

Effect of Age of Rootstock and Shade on Success of Microbudding in Sweet Orange CV. Sathgudi (*Citrus sinensis* L. Osbeck)

T. Teja¹, L. Mukunda Lakshmi², K.T. Venkata Ramana³ and G. Thanuja Sivaram⁴

¹HRS, Darsi, A.P Dr. Y.S.R.H. University

^{2,3}CRS, Tirupati, A.P Dr. Y.S.R.H. University

⁴HC & RI, Arpet Dr. Y.S.R.H. University

E-mail: ¹tejahorticulture16@gmail.com, ²lmukunda@gmail.com,

³ramanaktv@rediffmail.com, ⁴tejahorticulture16@gmail.com

Abstract—Sweet orange (cv. Sathgudi) is one among the major Citrus fruits grown in India. From decades sweet orange was commercially propagated by 'T' or Shield budding. Success of this method of propagation proved commercially viable but it takes much time to develop and maintain a successful budded plant which normally takes 1.5 to 2 years. To reduce the time span and maintainance of the rootstocks which is the main constraint in sweet orange industry, a new method of propagation, Microbudding, which involved grafting axillary bud (2 to 3 mm width and 1 to 1.5 cm length) on decapitated head of 4 to 6 month old root-stock, which resulted the grafted plants ready for planting within short period of 3-4 months was developed recently. Several factors influenced the success rate of Microbudded plants as it favors less aged rootstock with a very small scion (bud).

An experiment was conducted to standardize the optimum age (2 month to 6 month) of root stock seedling for micro-budding under open and shade net conditions and the study of results revealed that budlings prepared on six month old Rangpur lime root stock seedlings in open were found to take minimum time for sprouting (13 days) and recorded maximum success percentage (91.11 %) of budded plants, highest bud wood size (3.69 mm), maximum number of sprouts (1.90 no's), higher number of nodes and leaves (12.33 no's) with maximum plant height (16.30 cm) at 90 days after microbudding (DAM). Also Microbudding technique is found successful throughout the year under green house conditions. The process was unique as it bypasses the nursery phase besides require scions smaller than conventional trees suited for ultra high density planting.

Keywords: Microbudding, Sweet orange, age of rootstock

1. INTRODUCTION

Sweet orange is a profusely branched thorny shrubs or small tree belonging to the family Rutaceae. Globally sweet orange accounted for approximately 68% of citrus production. Andhra Pradesh occupies 1st place in citrus crop (Sathgudi and Acid lime) with the area 2.50 Lakhs ha. Among citrus crops in Andhra Pradesh sweet orange cv. Sathgudi occupies

an area of 2.04 Lakhs ha. producing 30.61 Lakhs MTs of fruits with a productivity of 15 MT per ha (SHM, 2013).

Microbudding is a unique technique developed by Luis Navarro, C.N. Roistacher, and Toshio Murashige at the University of California, Riverside, in 1975. It was a novel technique developed to produce citrus plants rapidly and inexpensively (Skaria and Zhang, 2000). This technique involved grafting axillary bud (2 to 3 mm width and 1 to 1.5 cm length) on decapitated head of 2 to 6 month old root-stock, which resulted the grafted plants ready for planting within short period of 3-4 months. Provision of shade during and after budding, was found to have beneficial effect on success of budding. Light is essential for triggering photosynthetic activity and thereby better nourishment of budded seedlings. The rate of photosynthetic activity varies with the level of shade (Swamy *et al.*, 1993). Microbudding technique is found successful throughout the year under green house conditions.

Success rate of the microbudded plants was mainly attributed to optimum temperature and relative humidity but detailed studies were not made to develop technology to produce the grafts throughout the year as demand for planting material is increasing every year. Hence, it is envisaged to maintain the optimum temperature and relative humidity conditions to improve the success rate of budlings throughout the year. Therefore, it is felt necessary to evaluate the effect of rootstock age and shade level on success of microbudding in sweet orange cv. Sathgudi in order to correlate with the prevailing temperature and relative humidity.

2. MATERIAL AND METHODS:

2-6 months old seedlings of Rangpur lime were used as a rootstock. The budwood taken from Sathgudi sweet orange (*C. sinensis* L. Osbeck) was used as a scion material. Healthy and elite mother trees with sufficient new flush of sweet orange cv.

