

Role of citrus rootstocks in modulating ‘Kinnow’ growth and physiology

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Abstract

A study was designed to determine the influence of different rootstocks on the growth, leaf nutrient composition, and physiological parameters of Kinnow (*C. nobilis* × *C. deliciosa*). In this experiment, two years old grafted plants of Kinnow on nine citrus rootstocks, including Trifoliate orange (*Poncirus trifoliata*), C-35 citrange (Ruby orange × Webber-Fawcett trifoliate), Troyer citrange (*C. sinensis* “Washington” × *Poncirus trifoliata*), Carrizo citrange (*C. sinensis* “Washington” × *Poncirus trifoliata*), Benton citrange (*Poncirus trifoliata* × *C. sinensis*), Cleopatra mandarin (*C. reshni*), Cox mandarin (*C. reticulata* ‘Scarlet mandarin’ × *Poncirus trifoliata* ‘trifoliate orange’), Rough lemon (*C. jambhiri*), and Sour orange (*C. aurantifolia*) was used. The research was carried out over two consecutive years in the Pothwar region, Islamabad, Pakistan. Results revealed that different citrus rootstocks significantly affected ‘Kinnow’ growth in both years at $P < 0.05$. ‘Carrizo’ rootstock resulted in significant increase in scion diameter, rootstock diameter, graft union diameter, leaf number, and leaf area of Kinnow in the year 2023. For physiological parameters, ‘Kinnow’ leaves had higher ‘An’, ‘ci’, ‘WUE’ values and Chlorophyll contents (SPAD) when plants were grafted onto Carrizo rootstock in 2022 and 2023, whereas VPD values were only higher on Carrizo rootstock during 2023. Regarding leaf nutrient compositions, ‘Kinnow’ leaves showed a notable increase in nitrogen (N) content when grafted onto Carrizo rootstock in 2023. Similarly, higher values of leaf potassium (K) and phosphorus (P) contents were observed when ‘Kinnow’ was grafted onto Carrizo rootstock. Overall, plants grafted on *Poncirus* had lower values of ‘VPD’, ‘gs’, ‘Ci’, ‘E’ compared to others during the studied period. Significant correlations among the morphological, physiological, and leaf nutrient compositions were identified at the $P < 0.01$ and $P < 0.05$ significance levels, showing that all the investigated parameters were strongly correlated. In conclusion, Kinnow had more growth on the ‘Carrizo’ rootstock, showing better results for growth vigor, nutrient uptake and physiological attributes.

Keywords: Citrus, Rootstocks, Kinnow, Carrizo, Nutrient composition, Cox, Physiological parameters, PCA

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Introduction

Citrus is one of the most significant crops in the global fruit industry, grown in the tropical and subtropical regions worldwide, because of its industrial and economic value (Shafqat et al., 2021). It is commercially grown in 53 countries across the globe, including China, Brazil, EU, USA, Turkey, Spain and Mexico (USDA, 2023). Pakistan also holds a key position in the citriculture covering an area of 155.9 thousand hectares, with 2334.4 thousand tons of production (GOP, 2024) and it has a considerable export potential in the international market. Although it is a commercially important fruit tree of the country, the country exported 236.73 thousand tons of fruit, generating a revenue of 80.75 million dollars (GOP, 2022) in the current year, which is half of the last year's exports i.e. 561.9 thousand tons and earned 119.5 million dollars. Moreover, the average yield of citrus in the country is reported at 12.5 tons per hectare and 2.46 million tons per season, which is two to three times less than the yield observed in leading citrus-producing countries.

Among various factors influencing the quality and yield of citrus, the choice of rootstock plays a crucial role in addressing both biotic and abiotic factors, particularly under changing climatic conditions (Martinez et al., 2024). Rootstock is the fundamental part of grafting influencing the water and nutrient uptake efficiency, photo-assimilate partitioning, root growth and conducting vessels distribution (Morales Alfaro et al., 2023). It modifies the structure of roots and improves the nutrient transport efficiency via changes in root architecture, interacts with soil conditions and root-to-shoot nutrient transport (Ibacache et al., 2020). A six-fold improvement was found in citrus production when right scion-rootstock combination was used (Sau et al., 2018; Snoussi et al., 2022). The impact of rootstock-scion interactions on fruit quality, growth and stress tolerance has been widely examined (Rasool et al., 2020).

Rootstock can influence the scion's biochemical, physiological and morphological characteristics, which particularly modify the vegetative growth of grafted plants (Hayat et al., 2020). The physiological parameters, including photosynthetic activity (Zhou et al., 2021); leaf gas exchange (Santos et al., 2024) and chlorophyll levels (Jiao et al., 2023) are also influenced by the rootstock choice, which leads to differences in fruit quality, plant growth and eventually the yield (Khan et al., 2020). Numerous

research studies have explored the effect of different rootstocks on growth of various citrus cultivars, such as 'Salustiana' sweet orange (Khan et al., 2020), Kinnow Mandarin (Qureshi et al., 2021), 'Shatangju' sicon (Hayat et al., 2022), 'Rio Red' (Turkmen et al., 2024), Lane late (Emmanouilidou and Kyriacou, 2017), Marsh seedless and Redblush (Morales Alfaro et al., 2023). Hence, a suitable rootstock selection is vital for improving different horticultural traits, viz., tree vigor, leaf nutrient status, adaptability to different soils, root distribution, fruit quality and resistance to pests and diseases (Tirado-Corbala et al., 2018; Shahkoomahally et al., 2021).

The cultivable area for citrus production is decreasing day by day because of the susceptibility of existing rootstocks towards biotic as well as abiotic factors, which is the main reason for the shortening of orchard life. In Pakistan, the citrus industry is dependent on two main rootstocks i.e. sour orange and rough lemon, both are well adapted to calcareous soils. However, plants on both these rootstocks are prone to different pests and diseases (Tietel et al., 2020; Carvalho et al., 2022). Therefore, the selection of an appropriate rootstock is imperative which has more compatibility with the existing scion variety. This research study aimed to investigate the effect of different rootstocks on the growth of 'Kinnow', its physiological traits, and the composition of leaf nutrients, with a particular emphasis on understanding the correlation among plant growth, leaf mineral contents and physiological parameters.

Material and Methods

Plant materials and growing conditions

The experiment was conducted in the orchard of Fruit Crops Research Program, Horticultural Research Institute (HRI), NARC, Islamabad, Pakistan, at an altitude of 507 meters with a latitude of 33.6844° N and longitude of 73.0479° E. The study was completed over two consecutive years (2021-22 to 2022-23) by using Randomized Completely Block Design (RCBD) with four replicates (each had five plants). The region has a humid subtropical climate with an annual rainfall of about 1,291.7 mm, which falls mainly in July and August. The dry season usually lasts from October to November. The minimum temperature can drop to -3.9°C (25.0°F), while the maximum temperature in June can reach 46.1°C (115.0°F). In September 2019, the 'Kinnow' (*C. nobilis* × *C. deliciosa*) scion was grafted onto one-year-old citrus rootstocks, including

Trifoliolate orange (*Poncirus trifoliata*), C-35 citrange (Ruby orange × Webber-Fawcett trifoliolate), Troyer citrange (*C. sinensis* × *Poncirus trifoliata*), Carrizo citrange (*C. sinensis* × *Poncirus trifoliata*), Benton citrange (*Poncirus trifoliata* × *C. sinensis*), Cleoptera mandarin (*C. reshni*), Cox mandarin (*C. reticulata* ‘Scarlet mandarin’ × *Poncirus trifoliata* ‘trifoliolate orange’), Rough lemon (*C. jambhiri*), and Sour orange (*C. aurantifolia*) (See characteristics Table-1). Plants

were grown in plastic pots filled with a well-balanced mixture of sand, silt, and rotten manure (1:1:1 v/v/v) under screen house. In October 2021, all scion/rootstock combinations were planted in a field using a square planting arrangement with 5.4 x 5.4 m spacing. Plants were irrigated via furrow irrigation every 3-4 days during summer and every two weeks in winter, with adjustments based on rainfall variations.

