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Growth and compatibility traits of different citrus rootstocks under mid-hill condition

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ABSTRACT

A study was carried out during 2022–23 at ICAR-Research Complex for North-Eastern Hills Region, Umiam, Meghalaya to find out the performance of Khasi mandarin (*Citrus reticulata* Blanco) on different rootstocks. The experiment was laid out in a randomized block design (RBD) with 10 treatments, viz. T_1, Karna khata; T_2, Trifoliate orange; T_3, Rough lemon; T_4, Rangpur lime; T_5, Khasi papeda; T_6, Taiwanica; T_7, Volkameriana; T_8, Tanzelodancy; T_9, Pummelo; and T_{10}, Cleopatra mandarin with 5 replications. With regards to grafting success, Rough lemon had the highest percentage, which was at par with Rangpur lime and Volkameriana, whereas Trifoliate orange had the lowest success rate. Maximum plant height, rootstock and scion diameter were noted in the rootstock of Rough lemon whereas highest number of leaves (17.33) and branches/plant (3.23) were recorded in Trifoliate orange. Through correlation analysis it was found that plant height was highly significantly correlated with rootstock diameter ($r^2 = 0.747$, $P<0.05$) and *vice versa*. Number of leaves/plant had high correlations with number of branches/plant ($r^2 = 0.923$, $P<0.01$) and scion diameter ($r^2 = 0.865$, $P<0.01$). Also, a significant correlation was found between scion diameter and number of branches/plant ($r^2 = 0.801$, $P<0.01$).

Keywords: Citrus, Graft success, Mandarin, Propagation, Rootstock

Citrus, a highly important fruit crop, is grown in tropical and sub-tropical regions globally. India stands as one of the leading producers of citrus fruits, with mango and banana being the predominant crops in terms of both area and production. The north-eastern region of India is recognized as the natural habitat for numerous citrus species, hosting approximately 17 different species, including *Citrus reticulata* Blanco (Bhattacharya and Dutta 1956). Khasi mandarins are particularly esteemed for their easy-to-peel fruit and high juice content. Despite the region's potential, the total citrus production in the north-eastern region for the year 2021–22 was 839.12 thousand tonnes from an area of 130.60 thousand hectares, with a productivity of 6.14 t/ha (Anonymous 2023). This productivity is significantly lower than the national average of 13.4 t/ha, primarily due to various factors such as poor-quality planting materials, unscientific orchard management, multiple nutritional deficiencies, disease and pest infestations, and the prevalence of old and unproductive orchards (Rymbai *et al.* 2024). Mandarin orchards in the region primarily consist of seedling origin plants, with limited use of budded and grafted plants in commercial orchards. It is well-known that plants raised from seeds undergo a prolonged juvenile phase with inconsistent performance in the field. On the contrary, vegetative propagation produces genetically uniform and true-to-type plants, offering advantages such as utilizing adapted rootstocks.

Rootstocks play a pivotal role in a plant's ability to absorb nutrients and water from the soil, influencing canopy development and photosynthesis (Richardson *et al.* 2003, Waqar *et al.* 2006). Additionally, rootstocks provide tolerance to various biotic and abiotic stresses, such as *Phytophthora* spp., nematodes, citrus tristeza virus, drought, salinity, waterlogging, and alkalinity. Given these considerations, there is a need to prioritize vegetative propagation to capitalize on the advantages offered by rootstocks and obtain genetically uniform, true-to-type, and high-quality planting material. However, the nursery performance of different citrus species as rootstocks varies significantly based on the scion variety and agro-climatic conditions (Sonkar *et al.* 2002). The success rates for conventional T-budding on different mandarin and rootstock combinations varied, 90% for Kinnow mandarin on Rough lemon (Joalka 1986); 68.31% for Nagpur mandarin on Rough lemon (Singh *et al.* 2012), and 89.67% for Coorg mandarin on Rangpur

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lime (Karunakaran *et al.* 2014). In view of these, a study was carried out on the growth and compatibility traits of different citrus rootstocks in mid-hill conditions, aiming to contribute valuable insights for enhancing citrus cultivation in the north-eastern region of India.

