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RESEARCH ARTICLE

Rootstock mediated enhancement of abiotic and biotic stress tolerance in acid lime *(Citrus aurantiifolia)*

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Abstract

The study aimed to evaluate the performance of different rootstocks for acid lime (*Citrus aurantiifolia*) under salinity and nematode stress, focusing on their impact on plant growth and biochemical parameters. Grafted combinations involving acid lime (PKM 1) scions with rangpur lime and wood apple rootstocks were tested for salinity tolerance and resistance to *Tylenchulus semipenetrans*. The study, conducted from 2023 to 2024 at Tamil Nadu Agricultural University, HC & RI, Coimbatore, Tamil Nadu, utilized varying sodium chloride (NaCl) concentrations to simulate salinity stress and nematode inoculation for biotic stress evaluation. Results indicated that the R5 combination (acid lime PKM 1 scion grafted onto rangpur lime) exhibited the highest salinity tolerance, evidenced by better chlorophyll retention, membrane stability, and enhanced activity of antioxidant enzymes such as catalase and superoxide dismutase. Similarly, T3 (acid lime PKM 1 scion grafted onto wood apple) plants demonstrated improved nematode resistance, marked by higher leaf phenol content and peroxidase activity, as well as a reduced nematode population. These findings suggest that grafting onto rangpur lime and wood apple rootstocks strengthens the physiological and biochemical mechanisms in acid lime, enabling better adaptation to environmental stresses. This study provides suitable rootstock options for enhancing acid lime productivity in areas impacted by nematode and salinity problems.

Keywords

abiotic stress; acid lime; biotic stress; *Citrus aurantiifolia*; grafting; rootstocks; stress tolerance

Introduction

Rootstocks are essential for growing fruit trees today and their value cannot be overstated. Rootstocks, the foundation of a tree's growth and productivity, are grafted onto scions to enhance their performance. The ability of rootstocks to adapt to biotic and abiotic challenges is crucial for citrus crops, as it greatly affects the trees' vigor, production and canopy (1). Citrus cultivation is declining as a result of these abiotic problems as well as biotic ones, such as disease and pest infestations. Both biotic and abiotic stressors pose a major threat to the global production and growth of plants.

Abiotic stress is extremely detrimental, leading to significant crop losses worldwide and a 50% reduction in the production of vital crops (2). Grafting is widely used in many plant species to enhance productivity, disease resistance and stress tolerance (3).

Due to nutritional imbalances caused by excessive soil salinity, osmotic stress and ion toxicity, citrus trees may experience stunted growth, leaf burn, defoliation and lower fruit yield and quality (4). One of the primary biotic limitations in citrus production is plant-parasitic nematodes, namely citrus nematodes *(Tylenchulus semipenetrans*). These nematodes can seriously harm roots, resulting in decreased absorption of nutrients and water, stunted development and reduced yield (5).

Rangpur lime *(Citrus × limonia*), a cross between a citron and a mandarin orange, is often referred to as lemandarin or mandarin lime. It is widely used as a rootstock in citrus orchards, especially in tropical and subtropical areas. Its capacity to provide the scion with advantageous features, such as increased productivity and stress tolerance, accounts for its appeal. Trees grown on rangpur lime rootstock generally exhibit rapid growth, early bearing and high production efficiency. Because of its adaptability and resilience to salinity stress, rangpur lime has become increasingly popular in various citrus-growing regions (6). Wood apple trees *Feronia limonia* (L.) Swingle, also called *Limonia acidissima* L., belongs to the Rutaceae family. Wood apple trees can be used as construction materials, rootstock for citrus crops, medicine, and raw materials for food and drink. Wood apples are endemic to the Indian subcontinent and Southeast Asia. It may be explored as a rootstock for susceptible crops, as it is drought-tolerant and resilient to a range of biotic and abiotic stresses. Wood apples can be used to create an acid lime graft that is resistant to various biotic and abiotic stresses that can reduce the yield of acid lime. Mandarins have been successfully grafted using wood apples as a rootstock. The Sathugudi cultivar, in particular, exhibits outstanding fruit quality despite the substantial dwarfing caused by this rootstock (7).

This study aimed to evaluate the performance of resistant rootstocks for salinity and nematode tolerance in acid lime. Additionally, it sought to identify the rootstock with the best growth and minimal toxicity symptoms under these stress conditions.

Materials and Methods

The experiment was conducted at the Tamil Nadu Agricultural University (TNAU), Horticultural College and Research Institute in Orchard, Coimbatore. With its loamy soils and high temperatures, this tropical region, which has moderate humidity, temperatures between 24°C and 35°C and 600–700 mm of annual rainfall, is a perfect place to research nematode tolerance and salinity under realistic agroclimatic conditions. Well-grown grafted combinations, which were 100 to 120 days old, were subjected to salt treatments. The experiment was carried out using a factorial completely randomized design (FCRD) with three replications.

Plant material and grafting procedure

The study materials collected (acid lime PKM 1, rangpur lime and wood apple rootstocks and seedlings) from Tamil Nadu Research Institutes and acid lime PKM 1 scions from TNAU Periyakulam. A scion shoot, 10-15 cm in length and similar in thickness to the rootstock, was selected. At the time of grafting, the rootstock measured 15-20 cm in height and had 17-20 leaves. The basal end of the scion was angled to create a wedge shape. The scions were wrapped in damp fabric and treated with a 0.1% Bavistin solution. A wedge grafting technique was used, cutting the rootstock longitudinally and inserting a wedge-shaped scion. The grafts were kept in a mist chamber with controlled humidity and temperature for 30 days post-grafting.