Table-1. Characteristics of Citrus rootstocks used for “Kinnow” grafting in the study (K. Bevington, “Know your Rootstocks” NSW Agriculture, Australia)

Rootstocks	Phytophthora	Collar rot	Sandy loam soils	Growth nature	Citrus nematodes	Citrus Greening (HLB)	CTV
<i>Poncirus trifoliata</i>	Tolerant	Tolerant	Sensitive	Dwarf	Tolerant	Tolerant	Highly tolerant
Carrizo Citrange	Tolerant	—	Moderate	Vigorous	Tolerant	Tolerant	Tolerant
Troyer citrange	Tolerant	—	Moderate	Vigorous	Tolerant	—	Tolerant
Benton citrange	Tolerant	—	Moderately tolerant	Medium	Better	—	Tolerant
Cleoptera mandarin	Moderately susceptible	Moderately susceptible	Well Adapted	Slow growing	Susceptible	—	Tolerant
Cox mandarin	Tolerant	Tolerant	Well adapted	Vigorous	—	—	—
C-35 citrange	Moderately susceptible	—	—	Vigorous	Tolerant	Tolerant	Tolerant
Rough lemon	Sensitive	Susceptible	Well adapted	Vigorous	—	—	Good
Sour Orange	Sensitive	Susceptible		Vigorous	—	Susceptible	Susceptible

Soil sampling and fertilizer application

Soil samples were collected and mixed from three depths, i.e. 0–15 cm, 15–30 cm, and 30–45 cm at six distinct locations within the experimental orchard (Coordinates: 33.6677908, 73.1311329) before planting the grafted plants. These samples were analyzed at the Soil Fertility Laboratory, Land Resources Research Institute, NARC, Islamabad. Based on the nutrient analysis detailed in Table-2, a fertilization plan was implemented for each plant over two years: 413 grams of nitrogen (N) from urea, 550 grams of phosphorus (P) from single super phosphate (SSP), and 200 grams of potassium (K) from sulfate of potash (SOP) was applied. Nitrogen was used in two splits: half in late December, along with the full doses

of P and K, and the other half during the fruit set in April (Atta et al., 2021).

Table-2. Soil Physico-chemical characteristics at the experimental site.

Soil Characteristics	0-20cm	20-40cm	40-60cm
pH	7.95	7.97	7.98
EC (ds/cm)	0.178	0.045	0.044
N (mg/kg)	1.42	1.4	1.35
P (mg/kg)	3.2	1.66	1.42
K (mg/kg)	152	132	128
OM (%)	2.24	1.76	1.72
Sand (%)	26.8	24.8	24.6
Silt (%)	26.4	26.4	26.6
Clay (%)	46.8	48.8	48.8
Texture	Loam	Loam	Loam

Growth parameters and leaf mineral analysis

From October 2021, growth-related parameters were recorded. Plant height (cm), scion length (cm), and rootstock length (cm) were measured using a measuring tape. The internode length (cm) was computed by dividing the scion's total length by the number of nodes. Stem diameter (mm) was recorded at three locations: 3 cm above the graft union (scion), 3 cm below the graft union (rootstock), and at the graft union itself, utilizing a Digital Vernier Caliper. Leaf area was measured with a leaf area meter (LI-3100C, USA) as described by Qureshi et al. (2022).

Leaf samples were collected in the March during both study years from different plant orientations (north, east, west, and south). Fully developed leaves, including both the blade and petiole, were taken from the midsection of the current season's shoots. The collected samples were cleaned, dried, and crushed before being digested in a double-acid solution of HClO₄ and HNO₃ (2:1). Nitrogen (N) contents were determined using the Kjeldahl methods (Bremner, 1965; Nelson and Sommers, 1980). Phosphorus (P) and Potassium (K) concentrations were analyzed with a Spectrophotometer (T80+ UV/VIS, China) and a flame photometer (FP910-4, UK), as described by Jackson (1980). To measure the contents of Zinc (Zn), Iron (Fe), Copper (Cu) and Manganese (Mn) the samples were similarly digested in the acid solution

and analyzed using an Atomic Absorption Spectrophotometer (AA-6300, Shimadzu, Japan), followed the procedure outline by Jackson (1980).

Physiological /gas exchange measurements

The photosynthesis-related parameters of fully matured leaves (4th or 5th from the shoot apex) were assessed with a moveable photosynthesis system (CIRAS-3, Amesbury, USA, www.ppsystems.com) between 9:00 AM and 11:30 AM. The environmental parameters included a leaf temperature ($25 \pm 2^\circ\text{C}$) and relative humidity ($65 \pm 5\%$). The cuvette temperature was regulated, and a standard CO₂ reference concentration of $390 \mu\text{mol mol}^{-1}$ was maintained. Parameters recorded included the net assimilation rate (An) [$\mu\text{mol (CO}_2\text{) m}^{-2} \text{ s}^{-1}$], intercellular CO₂ concentration (ci) [$\mu\text{mol (CO}_2\text{) mol}^{-1}$ (air)], stomatal conductance (gs) [$\text{mmol (H}_2\text{O) m}^{-2} \text{ s}^{-1}$], water use efficiency (WUE) [$\mu\text{mol (CO}_2\text{) mmol (H}_2\text{O)}^{-1}$] and transpiration rate (E) [$\text{mmol (H}_2\text{O) m}^{-2} \text{ s}^{-1}$]. Vapor Pressure Deficit (VPD, kPa) was also determined using the PP-System/Infrared Gas Analyzer (IRGA). Leaf chlorophyll content was determined with a chlorophyll meter 'SPAD-502' (Qureshi et al., 2022).

Experimental design and statistical analysis

Data analysis was conducted using two-way ANOVA to evaluate the effects of different rootstocks and years as fixed factors, with replicate blocks considered as random effects, employing a completely randomized block design. The analysis was completed with R software with packages such as stats, agricolae, and ggplot2 (R Core Team, 2018). Mean comparisons among rootstocks were carried out using Tukey's test at a 95% confidence interval. The Pearson correlation matrix method explored the interrelations among growth, leaf nutrient compositions, and physiological parameters across all rootstocks. Additionally, Principal Component Analysis (PCA) was used to examine the relationships and clustering patterns among the grafted plants (T1= Kinnow/C-35; T2=K/Carrizo; T3=K/Troyer; T4= K/Poncirus; T5=K/Cleoptera; T6=K/Cox; T7=Benton; T8=K/Rough lemon; T9= K/Sour orange).