MATERIALS AND METHODS

The study was carried out during 2022–23 at ICAR Research Complex for North-Eastern Hills Region, Umiam, Meghalaya. Approximately 90% of the total annual precipitation (2160.15 mm) takes place between May and October. The lowest recorded average temperature of 7.5°C occurred in January, while the highest temperature of 28.4°C was observed in June. Minimal relative humidity was noted during winter at 40.8%, with the maximum observed during the monsoon season at 90.5%.

Planting materials: The raising of rootstocks, scion preparation, and grafting procedures was followed according to the method described by Patel *et al.* (2010). Seeds of rootstocks were gathered from healthy fruits of elite mother plants. These seeds were thoroughly washed in running water and sown in a nursery bed between December–January. Seedlings attaining a height of 6–8 cm with 4–6 leaves, were transplanted into 4 × 6 inch black HDPE polybags containing a mixture of soil, sand, and farmyard manure in a 1:1:1 v/v ratio during April–May. Uniform-sized, healthy rootstocks, aged 6–7 months were utilized for grafting during July–August. Scion shoots of 3–4 mm thickness and 8–10 cm in length, containing 3–4 healthy buds, were collected from elite mother plants of Khasi mandarin. Defoliation of the selected scion shoots was carried out on the mother plants 7–10 days prior to detaching. Wedge grafting was conducted in the months of July–August. Heading back of rootstock at a height of 7.5–10 cm was done leaving 2–3 leaves on the lower side of rootstock. The beheaded rootstock was split to about 1.5–2 cm deep through the centre of stem. A wedge-shape cut of about 1.5–2 cm long was made on the lower side of the scion stick. The scion stick was then inserted into the split of the rootstock and aligned properly so that the cambium tissues of rootstock and scion come in good contact with each other. The union was tied with the help of150 gauge polythene strip. The care and management of grafted plants were carried out under polyhouse condition.

Experimental details: Wedge grafting of Khasi mandarin was performed on 10 different rootstocks, viz. T_1, Karna khata; T_2, Trifoliate orange; T_3, Rough lemon; T_4, Rangpur lime; T_5, Khasi papeda; T_6, Taiwanica; T_7, Volkameriana; T_8, Tanzelodancy; T_9, Pummelol; and T_{10}, Cleopatra mandarin. The experiment was conducted under low-cost polyhouse conditions. Data on seed germination percentage of different rootstocks and the number of days taken for the sprouting of grafted plants were recorded daily up to 40 days after grafting (DAG):

$$\text{Graft success (\%)} = \frac{\text{No. of grafted plants sprouted at 40 DAG}}{\text{Total no. of grafted plants}} \times 100$$

$$\text{Survivability (\%)} = \frac{\text{No. of grafted plants survived at 180 DAG}}{\text{Total no. of grafted plants}} \times 100$$

The experiment was laid out in randomized block design (RBD) with 5 replications, and each replication included 10 plants. A correlation analysis investigating plant growth parameters across various rootstocks of Khasi mandarin was conducted.

Statistical analysis: The replicated data underwent statistical analysis utilizing the Statistical Package for the Social Sciences (SPSS) version 14.0 software. Results were expressed as mean ± standard error (SE) through one-way analysis of variance (ANOVA) with a significance level set at $P<0.05$. Post-hoc analysis for significant differences was performed using Tukey's HSD (Honestly Significant Difference) test.

RESULTS AND DISCUSSION

Seed germination percentage: The results revealed a significant difference in seed germination for rootstocks (Table 1). The range of seed germination rates among different rootstocks was 70.34–88.00%. The highest germination percentage was noted in Khasi papeda (88.00%), which was at par with that of Rough lemon (86.10%). Trifoliate orange exhibited the lowest germination rate (70.34%). The variations were due to a distinction in the mechanisms governing germination and genetic differences among species (Lal *et al.* 2014, De and Patel 2019).

