}	0.58^{d}	1.25^{e}	0.30^{b}	1.62^{c}	0.83^{c}	0.84^{b}
JK	2.61^{e}	0.34^{d}	1.60^{e}	1.08^{b}	0.72^{b}	1.41^{d}	0.28^{c}	1.56^{d}	0.72^{d}	0.69^{d}
S × R										
Soil *p*H: 7.5										
RL	2.32^{g}	0.54^{a}	1.63^{ij}	1.02^{d}	0.84^{a}	1.57^{e}	0.46^{a}	1.36^{i}	0.75^{fg}	0.59^{m}
TC	1.85^{h}	0.37^{e}	1.85^{b}	1.21^{b}	0.70^{de}	1.26^{j}	0.31^{h}	1.98^{a}	0.99^{abc}	0.88^{d}
CM	2.66^{e}	0.35^{g}	1.76^{efgh}	1.10^{c}	0.66^{ef}	1.43^{h}	0.23^{l}	1.65^{de}	1.01^{ab}	0.95^{c}
X-639	1.79^{h}	0.37^{e}	1.83^{bc}	1.32^{a}	0.46^{i}	1.15^{l}	0.27^{j}	1.77^{c}	1.03^{a}	0.66^{kl}
JK	2.44^{f}	0.24^{k}	1.65^{ij}	1.21^{b}	0.77^{bc}	1.34^{i}	0.20^{m}	1.46^{h}	0.81^{e}	0.68^{jkl}
Soil *p*H: 5.35										
RL	2.63^{e}	0.46^{b}	1.94^{a}	0.88^{e}	0.77^{bc}	1.46^{gh}	0.34^{f}	1.53^{g}	0.73^{gh}	0.59^{m}
TC	3.33^{b}	0.34^{h}	1.90^{a}	1.12^{c}	0.75^{bc}	2.16^{a}	0.38^{b}	1.88^{b}	0.97^{bc}	0.79^{fg}
CM	3.08^{c}	0.4^{d}	1.64^{ij}	1.03^{d}	0.62^{fg}	1.82^{b}	0.34^{f}	1.62^{ef}	0.95^{c}	0.99^{c}
X-639	3.08^{c}	0.42^{c}	1.25^{k}	1.01^{d}	0.59^{gh}	1.12^{lm}	0.35^{e}	1.36^{i}	0.9^{d}	0.77^{gh}
JK	2.83^{d}	0.4^{d}	1.27^{k}	1.21^{b}	0.75^{bc}	1.12^{lm}	0.29^{i}	1.59^{efg}	0.79^{ef}	0.64^{l}
Soil *p*H: 4.6										
RL	3.28^{b}	0.37^{e}	1.81^{bcde}	0.81^{f}	0.73^{cd}	1.48^{fg}	0.29^{i}	1.69^{d}	0.68^{i}	0.59^{m}
TC	3.64^{a}	0.36^{f}	1.82^{bcd}	1.08^{c}	0.79^{b}	1.76^{c}	0.37^{c}	1.90^{b}	0.9^{d}	0.86^{de}
CM	3.30^{b}	0.3^{j}	1.62^{j}	0.92^{e}	0.66^{ef}	1.20^{k}	0.38^{b}	1.91^{b}	0.7^{hi}	1.17^{a}
X-639	3.00^{c}	0.37^{e}	1.8^{bcdefg}	0.88^{e}	0.64^{f}	1.62^{d}	0.34^{f}	1.92^{ab}	0.83^{e}	0.83^{ef}
JK	2.52^{f}	0.35^{g}	1.8^{bcdef}	1.01^{d}	0.64^{f}	1.57^{e}	0.35^{e}	1.56^{fg}	0.66^{i}	0.70^{ijk}
Soil *p*H: 3.8										
RL	3.56^{a}	0.35^{g}	1.81^{bcde}	0.77^{f}	0.55^{h}	1.51^{f}	0.36^{d}	1.23^{j}	0.68^{i}	0.53^{n}
TC	3.30^{b}	0.31^{i}	$1.75^{f\,h}$	0.99^{d}	0.84^{a}	2.13^{a}	0.32^{g}	1.81^{c}	0.88^{d}	0.72^{ij}
CM	3.36^{b}	0.34^{h}	1.77^{defgh}	0.88^{e}	0.44^{i}	1.82^{b}	0.27^{j}	1.91^{b}	0.68^{i}	1.08^{b}
X-639	3.33^{b}	0.34^{h}	1.79^{cdefgh}	0.88^{e}	0.62^{fg}	1.09^{m}	0.25^{k}	1.44^{h}	0.57^{j}	1.08^{b}
JK	2.66^{e}	0.36^{f}	1.67^{i}	0.90^{e}	0.73^{cd}	1.62^{d}	0.27^{j}	1.63^{de}	0.59^{j}	0.73^{hi}
LSD ($P \leq 0.05$)										
Soil *p*H (S)	0.038	0.004	0.021	0.013	0.013	0.02	0.004	0.025	0.012	0.016
Rootstock (R)	0.042	0.005	0.023	0.014	0.014	0.02	0.004	0.028	0.014	0.018
Interaction (S×R)	0.084	0.009	0.046	0.028	0.029	0.04	0.009	0.056	0.027	0.036