Results

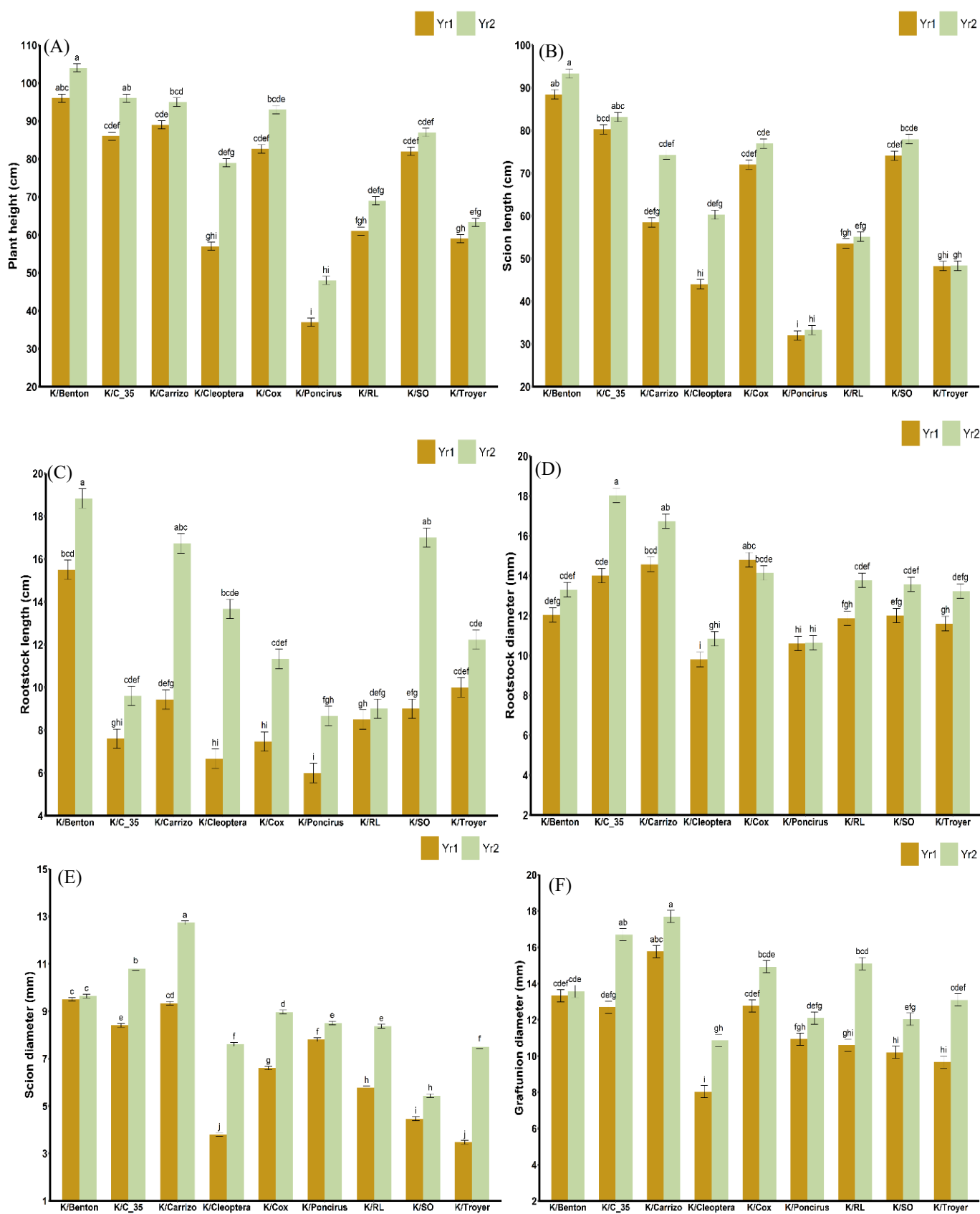
Soil analysis

Soil analysis revealed suboptimal conditions that may influence the rootstock and scion performance (Table 2). The soil showed a mildly alkaline pH, with consistent readings of 7.95 (0–20 cm), 7.97 (20–40 cm) and 7.98 (40–60 cm). This slight alkalinity suggests some potential challenges in nutrient availability, particularly for micronutrients like iron

and zinc, which are less soluble in alkaline soils. Organic matter contents ranged from 1.72% at 40–60 cm to 2.24% at 0–20 cm, indicating a decrease in depth. While these values are within the moderate range that they may limit soil fertility and microbial activity. Such conditions underscore the need for careful rootstock selection and soil management strategies to optimize nutrient uptake and support plant growth under these marginal soil conditions.

Growth parameters

The growth characteristics of 'Kinnow' were significantly affected by different rootstocks, years and their interactions at $P < 0.05$ significance level (Table 3). Specifically, 'Kinnow' grafted onto Benton rootstock exhibited increased plant height (105cm), scion length (94cm), rootstock length (18.9cm), and internode distance (3.3cm) in the year 2023 (Figure 1A, 1B, 1C and 1J). Conversely, 'Carrizo' rootstock resulted in a significant increase of scion diameter (13.3mm), rootstock diameter (18mm), graft union diameter (17.9mm), leaf number (530) and leaf area (37cm²) during the same year (Figure 1D, 1E, 1F, 1G and 1H). Shoot numbers for 'Rough lemon', 'Cox', and 'Carrizo' rootstocks remained similar, ranging from 2.3 to 2.4 in the year 2023 (Figure 1I). In our study, 'Poncirus' rootstock consistently exhibited reduced plant height, internode length, scion and rootstock length compared to all other rootstocks across both years.