Grafting combinations

T1	Wood apple alone
T2	Acid lime alone
T3	Rangpur lime alone
T4	Acid lime PKM 1 grafted on wood apple rootstock
T5	Acid lime PKM 1 grafted on Rangpur lime rootstock

Experiment 1: Screening for salinity tolerance

Plants were irrigated with sodium chloride (NaCl) dissolved in water as a stress therapy. The pot-weight-based approach was used to monitor the soil moisture content and plants were frequently watered with salt-dissolved water to reach the target concentration of NaCl, ensuring adequate leaching from the bottom of the polybags.

Salt treatment details

Treatment	NaCl concentration
S1	0 mM (Control)
S2	30 mM NaCl
S3	60 mM NaCl
S4	90 mM NaCl
S5	120 mM NaCl

Salinity levels of 30 mM, 60 mM, 90 mM, and 120 mM were achieved by dissolving different quantities of NaCl in one liter of water. Using an electrical conductivity (EC) meter, the salinity of each solution was confirmed. For 15 days, 100 mL of saline water was applied to each pot and irrigated with plants between 9:00 and 11:00 AM. In this Factorial Completely Randomised Design experiment with three replications, seedlings and grafted combinations were subjected to salt treatments (8).

Experiment 2: Screening for nematode tolerance

To identify citrus rootstocks resistant to *Tylenchulus semipenetrans*, rangpur lime (*Citrus limonia*) and wood apple (*Limonia acidissima*) were selected for screening based on their known resistance. Six-month-old, grafted seedlings of these rootstocks, grafted with acid lime (PKM 1), were maintained in the greenhouse.

Nematode collection and inoculation procedure

Nematodes were collected from 20-year-old citrus trees at TNAU, Coimbatore that were naturally afflicted. After digging up infected root samples, the nematodes were separated and identified as *Tylenchulus semipenetrans*. Using a modified Baermann funnel method, second-stage juveniles (J2) were extracted to create the inoculum. A solution containing a certain quantity of nematodes was placed around the root zone of each seedling to ensure successful infection.

Growth circumstances and combinations of grafting

The uniformly sized grafted plants were placed into earthen pots filled with a nutrient-rich medium. The medium included perlite for improved drainage and root oxygen, bark humus for added nutrients, sand to enhance drainage and peat moss to retain moisture, supporting healthy root growth. These components are commonly used in potting media and their effects on growth are typically tested in experiments to ensure optimal conditions. The pot culture experiment, which included three treatments and five replications, was set up in a completely randomized design using the 7-inch and 9-inch pots. Each day, water-soluble fertilizers were applied to the plants, along with daily irrigations using deionized water. At TNAU in Coimbatore, the greenhouse was kept at 30°C during the day and 26°C at night, with a relative humidity of 70–82%.

T1 Acid lime PKM 1 seedling alone susceptible (Control)

T2 Acid lime (PKM 1) grafted onto rangpur lime.

T3 Acid lime (PKM 1) grafted onto the wood apple.

Aftercare

Water was added to the pots throughout the trial. The pots were frequently moved to reduce the impact of sunslight and shadow. Four months after inoculation, the plants were carefully pulled out with a shovel and their roots were gently cleaned in a basin of water. The plants' biochemical changes and the number of nematodes in the soil (200 cc of soil) were recorded.

Biochemical analysis

For each replication in the salinity and nematode experiments, biochemical assays were conducted in triplicates to ensure precision and consistency.

Estimation of total chlorophyll content

Using Arnon's formula, determine the chlorophyll concentration and express it as mg/g fresh weight (9).

Estimation membrane stability index (MSI)

The membrane stability index (MSI) of the leaf sample was estimated and expressed in percentage (10).

Estimation of proline

The method for estimation of proline content was adopted from a previous study with slight modifications (11). Measure the intensity of the red colour at 520 nm. The proline content is expressed in mg/g.

Estimation of phenols

The total phenolic content was determined using the Folin -Ciocalteu reagent method (12). The amount of total phenolics was quantified and expressed in mg/g.

Estimation of catalase activity

Catalase activity was measured using a titration method with potassium permanganate and the results were expressed in units of µg H_2O_2 g^{-1} min^{-1} (13).

Estimation of peroxidase activity

Peroxidase (POD) activity in the samples was determined and expressed as absorbance units per gram of fresh weight (g^{-1} FW) (14).

Estimation of polyphenol oxidase

One gram of root and leaf samples was mixed with two milliliters of 0.1 M sodium phosphate buffer (pH 6.5), and the mixture was centrifuged for fifteen minutes at 4°C at 16,000 rpm. The supernatant obtained served as the enzyme source. To create the reaction mixture, 200 µL of the enzyme extract and 1.5 mL of sodium phosphate buffer (pH 6.5) were combined. To initiate the reaction, 200 µL of 0.01 M catechol was added and the change in absorbance at 495 nm per minute per gram of material was used to calculate the enzyme activity (15).

Superoxide dismutase activity (SOD)

The activity of superoxide dismutase (SOD) in leaf samples was measured using the method outlined by Fridovich and is reported as units mg^{-1} protein min^{-1} (16).

