Indian J. Hort. 71(3), September 2014: 311-314

Optimization of micro-budding technique in Coorg mandarin (*Citrus reticulata* Blanco)

G. Karunakaran[*], H. Ravishankar[**], T. Sakthivel[***] and D.K. Samuel[***]
Central Horticultural Experiment Station (IIHR), Chettalli 571 248, Kodagu, Karnataka

ABSTRACT

In the present study, 45- and 60-day-old Coorg mandarin micro-buds were budded on five- and six-month-old Rangpur lime rootstock seedlings. Higher success of micro-budded plants was recorded (30.71%) on six-month-old Rangpur lime rootstocks by using 60-day-old scion buds, which was higher than the five-month-old rootstock seedlings (18.66%) with same age of scion buds. Further, in order to increase the success, micro-budding was attempted under protected structures. Success of micro-budding on Rangpur lime was higher in polyhouse (56.49%) than shade nethouse (34.44%) and open conditions (21.50%) than other rootstocks. The number of days taken for sprouting was much earlier under protected conditions in Rangpur lime (13.36%) followed by Troyer citrange (14.82%) and trifoliate orange (19.54%) rootstocks. Micro-budded plants under open conditions took longer time for sprouting on Rangpur lime (18.32). Success of micro-budding was found to be significantly higher in protected structures than the open conditions for all the rootstocks. The results suggested that micro-budding technique could be gainfully exploited for shortening the propagation period and early biological indexing of citrus.

Key words: Citrus, propagation, micro-budding, Coorg mandarin.

INTRODUCTION

In Karnataka, Tamil Nadu and Kerala, an ecotype of mandarin, Coorg mandarin is quite popular and grown for over a century. It is mainly grown as one of the component crops in the coffee-based cropping system in these regions. Shield and T-budding are the age-old practice followed for commercial propagation in Coorg mandarin wherein rootstock used is 1½ to 2-year-old. Further, conventional propagation is season oriented and requires long time to produce budded plants. Micro-budding is a new 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. This technique was first developed by Skaria and Zhang (7) and later this technique was standardized in Nagpur mandarin, sweet orange and grapefruit (Vijayakumari and Singh, 8; Alam *et al*., 1; Mazhar *et al*., 5). Micro-budding facilitates year round multiplication of planting material to get the earliest marketable budded seedlings at lower cost as compared to conventionally budded plants. In addition, the technique has also the potential for early detection of virus and virus-like diseases in plants through the biological indexing (Ochoa *et al*., 6; Vijayakumari *et al*., 9, 10). Therefore, optimization of micro-budding technique in Coorg mandarin was attempted which is beneficial for commercial citrus growers and nurserymen to strengthen Coorg mandarin cultivation in Kodagu, Karnataka. Hence, the present study was undertaken.

*Corresponding author's E-mail: karanfruitsg@yahoo.co.in
**Director, Central Institute for Sub tropical Horticulture, Lucknow
***Indian Institute of Horticultural Research, Bengaluru

MATERIALS AND METHODS

Present study was conducted at CHES, Chettalli, Kodagu, Karnataka. Seeds of rootstocks Rangpur lime (*Citrus limonia* Osb.), Troyer citrange (*Poncirus trifoliata* (L.) Raf. × *C. sinensis* (L.) Osb.) and trifoliate orange (*Poncirus trifoliata* (L.) Raf.) were collected from the citrus germplasm maintained at CHES, Chettalli. Fresh seeds were sown in primary nursery and seedlings were raised in sterilized soil in trays following regular nursery practices. Forty-five day-old healthy seedlings from the primary nursery were selected and transplanted in polybags (30 cm × 10.5 cm) filled with farm yard manure, forest soil and sand (2:1:1). Uniform fertilizer schedule was adopted to attain required height (20-25 cm) of rootstock seedlings for micro-budding. The newly emerging buds were labeled in elite mother plants of Coorg mandarin maintained under polyhouse to serve as bud woods. Young delicate buds of uniform age (45-60 days) were carefully excised from the tender flush and used for micro-budding on five and six-month-old rootstock seedlings. In the beheaded (15-20 cm) rootstock seedlings, wedge shaped cut (2-2.5 cm) was given downward with sharp knife and tender scion

bud inserted and secured in place with polythene strip to ensure proper cambial contact and union. After 15-20 days, survival of micro-bud was recorded and polythene strip was removed.