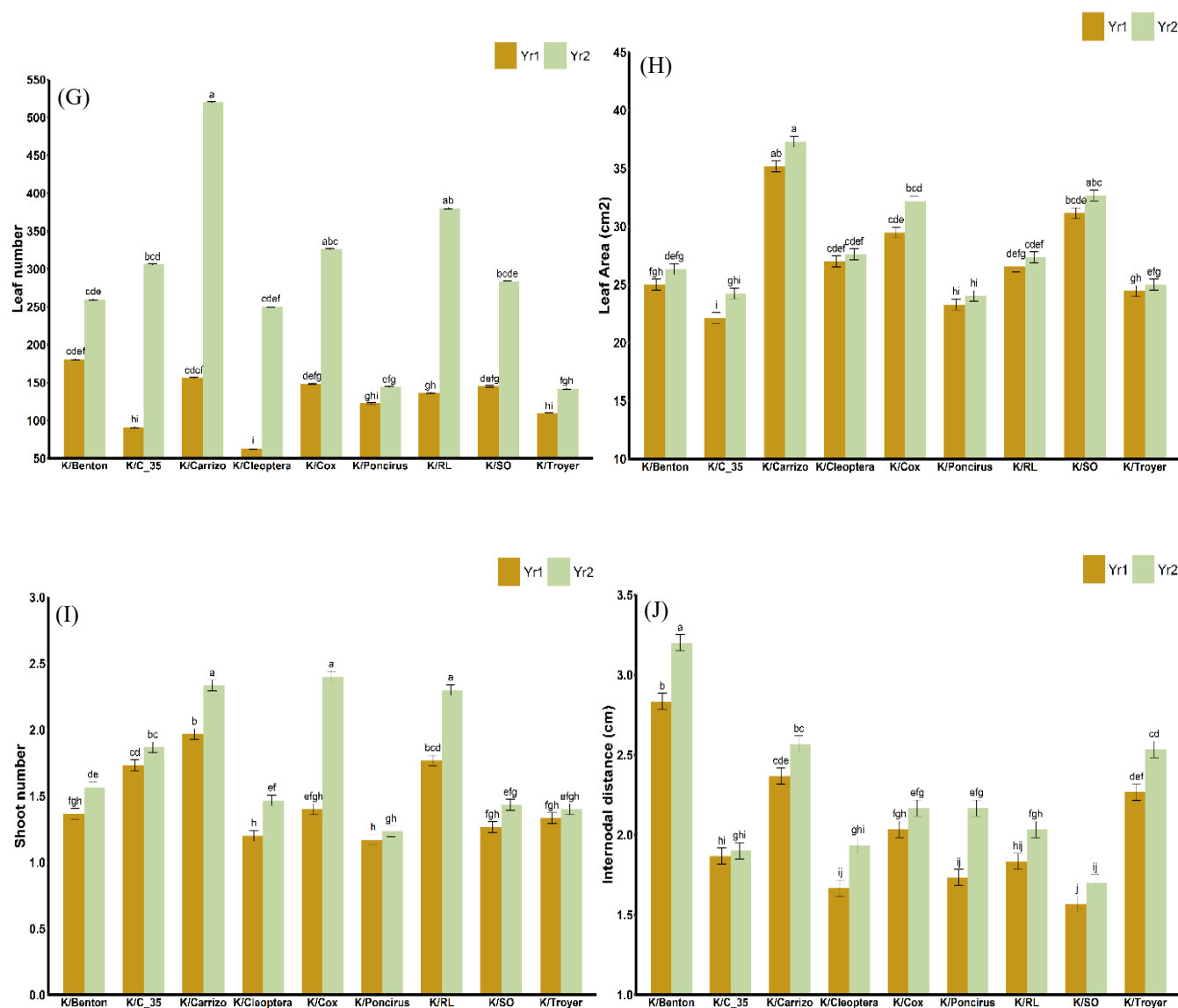


Figure-1. (A) Plant height (Scion-rootstock combinations (SC)= $P<0.001$; year (Y)= $P<0.002$; Scion-rootstock combinations $\times$ year (SC $\times$ Y)= $P<0.001$), (B) Scion length (SC= $P<0.001$; Y= $P<0.021$; SC $\times$ Y= $P<0.001$), (C) Rootstock length (cm) (SC= $P<0.001$; Y= $P<0.001$; SC $\times$ Y= $P<0.001$), (D) Rootstock diameter (SC= $P<0.001$; Y= $P<0.001$; SC $\times$ Y= $P<0.001$), (E) Scion diameter (SC= $P<0.001$; Y= $P<0.005$; SC $\times$ Y= $P<0.001$), (F) Graft union diameter (mm) (SC= $P<0.001$; Y= $P<0.001$; SC $\times$ Y= $P<0.0091$), (G) Leaf number (SC= $P<0.001$; Y= $P<0.001$; SC $\times$ Y= $P<0.001$), (H) Leaf area (cm²) (SC= $P<0.001$; Y= $P<0.002$; SC $\times$ Y= $P<0.001$), (I) Shoot number (SC= $P<0.001$; Y= $P<0.003$; SC $\times$ Y= $P<0.01$), (J) Internodal distance (cm) (SC= $P<0.001$; Y= $P<0.021$; SC $\times$ Y= $P<0.01$) for 'Kinnow' grafted on nine citrus rootstocks for two years. Error bars show standard error. Different letters account for significant differences among rootstocks using the Tukey's test ($P<0.05$) ($n = 4$).

Table-3. ANOVA for treatments (Scion-rootstock combinations), years and their interactions for all parameters.

Parameters	F-value		
	Year	Treat	Year:Treat
Plant Height (cm)	466.59*	690.9*	7.84*
Scion length (cm)	466.59*	690.92*	7.2**
Rootstock length (cm)	250.12*	67.82*	10.05Ns
Rootstock dia (mm)	376.93*	252.56*	7.17Ns
Scion dia (mm)	123.29**	264.46**	50.68*
Graft union dia (mm)	72.67*	42.85*	3.31*
Leaf number	135.1**	368.55*	94.38**
Leaf area (cm ²)	90.21*	157.31*	4.56*
Shoot number	130.06**	20.26Ns	12.92*
Internodal distance	194.97*	145.13**	7.55*
N (%)	601.5*	143.91*	23.5*
P (%)	70.09*	98.3*	3.36Ns
K (%)	275.3**	289.7**	12.2*
Fe (ppm)	284.48**	45.5*	6.33Ns
Zn (ppm)	165.6*	208**	18.8*
Mn (ppm)	269.1*	165.6*	12.5Ns
Cu (ppm)	93.9*	12.8*	3.19Ns
An	162.1*	289.3*	46.31*
E	52.2*	29.4Ns	1.44Ns
Ci	125.8*	306.3*	2.4Ns
gs	150.97*	255.99**	2.66*
WUE	908.5**	109.5*	29.7*
VPD	181.9*	217.8**	4.04*

*=significant; **=highly significant; NS= non-significant

Leaf mineral analysis

The choice of rootstock plays a significant role in determining the mineral composition of the scion leaves. Various studies have demonstrated the substantial impact of rootstocks on leaf mineral compositions in citrus trees. The foliar nutrient compositions of 'Kinnow' were significantly affected by the rootstocks, years, and their interactions, with a significance level of $P < 0.05$ (Figure 2). In the year 2023, leaves of 'Kinnow' displayed a notable increase in nitrogen content when grafted onto Carrizo rootstock (5.2%) compared to Poncirus rootstock (2.5%) (Figure 2A). Similarly, higher leaf potassium (K) and phosphorus (P) contents were observed when

'Kinnow' was grafted onto Carrizo rootstock, showing 1.7% for P and 3.3% for K in 2023 (Figure 2B & 2C). Moreover, plants grafted onto Cleopatra, Poncirus and Troyer rootstocks had lower leaf K contents across both years, ranging from 1.5% to 1.8%. Leaf zinc (Zn) contents were not significant in 'Kinnow' grafted onto Carrizo and Rough lemon rootstocks in 2023, measuring 31 ppm (Figure 2E). However, plants grafted onto Rough lemon rootstock exhibited higher leaf manganese (Mn) contents in both 2022 (47.3 ppm) and 2023 (51.2 ppm) compared to other rootstocks (Figure 2F). Copper (Cu) contents were not significantly different among Poncirus, Sour orange and Troyer rootstocks across both years (Figure 2G).

