Research Article

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Microbudding in mandarin (*Citrus reticulata* Blanco) as influenced by rootstocks, their age and growing media

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ABSTRACT

This study evaluates the influence of rootstock type, age and growing media on the success of microbudding in Mandarin (*Citrus reticulata* Blanco). The results indicated that Rough Lemon rootstock at six months of age grown in Cocopeat + Vermicompost (1:1) ($R_1G_4A_3$) exhibited the highest microbudding success (79.12%), fastest sprouting (12.98 days), and superior growth parameters, including seedling height (21.35 cm), stock diameter (3.59 mm), and leaf area index (1.17). Additionally, five-month-old Rough Lemon seedlings ($R_1G_4A_2$) achieved the highest survival percentage (95.84%), confirming its viability as an alternative treatment. The findings suggest that Cocopeat + Vermicompost (1:1) is an ideal growing medium for maximizing microbudding success and seedling vigor, making it a promising strategy for commercial Mandarin propagation.

Introduction

Citrus is the third most important fruit crop in India after mango and banana, cultivated across tropical and subtropical regions. It belongs to the Rutaceae family and is grown in over 100 countries worldwide (Choudhary *et al.*, 2023). Rough lemon (*Citrus jambhiri*) and Rangpur lime (*Citrus limonia* Osbeck) is the most favourable rootstock for mandarin. Rough lemon is a cold-hardy citrus and commonly known as Jamberi is widely used rootstocks for propagating mandarin in Central India and it is quicker growing rootstock as compared to other rootstocks as well as having better success rate. Rangpur is a cross of Citrus *reticulata* × *medica* and it is the most utilized rootstock for its specific qualities i.e. due to its heavy and early bearing, and drought resistance.

A good quality growing media plays an important role for obtaining luxuriant vegetative growth in various fruit crops and successful propagation of fruit crops in nurseries. The success of plant propagation depends largely on the quality of the growing media used (Choudhary *et al.*, 2023). Vermicompost, Azatobactor, Cocopeat, etc. are commonly used as growing media and soil amendments. Vermicompost is a product of a non-thermophilic bio-degradation of organic materials through interactions between earthworms and microorganisms. Cocopeat is an excellent organic component for container growing media and it has high water-holding capacity; excellent drainage; absence of weeds and pathogens; slow decomposition; easy wettability; and acceptable levels of pH, high cation exchange capacity, and electrical conductivity. Cocopeat contains natural *Trichoderma* which acts as a Bioagent against harmful pathogens. It provides a great environment for beneficial fungi and bacteria to grow. Better root growth results in

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better plant growth.

Mandarins are easily peelable fruits and segments are conveniently consumed by hands. Among the mandarins, 'Nagpuri' or 'Nagpur' mandarin is cherished for its unique thirst quenching sweet and sour taste. Due to this uniqueness, it is considered as one district one product of Chhindwara district by the government of Madhya Pradesh. Vidarbha region of Maharashtra and adjoining parts of Madhya Pradesh and Rajasthan (Jhalawar district) have more or less similar agro-climatic conditions and hence cultivation of this mandarin cultivar is blooming and expanding in these areas (Ladaniya, 2021).

Microbudding is a novel propagation technique and standardized in Citrus spp. This propagation method produces the infant citrus trees, which could revolutionize the commercial citrus propagation industry by saving grower's time, space and money (Choudhary *et al*., 2020). This technique was first developed by Skaria and Zhang. Microbudding is a grafting technique used in the development of citrus trees. Microbudding is done at a younger age, and because of apical hormonal dominance. Microbudding technique was standardized in *Citrus reticulata* Blanco cv. Nagpur mandarin which is also applicable for other Citrus species for fast and round the year multiplication of disease free planting material and also commercial citrus propagation for getting the marketable budded plants earlier with reduced cost (Vijayakumari and Singh, 2003). In the view of above facts the present study the effect of rootstocks age and growing media for success of microbudding in Mandarin (*Citrus reticulata* Blanco)" on different root stock was carried out.

Material and Methods

Experimental site

The experiment was carried out at JNKVV, Zonal Agricultural Research Station, Chandangaon, Chhindwara (M.P.) in 2021-22. It is situated at 22°3'26.77" North (latitude) and 78°56'17.42" East (longitude) at an altitude of 675 m above the mean sea level.

