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Research Article

MICRO BUDDING OF INDIGENOUS & EXOTIC CITRUS CULTIVARS: A BOON TO INDIAN FARMERS TO SHORTEN THE CITRUS NURSERY PHASE

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ABSTRACT

Commercial Citrus propagation in India, by shield/T budding is an age old practice and is confronted with the limitations of appropriate rootstock size/age (1 year old Rough lemon(*C. jambhari* Lush), 1 ½ to 2 year old Rangpur lime (*C. limonia* Osbeck)), scion wood (round twigs having white streaks which are season restricted) and favorable climate for budding. Further traditional budding takes longer time (about 22 months) to produce plantable bud grafts. A rapid and year round propagation technique known as "micro budding" that can reduce the nursery phase was developed first time in India at CCRI in *Citrus reticulata* Blanco Nagpur Mandarin on just 5 month old commercial Citrus rootstocks of Central India. This method is reliable & reproducible for all Citrus species (Sweet orange (*C. sinensis* Osbeck), Kinnow mandarin (*C. nobilis* x *C. deliciosa*) and other exotic cultivars also. This biotech break has tremendous scope in commercial propagation and research. It can be done in a low cost green house and reduces huge cost on labour and maintenance during the nursery phase. Through this technique CCRI has multiplied around 15,000 quality planting material of Nagpur mandarin in a time span of 11 - 12 months and released grafts to Citrus growers of Central India. Before releasing, the micro budded plants of *Citrus reticulata* Blanco Nagpur mandarin were evaluated for their performance in replicated field trials both at CCRI and on farmers fields. This paper reports the protocol and application of microbudding with promising citrus cultivars for early market supply to the farmers and initial field performance.

INTRODUCTION

Citrus spp. are traditionally propagated by shield/T budding where in an active single bud collected from a selected mother tree (scion) is budded on to a desirable rootstock seedling of suitable size. Successful bud union requires proper contact between the tissues of scion and root stock, meristematic growth and a favourable environment (high humidity and proper temperature for cambial activity). Seedlings of popular root stock spp namely Rough lemon, and Rangpur lime attain buddable size at the age of 1 year and 1 ½ to 2 years. Buds with active meristematic cells/phloem can only be excised from scion wood of proper size in right season. Budding period of a locality is limited by seasonal variations in humidity and temperature.

In Central India, nursery men traditionally raise the root stocks in nursery beds in the open field and after attaining suitable size, budding is done in the winter season from November to January. The budding requirements mentioned above restrict the quantum of budgrafts produced in a year. A simple, economical budding technique named as microbuddding was first developed by Dr. M. Skaria, Texas A&M University,U.S.A and later standardized in Nagpur mandarin for the first time in India.(Vijayakumari and Shyam Singh 2000a, 2000b, 2002a, 2002b and 2003, Vijayakumari, 2008). At CCRI the Shoot Tip Grafting and Microbudding techniques were dovetailed to produce promising, disease free, marketable bud-grafts on five month old root stocks economically in a shorter period and plants were supplied to farmers. The performance of micro-budded plants was evaluated both at nursery stage and after field planting and the results are presented in this paper.

MATERIALS AND METHODS

Seeds of commercial root stock species viz., Rough lemon and Rangpur lime were sown in sterilized soil in trays. Two months old seedlings were transferred to polybags (12"x6") having sterilized soil mixture and grown till the required budding height of more than 10" was obtained. Shoot Tip grafting (STG) derived disease free plants maintained in bigger cement pots were used as a source of scion wood. Delicate young buds carefully cut from the tender new flush were used for

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microbudding. The rootstock (both Rough lemon and Rangpur lime) seedlings were given a 'Wedge' shaped cut after decapitating at a height of 10" from the bottom and the tender scion bud of commercial citrus scions like sweet orange, kinnow mandarin, Nagpur mandarin , cala mondin , acid lime and some potential exotic citrus cultivars were inserted inside the wedge cut and covered with a microtip of micropipette to maintain high humidity level. After a week, micro-budded plants were observed for survival/ sprouting and the microtip caps were removed within 12-16 days. The performance in the nursery phase, in terms of budding success, days taken for sprouting, length of the shoot and number of leaves, of micro-budded Vs conventional budded grafts were recorded. After field planting Plant Height, Stem height, Stock girth, Scion girth, canopy volume and fruit number were recorded. The data was statistically analyzed (using CRD / RBD designs) and results presented.