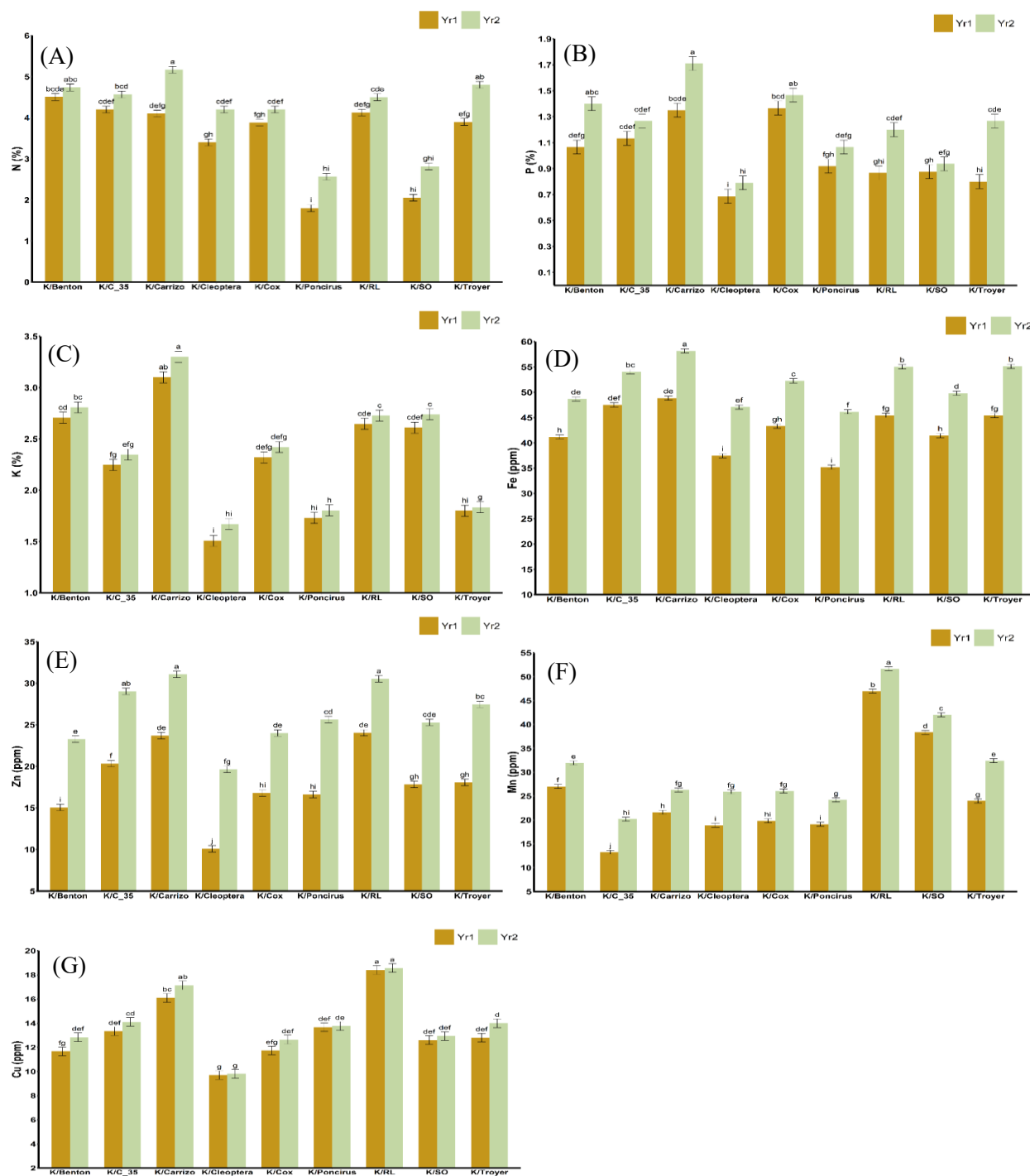
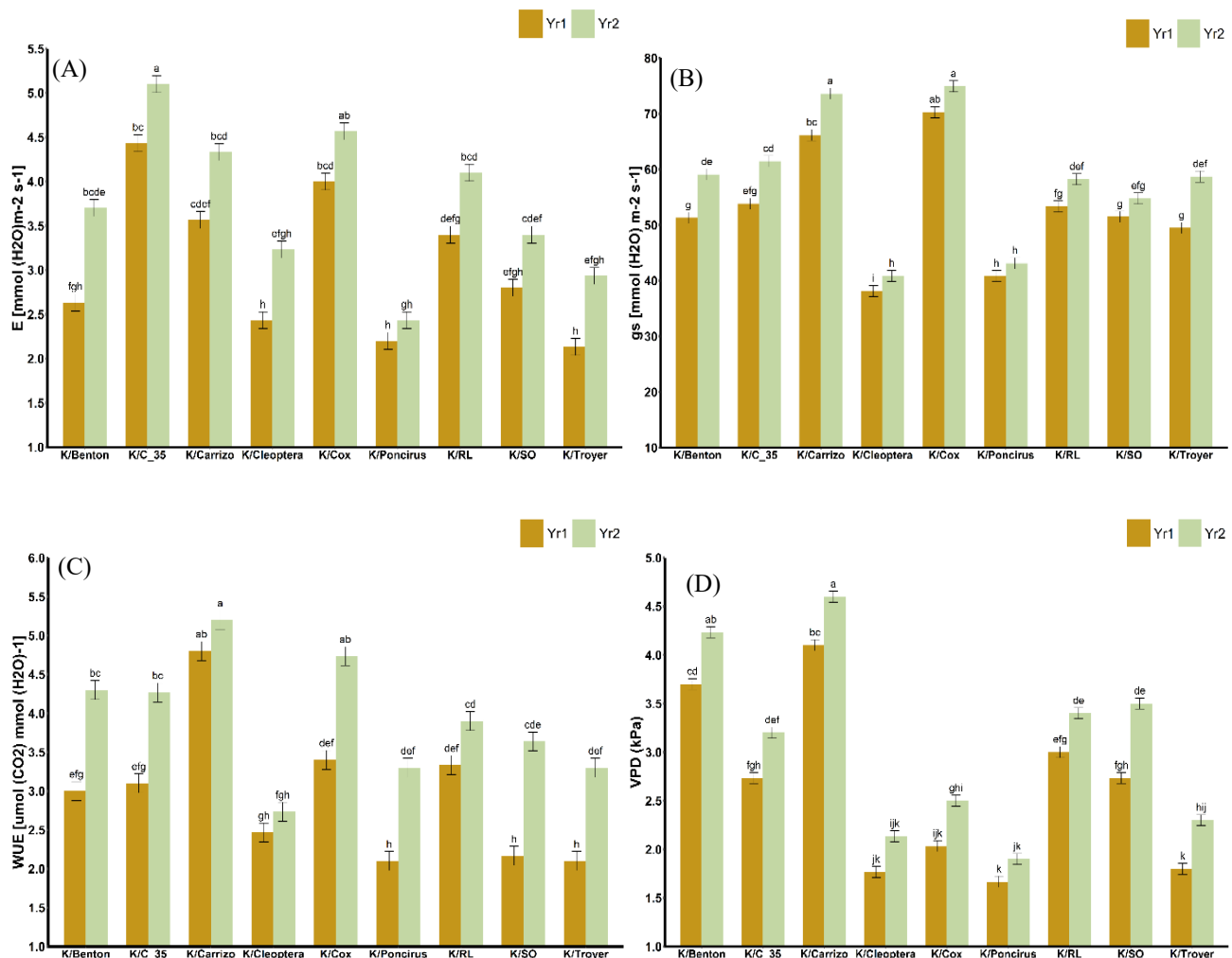


Figure-2. (A) Nitrogen (%) (Scion-rootstock combinations (SC)= $P<0.001$; year (Y)= $P<0.021$; Scion-rootstock combinations $\times$ year (SC $\times$ Y)= $P<0.001$), (B) Phosphorus (%) (SC= $P<0.001$; Y= $P<0.001$; SC $\times$ Y= $P<0.001$), (C) Potassium (%) (SC= $P<0.001$; Y= $P<0.041$; SC $\times$ Y= $P<0.001$), (D) Iron (ppm) (SC= $P<0.001$; Y= $P<0.001$; SC $\times$ Y= $P<0.001$), (E) Zinc (ppm) (SC= $P<0.001$; Y= $P<0.001$; SC $\times$ Y= $P<0.001$), (F) Manganese (ppm) (SC= $P<0.001$; Y= $P<0.021$; SC $\times$ Y= $P<0.05$), (G) Copper (ppm) (SC= $P<0.01$; Y= $P<0.02$; SC $\times$ Y= $P<0.05$), of 'Kinnow' leaf grafted on nine citrus rootstocks for two years. Error bars represent standard error. Different letters account for significant differences among rootstocks using the Tukey's test ($P<0.05$) ($n = 4$).