Nitrate reductase activity (NRase activity)

Nitrate reductase activity was determined in fully expanded functional leaves (17). The enzyme activity was expressed as µg NO2 g^{1} h^{1} using KNO2 as a standard.

Statistical analysis

Experiment I was conducted under a factorial completely randomised design (FCRD), while Experiment II was established and conducted using a completely randomized design (CRD). The significance of data was established at $p = 0.05$. The statistical analysis was carried out using OP STAT (Operational Statistics) and ICAR – WASP (Web Agri Stat Package).

Results

Experiment I: Salinity tolerance

Morphological characters

Plant height and leaf area

As shown in Table 1, the impact of salt on plant biomass was noted for five distinct rootstocks at varying salinity concentrations. The plant height of R1 (wood apple seedlings) gradually decreased from 35.15 cm at 0 mM to 27.94 cm at 120 mM, indicating a definite adverse effect of higher salinity. The plant height of R2 (acid lime seedling) decreased from 38.35 cm at 0 mM to 29.76 cm at 120 mM, indicating a similar trend. Under no stress, R3 (rangpur lime seedling) showed the greatest height (43.77 cm), but at 120 mM, this value dropped to 31.45 cm. A steeper

Table 1. Effects of salinity on plant growth parameters of various citrus rootstocks

Rootstock / seedings	Salinity conc.	Plant height (cm)	Leaf area (cm²)	Root length (cm)	Root fresh weight	Root dry weight	Stem diameter (mm)
R1	0 mM	35.15	17.89	15.72	1.9	0.59	14.1
	30 mM	34.31	17.69	15.56	1.82	0.41	13.5
	60 mM	33.52	17.3	15.23	1.67	0.32	12.1
	90 mM	30.71	16.67	14.37	1.5	0.27	10.9
	120 mM	27.94	15.06	13.37	1.31	0.19	9.8
R2	0 mM	38.35	23.67	17.7	2.37	0.73	18.2
	30 mM	36.54	23.1	17.67	2.26	0.5	17.7
	60 mM	35.37	20.19	17.5	2.03	0.41	16.5
	90 mM	32.11	19.27	15.25	1.86	0.29	15.4
	120 mM	29.76	19.01	14.75	1.63	0.25	13.1
R3	0 mM	43.77	25.35	19.09	2.76	0.94	20.5
	30 mM	42.05	24.81	18.21	2.61	0.82	19.4
	60 mM	40.23	22.66	18.01	2.32	0.7	18.2
	90 mM	36.06	21.43	17.04	1.97	0.45	17
	120 mM	31.45	19.01	15.69	1.67	0.31	15.2
R4	0 mM	32.78	26.55	18.02	2.54	0.89	19.6
	30 mM	29.49	25.67	17.43	2.37	0.75	18.8
	60 mM	28.3	24.42	17.16	2.19	0.63	17.4
	90 mM	23.95	20.26	16.29	1.9	0.46	16.1
	120 mM	19.88	18.22	14.22	1.46	0.23	15.7
R5	0 mM	45.36	27.19	18.63	2.98	1.07	123.6
	30 mM	43.62	26.42	18.2	2.73	0.81	20.3
	60 mM	42.53	25.13	18.05	2.51	0.69	19.5
	90 mM	40.33	23.04	17.87	2.22	0.5	18.2
	120 mM	37.02	21.54	15.77	1.79	0.34	17.4
SE d	Rootstock	0.28	0.17	0.16	0.02	0.006	0.01
	Salinity	0.28	0.17	0.16	0.02	0.006	0.01
Interaction	R×S	0.64	0.39	0.37	0.04	0.013	0.07
CD	Rootstock	0.58	0.35	0.33	0.04	0.012	0.03
	Salinity	0.58	0.35	0.33	0.04	0.012	0.03
Interaction	R×S	1.29	0.79	0.75	0.09	0.026	0.03

Significance at p = 0.05; treatment details: R1– wood apple seedlings, R2 - acid lime seedling, R3 - rangpur lime seedling, R4 - acid lime (scion) grafted on wood apple (rootstock), and R5 - acid lime (scion) grafted on rangpur lime (rootstock).

reduction was noted for R4 (acid lime grafted on wood apple rootstock), from 32.78 cm at 0 mM to 19.88 cm at 120 mM. R5 (acid lime grafted on rangpur lime rootstock) also followed a decreasing trend, from 45.36 cm to 37.02 cm under maximum salinity stress. The leaf area in R1 decreased from 17.89 cm^2 at 0 mM to 15.06 cm^2 at 120 mM. R2, which peaked at 23.67 cm^3, decreased to 19.01 cm^2 at the maximum salinity. At 25.35 cm^2, R3 had the largest initial leaf area, which decreased to 19.01 cm^2. With rising salinity, the R4 grafting combination similarly declined from 26.55 cm^3 to 18.22 cm^3, although it exhibited less steep reduction than R3 under optimal conditions. At 0 mM salinity, R5 had the largest leaf area, measuring 27.19 cm^2; however, this dropped to 21.54 cm^2 at 120 mM.