RESULTS

Budding Success : Microbudding was successfully done on Rough lemon and Rangpur lime rootstock seedlings of five month age, 10" height, measuring 3 mm in diameter by inserting new flush of scion in a wedge cut given at the decapitated top of the seedling and immediately covering with a micropipette tip (Fig. 1). Bud sprouting was observed within 12-14 days. Days taken for bud emergence is significantly lower in microbudded rough lemon compared to conventional budding (Fig. 2). The time taken for the emergence of sprouted shoots in microbudding is half of the duration required in conventional shield budding. In rough lemon maximum mean success (95.71%) obtained with microbudding in comparison with propagation by conventional shield budding (90.57%). No significant difference was observed between the treatments for plant height and number of leaves both at 4 ½ and 9 months after budding in rough lemon rootstock (Table 1.).

Fig 1 Micro budding with micropipette tip.

Fig 2 Micro vs conventional budding one month after microbudding.

In case of Rangpur lime also significantly higher percentage of budding success (92.41%) was obtained with microbudding propagation compared to conventional shield budding (73.81%). Significantly earliest sprouting bud was obtained in microbudding compared to shield budding (Table 2).

Table 1 Comparative performance of micro budding vs conventional budding on Rough lemon

Sr. No	Rootstock	Type of budding	Days of sprouting	Plant height (4½ months)(cm)	No. of leaves (4 ½ months)	Plant height (9 months)(cm)	No. of leaves (9 months)	Success of micro budding (%)
1.	Rough lemon	Microbudding	12.90	23.80	20.66	47.95	36.57	95.71 (78.03)
		Commercial budding	30.14	23.31	20.34	50.15	36.93	90.57 (72.05)
	CD 1%		0.728	NS	NS	NS	NS	NS
	5%		0.522					

Table 2 Comparative performance of micro budding Vs conventional budding on Rangpur lime

Sr. No	Rootstock	Type of budding	Days of sprouting	Plant height (4 ½ months) (cm)	No. of leaves (4 ½ months)	Plant height (9 months) (cm)	No. of leaves (9 months)	Success of micro budding (%)
1.	Rangpur lime	Micro budding	13.40	21.42	20.14	45.21	35.26	92.41 (74.00)
		Commercial budding	30.57	24.22	21.36	55.34	44.42	73.81 (59.21)
	CD 1%		0.470	-----	NS	-----	6.87	NS
	5%		0.337	2.683		7.383	4.93	

Figures given in parenthesis are transformed (angular) values

Significant difference was recorded between the treatments for number of leaves and plant height at 9 months after budding (Fig 3), but the difference observed in the number of leaves and planr height is not significantly high between the two types of budding. The budding success was more in Rough lemon than Rangpur lime.

Figures given in parenthesis are transformed (angular) values

Fig 3 Micro vs conventional one month after budding in Rangpur lime

Nursery performance: A total of 19 exotic and indigenous Citrus cultivars were propagated via microbudding for early market supply of newly introduced/ released varieties, which fall in the category of mandarin, Sweet orange, Grapefruit, pummelo and Acid lime. The data revealed in mandarin group the success varied between 70-100%, in Sweet orange the microbudding success varied between 65-100%, in grapefruit between 81.5 to 100%, in pummelo both the indigenous & exotic pummelo cultivars microbudded with 100% success. Poor microbudding success was obtained in Acid lime (20%). Significant difference was recorded in days taken for sprouting, total plant height and number of leaves 3 month after microbudding.

Maximum mean plant height (47.50 cm) was recorded in Nagpur mandarin followed by N4-seedless (42.90), Cutter Valencia (42.73), USA-145(41.93), Calamondin (41.80). Over all mandarins performed better because, mandarins have a good compatibility with roughlemon. Significantly lowest values in terms of micro budding success, plant height and number of leaves were recorded in Acid lime (Table 3) The procedure was successful throughout the year in low cost screen house. (Ochoa *et al*, obtained a bud take of 61% and 80% under temperature regime of 24 to 32℃ and 21 to 27℃ respectively, when 'Hamlin' sweet orange (*Citrus sinensis*(L.)Osb.) microbudded on to sour orange (*C. aurantium* L.) rootstock for biological indexing purpose in USA).

