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Impact of various citrus rootstocks on growth, biochemical characteristics, and nutrient accumulation in Darjeeling Mandarin

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Abstract

An experiment was carried out to study the influence of three commercial rootstocks, *i.e.*, Rangpur Lime, Rough Lemon and Trifoliate Orange on the growth characteristics, grafting success, biochemical and nutrient accumulation in the leaves of Darjeeling mandarin. Data were collected 180 days after grafting for growth parameters and one year after grafting for biochemical and nutrient accumulation by the scion of Darjeeling mandarin. The results revealed significant variation across the rootstocks under study. Rough lemon recorded the highest grafting success (91.25 %) followed by Rangpur lime (87.50 %). In terms of growth parameters rough lemon demonstrated the highest rootstock length (22.26 cm), scion length (11.72 cm), and leaf area (39.59 cm²). Darjeeling mandarin was recorded highest starch content (2.9 mg/g), total sugar (1.31 mg/g), total protein (3.98 mg/g), and total phenol content (0.941mg/g). Higher proline content (1.9 mg/g) in rough lemon indicates a high stress tolerance. Trifoliate Orange recorded the lowest scion length (4.87 cm), leaf area (17.92 cm²) and total sugar content (0.89 mg/g). Concerning macronutrient and micronutrient accumulation, Rangpur Lime recorded the highest calcium (2.35 %), magnesium (0.42%) and iron content (149.8 %). Thus, the findings revealed that Rough Lemon and Rangpur Lime are the most promising rootstocks for improving growth, nutrient uptake, and biochemical traits of Darjeeling Mandarin, making them ideal rootstocks for commercial propagation on Darjeeling mandarin.

Key words: Darjeeling mandarin, biochemical composition, rootstocks, grafting success, nutrient accumulation, Rangpur lime, Rough lemon, Trifoliate orange.

Introduction

Mandarin (*Citrus reticulata* Blanco), sweet orange (*Citrus sinensis* Osbeck), and acid lime (*Citrus aurantifolia* Swingle) are the three main commercial citrus groups grown in India. Various ecotypes of mandarin, including those from Sikkim, Darjeeling, and Khasi, are highly valued for their exceptional quality and have substantial export potential (Singh and Singh 2006). Mandarins have been grown for centuries in West Bengal's Darjeeling and Kalimpong hills. According to recent estimates, in West Bengal alone, mandarins are cultivated across 4.18 thousand hectares, yielding approximately 39.19 thousand tons, with a productivity rate of 9.36 tons per hectare (Anonymous 2023). Numerous biotic and abiotic factors are to blame for the declining production, including pest infestations (such as fruit flies and citrus trunk borer), ageing orchards, poor cultural practices, diseases like citrus gummosis, citrus greening, and Citrus Tristeza Virus (CTV), as well as the lack of high-quality planting materials.

In the Darjeeling and Kalimpong regions, mandarins are predominantly propagated through seeds, often leading to seedlings from diseased mother plants, which hampers productivity (Gurung *et al.*, 2020). Vegetative propagation techniques like budding and grafting ensure true-to-type propagation from the mother plant. Rootstocks play a critical role in citrus cultivation, affecting the growth, yield, and quality of the scion. Rootstocks also play a pivotal role in enhancing the vigour, precocity, productivity, fruit quality, and longevity of grafted scions (Richardson *et al.*, 2003, Waqar *et al.*, 2006). They also influence tree susceptibility to diseases and pests. Studies show that rootstocks like Rough Lemon are vigorous with extensive root systems and exhibit tolerance to diseases such as tristeza, exocortis, and xyloporosis. Similarly, Rangpur Lime is recognized for its tolerance to soil salinity. Therefore, selecting appropriate rootstocks is crucial, accounting for distinct environmental conditions (Warchefsky *et al.*, 2016; Carvalho, D. *et al.*, 2021). In Darjeeling Mandarin, selecting the right rootstock can significantly impact its performance, yield *etc.*, Considering the critical role rootstocks play in citrus cultivation, the study aimed to evaluate the influence of three important rootstocks *i.e.,* Trifoliate Orange, Rangpur Lime, and Rough Lemon on the growth, biochemical profile and nutrient accumulation in Darjeeling Mandarin.

Materials and methods

Experimental Site: The experiment was conducted at the Indian Agricultural Research Station (ICAR) in Kalimpong, West Bengal, with an altitude of 1127.26 m above mean sea level during 2022-23. It lies on 27.06°N latitude and 88.46°E longitude. The area also experiences high annual rainfall, ranging from 2,000 to 3,500 mm and the soils are acidic.