Root length and stem diameter

Root length in each rootstock was also influenced by salinity. At 0 mM, the root length in R1 was 15.72 cm, which decreased to 13.37 cm at 120 mM. The reduction in R2 root lengths from 17.7 cm to 14.75 cm, was significant. R3 maintained the longest roots, showing a reduction from 19.09 cm to 15.69 cm. Under 120 mM salinity, R4 experienced a consistent decrease in root length, from 18.02 cm to 14.22 cm, whereas R5 exhibited the least reduction, decreasing from 18.63 cm to 15.77 cm. The influence of salinity was also observed on stem diameter. The stem diameter in R1 decreased from 14.1 mm at 0 mM to 9.8 mm at 120 mM. R2 demonstrated a reduction, dropping from 18.2 mm to 13.1 mm. R3 had the largest stem diameter, which decreased from 20.5 mm at 0 mM to 15.2 mm at 120 mM. In R4, the stem diameter dropped from 19.6 mm to 15.7 mm and in R5, it decreased from 23.6 mm to 17.4 mm, respectively.

Root fresh and dry weight

As the salinity increased, the fresh weight of the roots decreased proportionately. At 0 mM, R1 weighed 1.9 g, but at 120 mM, it weighed 1.31 g. The root fresh weight of R2 declined to 1.63 g at 120 mM, whereas the root fresh weight of R3, which was the greatest at 2.76 g, decreased to 1.67 g. Reductions from 2.54 g and 2.98 g to 1.46 g and 1.79 g, respectively, were seen in the R4 and R5 combinations.

The effect of salinity on root dry weight followed a decreasing trend. In R1, dry weight dropped from 0.59 g at 0 mM to 0.19 g at 120 mM. R2 experienced a decrease from 0.73 g to 0.25 g, and R3 from 0.94 g to 0.31 g. R4 and R5 also exhibited a decrease, from 0.89 g and 1.07 g to 0.23 g and 0.34 g, respectively (Table 1).

Biochemical parameters

Total chlorophyll content and membrane stability index (%)

Chlorophyll levels decreased significantly with increasing salinity in all rootstocks. According to an analysis of total chlorophyll content, rootstock R5 consistently showed the highest chlorophyll content, suggesting superior salinity tolerance. Conversely, at higher salinity levels, rootstock R1 exhibited the lowest chlorophyll content, indicating a notable susceptibility to salinity stress. A similar pattern was also observed in the membrane stability index, where R1 showed significant decreases as salinity levels increased, despite retaining the highest stability at 0 mM salinity. Rootstock R5 exhibited the greatest reduction in stability with increasing salinity, while rootstock R2 displayed intermediate membrane stability (Fig. 1). These results demonstrate how different rootstocks respond to salinity, with R5 demonstrating the highest resistance in terms of membrane stability and chlorophyll retention.

Proline content and total phenols content

Proline and phenol contents of different rootstocks responded differently to rising salt levels. Proline levels generally increased as salinity rose, with R4 accumulating proline at the fastest rate, suggesting that it plays a role in osmotic adjustment under stress. Demonstrating improved defense mechanisms against oxidative stress, R5 consistently showed the highest phenol concentration across all treatments. In contrast, phenolic compounds increased significantly in response to salt stress and this trend was observed consistently across all rootstocks. The physiological responses of rootstocks to salt vary, as demonstrated by our data, which also highlight the capability of R4 to accumulate proline and R5 to strengthen phenolic responses (Fig. 2).

Catalase activity and peroxidase activity

Rootstock R5 exhibited the greatest enzymatic activity, indicating its greater ability to mitigate oxidative stress under saline conditions. Catalase activity increased with rising salt levels, with the range expanding to 1.39-1.96 (μ moles H_2O_2 hydrolysed mg^{-1} protein min^{-1}). Similarly, peroxidase activity also increased gradually with rising salinity, with rootstock R5 again demonstrating the highest activity, and highlighting its enhanced capacity to scavenge reactive oxygen species. The rootstocks R1 and R2 showed the lowest levels of catalase and peroxidase enzymatic activity, indicating a decreased ability to respond to stress (Fig. 3). These findings emphasize the importance of rootstock selection for optimizing enzymatic responses to salt stress, with R5 demonstrating the strongest defense systems.

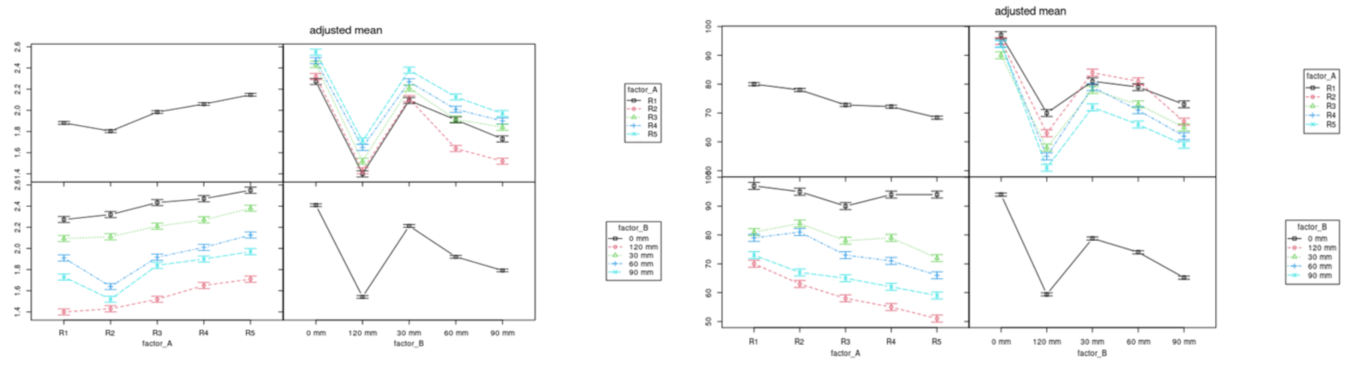
Superoxide dismutase and nitrate reductase activity