Table 3 Effect of exotic and indigenous cultivars on micro budding success

S.no.	Treatment	% Success	Days taken for sprouting	scion height(cm)	girth of scion(cm)	total height(cm)	no of leave
1	Nagpur mandarin	100(90)a	13.30h	23.90a	0.98bc	47.50a	20.60a
2	Sweet orange	90(73.62)bc	16.50g	17.80bc	0.83cde	39.00bcde	17.70ab
3	Cala mondin	80(66.38)cde	16.20g	20.70ab	0.74defg	41.80abc	17.60ab
4	Kinnow mandarin	70(59.87)de	16.50g	15.40cd	0.64g	31.80efg	14.30bc
5	Acid lime	20(26.56)f	19.00efg	2.4i	0.14h	6.6h	2.8g
6	N4 seedless	100(90)a	15.90g	17.46bc	0.82cdef	42.90ab	14.66bc
7	CCRI pummelo 5	100(90)a	19.80de	7.66gh	0.88bcde	32.45defg	6.46fg
8	Cutter Valencia	100(90)a	16.40g	15.79bc	1.03b	42.73ab	11.93cd
9	Flame Grapefruit	100(90)a	20.40cde	14.39cdef	0.91bcd	39.19bcd	10.59de
10	Frost owari	86.66(71.58)bcd	16.80fg	10.33efgh	0.64fg	33.73defg	9.26def
11	USA-145 Pummelo	100(90)a	20.50cde	14.86cde	134a	41.93abc	10.06def
12	Mar's early	86.48(70.80)bcd	21.10bcd	10.60defg	0.88bcde	36.10bcdef	9.85def
13	Salustiana	97.10(82.38)ab	22.25abc	9.8fgh	0.88bcde	35.07cdef	9.70def
14	Delta	92.59(77.74)abc	20.60cde	8.22gh	0.73efg	31.92defg	9.0def
15	Trovita Strain	96.55(83.13)ab	23.65a	5.57hi	0.64g	27.64g	6.55f
16	Cara cara	90(75.98)bc	23.35ab	7.85gh	0.71efg	29.90fg	8.95def
17	Rio red	85(72.12)bcd	20.60cde	8.60gh	0.83cde	32.00defg	8.75def
18	Diller	65.71(54.60)de	22.40abc	8.24gh	086bcde	32.72defg	9.60def
19	Star Ruby	81.57(70.27)bcd	18.20efg	8.0gh	0.85cde	30.57fg	7.65ef
CD %	0.01%	17.905	3.194	6.592	0.235	9.656	4.865
	0.05%	13.500	2.408	4.970	0.177	7.280	3.668

Figures in parenthesis are transformed values

Fig 4 Micro budding of promising exotic Citrus scion cultivars

Field performance*:* Studied the comparative field performance of Microbudded (propagated on 5 month old rootstocks) vs. Conventional budgrafts (propagated one year old rootstock) in on farm demonstration trail at CCRI, Nagpur. The statistically analyzed data on initial bearing performance indicated microbudded trees are either at par or better in vegetative growth and yield parameters viz. plant height, stem height, scion growth, stock growth and canopy volume and number of fruits compared to conventionally propagated trees by shield budding even through both were field transferred at the same time(Table 4.) The microbudded plants reached the field earlier by at least 10 -12 months, but performed true to type started bearing from 3rd year onwards confirming by absence of juvenile phase.

Table 4 Comparative field performance of microbudded Vs commercial budded plants on Rough lemon rootstock

Rootstock	Type of budding	Plant height (m)	Stock girth (cm)	Scion girth (cm)	Stem height (cm)	Canopy volume (m3)	No. of fruits
Rough lemon	Microbudding	4.24	58.85	55.92	60.35	14.05	524.28
	Commercial budding	3.81	53.28	50.57	57.07	13.73	402.50
	CD						
	1%	0.37	4.11	----	NS	NS	84.71
	5%	0.27	2.95	4.54			60.75

Commercial citrus nurseries use shield / T budding method for nursery stock production. Rootstock seedlings of appropriate size/age (1 year old in case of rough lemon and $1^{1}/_{2}$ to 2 year old in case of Rangpur lime), suitable bud wood and favorable climate /season are essential. Further bud grafts

requires longer time (18-20 months) for field release. These requirements limit the rate of multiplication.