Planting materials: Three rootstocks *i.e.,* Trifoliate Orange, Rangpur Lime, and Rough Lemon were grafted with Darjeeling

Mandarin scions. Data for growth parameters and grafting success were collected at 180 DAG (Days after grafting). From one-year-old successful grafts, leaves were collected randomly for biochemical and nutrient accumulation. Biochemical analysis involved starch, total sugar, reducing sugar, non-reducing sugar, protein, proline and phenol content. Macronutrient (N, P, K, Ca, Mg) and micronutrient (Fe, Mn, Cu, Zn) levels were also measured.

Growth parameters: Growth parameters such as rootstock length (cm), scion length (cm), number of internodes, number of leaves and leaf area (cm^2) were recorded after 180 days of grafting (DAG).

Grafting success: Grafting success (%) was calculated by counting the total number of grafts that sprouted and grown divided by the total number of grafted plants.

Grafting success (%)= [Total number of grafts sprouted and grown / Total number of grafted plants] x 100

Biochemical parameters: Biochemical analysis involved analysis of starch content, total sugar content, reducing sugar, non-reducing sugar, protein, proline and phenol content. Leaves from the successful grafts were randomly collected one year after grafting and were analyzed by the methods given by (Sadasivan and Manickaran, 2007).

Nutrient accumulation: Macronutrients and Micronutrients in the leaves sample were analyzed following the guidelines given by the ICRADA manual 3rd edition (Ryan *et al.,* 2001).

Data Analysis*:* Statistical analysis of data was done by following Fisher's Analysis of Variance (ANOVA) as following Web Agri Stat Package 2.0 (WASP) given by Jangam and Thali (2004)

Results and discussion

Growth parameters and grafting success: Rough Lemon exhibited the highest rootstock length (22.26 cm) and scion length (11.72 cm), followed by Rangpur Lime (Table 1). Rangpur Lime (7.71), (9.01) and Rough Lemon (7.07), (8.1) demonstrated significantly higher numbers of internodes, respectively and leaves compared to Trifoliate Orange (2.70), (3.7). Leaf area was also significantly larger for Rough Lemon (39.59 cm^2) and Rangpur Lime (31.90 cm^2). Similar results were also observed when the grafted scion on the Rough lemon was found to be the more vigorous height of the plant (Waqar *et al.*, 2006, Nasir *et al.*, 2011; Singh *et al.*, 2012; Dubey and Sharma 2016). Maximum scion length was seen in the Darjeeling and Sikkim mandarins when they were budded over the rough lemon rootstock according to Dubey and Singh (2003). Gurung *et al.* (2020) also observed the highest rootstock length of 12.06 cm in Rough lemon and 11.99 cm in Rangpur lime. The longer scion shoot on rough lemons may be the result of a strong and rapid union between the bud and rootstock, as well as a stronger nutritional uptake by the sprouting branch. Grafting success was recorded highest in the case of Rough Lemon (91.25%) and lowest in Trifoliate orange (63.17 %). Rangpur lime and Rough lemon exhibited the highest rates of graft survival (Talukder *et al.*, 2015). These may result from a particular stock-scion combination's unique compatibility behaviour, which differs depending on the species and hence promotes greater cambium production and vascular tissue growth in the graft union between the scion and stock (Rymbai *et al.*, 2023).

Table 1. Effect of different rootstocks on plant growth characteristics and grafting success at 180 DAG (Days after grafting)

Rootstock	Trifoliate Orange	Rangpur Lime	Rough Lemon	CV	CD@5%
Rootstock length (cm)	15.17	20.61	22.26	17.38	NS
Scion length (cm)	4.87	10.81	11.72	31.13	NS
Number of Internodes	2.70b	7.71a	7.07a	21.84	2.89
No. of leaves	3.7b	9.0a	8.1a	18.36	2.89
Leaf area (cm^2)	17.92b	31.90a	39.59a	16.87	11.39
Grafting success (%)	63.17c	87.50ab	91.25a	4.28	6.41

Means in the columns followed by different letters are significantly different at 5% level of significance

Biochemical Parameters: Fig. 1 illustrates the estimation of various biochemical compounds present in the leaves of Darjeeling Mandarin grafted onto three different rootstocks: Rough Lemon, Rangpur Lime, and Trifoliate Orange. The biochemical parameters evaluated include starch, total sugars, reducing sugars, non-reducing sugars, total protein content, proline content and total phenol content. Among the rootstocks, Rough Lemon demonstrated the highest starch content (2.9 mg/g), followed by Rangpur Lime (2.13 mg/g) and Trifoliate Orange