The study's findings showed that, as salt levels increased, there were notable differences in the activities of nitrate reductase (NR) and superoxide dismutase (SOD) among various rootstocks. Throughout all salinity treatments, Rootstock R5 consistently showed the stronger SOD activity, indicating greater enzymatic defense against oxidative stress. However, rootstock R2 had the lowest SOD activity, suggesting that it is less able to withstand oxidative damage caused by salinity. Nitrate reductase activity declines at higher salinity levels because excess salts disrupt nutrient uptake and induce osmotic stress, limiting the enzyme's function. Even in R5, these factors hinder optimal growth and enzyme activity, highlighting its efficient nitrogen absorption under stress (Fig. 4). On the other hand, rootstock R2 exhibited the lowest nitrate reductase activity, particularly at high salinity levels, indicating deficient nitrogen metabolism.

Experiment II – Neamtode tolerance

Morphological parameters

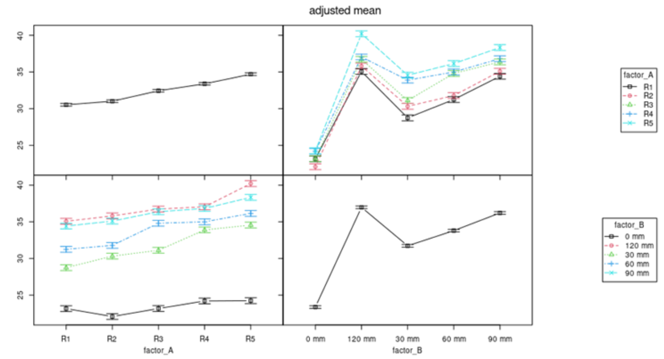
Table 2 presents data regarding plant biomass. The tallest plant, T2, measured 42.23 cm. T3 and T1 (acid lime), followed next measuring 37.80 cm and 29.34 cm, respectively. T1 (acid lime) exhibited far fewer leaves (9.00), whereas T3 had the most leaves (19.54), closely followed by T2 (18.25). The root length in T3 was longest, measuring 16.66 cm, with a statistically significant



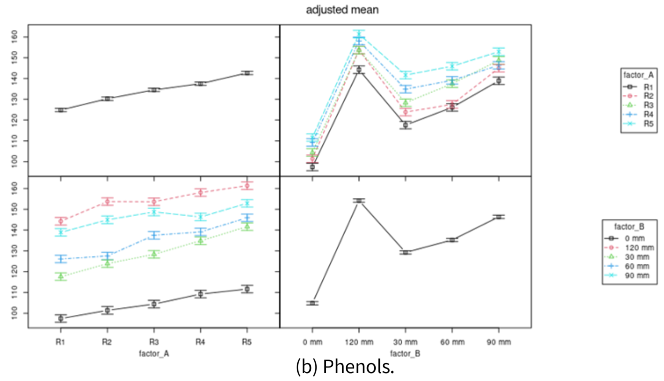
(a) Total chlorophyll content.

(b) Membrane stability index (%).

Fig. 1. Effect of salinity stress on (a) total chlorophyll content ($mg\ g^{-1}$) and (b) membrane stability index (%) in different citrus seedling and rootstock treatments.

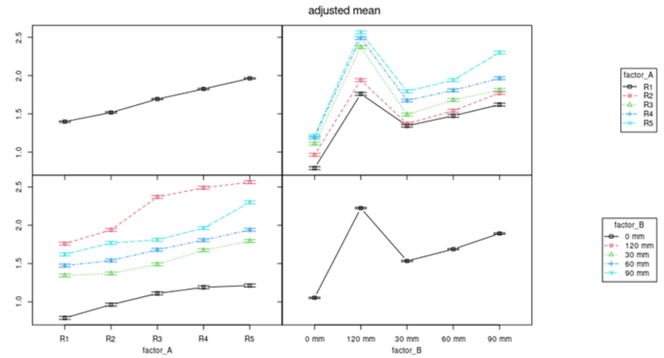


(a) Proline content.

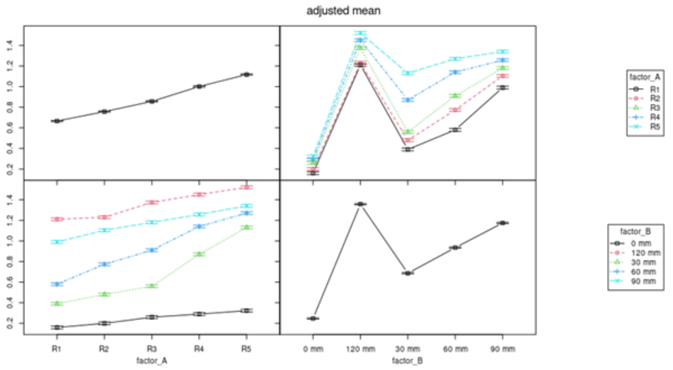


(b) Phenols.