No. of days to graft sprouting: The duration for sprouting showed significant variations among rootstocks. Specifically, Khasi mandarin grafted on Rough lemon exhibited the shortest time for both first sprouting (15.05%) and 50% sprouting (26.00 days). Conversely, Khasi mandarin grafted on Cleopatra mandarin required the longest period for first sprouting (29.40 days), and Karna khata had the lengthiest time for 50% sprouting (35.62 days). These findings closely resemble those reported by Singh *et al.* (2012). The higher success in grafting can be attributed to the expedited union of xylem and cambium tissues between the scion and rootstock, fostering the growth of new shoots (Hartmann *et al.* 1997, Deshmukh *et al.* 2017, Rymbai *et al.* 2023). Similarly, Patel *et al.* (2010) noted that early sprouting occurs due to rapid callusing and early cambial contact, facilitating a quicker graft union.

Grafting success percentage: The success of grafting in Khasi mandarin on different rootstocks has a notable impact on the overall graft success (Fig. 1). Rough lemon demonstrated the highest success rate (93.58%), which was at par with Rangpur lime (90.60%) and Volkameriana (90.00%), while Trifoliate orange exhibited the lowest success rate at 80.70%. This aligns with the findings of Singh and Chahal (2021), who noted that Rough lemon, Volkameriana lemon, and Rangpur lime achieved the highest budding success rates at 81.9%, 78.9%, and 77.5%, respectively. Similarly, Singh *et al.* (2012a) reported that the highest budding success rate (68.31%) occurred on Rough

lemon rootstocks compared to those of Rangpur lime, Sour orange, and Carrizo citrange. Nasir *et al.* (2006) reported the highest budding success rate of Kinnow mandarin on Rangpur lime, followed by Rough lemon. The successful budding on Rough lemon, Rangpur lime and Volkameriana can be attributed to their evergreen nature, enabling them to retain leaves, and ensuring consistent movement of photosynthates and better sap flow during the budding period. Deshmukh *et al.* (2021) suggested that a higher graft success percentage may result from better compatibility between rootstock and scion, coupled with favourable sugar content and an optimal C: N ratio. On the other hand, species such as Trifoliate orange and their hybrids exhibit a poor graft success rate due to their deciduous nature, fewer leaves, and thinner stem size (Singh and Chahal 2021). Also, in the process of grafting, two genetically different plant materials are usually combined to form the citrus tree. The relationship between scion and stock, which is commonly termed affinity or compatibility, is of fundamental importance to successful long-term commercial performance. This phenomenon may be owing to genetic or physiological in-congeniality and can be the major reason for fluctuating budding/grafting success among different rootstocks (Singh and Chahal 2021). Furthermore, Deshmukh *et al.* (2017) proposed that the formation of the graft/bud union may be hindered by a limited number of active buds, poor sap flow, and physiological conditions of the rootstock, leading to poor graft success observed in some rootstocks.

Graft survivability percentage: The graft survivability was also significantly varied among rootstock (Fig. 2). The highest graft survivability percentage was obtained on Rough lemon followed by Rangpur lime, while the lowest was recorded on Khasi papeda. Similarly, it was earlier reported that Rough lemon had the highest graft survivability rate, followed by Rangpur lime (Talukder *et al.* 2015). These might be due to the specific compatibility behaviour of a particular stock-scion combination, which varies with species and thus facilitates better cambium formation and vascular tissue development in graft union between scion and stock (Rymbai *et al.* 2023). Furthermore, the higher tissue regeneration of stock-scion interaction ensures a good movement of water, nutrients, and growth hormones (Perez-alfocea *et al.* 2010, Mestre *et al.* 2017), thus leading to higher plantlet growth and survivability.

Table 1 Effect of different rootstocks on grafting and sprouting of Khasi mandarin