Physiological /gas exchange measurements

Significant variations in leaf 'gs', 'ci', 'E', WUE and VPD of 'Kinnow' were observed among all rootstocks across both years at a significant level of $P < 0.05$ (Figure 3). In 2023, 'Kinnow' leaves showed higher net photosynthetic rate (An) values [$8.67 \mu\text{mol} (\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$] when grafted onto Carrizo rootstock, with no statistically significant differences observed among other rootstocks in either year (Figure 3A). Higher 'ci' values [290 and $285 \mu\text{mol} (\text{CO}_2) \text{mol}^{-1} (\text{air})$] (Figure 3B) and WUE [5.3 and $5 \mu\text{mol} (\text{CO}_2) \text{mmol} (\text{H}_2\text{O})^{-1}$] (Figure 3C) were consistently recorded in plants grafted onto Carrizo rootstock in 2022 and 2023, respectively, whereas VPD values were only higher (4.6 kPa) on Carrizo rootstock during 2023 (Figure

3D). Carrizo and Cox rootstocks revealed no significant changes in 'gs' values of 'Kinnow' leaves throughout both years (Figure 3E). C-35 and Cox rootstocks demonstrated higher transpiration rate (E) values [$5.2 \text{ mmol} (\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$ and $4.6 \text{ mmol} (\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$] in 2023 (Figure 3F). Higher leaf chlorophyll concentration (SPAD) was observed in plants grafted onto Carrizo rootstock during 2023, while differences in leaf chlorophyll concentration (SPAD) were considered insignificant for all other rootstocks across both years. Notably, plants grafted onto Poncirus rootstock consistently exhibited lower values of VPD, gs, ci, and E compared to the other rootstocks across both years.



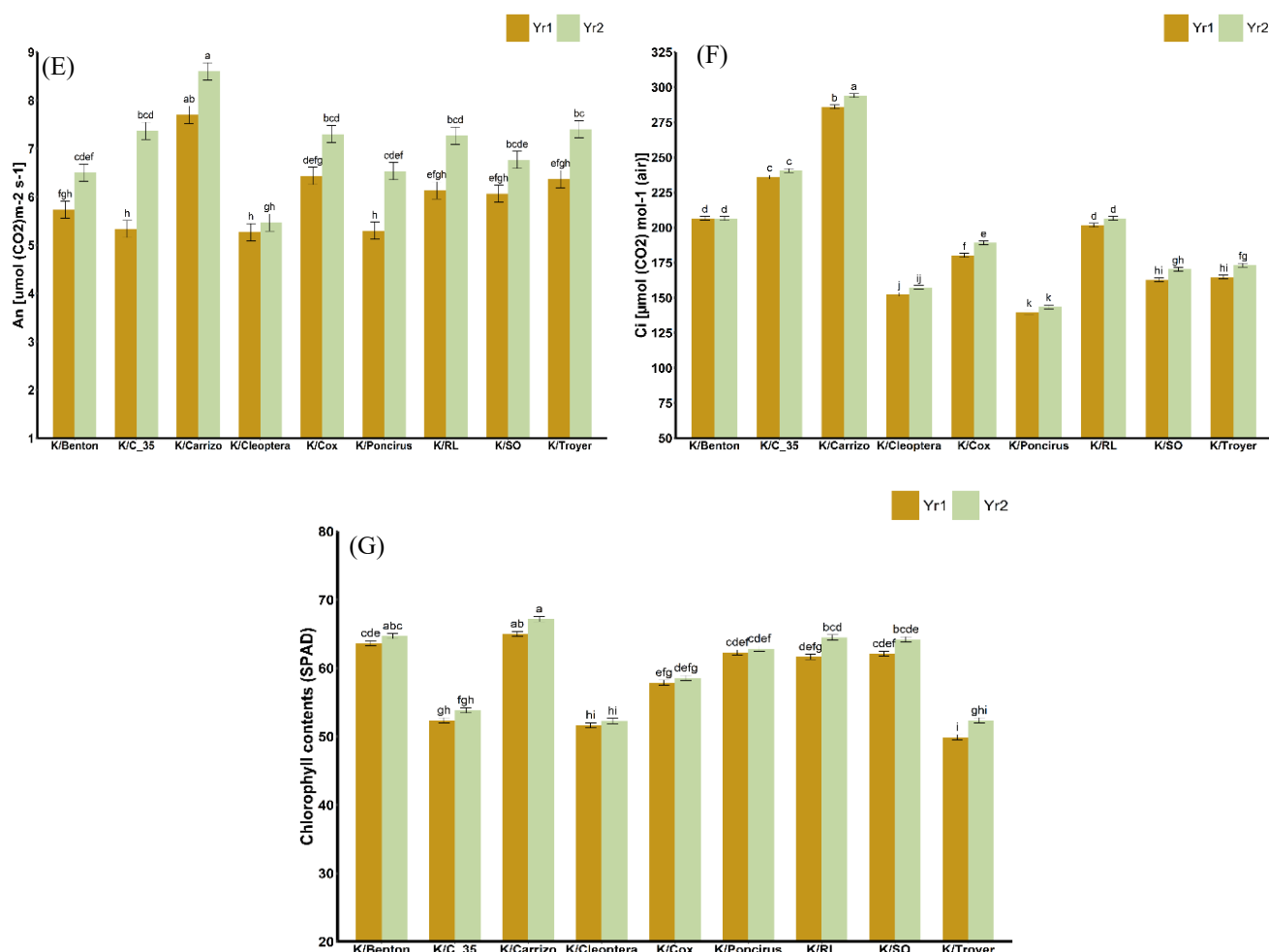


Figure-3. (A) Net assimilation rate, 'An' [$\mu\text{mol} (\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$] ($SC=P<0.001$; $Y=P<0.001$; $SC \times Y=P<0.01$); (B) Sub-stomatal conductance 'ci' [$\mu\text{mol} (\text{CO}_2) \text{mol}^{-1} (\text{air})$] ($SC=P<0.001$; $Y=P<0.001$; $SC \times Y=P<0.02$); (C) WUE [$\mu\text{mol} (\text{CO}_2) \text{mmol} (\text{H}_2\text{O})^{-1}$] ($SC=P<0.001$; $Y=P<0.001$; $SC \times Y=P<0.011$); (D) Vapour pressure deficit (kPa) ($SC=P<0.001$; $Y=P<0.001$; $SC \times Y=P<0.0029$); (E) Stomatal conductance, 'gs' [$\text{mmol} (\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$] ($SC=P<0.001$; $Y=P<0.001$; $SC \times Y=P<0.0301$); (F) Transpiration rate, 'E' [$\text{mmol} (\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$] ($SC=P<0.001$; $Y=P<0.001$; $R \times Y=P<0.022$); and (G) Chlorophyll contents (SPAD) ($SC=P<0.001$; $Y=P<0.001$; $SC \times Y=P<0.01$) in 'Kinnow' leaf grafted on nine citrus rootstocks for two years. Error bars represent standard error. Different letters account for significant differences among rootstocks using the Tukey's test ($P<0.05$) ($n = 4$).