Sathgudi were selected in the bud wood block of AICRP on Fruits, Tirupati. The terminal shoots of current season growth with 15-20 cm length and nodal buds not less than 8, having pencil thickness were selected, pre-cured on the same day of microbudding. The scion shoots were collected from mother trees early in the morning on the day of budding. The micro budding was done in the month of March 2014. The root stocks were budded by inserting axillary bud (2 to 3 mm width and 1 to 1.5 cm length) into the vertical slit (2-3 cm long) on decapitated head of 2 to 6 month old root-stock. To achieve close physical contact between scion and stock tissues, a plastic micropipette tip (0-200 μ l) was placed with slight pressure over top of bud union. After 7 days period, when bud take was initiated, buds were observed for their survival then microtip caps were removed from bud top. The plant growth of sweet orange budlings were periodically assessed at monthly intervals upto 90 days after budding (DAB) under open and shade net conditions. Data was collected for minimum number of days taken for sprouting and recorded maximum success percentage of budded plants, highest bud wood size, maximum number of sprouts, higher number of nodes and leaves with maximum plant height at 90 days after microbudding(DAM). Means for the data collected were compared by standard error.

3. RESULTS AND DISCUSSION:

3.1 Number of days to emerge first leaf after budding:

Earliest leaf emergence of 14.69 days was noticed in budlings kept under shade condition, whereas maximum days (20.25) were taken for the emergence of first leaf on budlings in open condition (Table 3.1).Among different age of the root stock seedlings six months old seedlings recorded significantly the lowest number of days for the emergence of first leaf (14.98) whereas, significantly highest number of days were recorded in two months old seedlings (19.87). Six months old seedlings in shade recorded significantly the lowest number of days for first leaf emergence (13.00) whereas, maximum number of days (23.00) were recorded in 2 months old seedlings in open.

Table 3.1: Effect of age of the root stock seedling and growing condition on Number of days to emerge first leaf after budding in sweet orange (*Citrus sinensis* L.Osbeck) cv. Sathgudi.

Age of Rootstock	Age of Rootstock					
	2 months	3 months	4 months	5 months	6 months	Mean
Open(%)	23.00	20.77	20.70	19.80	16.97	20.25
Shade(%)	16.73	15.13	14.80	13.80	13.00	14.69
Means	19.87	17.95	17.75	16.80	14.98	
	F-test	SEm \pm	C.D. (5%)			
Growing condition(A)	*	0.24	0.72			
Age of Rootstock(B)	*	0.38	1.13			
Interaction(A x B)	*	0.54	1.64			
ARS - Age of the Rootstock Seedling			GC - Growing Condition			

3.2 Number of Sprouts on the scion:

Mean number of sprouts on the scion increased from 1.42 at 60 DAM to 1.54 at 90 DAM under two different growing conditions(Table 3.2). At 90 DAM the highest number of

sprouts on the scion (1.54) under shade condition is on par with the lowest value (1.51 cm) recorded in open condition. Six months old root stock seedlings produced significantly more number of sprouts on the scion (1.42), whereas, the lowest number of number of sprouts on the scion (1.05) were observed on two months old root stock seedlings at 60 DAM. Similar results were also observed at 60 days (1.05 and 1.68) and 90 DAM (1.08 and 1.85).Among the interaction effects, significantly At 90 days after microbudding significantly highest (1.90) number of sprouts on the scion were produced on six months old root stock seedlings under shade condition while the lowest (1.00) value recorded by two months old root stock seedlings under shadenet condition.The increase in the number of sprouts most probably due to increase in the length of the sprout, plant height and consequently more number of nodes were also developed. These results coincide with the findings of Sivudu *et al.* (2014) in mango and Abbas mazhar *et al.*(2006) in sweet orange.

Table 3.2: Effect of age of the root stock seedling and growing condition on Number of sprouts on the scion in sweet orange (*Citrus sinensis* L.Osbeck) cv. Sathgudi

ARS	60 Days after microbudding						90 Days after microbudding					
	2 MRS	3 MRS	4 MRS	5 MRS	6 MRS	Mean	2 MRS	3 MRS	4 MRS	5 MRS	6 MRS	Mean
Open(A ₁)	1.10	1.53	1.30	1.43	1.73	1.42	1.17	1.63	1.43	1.50	1.80	1.51
Shade(A ₂)	1.00	1.47	1.43	1.57	1.63	1.42	1.00	1.57	1.53	1.70	1.90	1.54
Means	1.05	1.50	1.37	1.50	1.68		1.08	1.60	1.48	1.60	1.85	
	60 DAM						90 DAM					
	F-test		SEm \pm		C.D. (5%)		F-test		SEm \pm		C.D. (5%)	
Growing condition (A)	NS		0.03		-		*		0.03		0.08	
Age of the root stock (B)	*		0.04		0.12		*		0.04		0.12	
Interaction (A x B)	*		0.06		0.18		*		0.06		0.17	
ARS - Age of the Rootstock Seedling						GC - Growing Condition						
MRS - Months Rootstock Seedling						DAM - Days After Microbudding						