RL, Rangpur lime; TC, Troyer citrange; CM, Cleopatra mandarin; and JK, *Jatti khatti*.

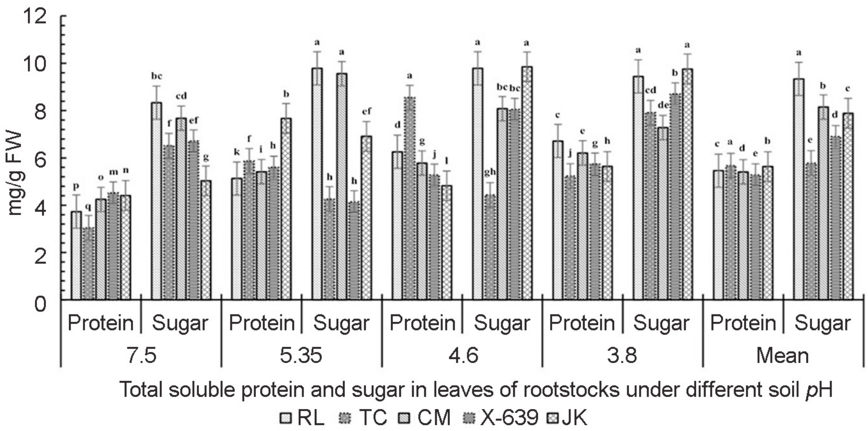

Fig. 1 Total soluble protein and sugars in 5 citrus rootstocks at varying soil *p*H values. RL, Rangpur lime; TC, Troyer citrange; CM, Cleopatra mandarin; JK, Jatti khatti.

to different sinks via phloem loading (Hawkesford *et al.* 2023). The finding elucidates the observation of Balal *et al.* (2011), who reported higher accumulation of sugars in Rangpur lime and lowest contents in Sanchton Citrumelo as well as Carizzo citrange rootstocks under salt stress.

Phenolic compounds are secondary metabolites and their biosynthesis is reported to be triggered both under biotic and abiotic stresses. Similarly, proline is one of the most abundantly synthesized amino acids in citrus leaves under stress conditions. In the present study, it was observed that different rootstocks responded differently at varying levels of soil *p*H (Fig. 2). Among all the rootstocks studied irrespective of soil *p*H, the maximum total phenols and proline contents were recorded in CM (6.76 GAE mg/g and 40.27 μmol/g) and the lowest values were obtained in TC (4.37 GAE mg/g) and X-639 (31.27 μmol/g), both of which have *Poncirus trifoliata* as pollen parent.

These findings are in accordance with those of Argamasilla *et al.* (2014) who had reported higher levels of phenylpropanoid, a phenolic compound, as well as proline in Cleopatra mandarin than in Carrizo citrange under drought stress. In the present experiment, the optimum absolute growth rates (AGR) for these rootstocks were obtained under soil *p*H 4.6 (data not given). Therefore, the ratios of proline content in leaves of these rootstocks under soil *p*H 3.8 and 4.6 are: 0.89, 1.10, 0.66, 0.92, and 0.97 for RL, TC, CM, X-639, and JK, respectively. The smallest ratios pertained to CM (0.66) and RL (0.89), indicating that these are more tolerant citrus genotypes.