Rootstock	Seed germination (%)	Days taken to first sprout	Days taken to 50% sprouting
Karna khata	75.75 ± 0.80^{bcd}	25.12 ± 0.84^{ab}	35.62 ± 0.96^{a}
Trifoliate orange	70.34 ± 2.02^{d}	21.05 ± 0.72^{bc}	32.10 ± 1.20^{abc}
Rough lemon	86.10 ± 0.55^{a}	15.05 ± 1.05^{d}	26.00 ± 0.80^{d}
Rangpur lime	78.80 ± 1.15^{bc}	17.52 ± 1.13^{cd}	28.78 ± 1.19^{bcd}
Khasi papeda	88.00 ± 0.98^{a}	16.10 ± 1.29^{d}	27.50 ± 1.33^{cd}
Taiwanica	76.80 ± 0.60^{bcd}	17.66 ± 0.71^{cd}	28.60 ± 0.66^{bcd}
Volkameriana	75.97 ± 0.77^{bcd}	16.52 ± 1.58^{cd}	26.56 ± 1.10^{d}
Tanzelodancy	74.72 ± 2.26^{cd}	18.38 ± 0.57^{cd}	28.93 ± 0.68^{bcd}
Pummelo	81.96 ± 1.98^{ab}	27.15 ± 0.92^{a}	33.37 ± 0.66^{ab}
Cleopatra mandarin	81.57 ± 1.82^{abc}	29.40 ± 0.51^{a}	34.07 ± 1.04^{a}

Values given are mean (n=3) with ± SE. One-way analysis of variance (ANOVA) plus posthoc Tukey test was done to compare means. Superscript lower case letters on each column indicated statistical significance (P<0.05).

Therefore, the better graft survivability performance of Rough lemon and Rangpur lime as rootstocks for mandarin in comparison to other citrus species is the reason for their more common usage (Ghosh and Singh 1993). Interestingly, graft on Khasi papeda had the lowest survivability, despite early sprouting and high graft success. This might be owing to the unique genetic behaviours of the rootstock Khasi papeda on adaptability and survivability. Further, Asahina and Satoh (2015) and Rasool *et al.* (2020) reasoned that low percentage of graft survivability at later stage might be due to the disruption of vascular system caused by the wound given to the stock and the scion during the grafting

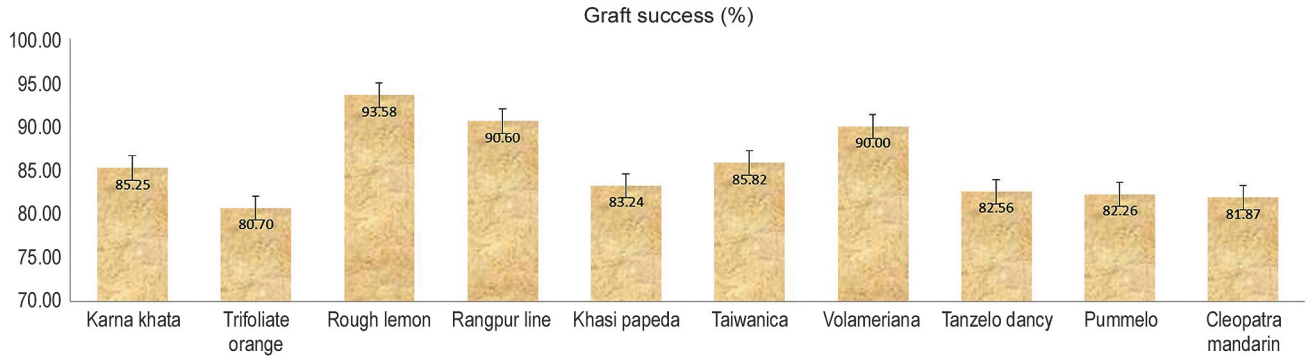

Fig. 1 Effect of different rootstocks on graft success of Khasi mandarin.