Correlation

Statistical analysis revealed significant correlations among the studied parameters at both $P<0.01$ and $P<0.05$ levels (Figure 4). A strong association was identified among graft union diameter, WUE, ci, and P content ($P<0.01$), as well as among Fe contents, WUE and graft union diameter ($P<0.05$) across all grafted plants. Positive correlations were also noted among An, ci, WUE, and gs in leaf samples at $P<0.05$.

Similarly, An exhibited a positive relationship with K contents, leaf number, shoot number and graft union diameter at $P<0.05$. For nutrient elements, K contents demonstrated strong correlations with Fe and Zn contents ($P<0.01$), while P contents showed a notable relationship with Fe contents ($P<0.05$). Furthermore, ci was strongly associated with rootstock, scion, graft union diameter, WUE and VPD at $P<0.01$ for all evaluated grafted plants.

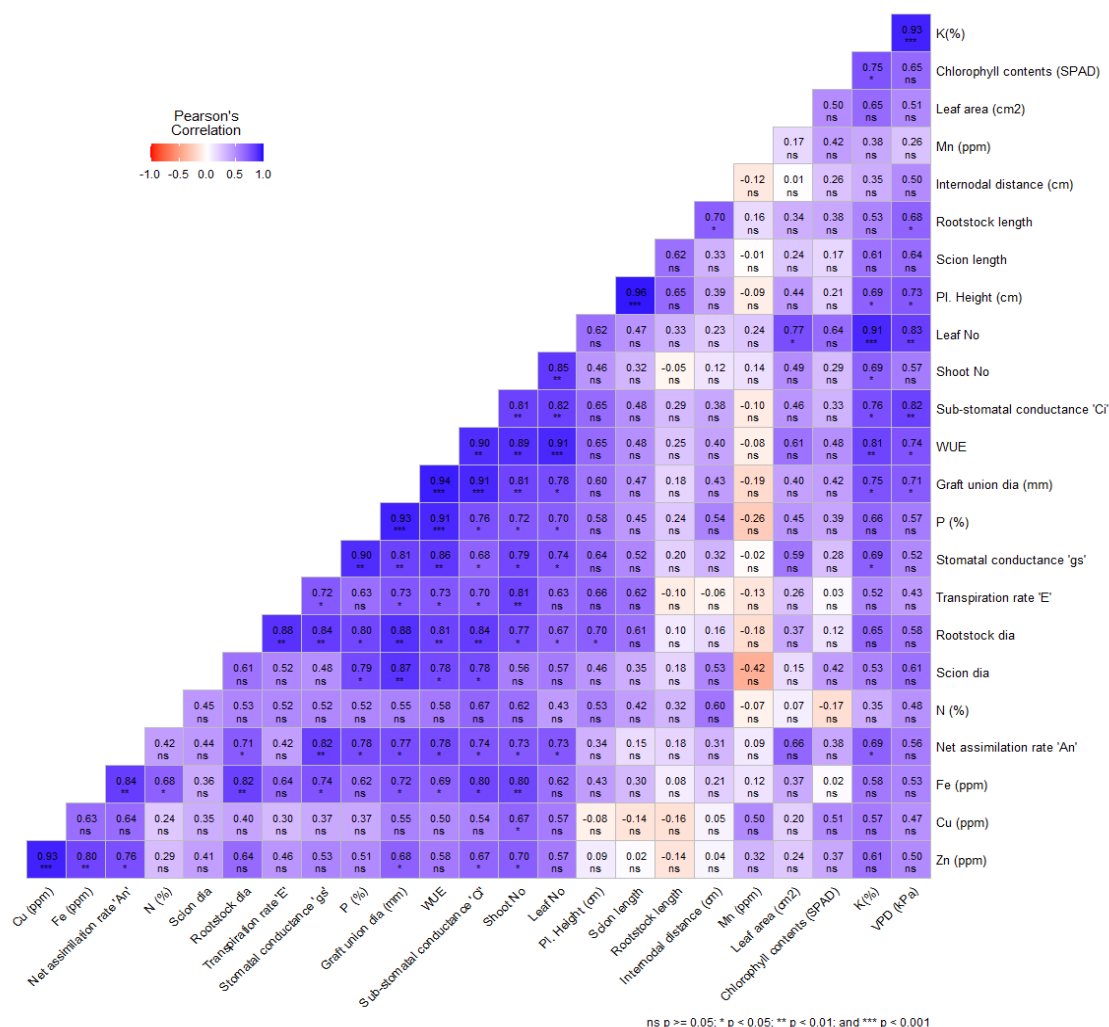


Figure-4. Correlations among plant growth, leaf nutrient compositions and physiological measurements ($*$ = $P<0.05$; $**$ $P<0.01$) for 'Kinnow' grafted on nine rootstocks. Each data point represents the mean for both years for each grafted plant.

Principal component analysis

To gain a comprehensive understanding of the performance of the nine rootstocks and identify the most appropriate one for the scion varieties, a PCA was conducted. The first PCA1 accounted for 55.2% of the total variance, showing that it represents most of the variability among the evaluated parameters. PCA1 was strongly associated with rootstock length, plant height, vapor pressure deficit (VPD), nitrogen content (N%), scion diameter and phosphorus contents (P%), suggesting that these traits are key indicators of rootstock performance (Figure 5).

The second principal component (PCA2) explained an additional 12.7% of the variance, contributing to a cumulative 67.9% of the total variance explained by the first two components. The PCA1 and PCA2 analysis clearly distinguished the rootstocks, with separate clustering patterns based on their growth and physiological attributes. For illustration, rootstocks with more values of P% and VPD were grouped, whereas those with greater scion diameter and plant height molded another cluster. These findings highlight the heterogeneity among the rootstocks and show the traits that contribute ominously to their differentiation.

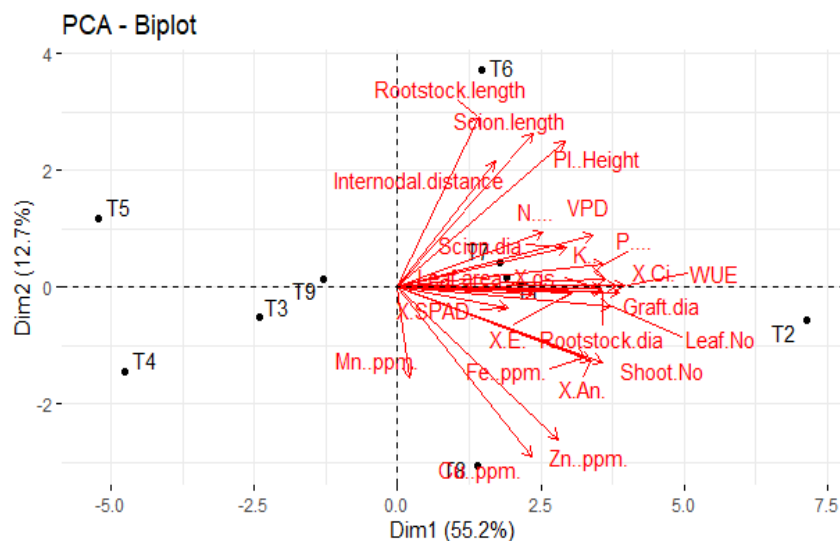


Figure-5. Principal Component Analysis for plant growth, nutrient composition, and physiological measurements of 'Kinnow' grafted on nine citrus rootstocks (T1= Kinnow/C-35; T2=K/Carrizo; T3=K/Troyer; T4= K/Poncirus; T5=K/Cleopatra; T6=K/Cox; T7=K/Benton; T8=K/Rough lemon; T9= K/Sour orange).