Fig. 2. Effect of salinity stress on (a) leaf proline content (μg^{-1} of FW) and (b) total phenols content (mg g^{-1}) in different citrus seedling and rootstock treatments.

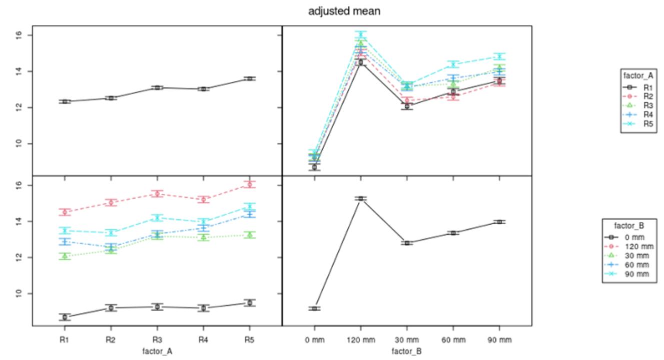


(a) Catalase activity.

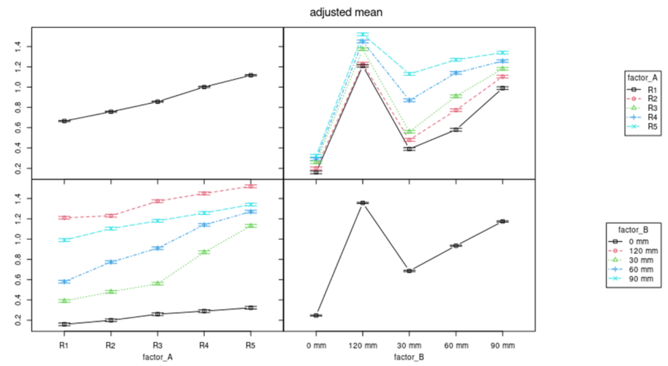


(b) Peroxidase activity.

Fig. 3. Effect of salinity stress on (a) catalase activity (μ moles H_2O_2 hydrolyzed mg^{-1} protein min^{-1}) and (b) peroxidase activity (absorbance units g^{-1} FW) in different citrus seedling and rootstocks treatments.



(a) Superoxide dismutase activity.



(b) Nitrate reductase activity.

Fig. 4. Effect of salinity stress on (a) superoxide dismutase (units mg^{-1} protein min^{-1}) and (b) nitrate reductase activity (μg NO_2 g^{-1} hr^{-1} FW) in different citrus seedling and rootstocks treatments.

Table 2. Effect of *T. semipenetrans* on plant growth parameters

Treatment	Plant height (cm)	Number of leaves	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Root dry weight (g)
T1	29.34	9.00	10.21	20.33	1.67	0.47
T2	42.23	18.25	15.34	29.01	2.11	1.34
T3	37.80	19.54	16.66	22.87	2.55	1.61
SE d	0.67	0.51	0.20	1.41	0.12	0.05
CD	1.524	1.16	0.46	3.20	0.27	0.13

Significance at p = 0.05;
Treatment Details: T1 –acid lime PKM 1 seedling alone susceptible (Control), T2 –acid lime (PKM 1) grafted onto rangpur lime, T3 –acid lime (PKM 1) grafted onto wood apple.

difference. T2 followed in next at 15.34 cm, while T1 (acid lime alone) followed in last at 10.21 cm. When compared to T1 (acid lime) recorded a shoot length of 20.33 cm. T2 had the longest shoot length (29 cm), followed by T3 (22.87 cm). These differences were statistically significant. T3 had the largest fresh and dry root weights, followed by T2, while T1's acid lime had the lowest.

Biochemical analysis

Total chlorophyll content and phenols

Across the three treatments, a decline in the overall chlorophyll content was recorded. T1 (acid lime) exhibited minimum chlorophyll, however, T2 recorded maximum, and it was closely followed by T3 (Fig. 1). T3 leaves recorded the highest phenol concentration which was comparable to T2's and lowest in T1 acid lime (Fig. 5). T3 exhibited the highest phenol level among the roots, followed by T2 and T1.

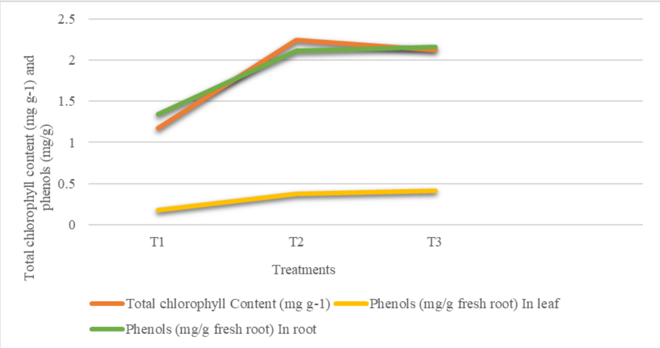


Fig. 5. The effect of *Tylenchulus semipenetrans* inoculum on chlorophyll content in leaf and phenols activity in both leaves and roots.

Peroxidase activity, polyphenol oxidase activity, and proline

Significant differences were seen in the peroxidase activity between the treatments for both the roots and the leaves. T3 (acid lime grafted onto wood apple) showed the highest peroxidase activity in leaves. In roots, T3 exhibited the maximum peroxidase activity again, followed by T2 and T1. Statistics showed that the variations in root peroxidase activity were also significant (Fig. 6).