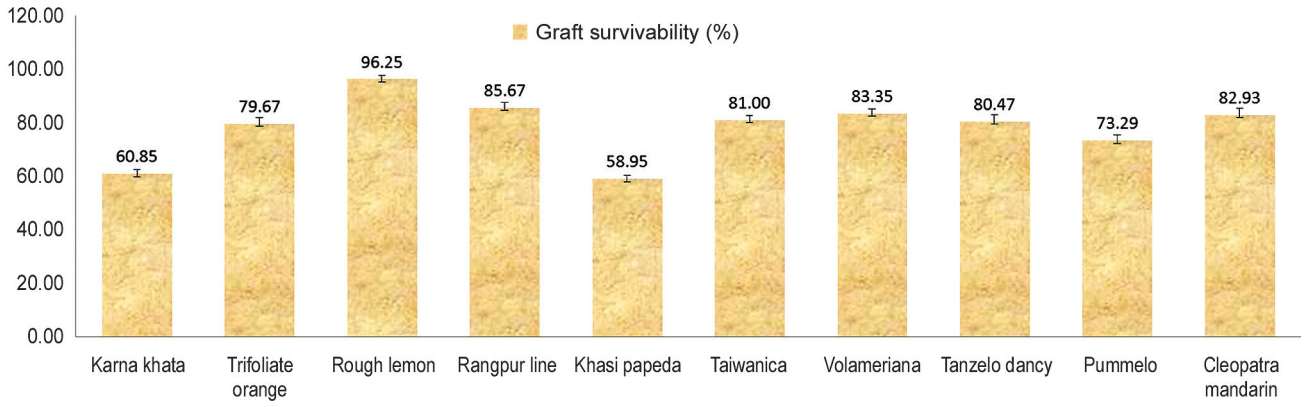

Fig. 2 Effect of different rootstocks on graft survivability of Khasi mandarin.

process which in turn might have interrupted in the water uptake and nutrient transport to the graft junction to ensure the continuity of plant growth.

Plant height: Results revealed that the maximum plant height was noted in Rough lemon followed by Pummeloand Tanzelodancy which was significantly higher than that of other rootstocks. Volkameriana attained a minimum plant height which was on par with Taiwanica and Khasi papeda (Table 2). Similar findings have also been reported in which the scion grafted on Rough lemon was more vigorous with regards to plant height (Waqar *et al.* 2006, Nasir *et al.* 2011, Singh *et al.* 2012a, Dubey and Sharma 2016). The effects of rootstocks on citrus plant height have also been noted by Jover *et al.* (2012). According to Shafieizargar *et al.* (2012), plant height can be managed by using suitable rootstocks due to its significant influence on many of the derived characters. Further, Dubey and Sharma (2016) stated that better absorption of macronutrients by the rootstocks may have resulted in an increase plant height.

Rootstock and scion diameter: The maximum diameter of both the rootstock and scion was observed in Rough lemon, while the minimum was recorded in Volkameriana (Table 2). This discrepancy may be attributed to the rapid and robust union between the rootstock and scion, facilitating accelerated growth of the scion on the rootstock (Skene *et al.* 1983). Malasi *et al.* (2017) proposed that highly compatible rootstock and scion combinations lead to increased metabolic activity after the formation of vascular tissues between the scion and rootstock, ultimately resulting in an augmentation of both rootstock and scion diameter. In cases where rootstocks are deemed incompatible with the scion, it can lead to an imbalance in physiological processes, hindering overall plant growth and productivity. Maintaining a harmonious relationship, as indicated by comparable diameter of the scion and stock, is essential for achieving a healthy yield of good quality (Waqar *et al.* 2006).

Number of leaves and branches/plant: Trifoliate orange had the highest number of leaves and branches/plant (Table 2). On the other hand, Karna khata had the lowest number of leaves and Khasi papeda had the lowest number of branches/plant. This may be because certain plant genotypes have fewer leaves as a characteristic, leading to noticeable differences in plants grafted over rootstocks. Additionally, some rootstocks may exhibit slower growth which can result in a lower number of leaves and branches. According to Ginandjar and Subandi (2018), the age of the rootstock and the manner of budding/grafting had a very substantial impact on the number of leaves and the

Table 2 Effect of different rootstocks on plant growth characteristics at 180 days after grafting