Discussion

Rootstocks/scion combinations regulate the plant size, yield and fruit quality traits and used to improve tolerance to abiotic as well as biotic stresses, helps the plant to adapt to different environmental conditions (Forner-Giner et al., 2020; Salih and Al-Jeboory, 2023). In this study, a significant impact of varied citrus rootstocks on the growth performance of 'Kinnow' variety was noticed across both years. Overall, Carrizo rootstock showed more compatibility with the Kinnow and improved its growth traits including, leaf area, leaf number, scion, rootstock and graft union diameter across both the years. Our observations are in line with the findings of Hayat et al. (2022), who observed a noticeable impact of Citrus rootstocks on the vegetative growth of grafted 'Shatangju' plants. Likewise, Qureshi et al. (2022) described differences in morphological traits, fruit quality and yield in 'Kinnow' mandarin when grafted onto different citrus rootstocks. Moreover, an earlier study examined the influence of varied rootstocks on vegetative growth and yield of lemon cultivars, and it was found that 'RLC-4' and 'Rough Lemon' rootstocks resulted in more canopy size and tree height, while, the 'Billikichlli' produced the increased trunk cross-sectional area "TCSA" (Dubey and Sharma, 2016). In another study, different rootstocks including 'Pujiang Xiangcheng', sour pummelo and trifoliate orange were assessed for photosynthesis, phytohormones, fruit

quality, growth performance of *Citrus maxima* (Burm.) var. 'Guanxi Miyou'. It was suggested that the 'Pujiang Xiangcheng' was more suitable rootstock as compared to the other rootstocks (Xie et al., 2022; Mahmood et al., 2021). Our findings indicate that the use of Poncirus rootstock led to growth reduction, which is in line with the previous reports showing Poncirus as a semi-spreading/dwarfing rootstock with moderate vigor (Hayat et al., 2022; Khan et al., 2022). Earlier studies demonstrated reduced productivity in citrus trees grafted onto Cleopatra rootstock, which showed that the type of rootstocks is important in determining the plant growth and vigor (Jayswal et al., 2020).

The selection of rootstock impacts the micro- and macronutrient transport and absorption in fruit crops (Shahkoomahally et al., 2020). Citrus nutrient deficiency resistance is mainly determined by the specific rootstock type and its capacity to activate biochemical, physiological and morphological mechanisms at root level (Pestana et al., 2023). In this study, all rootstocks showed significant effects on the uptake of both macro and micronutrients in the grafted plants over both experimental years. Research by Shahkoomahally et al. (2020) and Sarkhosh et al. (2021) highlighted the diverse genetic capabilities of different rootstocks, particularly in terms of mineral element absorption and hydraulic conductance capacity, which contribute to variations in leaf mineral compositions. The mineral contents increased by

different rootstocks might be due to the more enhanced root absorption and efficient minerals translocation to the above parts (Khan et al., 2020). Rootstock significantly influenced the leaf macro-micro elements compositions of different plants, such as cherries, peaches and plum (Reig et al., 2018). Our results showed that, plants on Carrizo rootstock had higher Fe contents during 2023, whereas, plants on Rough lemon had higher leaf Mn contents during 2022 and 2023. The impact of trifoliate orange and citrange rootstocks on nutrient uptake of navel oranges was determined by Liu et al. (2020), who noted that the nutrient accumulation increased steadily, and the seasonal patterns of nutrients were similar for both rootstocks. In another study, Navel oranges had more leaf number, scion and root length, and the higher contents of N, K, P, Fe, Mg, Mn, Zn when budded on *C. volkamariana* (Yahia et al., 2019; Khan et al., 2024). Moreover, Qureshi et al. (2022) found that the rootstock affected the mineral distribution and uptake in Kinnow mandarin.

In our investigation, different rootstocks had marked variations in Kinnow physiological parameters viz., An, ci, E, WUE, VPD, and gs. Our findings are in line with the previous studies who reported notable differences in photosynthetic performance, physiological characteristics and growth of 'Yuanxiaochun' scion grafted onto various rootstocks (Wang et al., 2020). Similarly, physiological differences, including chlorophyll contents and stomatal conductance were reported when different orange varieties were grafted onto diverse rootstocks (Santos et al., 2019). Changes in photosynthetic activity were noticed when sweet oranges were budded onto different rootstocks (Ribeiro et al., 2014). Changes in the photosynthetic behavior and growth of orange trees was also studied on different rootstocks (Machado et al., 2010; Cimen et al., 2014).

Significant correlations were identified among the tested variables, for example, a strong correlation among WUE, graft union diameter, P contents and ci was noted across all the grafted plants. The findings by Zhang et al. (2017) align with our results, highlighting the impact of P availability on leaf area. Insufficient phosphorus levels reduced leaf area, subsequently affecting the photosynthetic capacity and plant growth. Moreover, the studies by Li et al. (2022) emphasized the correlation between leaf N, P contents and WUE in *M. sibirica*. A positive correlation was noticed among different physiological parameters viz., An, gs, ci and WUE. Previous studies by Hatfield and

Dold (2019) determined the complex relationship between transpiration and photosynthesis, particularly for water-deficit conditions, showing its applicability for WUE of genetic materials. Our results align with the findings of Medrano et al. (2002) and Flexas et al. (2002). Additionally, the correlation between 'gs' and 'An' was found in different barley genotypes by Condon et al. (2002) agrees with our results. Moreover, Jiang et al. (2006) described vigorous relationships, not only between gs' and 'ci', but also between 'gs' and 'An' or 'An' and 'ci', among fourteen barley genotypes. Likewise, our consequences revealed a strong correlation between vital nutrients such as phosphorus and 'An' among all grafted plants. These findings emphasize the substantial influence of nutrient balance on photosynthesis, showing the impact of macro-micronutrient availability on these essential physiological processes (Therby-Vale et al., 2022).

Conclusions

In conclusion, the study emphasizes how different citrus rootstocks influenced the growth performance and physiology of 'Kinnow' significantly. 'Carrizo' rootstock resulted in improved physiological attributes, plant growth and nutrient uptake remarkably, which showed that this rootstock can be a promising alternative for 'Kinnow' cultivation. These findings describe the valuable understandings for citrus cultivation, focusing on the importance of selecting appropriate rootstock, which is more compatible with the existing scion variety.

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Contribution of Authors

Mumtaz S, Khan MA & Shahzad A: Conceived idea, planned the experiments and edited manuscript.
 Mumtaz S: Conducted the experiments, collected data, prepared the initial draft and edited manuscript.
 Rana MR: Assisted in data analysis and reviewed manuscript.
 Shahzad A & Ali I: Managed the essential materials, reagents, analysis tools and reviewed manuscript.
 Khan MA: Reviewed & edited manuscript and provided funds.

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