Leaf polyphenol oxidase activity

In both leaves and roots, T3 exhibited the highest polyphenol oxidase activity, followed by T2 and T1. All differences were statistically significant. T3 again showed the highest polyphenol oxidase activity in the roots. Grafted plants had higher proline content in their roots and leaves. Following T2, T1, and T3 exhibit the highest proline activity in both leaves and roots. The variations were statistically significant.

Juvenile population/200 cc of soil and female nematode population/g of root

The corresponding population numbers for juveniles and total females of the treatments T1, T2 and T3 are shown in Fig. 7. T1 recorded the highest number of females (18) and juveniles (365). T2 exhibited a total of 220 juveniles and 7 females. T3 contained the minimum number of juveniles (143) and females (5). The significant difference in the number of juveniles suggests that the treatment have a major effect on juvenile survival rates or reproduction.

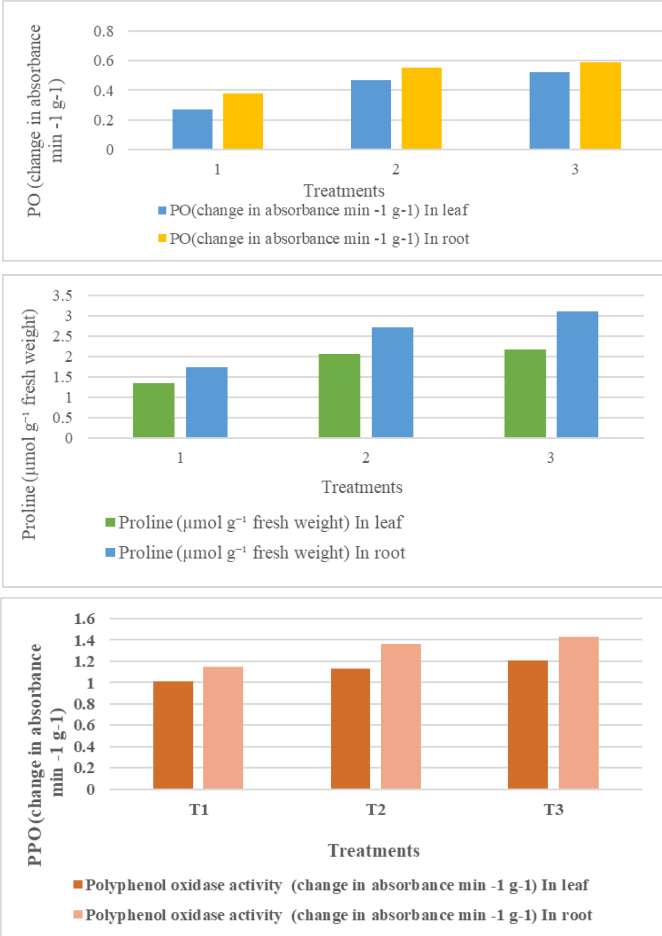


Fig. 6. The effect of *Tylenchulus semipenetrans* inoculum on peroxidase activity, proline, and polyphenol oxidase activity in both leaves and roots with juvenile population in 200 cc of soil and female nematode population per gram of root.

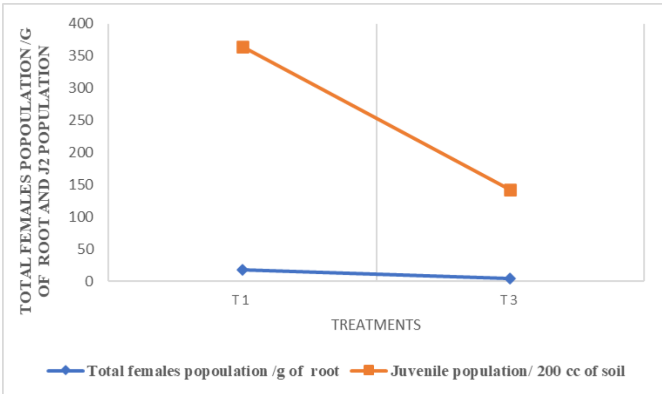


Fig. 7. Total female nematode population per gram of root in *Tylenchulus semipenetrans* infected acid lime and grafting.

Discussion

Effect of salinity on plant growth parameters and biochemical parameters

In a salty environment, an overabundance of sodium and chloride ions can alter plants' nutritional balance, resulting in reduced biomass. The detrimental effects of salt include altered plant development, leaf damage, and reduced nutrient absorption, all of which contributing to a decline in plant growth (18). The results of the experiment are in consistent with research findings from studies conducted on mangoes. The findings revealed that when saline levels increased in all soil types, plant morphological traits including height, number of leaves and spread were adversely impacted (19).

This rootstock combination might have improved salt tolerance mechanisms, as evidenced by R5 (acid lime grafted onto rangpur lime), which maintaining higher chlorophyll levels under stress. One of the most important factors in determining a plant's resistance to salt stress is membrane stability, which reflects, how cellular membranes functions under stressful conditions. Remarkably, the grafted combinations (R4 and R5) showed lower MSI levels, despite better performance in chlorophyll-related parameters. This suggests that multiple pathways contribute to preserving photosynthetic efficiency and membrane stability during salt stress, indicating that chlorophyll retention and membrane stability may not always be directly correlated (20).