Rootstock	Plant height (cm)	Rootstock diameter (cm)	Scion diameter (cm)	No. of leaves/ plant	No. of branches/ plant
Karna khata	23.20 ± 0.85^{de}	0.29 ± 0.01^{ab}	0.25 ± 0.01^{a}	10.67 ± 1.27^{c}	2.10 ± 0.12^{ef}
Trifoliate orange	25.97 ± 0.42^{cde}	0.30 ± 0.06^{ab}	0.28 ± 0.01^{a}	17.33 ± 0.85^{a}	3.23 ± 0.14^{a}
Rough lemon	38.10 ± 1.17^{a}	0.37 ± 0.02^{a}	0.30 ± 0.01^{a}	11.67 ± 0.73^{bc}	$3 .02 \pm 0.11^{ab}$
Rangpur lime	30.64 ± 1.24^{bc}	0.34 ± 0.03^{ab}	0.28 ± 0.02^{a}	16.67 ± 1.23^{a}	2.75 ± 0.11^{bcd}
Khasi papeda	22.53 ± 1.07^{de}	0.35 ± 0.01^{ab}	0.30 ± 0.06^{a}	13.67 ± 0.81^{abc}	1.93 ± 0.08^{f}
Taiwanica	21.63 ± 1.13^{e}	0.33 ± 0.02^{ab}	0.26 ± 0.07^{a}	12.00 ± 0.95^{bc}	2.42 ± 0.11^{de}
Volkameriana	20.88 ± 0.98^{e}	0.24 ± 0.02^{b}	0.18 ± 0.04^{a}	14.00 ± 1.15^{abc}	2.50 ± 0.06^{cde}
Tanzelodancy	34.30 ± 1.20^{ab}	0.29 ± 0.004^{ab}	0.24 ± 0.002^{a}	15.23 ± 0.20^{ab}	2.92 ± 0.02^{abc}
Pummelo	35.80 ± 1.76^{ab}	0.35 ± 0.004^{ab}	0.28 ± 0.002^{a}	14.49 ± 0.27^{abc}	2.96 ± 0.06^{abc}
Cleopatra mandarin	27.38 ± 0.67^{cd}	0.32 ± 0.004^{ab}	0.29 ± 0.01^{a}	15.01 ± 0.41^{abc}	2.64 ± 0.06^{bcd}

Values given are mean (n=3) with ± SE. Superscript lowercase letters on each column indicated statistical significance (P<0.05).

Table 3 Correlation among the plant growth characteristics of Khasi mandarin

	Plant height (cm)	Rootstock diameter (cm)	Scion diameter (cm)	No. of leaves/ plant	No. of branches/ plant
Plant height (cm)	1.000**	0.747*	0.374^{NS}	0.484^{NS}	0.279^{NS}
Rootstock diameter (cm)		1.000**	0.326^{NS}	0.539^{NS}	0.446^{NS}
Scion diameter (cm)			1.000**	0.865**	0.801**
No. of leaves/plant				1.000**	0.923**
No. of branches/plant					1.000**

high percentage of shoots. Furthermore, Matheron *et al.* (1998) reported that plant species such as Rough lemon and Volkameriana have a high growth rate and minimal branching, which suggests they are highly juvenile and also possess a substantial number of leaves.

Correlation: It was observed that plant height had the highest positive correlation ($r^2 = 0.747$, $P<0.05$) with rootstock diameter and non-significant correlations with scion diameter ($r^2 = 0.374^{NS}$), number of leaves/plant ($r^2 = 0.484^{NS}$) and number of branches/plant ($r^2 = 0.279^{NS}$) (Table 3). Similarly, rootstock diameter also had non-significant correlations with scion diameter ($r^2 = 0.326^{NS}$), number of leaves/plant ($r^2 = 0.539^{NS}$) and number of branches/plant ($r^2 = 0.446^{NS}$). A study by Kumar *et al.* (2016) stated that rootstock had significant effect on the plant height. Scion diameter had the highest positive correlation with number of leaves/plant ($r^2 = 0.865$, $P<0.01$) followed with number of branches/plant ($r^2 = 0.801$, $P<0.01$). Number of leaves/plant had the highest significant positive correlation with number of branches/plant ($r^2 = 0.923$, $P<0.01$) and *vice versa*. Similar findings were reported by Kamanga *et al.* (2017) where a strong positive correlation was found between number of branches and number of leaves ($r^2 = 0.898$, $P<0.01$).

The experiment showed that Rough lemon exhibited better graft success, survivability as well as growth performance of the grafted plants followed by Rangpur lime while the lowest was recorded in Taiwanica. Thus, it may be concluded that Rough lemon and Rangpur lime are suitable rootstock for vegetative propagation of Khasi mandarins in mid-hill conditions.

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