Experiment I comprised of standardization of the age of stock and scion for micro-budding technique, laid out in completely randomized design, while Experiment II was conducted to enhance success rate of micro-budding was also studied in three different commercial rootstocks, *viz*., Rangpur lime, Troyer citrange and trifoliate orange under different protected structures (polyhouse, shade nethouse and open conditions). There were 10 plants per treatments per replications in a factorial completely randomized design, replicated five times. The mean minimum and maximum temperatures that prevailed (19.1 & 28°C) in open conditions, shade nethouse (21 & 28.8°C) and polyhouse (23.7 & 29.5°C) were recorded. The detailed observations on percentage of success, days taken for sprouting and growth characters were recorded and data analysed statistically.

RESULTS AND DISCUSSION

In Experiment I, higher success of micro-budded plants were recorded (30.71%) on six-month-old Rangpur lime rootstock seedlings by using 60-day-old scion buds, which was higher than the five-month-old rootstock seedlings (18.66%) with same age of scion bud. The least success (14.37%) was noticed in the five-month-old rootstock with 5-day-old micro-buds. The number of days taken for the micro-budded plants to sprout showed significant results among the age of stock and scion. The sprouting of micro-bud was faster (14.90 days) on six-month aged rootstocks with 60-day-old micro-bud, followed by the same age of rootstock with 45-day-old micro-bud. Forty five-day-old scion buds with five-month age of rootstock took maximum number of days (20.33) to sprout (Table 1). Significant results were observed with different age of stock and scion in respect of success percentage and sprouting of micro-buds. Kadam *et al.* (3) reported that micro-grafting success was significantly influenced by the rootstock age in wood apple. It is apparent that success of microbudding depends on the rate of tissue differentiation and proliferation of rootstock and scion, which is affected by age. Performance of rootstock age six-month with 60- and 45-day-old scion was significantly superior as compared to five months with 60- and 45-day-old scion buds. Vijayakumari *et al.* (9) reported that better performance in success percentage and growth characters in Nagpur mandarin was recorded in five and six-month-old rootstocks than the four months. Lowest success rate was recorded in five-month-old rootstock with 45-day-old scion could be due to quick cell dehydration, proliferation of callus tissues by both the graft components (stock and scion) leading to vascular discontinuity arising from inadequate physiological maturity of rootstock (Wang and Kollmann, 11).

Table 1. Performance of micro-budding with different age of Rangpur rootstock and Coorg mandarin.

Treatment	Success (%)	Days taken for sprouting
5-month-old rootstock and 45-day-old micro-bud	14.37 (22.28)*	20.33
5-month-old rootstock and 60-day-old micro-bud	18.66 (25.59)	17.90
6-month-old rootstock and 45-day-old micro-bud	22.89 (28.58)	16.48
6-month-old rootstock and 60-day-old micro-bud	30.71 (33.66)	14.90
CD at 5%	1.70	1.51

*Figures in parentheses indicate Arcsine transformed values

The comparative evaluation (Table 2) of microbudding and T-budding (conventional) revealed that the success percentage of micro-budding (49.56) was lower than the conventional budding (89.67) under polyhouse. Micro-budding gave moderate success (49.56%), but in Nagpur mandarin with six-month-old rough lemon rootstock gave 70 per cent success (Vijayakumari and Shyam Singh, 8; Vijayakumari *et al*., 9).

In Experiment II, micro-budding technique was attempted on different commercial rootstocks, to increase the success percentage of micro-budding evaluated under different structures indicated that the success percentage, days taken for sprouting and growth was significantly better on Rangpur lime, followed by Troyer citrange and trifoliate orange, which were at par for all the parameters except number of leaves (Fig. 1). Rangpur lime recorded the highest micro-budding success and growth characters,

Table 2. Comparative performance of micro-budding vs conventional budding on Rangpur lime rootstock.