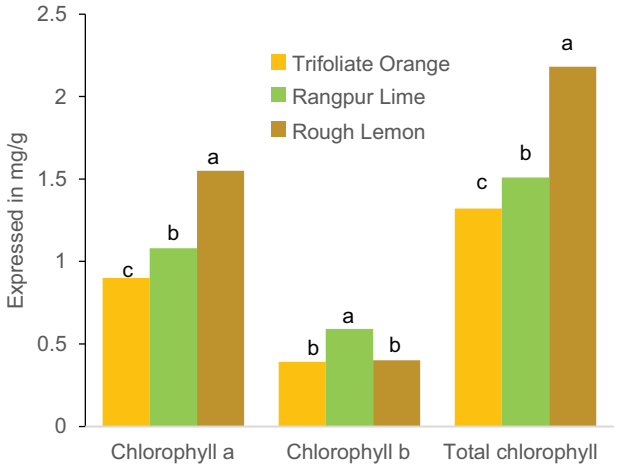

Fig. 1. Estimation of biochemical on the leaves of Darjeeling mandarin (1 year after grafting).

(2.10 mg/g), both of which exhibited comparable levels. In terms of total sugar content, Rough Lemon again outperformed the other rootstocks, recording 1.31 mg/g, while Trifoliate Orange exhibited the lowest concentration (0.89 mg/g). Similarly, the highest reducing sugar content was observed in Rough Lemon (0.87 mg/g), with Trifoliate Orange and Rangpur Lime showing lower but similar levels. Non-reducing sugars were consistently low across all three rootstocks, although Rough Lemon registered a slightly higher content (0.44 mg/g). Rough Lemon also exhibited superior total protein content (3.98 mg/g), proline content (1.9 mg/g), and total phenol content (0.94 mg/g) compared to the other rootstocks. This indicates that grafting onto Rough Lemon may enhance antioxidant properties in Darjeeling Mandarin.

Fig. 2 demonstrates chlorophyll a, chlorophyll b and total chlorophyll across the Darjeeling mandarin grafted on trifoliate orange, Rangpur lime and Rough Lemon. Chlorophyll a and

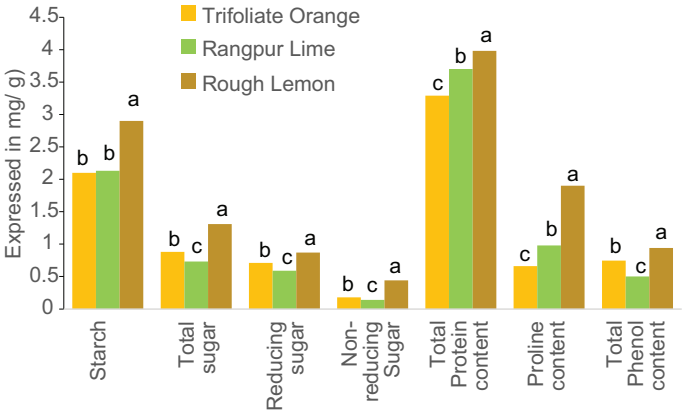

Fig. 2. Estimation of chlorophyll content on the leaves of Darjeeling mandarin (1 year after grafting).

Total Chlorophyll were most abundant in Rough Lemon with values of 1.55 mg/g and 2.18 mg/g respectively, suggesting enhanced photosynthetic efficiency in this rootstock. These findings collectively highlight the potential of Rough Lemon as a superior rootstock for optimizing the biochemical profile and physiological performance of Darjeeling Mandarin.

Leaf macronutrients and micronutrients: Rootstocks had a significant impact on the levels of nutrients in leaves. (Fig. 3). Darjeeling mandarin grafted on Rough Lemon accumulated the highest levels of nitrogen (2.16 %), potassium (2.12%) and calcium (2.34%) in the leaves. Rangpur Lime showed the highest accumulation of magnesium (0.42%) and nitrogen (2.11%) indicating superior nutrient uptake in these rootstocks compared to Trifoliate Orange. While Trifoliate orange recorded lowest potassium content (1.45%), calcium content (1.95 %) and magnesium content (0.36%). Numerous researchers have