3.3 Length of the initial sprout:

Length of the initial sprout of the budling increased from 2.73 cm at 60 days after microbudding to 3.24 cm at 90 DAM for growing conditions (Table 3.3). The length of the initial sprout per budling was found to be highest at 60 DAM (4.65 cm) and 90 DAM (5.16 cm). Microbudding on two months old root stock seedlings recorded the lowest length of the initial sprout per budling (1.21 cm). Similar trend was recorded at 60 DAM (1.73 cm) and 90 DAM (2.23 cm). The highest length of the initial sprout was observed by microbudding on six months old root stock seedlings at 60 DAM (4.65 cm) whereas the lowest value (1.69 cm) was recorded in two months old root stock seedlings in open condition. At 90 DAM the highest (5.19 cm) length of the initial sprout was observed on six months old root stock seedlings under open condition while the lowest (2.22 cm) value was recorded by microbudding on two months old root stock seedlings in shade condition. Highest values in respect of these parameters were recorded by the budlings prepared under open condition which were on par with shadenet condition. Warm conditions coupled with

high light intensity in open and warm conditions coupled with humid conditions in shadenet house had almost shown the same effect on the sprout length and plant height in the budlings. These results are in agreement with findings of Mulla *et al.* (2011) in Jamun and Sivudu *et al.* (2014) in mango.

3.4 Scion stem diameter

There was no significant difference between open and shade with regard to scion stem diameter recorded at the three intervals. Scion stem diameter of the budling increased from 1.79 cm at 60 DAM to 2.24 cm at 90 DAM under different growing conditions (Table 3.4). Results recorded were on par with each other at 60 (1.79 and 1.78 cm) and 90 DAM (2.24 and 2.22 cm). The scion stem diameter per budling was found to be highest at 60 (2.29 cm) and 90 DAM (2.81 cm). Microbudding on two months old root stock seedlings was found to record the lowest scion stem diameter (1.04 cm). Similar trend was recorded at 60 days (1.41 cm) and 90 days after microbudding (1.73 cm). The results were on par with each other in different growing conditions. The variations in scion stem diameter might be due to more number of shoots per plant under shadenet and increased diameter of shoot (scion). Similar findings have been reported Sertkaya (2004) in Washington navel orange, Naeem alam *et al.* (2006) in sweet lime and grapefruit.

Table 3.3: Effect of age of the root stock seedling and growing condition on Length of the initial sprout in sweet orange (*Citrus sinensis* L.Osbeck) cv. Sathgudi.

ARS \ GC	60 Days after microbudding						90 Days after microbudding					
	2MRS	3MRS	4MRS	5MRS	6MRS	Mean	2MRS	3MRS	4MRS	5MRS	6MRS	Mean
Open(A _i)	1.69	2.39	2.02	2.89	4.65	2.73	2.24	2.69	2.48	3.61	5.19	3.24
Shade(A _i)	1.76	2.53	1.93	2.97	4.65	2.77	2.22	3.01	2.36	3.48	5.12	3.24
Means	1.73	2.46	1.98	2.93	4.65		2.23	2.85	2.42	3.55	5.16	
	60 DAM						90 DAM					
	F-test		SEm ±		C.D. (5%)		F-test		SEm ±		C.D. (5%)	
Growing condition (A)	*		0.10		0.29		NS		0.09		-	
Age of the root stock (B)	*		0.15		0.46		*		0.15		0.44	
Interaction (A x B)	*		0.22		0.65		*		0.21		0.62	

ARS - Age of the Rootstock Seedling
MRS - Months Rootstock Seedling
GC - Growing Condition
DAM - Days After Microbudding
NS - Non Significant

Table 3.4: Effect of age of the root stock seedling and growing condition on Scion stem diameter in sweet orange (*Citrus sinensis* L.Osbeck) cv. Sathgudi

ARS \ GC	60 Days after microbudding						90 Days after microbudding					
	2MRS	3MRS	4MRS	5MRS	6MRS	Mean	2MRS	3MRS	4MRS	5MRS	6MRS	Mean
Open(A _i)	1.44	1.50	1.68	2.05	2.26	1.79	1.77	2.03	2.14	2.49	2.78	2.24
Shade(A _i)	1.37	1.52	1.69	2.01	2.31	1.78	1.68	2.04	2.17	2.38	2.85	2.22
Means	1.41	1.51	1.68	2.03	2.29		1.73	2.03	2.16	2.43	2.81	
	60 DAM						90 DAM					
	F-test		SEm ±		C.D. (5%)		F-test		SEm ±		C.D. (5%)	
Growing condition (A)	NS		0.04		-		NS		0.05		-	
Age of the root stock (B)	*		0.07		0.21		*		0.08		0.23	
Interaction (A x B)	*		0.10		0.30		*		0.11		0.32	