Propagation	Age of the rootstock (month)	Success (%)	Days taken for sprouting
Micro-budding	6	49.56 (44.75)*	16.57
Conventional budding	12-14	89.67 (71.26)	25.19
CD at 5%	-	3.13	1.32

Age of scion (60-day-old micro-bud)
*Figures in parentheses indicate Arcsine transformed values

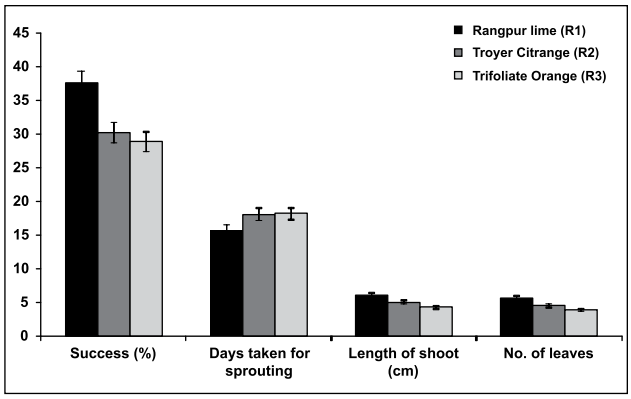

Fig. 1. Performance of micro-budding on three different rootstocks. (Data pooled over all protected structures and open conditions). *The error bars indicate SE of percentage

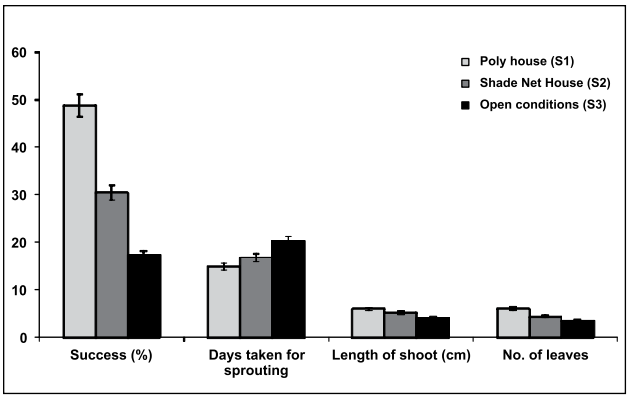

Fig. 2. Performance of micro-budding under protected structures (pooled data).

which showed it as suitable stock-scion combination and seems that this species have peculiar growth characteristics. Further, Karunakaran *et al.* (4) reported that Rangpur lime rootstock attributed the better compatibility with Coorg mandarin.

Among the protected structures, the maximum success percentage, growth parameters and lesser time for sprouting of buds was registered under polyhouse which were significantly superior to shade nethouse and open conditions (Fig. 2). These findings showed similarity with that of Vijayakumari and Singh (8) in Nagpur mandarin. Earlier, Skaria and Zhang (7) reported that successful micro-budding on several rootstocks throughout the year under greenhouse conditions. Protected environment helps to boost up the success of micro-budding. This might be due to optimum temperature prevailed in protected growing conditions which in turn results better parenchyma fill space between stock and scion.

Success of micro-budding on Rangpur lime was significantly higher in polyhouse (56.49%) followed by shade nethouse (34.44%) and open conditions (21.50%). Similar trend was observed in respect of micro-budding on Troyer citrange and trifoliate orange (Table 3). Days taken for sprouting was earlier under polyhouse in Rangpur lime (13.36) followed by Troyer citrange (14.82) and trifoliate orange rootstock (16.54) when compared to the shade nethouse conditions. Length of shoot and number of leaves were higher in Rangpur lime rootstock under polyhouse than the shade nethouse and open conditions irrespective of the rootstocks. This is corroborated by the findings of Vijayakumari and Singh (8) and Vijayakumari *et al*. (10) that success of micro-budding and growth characters in Rangpur lime was higher in both screen as well as glasshouse than open unprotected conditions. This could be attributed to optimum microclimate prevailing in polyhouse and better wound healing response in Rangpur lime, which in turn resulted in quicker formation of callus and better cell recognition.

Table 3. Performance of microbudding in Coorg mandarin on different rootstocks under protected structures.

Treatment	Success (%)			Days taken for sprouting			Shoot length (cm)			No. of leaves		
	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Polyhouse (S_1)	56.49 (48.73)*	49.71 (44.83)	40.16 (39.33)	13.36	14.82	16.54	7.21	6.25	4.48	7.55	5.94	4.80
Shade nethouse (S_2)	34.44 (35.20)	26.03 (33.11)	30.74 (34.05)	15.58	17.23	17.69	6.21	5.13	4.51	5.46	4.26	3.62
Open conditions (S_3)	21.50 (26.65)	14.91 (22.94)	15.61 (23.28)	18.32	22.27	20.26	4.76	3.77	3.75	4.00	3.36	3.16
CD at 5%	6.09	7.94	4.50	2.47	2.74	3.01	1.29	1.04	NS	1.21	1.22	1.04

Age of scion (60-day-old micro-bud) and rootstock (6-month-old): R_1 = Rangpur lime, R_2 = Troyer citrange, R_3 = Trifoliate orange
*Figures in parentheses indicate Arcsine transformed values

When micro-budded plants were kept under open conditions there was no influence on the success, sprouting and growth of microbuds, possibility due to lack of cambial activity at point of bud union (Ana Pina and Errea, 2; Wang and Kollmann, 11). Trifoliate orange rootstock took slightly longer time to sprout and its performance was on par irrespective of the kind protected structures.