Proline helps protect against osmotic dehydration, and under extreme salt stress (120 mM NaCl), its accumulation notably increases in R5, likely due to enhanced proline biosynthesis or regulation of proline degradation pathways. These results are align with the previous studies, which observed that phenolic compounds accumulated in various plants, including citrus, under diverse stressors (21). Previous research indicates that abiotic stressors often lead to an increase in phenol synthesis, which serves as a protective mechanism (22).

Grafted plants, particularly R5, showed increased activity of antioxidant enzymes like catalase, peroxidase, and superoxide dismutase (SOD) under salt stress, indicating enhanced salt tolerance. The increase in catalase and peroxidase activity suggests that grafting enhanced the plant's ability to scavenge reactive oxygen species (ROS), reducing oxidative damage. The elevated SOD activity further indicates that grafting strengthens the plant's initial defense against superoxide radicals, a key aspect of the antioxidant defense system. These findings align with previous research showing that grafting improves a plant’s resilience to stress (23, 24). NRase activity, the primary source of inorganic nitrogen for plants, is highly sensitive to stress, with even small amounts reducing its level. Salinity-induced inhibition of NRase activity in leaves is primarily due to reduced NO xylem transport. NRase activity can serve as an indicator of a plant's ability to tolerate stress. Grafting, particularly R5, is beneficial for preserving nitrogen metabolism under salt stress (25).

Effect of *T. semipenetrans* on plant growth parameters and biochemical parameters

Grafted plants exhibit better performance in terms of height, leaf count, root length, and other metrics, indicating that both rootstocks offer superior resistance to *T. semipenetrans*, thus facilitating enhanced growth. Compared to T1, which is susceptible, the study showed that an inoculum level of 1000 J2 per plant caused a significant reduction in plant height, leaf number, and root and shoot length, with the decline being particularly pronounced. The current research confirmed the findings that injecting of 1000 (J2) of nematodes per pot significantly reduced plant height, fresh and dry weight of shoots and roots, and root length (26).

Grafting onto resistant rootstocks considerably strengthens the plants' defense systems against *T. semipenetrans* infection, according to the biochemical study. Chlorophyll concentration increased in the grafted plants (T2 and T3) (27). The reduced yield observed in T1 acid lime PKM 1 is caused by a reduction in the production of important enzymes like Rubisco, which is essential for photosynthesis. These results correspond with studies on citrus conducted (28). The infected plants have higher levels of proline because nematodes hinder the roots' capacity to absorb nutrients and water. In addition to its many other functions as a redox buffer, a chelator of cytoplasmic minerals, a scavenger of reactive oxygen species (ROS), and an osmo regulator, proline is also known to enhance protein stability, maintain membrane integrity, and facilitate the activation of enzymes (29).

In this investigation, nematode-inoculated plants exhibited elevated levels of total phenols, peroxidase (PO), and polyphenol oxidase (PPO) activity in their roots and leaves. Nematodes produce β-glycosidases in the host tissue, which release phenols, that are often bound as glycosides in plants (30). In addition to oxidizing substrates with hydrogen peroxide, peroxidases can help scavenge peroxide and synthesize polyphenolic compounds (31). Wood apple rootstock (T3) has increased PO and PPO activity, indicating that these enzymes are important for its improved resistance to nematode stress (32).

Juvenile survival rates or reproduction may be significantly impacted by the treatments, as indicated by the notable change in juvenile population numbers (365 in T1 versus 143 in T3) in Fig. 7. The clear symptoms of nematode infections, such as yellowing of the leaves and decreases in plant height and weight, have been documented by several researchers (33). The increases in nematode populations and the resulting decline in crop yields, or other signs of negative effects, is a physiological response (33). Similar results suggests that the low final population densities were caused by the severely damaged root systems' inability to support large nematode populations (34, 35).

Conclusion

This study evaluates the salinity and nematode tolerance of various rootstocks to identify the best performers for acid lime cultivation. The results demonstrated that grafting acid lime onto rangpur lime (R5) and wood apple (T3) provided superior resilience under both stress conditions. The R5 combination displayed the best salinity tolerance by retaining higher chlorophyll levels, membrane stability, and antioxidant enzyme activity. Similarly, T3-grafted plants exhibited greater tolerance to nematode stress, with higher chlorophyll content, phenol concentration, and enzymatic activity, leading to improved growth metrics. Selecting stress-resistant rootstocks and implementing integrated management strategies can help sustain citrus production. For increased nutrient efficiency, disease resistance, stress tolerance, yield, and climate change adaptability, rootstocks are essential. By enhancing resilience in harsh environments and reducing dependence on chemical treatments, rootstocks are indispensable for promoting sustainable agriculture.

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Authors' contributions

KAS conceptualized the idea, research layout, and revised the draft. MG supplied the planting material, specifically the rangpur lime rootstock. MVJ planned, drafted the manuscript, used software, conducted the investigation, and analyzed the data. JG contributed to the planning and guided the nematode stress experiments. IM facilitated the provision of laboratory resources, nursery facilities, and other essential inputs. All the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Grammarly for grammar check and spelling mistakes, and paraphrase to reword text by replacing words with synonyms in order to correct spelling mistakes and grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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