ARS - Age of the Rootstock Seedling
MRS - Months Rootstock Seedling
GC - Growing Condition
DAM - Days After Microbudding
NS - Non Significant

3.5 Microbudding success percentage:

The microbudded plants after 90 days were growing normally and manifested good health (Table 3.5). Results indicated that 4 mm of scion bud Results indicated the highest mean success rate in open condition (71.33 %) and the lowest in shade condition (59.77 %). With regards to the age of the seedlings, highest success rate of microbudding was recorded in six months old root stock seedlings (84.44 %) and the lowest (33.89 %) was recorded in two months old root stock seedlings. The success rate of microbudding in four (72.77 %) and five months old root stock (76.11 %) seedlings was on par with each other. However, interaction effect due to both factors was non-significant. Among the interactions, the highest success rate was recorded in six months old root stock seedlings in open condition (91.11 %) whereas the lowest was recorded in two months old root stock seedlings under shade (33.33 %).

Table 3.5: Effect of age of the root stock seedling and growing condition on Success percentage of microbudding in sweet orange (*Citrus sinensis* L.Osbeck) cv. Sathgudi.

Age of Rootstock \ Growing condition	2 months	3 months	4 months	5 months	6 months	Mean
Open(%)	34.44	70.00	80.00	81.11	91.11	71.33
Shade(%)	33.33	51.11	65.55	71.11	77.77	59.77
Means	33.89	60.55	72.77	76.11	84.44	
	F-test		SEm ±		C.D. (5%)	
Growing condition(A)	*		0.14		0.43	
Age of Rootstock(B)	*		0.22		0.68	
Interaction(A x B)	NS		0.32		-	

3.6 Plant height of microbudded plants:

Height of the microbudded plant increased from 6.73 cm at 30 days after microbudding to 11.56 cm at 90 DAM (Table 3.6). The recordings taken in both the growing conditions were non-significant. At 30 DAM the highest height of the microbudded plant (6.87 cm) was recorded in shade whereas, the lowest (6.73 cm) in open. Similar non-significant results on height of the microbudded plant were recorded in intervals at plant per budling was found to be highest (9.62 cm) in 6 months old root stock seedlings at 30 days after microbudding with a similar trend at 60 and 90 days after microbudding. Microbudding in 2 months old root stock seedlings was found to record the lowest height of the microbudded plant per budling (5.10 cm). Similar trend was recorded at 60 and 90 DAM. Among the interactions

Table 3.6: Effect of age of the root stock seedling and growing condition on Plant height in sweet orange (*Citrus sinensis* L.Osbeck) cv. Sathgudi.

ARS	60 Days after microbudding						90 Days after microbudding					
	2MRS	3MRS	4MRS	5MRS	6MRS	Mean	2MRS	3MRS	4MRS	5MRS	6MRS	Mean
Open(A ₁)	7.50	8.60	8.03	10	12.47	9.42	9.70	9.90	8.10	12.23	16.30	11.25
Shade(A ₂)	7.50	9.47	7.20	8.80	13.00	9.19	9.80	11.63	9.80	12.13	14.43	11.56
Means	7.50	9.03	7.62	9.65	12.73		9.75	10.77	8.95	12.18	15.37	
	60 DAM						90 DAM					
	F-test		SEm ±		C.D. (5%)		F-test		SEm ±		C.D. (5%)	
Growing condition (A)	*		0.35		1.03		*		0.38		1.14	
Age of the root stock (B)	*		0.55		1.63		*		0.61		1.80	
Interaction (A x B)	*		0.78		2.12		*		0.86		2.51	
ARS - Age of the Rootstock Seedling						GC - Growing Condition						
MRS - Months Rootstock Seedling						DAM - Days After Microbudding						

60 and 90 DAM. The height of the microbudded, at 90 DAM significantly the highest of 16.30 cm and lowest of 9.70 cm height of the microbudded plant was observed by microbudding on six months and two months old root stock seedlings in open condition.

4. CONCLUSION

From the present study that the warm, growing temperatures in the open conditions found to favour good survival percentage whereas warm, humid conditions under shadenet favour luxurious vegetative (and pathogen) growth yielding stouter budlings. Microbudding in sweet orange on six months old root stock seedlings proved to be the most suitable stage for propagation of sweet orange through microbudding technique. The use of root stocks of Rangpurlime and involvement of micropipette tip on top of graft union remained as key factors in successful microbudding under open conditions. Six months old rootstocks not only offer technical simplicity and economic feasibility but also showed promise for commercial application due to shortest nursery duration (which otherwise normally be 2 years period required for conventional T-budding) with 91 per cent bud success rate.

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