Technical programme of the work

The experiment was laid out in an Asymmetric Factorial Completely Randomized Block Design with three replications. The study included three factors viz., Rootstock: (R_1: Rough Lemon and R_2: Rangpur Lime), Growing Media: (G_1: Soil + Vermicompost in 1:1 ratio), G_2: Soil + Azotobacter in 1:1 ratio, G_3: Soil + Vermicompost + Azotobacter in 1:1:1 ratio and G_4: Cocopeat + Vermicompost in 1:1 ratio) and Age of Rootstock: (A_1: 4 months, A_2: 5 months and A_3: 6 months). This design resulted in 24 treatment combinations. For microbudding, bud sticks were taken from Nagpur Santra (Mandarin) - an important variety grown in the Madhya Pradesh region.

Preparation of rootstock seedlings

The 2 month (60 days) old seedlings were transplanted in poly bags (20 cm x 30 cm). Carefully uprooted the Rough lemon and Rangpur lime seedlings from tray after that roots were washed under running tap water and treated with fungicide (0.2 % Bavistin) solution for 5 minutes before transferring to poly bags. These seedlings were transplanted in polybag filled with different growing media as per treatment. 15 seedlings were transplanted in polybags, which contained different growing media as per the treatments. The recommended intercultural operations like hoeing, fertilizer application, foliar spray of urea, irrigation and spraying of pesticide were applied to raise vigorous and healthy rootstock seedlings.

Planting material for microbudding

For microbudding two different rootstocks (Rough lemon and rangpur lime) were transplanted in various growing media. Bud sticks of Mandarin cv. Nagpur Santra were taken in microbudding. Healthy and elite mother trees with sufficient new flush of Mandarin cv. Nagpur Santra were selected in the mother block of Zonal Agricultural Research Station, Chhindwara and the trees were tagged for collection of bud. The terminal shoots of current season growth with 15-20 cm length and nodal buds having pencil thickness were selected. Pre-curing such as removal of leaves was done with sterilized secateurs on the same day of microbudding. The scion shoots were collected from mother trees early in the morning on the day of budding. Immediately after separation of scions from mother trees, they were wrapped in moist cloth and carried in polythene cover to the site of budding.

Procedure of microbudding

The leaf petiole is cut off just above the bud, and the bud is removed from the bud stick with a razor-sharp knife. A flat cut is made just underneath the bud. Only the bud and a small piece of wood under it are used. Selected rootstock seedling is beheaded leaving 15-20 cm. On beheaded portion a wedge-shaped cut (2-2.5 cm) is given downward with a sharp knife and tender scion bud (3-4 mm width and 1 to 1.5 cm length) inserted on the de topped root stock in a wedge cut and immediately protected by covering with a micropipette tip to achieve close physical contact between scion and stock tissues was placed with slight pressure over top of bud union. After a week, microbuds are observed -and then micro tips are removed after their sprouting within

12-14 days. The plant growths of mandarin buddings were periodically assessed at monthly intervals up to 90 days after microbudding (DAM).

Observations recorded

Physiological parameters regarding growth were recorded i.e. seedling height (cm), stem diameter (mm), fresh weight of shoots (g), dry weight of shoots (g), fresh weight of roots (g), dry weight of roots (g), survival of seedlings (%) and leaf area index at the time of microbudding. Budding parameters observations was recorded i.e. days taken to first sprout, Microbudding success (%), length of micro budded sprout (cm), number of leaves/ plant and stock diameter (mm) at 90 days after microbudding. The data collected during the investigation were analyzed statistically by the method of analysis of variance. The significance of various treatments was judged as suggested by Fisher (1937) applying 'F' test.

Results and Discussion

The highest seedling height (21.35 cm), stem diameter (3.59 mm), fresh shoot weight (2.25 g), dry shoot weight (0.79 g), and leaf area index (1.17) were recorded in six-month-old Rough Lemon rootstocks ($R_1G_4A_3$) grown in Cocopeat + Vermicompost (1:1) at the time of microbudding (Table 1). The highest survival percentage (95.84%) was observed in four-month-old Rough Lemon seedlings ($R_1G_4A_1$) grown in the same medium, demonstrating its effectiveness in early-stage seedling establishment (Fig. 1). These results indicate that Cocopeat + Vermicompost provides an optimal growing environment for rootstock development, improving seedling vigor and adaptability for microbudding success. The findings align with Bhagat *et al.* (2013), who reported that a combination of Soil + FYM + Cocopeat (2:1:1) significantly enhanced seedling growth parameters, including stem diameter, leaf area, and root system development in Rough Lemon rootstocks. Thus, adopting Cocopeat + Vermicompost (1:1) as a growing medium can significantly improve the physiological attributes of citrus rootstocks, ensuring better survival rates and successful propagation.