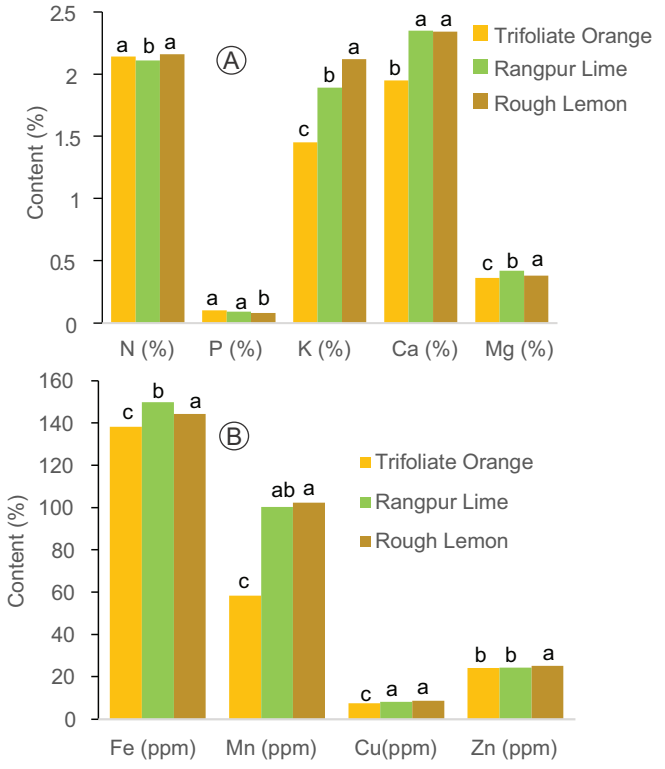

Fig 3 (A) Performance of Darjeeling mandarin in terms of leaf macronutrients accumulation. (B) Performance of Darjeeling mandarin in terms of leaf micronutrients accumulation.

also documented variations in N and P accumulation by various rootstocks in citrus cultivars (Dubey & Sharma, 2016; Toplu *et al.*, 2012; Kumar *et al.*, 2017). Rangpur Lime exhibited the highest manganese (100.3 ppm) and iron (149.8 ppm) content, with Rough Lemon closely following. Trifoliate orange recorded the lowest iron content of (138.02 ppm) and manganese content (58.28 ppm). All three rootstocks demonstrated comparable zinc and copper accumulation. Variations in the foliar micronutrient concentration have also been documented previously, attributed to the rootstocks of various fruit crops, which differ in their ability to absorb nutrients through the roots (Kayon, 2008).

Correlation matrix: Variables like rootstock length, scion length, leaf area and grafting sucess have very high positive correlations (close to 1) (Table 2), suggesting that these growth-related variables are highly related. For example, Rootstock length and scion length have a correlation of 0.995, meaning that as one increases, the other tends to increase proportionally. With regard to grafting success (%), it shows a strong positive correlation with rootstock length (0.995), leaf area (1.00) and Leaf area (0.973).

This indicates that grafting success is highly influenced by the overall growth metrics of both scion and rootstocks lengths and leaf area. A study by Kumar *et al.* (2016) stated that rootstock had significant effect on the plant height and grafting success (%). Leaf area showed highly positive correlations with rootstock length (0.991), Scion length (0.973) and grafting success (0.973). It also correlated positively with biochemical parameters like starch content (0.792) and highly positively correlated with total protein content (0.998), suggesting that higher leaf area might enhance starch and protein content. Protein content shows a very strong positive correlation with almost all the growth characteristics, emphasing the importance of protein in plant growth and grafting success. Satisha *et al.* (2007) observed that while there was a negative association found between success % and total phenols, a substantial positive correlation was found between rooting percentage, total proteins, and WUE. Biochemical traits such as starch, protein and proline content showed significant positive correlations with variables like Scion length, leaf area and grafting success, while Total phenol content showed weaker correlations or non-significant correlations with most variables.

Rough Lemon and Rangpur Lime outperformed Trifoliate Orange in most parameters, showing higher growth rates, biochemical activity and nutrient accumulation. These rootstocks are better suited for Darjeeling Mandarin cultivation, offering potential improvements in fruit quality and yield. The higher proline and phenol content in Rough Lemon suggests better stress tolerance, which is crucial for cultivation in the Eastern Himalayan region. Rangpur Lime and Rough Lemon are promising rootstocks for enhancing the growth, biochemical traits, and nutrient absorption of Darjeeling Mandarin. These findings provide valuable insights for selecting these rootstocks to improve the performance of this important citrus cultivar in challenging hilly terrains.

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Table 2. Correlation among the plant growth parameters and biochemical parameters on Darjeeling mandarin

Parameter	Scion length (cm)	Leaf area (cm^2)	Grafting success (%)	Starch (mg/g)	Total sugar (mg/g)	Total protein content (mg/g)	Proline content (mg/g)	Total phenol content (mg/g)
Rootstock length	0.995**	0.991**	0.995**	0.704*	0.460^{NS}	0.982**	0.841**	0.159^{NS}
Scion length	1.000**	0.973**	1.000**	0.628*	0.367^{NS}	0.957**	0.782*	0.058^{NS}
Leaf area		1.000**	0.973**	0.792*	0.574^{NS}	0.998**	0.905**	0.289^{NS}
Grafting success			1.000**	0.629*	0.368^{NS}	0.958**	0.782*	0.059^{NS}
Starch				1.000**	0.954**	0.826**	0.976**	0.813**
Total sugar					1.000**	0.620*	0.867*	0.950**
Total protein						1.000**	0.928**	0.344^{NS}
Proline content							1.000**	0.668*
Total phenol								1.000**
Total chlorophyll								

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