Study indicated that better performance of micro-budding under protected structures which is due to optimum temperature regime (23.7-29.5°C) and relative humidity prevailing under polyhouse during the period. The results are also in close conformity with the findings of Ochoa *et al.* (6) who obtained a bud take of 61 and 80% under temperature regimes of 24-32° and 21-27°C respectively, when sweet orange was microbudded on sour orange rootstock for biological indexing purpose. This phenomenon can be well explained in light of the fact that active scion showed quick sprouting on account of proper callusing and union, physiological state and stored food materials under favourable environment conditions as could be made available under polyhouse. The results pointed out the possibilities of exploiting micro-budding in Coorg mandarin and could prove useful in shortening the propagation period and early orchard establishment. Furthermore, this approach may also reduce the time for biological indexing of virus and virus-like diseases in citrus plants. The study also suggested that in order to improve success percentage over conventional budding manipulation of physical factors affecting micro-bud necrosis and healing of graft union needs critical attention.

REFERENCES

1. Alam, N., Naveed, F., Khan M.M., Abbas, M. and Ahmad, S. 2006. Early age propagation of three commercial C*itrus* species through microbudding technique. *Pakistan J. Agri. Sci.* **43**: 42-44.

2. Ana Pina and Errea, P. 2005. A review of new advances in mechanism of graft compatibility-incompatibility. *Scientia Hort.* **106**: 1-11.

3. Kadam, J.H., Karale, A.R., Garad B.V. and Desai, U.T. 2007. Micro-grafting in wood apple. **In**: *Recent Trends in Horticultural Biotechnology*, Raghunath Keshvachandran (Eds.), New India Publishing Agency, New Delhi, pp. 371-73.

4. Karunakaran, G., Ravishankar, H., Samuel D.K. and Sathiya Priya, N. 2006. Standardization of shoot tip grafting in Coorg mandarin (*Citrus reticulata* Blanco). **In**: *Proc. Natl. Symp. Citriculture: A Road Map*. Shyam Singh, A.K. Srivastava, V.J. Shivankar and C.N. Rao (Eds.), pp. 2-4.

5. Mazhar, A., Khan, M.M., Mughal, S.M., Jaskani, M.J. and Haider, A. 2006. Propagation of CTV-free sweet orange [*Citrus sinensis* (L.) Osb.] plants through microbudding technique. *Pakistan. J. Bot*. **38**: 583-87.

6. Ochoa, P.M., Dekkers, M.G.H., Skaria, M. and Lee, R.F. 2000. Use of microbudding to expedite production of experimental citrus hosts for use for biological indexing of citrus pathogens. **In**: *ISC Congress-2000*, Orlando, Florida, pp. 129.

7. Skaria, M. and Zhang, T. 2000. Field performance of micro-budded citrus trees in Texas. **In**: *ISC Congress-2000*, Orlando, Florida, pp. 130.

8. Vijayakumari, N. and Singh, S. 2003. Standardization of microbudding technique in citrus. *Indian J. Hort. Sci.* **60**: 127-30.

9. Vijayakumari, N., Singh, S. and Thote, S.G. 2008. Application of micro-budding: A faster propagation technique in citrus. *J. Soils Crops*, **18**: 89-91.

10. Vijayakumari, N., Singh, S., Ghosh, D.K., Das, A. K. and Dhurjati, T. 2001. Performance of micro-budding under different greenhouse structures in *Citrus reticulata* cv. Nagpur mandarin. *South Indian Hort*. **49**: 335-37.

11. Wang, Y. and Kollmann, R. 1996. Vascular differentiation in the graft union of *in vitro* grafts with different compatibility. *J. Plant Physiol.* **147**: 521-33.

Received: August, 2012; Revised: July, 2014;
Accepted: August, 2014