The six-month-old Rough Lemon rootstock ($R_1G_4A_3$) grown in Cocopeat + Vermicompost (1:1) exhibited superior budding performance, recording the shortest time to first sprouting (12.98 days) and the highest microbudding success rate (79.12%) (Table 2 & Fig. 2). This treatment also resulted in the longest micro-budded sprout (10.32 cm), the highest number of leaves per plant (3.54) at 30 days, and the largest stock diameter (3.49 mm) at 90 days post-microbudding, confirming its efficacy in citrus propagation. These findings align with Vijayakumari (2019), who reported that six-month-old Rough Lemon seedlings showed earlier sprouting and higher microbudding success. Similarly, Bhagat *et al.* (2013) and Dongre *et al.* (2014) observed that Rough Lemon seedlings grown in Soil + FYM + Cocopeat (2:1:1) had the highest proportion of early buddable seedlings and successful budding rates. Additionally, Singh *et al.* (2018) demonstrated improved growth and budding success in Rough Lemon when using Soil + FYM + Cocopeat (SFC) combined with Azospirillum and AM fungi consortium.

These results emphasize that using Cocopeat + Vermicompost (1:1) with six-month-old Rough Lemon rootstocks optimizes microbudding success, promoting faster sprouting, better seedling growth, and increased budding efficiency in citrus propagation systems.

Table 1. Growth and survival of rootstock as influenced by growing media and age at the time of microbudding

Treatment	Seedling height (cm)	Stem diameter (mm)	Fresh wt. of seedling (g)	Dry wt. of shoots (g)	Leaf area index	Survival of seedlings (%)
$R_1G_1A_1$	10.10	2.15	1.48	0.41	0.41	61.05
$R_1G_1A_2$	15.68	2.64	1.79	0.57	0.43	89.87
$R_1G_1A_3$	16.64	2.91	1.83	0.64	0.64	77.82
$R_1G_2A_1$	10.93	2.18	1.52	0.42	0.56	65.82
$R_1G_2A_2$	17.64	2.98	1.85	0.59	0.42	91.44
$R_1G_2A_3$	20.01	3.44	2.02	0.71	0.67	79.49
$R_1G_3A_1$	12.81	2.22	1.59	0.44	0.93	70.07
$R_1G_3A_2$	16.20	2.85	1.80	0.57	0.52	92.84
$R_1G_3A_3$	20.95	3.53	2.22	0.78	0.60	81.24
$R_1G_4A_1$	13.82	2.33	1.63	0.46	1.10	70.85
$R_1G_4A_2$	19.43	3.36	1.93	0.62	0.56	95.84
$R_1G_4A_3$	21.35	3.59	2.25	0.79	0.83	82.44
$R_2G_1A_1$	9.00	1.98	1.37	0.38	1.17	73.11

$R_2G_1A_2$	14.25	2.42	1.68	0.54	0.38	84.02
$R_2G_1A_3$	18.03	3.00	1.85	0.65	0.58	73.52
$R_2G_2A_1$	9.34	2.12	1.41	0.39	0.72	62.82
$R_2G_2A_2$	14.41	2.51	1.71	0.55	0.43	85.86
$R_2G_2A_3$	16.85	2.96	1.83	0.64	0.60	75.48
$R_2G_3A_1$	16.42	2.87	1.81	0.51	0.66	63.86
$R_2G_3A_2$	14.97	2.56	1.78	0.57	0.62	89.25
$R_2G_3A_3$	18.56	3.11	1.88	0.66	0.52	77.16
$R_2G_4A_1$	13.39	2.25	1.57	0.44	0.70	64.86
$R_2G_4A_2$	19.12	3.31	1.91	0.61	0.54	90.90
$R_2G_4A_3$	20.51	3.50	2.13	0.75	0.74	78.57
SEm±	0.188	0.039	0.021	0.006	1.04	68.13
CD at 5%	0.527	0.108	0.059	0.017	0.009	0.331