In an experiment of salt stress by Balal *et al.* (2011), Rangpur lime was observed with the highest accumulation of proline, indicating the greatest salt tolerance among the 10 rootstocks studied. In the present study, the higher contents of both total phenols (5.98 GAE/mg) and proline (45.98 GAE μmoles/g) in leaves were observed in rootstocks grown in soils with 7.5. This suggests that soil *p*H 7.5 is perceived to be sub-optimal for citrus rootstocks, affecting their optimal growth and development.

Principal component analysis (PCA): PCA was executed to understand the relationships among critical variables and the performance of the five rootstocks under varying soil *p*H values of 7.5, 5.35, 4.6, and 3.8. In the biplot with four levels of soil *p*H (Fig. 3A), the first principal component 1 (PC1) accounted for 58.03% of the total variation, depicting factors 3 and 4 i.e. soil *p*H values of 4.6 and 3.8, respectively, as more favourable environments for citrus irrespective of genotypes in general, which is an acidic loving crop (Zhang *et al.* 2020). N leaf, N root, Mg leaf, Mg root, Ca leaf, Ca root, and protein leaf were the major contributing variables to PC1, whereas K leaf, P leaf, P root, Proline leaf, and Sugar leaf contributed significantly to PC2, accounting for 25.90% of the total variation. The biplot pertaining to five rootstocks (Fig. 3B) revealed that PC1 accounted for 37.8% of the total variation and clearly demonstrated separation based on rootstock genotype, i.e., 3, 4, and 5 i.e. CM, X-639, and JK, in cluster alienating rootstocks (RL) and 2 (TC). The variables viz. N leaf, N root, K leaf, Mg leaf, R root, and protein leaf were positioned on the positive plane on both axes, indicating a positive

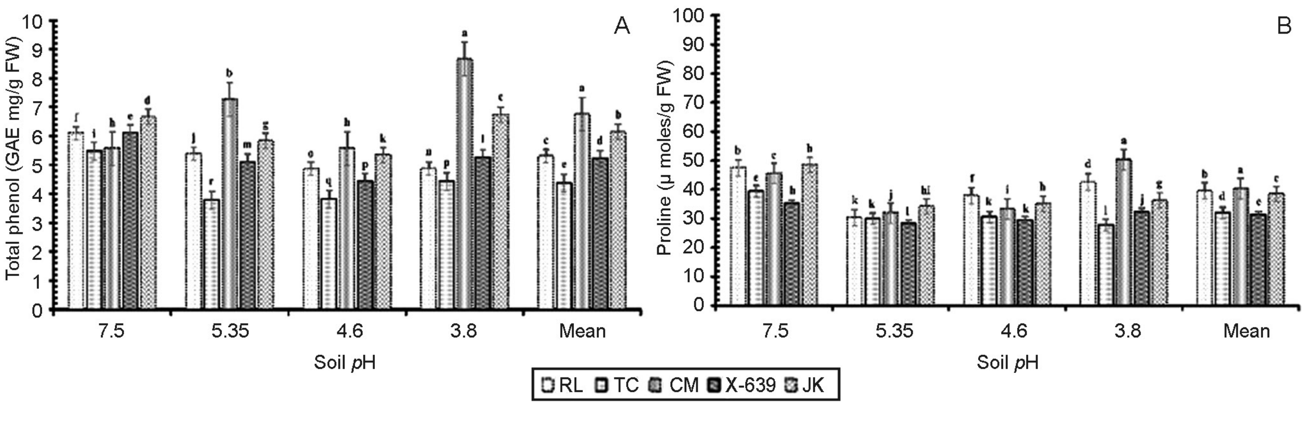

Fig. 2 Total phenol (A) and proline (B) contents in 5 citrus rootstocks at varying soil *p*H values. RL, Rangpur lime; TC, Troyer citrange; CM, Cleopatra mandarin; JK, Jatti khatti.