To overcome the above limitations, micro budding protocol is standardized and successfully employed for faster, economical and year-round multiplication of planting material of Nagpur mandarin and other *Citrus* species under humidity controlled screen house/ low cost shade net structure and requires lesser space . Micro budding is a miniature budding in which young citrus root stock measuring 3 mm diameter are de-topped and scion bud is inserted in a wedge cut on the root stock. The budded portion is immediately covered with a micropipette tip to modulate the climate and to protect it. The micro pippets tips are removed after 12-14 days from the sprouted bud grafts. The bud grafts attain plantable size in 10 months. Sourcing of scion from STG derived disease free plants assures health of the plants. It was apparent from the data of nursery performance of microbudded vs conventional budded plants. The growth in terms of length of shoot and number of leaves is either at par or faster in microbudded plants than in conventional budded plants of Rangpur lime and Rough lemon. The growth (number of leaves after 41/2 and 9 months of budding) of microbudded plants (both on Rough lemon and Rangpur lime) was at par with the growth of conventional budded plants even though microbudding was done on 5 month old rootstocks and conventional budding was done on 12 month old rootstocks. The microbudded plants reached plantable size within a year after sowing the rootstock seeds.

Rangpur lime which is inherently slow growing rootstock, also attained the plantable size after microbudding with in a year from the date of sowing of rootstock seeds [Position of Table 2]. The microbudding propagation shortens the nursery phase from almost 2 years to 1 year depending on the rootstock and the region, which is evident from the data. Skaria (2000) reported faster production of budded plants at a low cost compared to conventionally budded trees.

Microbudding was explored with different indigenous and exotic citrus cultivars for early market supply of bud grafts. The data revealed highest microbudding success and plant growth in terms of total plant height, number of leaves & girth of scion 3 months after microbudding was more in Nagpur mandarin compared to rest of the cultivars. Overall mandarin performed better because of their good compatibility with rough lemon rootstock. Mandarin group microbudded with70-100% success and sweet orange group cultivars microbudded with 65-100% success. Ochoa *et al*, obtained a bud take of 61% and 80% under temperature regime of 24 to 32°C and 21 to 27°C respectively, when 'Hamlin' sweet orange (*Citrus sinensis* (L.)Osb.) microbudded on to sour orange (*C. aurantium* L.) rootstock for biological indexing purpose in USA).

From the field evaluation it is clear that microbudded plants reached the field earlier by at least 10 -12 months, but performed true to type started bearing from 3rd year onwards confirming by absence of juvenile phase. In Texas microbudded grape fruit and lemon trees produce the fruits within 2 months after microbudding (Skaria 2000).

Micro budding was successful with different commercial scion and rootstock combinations (Skaria, 2000; Vijayakumari, 2000b). Micro budding was also used in *in vitro* rescue of genetically modified Rio Red grape fruit explants in Texas (Skaria,2000), which were otherwise difficult to root & develop into complete plants. In Texas, USA, one private nursery successfully produced microbudded trees commercially with good growth both in nursery as well as in the field (Skaria, 2000). In India, even though some progressive farmers are coming forward to learn the microbudding technique for implementation in the commercial propagation, it is yet to reach to the commercial nursery men/growers for wider adoption due to lack of awareness regarding benefits. Newerthless the technology on the microbudding was transferred for commercial implementation to the entrepreneur farmer of Tamilnadu in January 2018 by CCRI, Nagpur.

CONCLUSION

Standardization of micro budding on just 5 months old root stock is a significant mile stone in the propagation of Citrus trees that enables faster, low cost, year round multiplication which is a boon for farmers and researchers (for disease investigation). Microbudding technology was explored by early market release of promising indigenous and exotic citrus cultivars at CCRI, Nagpur. The field performance of microbudded trees showed on par survival, growth, true to type character and precocity with a potential for high density planting.

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