Table 2. Effect of rootstock, growing media and age of rootstock at 90 days after microbudding

Treatment	Days taken to first sprout	Microbudding success (%)	Length of sprouted shoot after microbudding 90 days	Number of leaves/ plant days after microbudding at 90 days	Stock diameter (mm) days after microbudding at 90 days
$R_1G_1A_1$	19.88	47.65	9.06	9.49	3.01
$R_1G_1A_2$	18.52	50.42	9.34	9.76	3.26
$R_1G_1A_3$	17.32	52.98	9.46	9.87	3.31
$R_1G_2A_1$	19.75	44.75	9.13	9.52	3.13
$R_1G_2A_2$	16.78	57.24	9.67	9.94	3.34
$R_1G_2A_3$	14.65	68.33	10.09	10.15	3.44
$R_1G_3A_1$	19.62	46.19	9.13	9.55	3.14
$R_1G_3A_2$	18.32	51.20	9.35	9.80	3.27
$R_1G_3A_3$	13.55	73.37	10.20	10.26	3.46
$R_1G_4A_1$	19.35	48.94	9.17	9.62	3.18
$R_1G_4A_2$	15.22	65.62	9.98	10.11	3.43
$R_1G_4A_3$	12.98	79.12	10.32	10.43	3.49
$R_2G_1A_1$	20.15	42.60	8.92	9.40	3.07
$R_2G_1A_2$	19.22	49.03	9.20	9.65	3.21
$R_2G_1A_3$	16.48	59.01	9.85	9.97	3.36
$R_2G_2A_1$	19.98	43.31	8.95	9.45	3.09
$R_2G_2A_2$	18.95	49.44	9.27	9.68	3.22
$R_2G_2A_3$	16.92	53.30	9.49	9.91	3.32
$R_2G_3A_1$	17.78	53.08	9.41	9.84	3.28
$R_2G_3A_2$	18.82	49.98	9.28	9.72	3.24
$R_2G_3A_3$	16.08	61.34	9.89	10.00	3.38
$R_2G_4A_1$	19.52	50.07	9.13	9.58	3.16
$R_2G_4A_2$	15.65	64.25	9.98	10.03	3.40
$R_2G_4A_3$	14.02	72.07	10.15	10.23	3.46
SEm±	0.168	0.375	0.070	0.136	0.042
CD at 5%	0.471	1.051	0.195	0.380	0.117

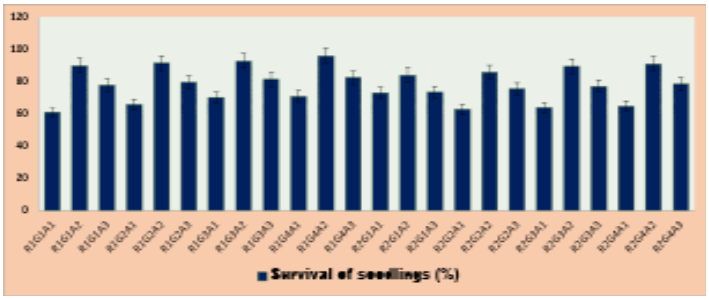

Fig. 1: Survival of seedlings (%) as influenced by growing media and age at the time of microbudding

Fig. 2: Effect of rootstock, growing media and age of rootstock on microbudding success (%)

Preparation of rootstock

Wedge cut for inserting bud

Covering with micropipette cap

Conclusion

The study demonstrates that Cocopeat + Vermicompost (1:1) is the best growing medium for Rough Lemon rootstocks, with the age of six month old ($R_1G_4A_3$) showing superior performance in both physiological and post-budding parameters. This treatment exhibited 79.12% microbudding success, minimum days to first sprout (12.98 days), and maximum seedling height (21.35 cm), stock diameter (3.59 mm), and leaf area index (1.17). These results provide a strong basis for recommending Cocopeat + Vermicompost (1:1) as a sustainable and efficient growing medium for large-scale Mandarin propagation, reducing the nursery phase while improving overall plant health and success rates.

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Conflict of Interest

The authors have no conflict of interest.

Data Sharing

All relevant data are within the manuscript.

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