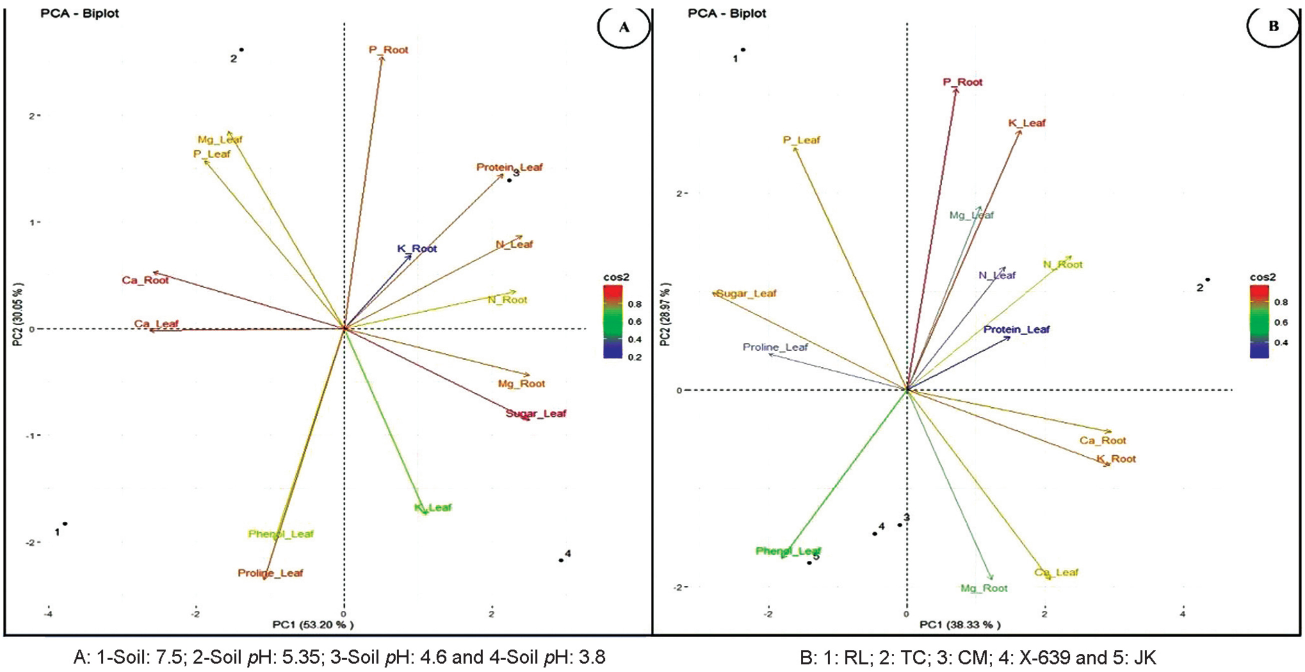

Fig. 3 PCA biplot for different rootstocks (A) and soil *p*H levels (B) with respect to traits. RL, Rangpur lime; TC, Troyer citrange; CM, Cleopatra mandarin; JK, Jatti khatti.

correlation. Our results corroborated with Ahmad *et al.* (2022) who had reported more than 50% variation among the studied parameters of citrus leaf nutrients.

Based on the results of the present study, citrus rootstocks are found to exhibit optimal nutrient uptake and accumulation of biomolecules at soil *p*H 5.35 and 4.6. Whereas, soil *p*H 7.5 and 3.8 were found to be sub-optimal for studied citrus rootstocks. Among the 5 rootstocks studied, RL presented the maximum total soluble sugars, the important osmolytes across varying soil *p*H values of 5.35, 4.6, and 3.8. Additionally, proline content was also relatively higher in leaves of RL in soils with lower *p*H. Therefore, Rangpur lime which is already a compatible rootstock for *Citrus sinensis* Osbeck and *C. reticulata,* can be recommended as a preferred rootstock for citriculture in regions having acidic soils. On the other hand, citrus cultivars grafted upon Rangpur lime can be utilized for establishment and expansion of orchards in such regions. This recommendation could also support citrus rootstock improvement programmes, particularly in areas with very low soil *p*H.

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