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Effect of different method of placement of shoot tips on *in vitro* shoot tip grafting in Nagpur mandarin

Ekta D. Shinde* and N.D. Jogdande
Tissue Culture Laboratory, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidypeeth, Akola 444104

ABSTRACT

Shoot tip grafting (STG) comprises of *in vitro* grafting of meristem along with leaf primordia on the rootstock. Success of STG depends upon the method of placement of this meristem. Three different grafting techniques were followed in the present study using Nagpur Mandarin as scion on Rough lemon stock. Sterilized decoated seeds of Rough lemon were germinated in MS medium at 27± 1^{0}C in complete darkness for two weeks. Surface sterilized shoot tips of Nagpur Mandarin of 0.1 to 0.3 mm were placed by top cut surface, window cut and apical triangle cut method, so as to record the success percentage. Although early sprouting of grafts was observed by top cut surface method in 13.25 days with the grafted shoot length of 1.88 cm but maximum success of 46.66% was observed by apical triangle cut method, The successful grafts with 2-4 expanded leaves were transplanted to soilrite for hardening.

Key words: *In vitro,* shoot tip grafting, method, Nagpur mandarin.

INTRODUCTION

Citrus is grown in the tropical and sub-tropical climate and has wide distribution in the world. The genus *Citrus* comprises of mandarin orange, sweet orange, lime and lemon, grapefruit and much more species. These different species are propagated both by seeds and vegetative means like cutting and budding. Mandarin orange (C*itrus reticulata* Blanco.), cv. Nagpur mandarin is well-known in Central India as 'Nagpur Santra' and is popular for its sugar-acidity blend. But productivity and longevity of the santra orchards is declining due to the various factors like prevalence of viruses and virus – like diseases and their inadvertent multiplication in commercial nurseries. (Vijayakumari *et al.,* 9). As Mandarin orange is commercially propagated by shield budding, disease – free planting material in the nursery is the pre-requisite for the establishment of a viable and productive santra industry. Tissue culture is being used effectively in several countries for asexual propagation and recovery of pathogen – free plants in citrus. Shoot tip grafting (STG) or micrografting, a microscopic version of normal grafting technique followed *in vitro*, is used for elimination of virus and virus – like diseases of citrus plants (Navarro and Juarez, 4). However STG requires much dexterity and special skills. A number of scientist till now have contributed in finding out different ways to enhance success percentage of STG (Navarro *et al., 5,* Mukhopadhyay *et. al.,* 3 and Vijayakumari and Singh, 8). On this line the present investigation was carried out so as to study the effect of method of placement of shoot tip on grafting success of Nagpur mandarin.

*Corresponding authors E-mail: ekta248@reddifmail.com

MATERIALS AND METHODS

In vitro STG was done by grafting the shoot tips of Nagpur Mandarin on Rough lemon rootstock. Fruits of Rough lemon were obtained from main garden of the university. Seeds were extracted from the mature fruits and washed thoroughly, cleaned, dried and treated with fungicide and then packed in plastic bags. These treated seeds were stored in refrigerator at 4^0 C. The treated seeds were first thoroughly rinsed with water and dried. Both the seed coats were removed and surface sterilized with 0.5 percent sodium hypochlorite solution followed by 3-4 washings with sterile distilled water. Seeds were then cultured on MS basal salts with 0.8% agar. Culture tubes were incubated at 27± 1^0 C in dark. Two-week old etiolated seedlings were used as rootstock for STG.

Shoot tips of Nagpur Mandarin were isolated from actively growing branches of field-grown trees. New flushes of 3 cm length were collected and large leaves were stripped off leaving 1 cm long shoot tips. These shoots were soaked in water containing 1-2 drops of labolene for 10 minutes. Thoroughly washed shoot tips were surface sterilized by 0.25% sodium hypochlorite followed by washing with sterile distilled water. Two – week old rootstock seedlings were decapitated under sterile condition leaving about 2-3 cm of epicotyl. Shoot tips of 0.1-0.3 mm size comprising of apical meristem

and 2-3 leaf primordial were excised with a fine scalpel blade aseptically under stereoscopic microscope. These dissected shoot tips were placed on the rootstock in three different ways. Top cut surface method comprised of placing the meristem on the top surface of epicotyl in contact with the pith. In the apical triangle cut method, a triangular cut was given at the tip of the decapitated rootstock and the cortex was scooped out. Shoot tip was placed inside the triangular hole on the cortex in contact with cambium. For the window cut, a rectangular cut was made and the tissue cylinder of 1-2 mm depth was lifted. The shoot tip was placed inside the window cut in contact with the basal cut. The decapitated rootstocks were kept moistened and grafting was performed in quick succession by placing the meristem on the exposed part of decapitated rootstock.

The micrografts were then aseptically cultured in liquid MS media containing modified Whites vitamins and sucrose 7.5% and then incubated at 27$\pm$ 1^0C by exposing daily to 1000 lux illumination for 16 hours photoperiod. Observations on number of days taken for sprouting, number of leaves, length of sprouts etc were recorded. The data was analyzed by Completely Randomized Block design (Panse and Sukhatme, 6). The successful grafts with 2-4 expanded leaves were kept for hardening.

RESULT AND DISUCSSION

The method of placement of the excised shoot tip onto the rootstock seedling significantly influenced the degree of grafting success (Table 1). Significantly early sprouting of grafts was observed by top cut surface method in 14.75 days. Although this method gave the earliest sprouting with maximum shoot length 1.75 cm but percent success of STG grafts was low. This may be due to the development of adventitious shoots from the top surface which quickly proliferate and overgrow the micrografted apex. Similar findings were reported by Starrantino and Caruso (7) and Kumar and Dhatt (2) who suggested lateral micrografting. Edriss and Burger (1) also reported that adventitious shoots from the rootstock seemed to arise more readily from tissues at the top of the decapitated epicotyl.

Further it is clear for the data presented in Table 1, that maximum grafting success of 37.25 % was achieved by apical triangle cut method. Previously Vijayakumari *et al.* (9) also reported that apical triangle cut method yielded more than inverted T method. This is because of easy placement of the tender shoot tip inside the apical triangle cut and less damage as compared to inverted T – cut method. Since success percentage of STG plants is more important criterion than other growth characteristics, apical triangle cut method was considered as the superior method over the rest.

Thus in this study it was found that method of cut and the placement of shoot tip on the rootstock greatly affects the frequency of successful grafts. This study will be of great help in establishing a disease-free nursery for large scale multiplication of Nagpur mandarin.

REFERENCES

1. Edriss, M.H. and Burger, D.W. 1984. Micrografting shoot tip culture of citrus on three trifoliate rootstocks. *Scientia Hort.* **23**: 255-59.
2. Kumar, K. and Dhatt, A.S. 2004. Overcoming problems in citrus micrografting. *Indian Hort.* **49**: 14-15.
3. Mukhopadhyay, S., Pradhan, Jaishree R., Sharma B.C., Gurang, Anita and Sengupta, R.K. 1996. Production of disease free Darjeeling orange plants by shoot tip grafting technique. *Proceedings Int. Soc. Citriculture,* **2**: 855-57.
4. Navarro, L. and Juarez, J. 1977. Tissue culture technique used in Spain to recover virus free citrus plants. *Acta Hort.* **73**: 425-35.
5. Navarro, L., Roistacher, C.N. and Murashige, T.

Table 1. Effect of different methods of placement of shoot tips on STG in Nagpur mandarin•

Treatment No.	Method of placement of shoot tips	Mean No. of days required for sprouting	Mean length of grafted shoots (cm)	Mean No. of leaves on grafted shoots	% success*
M1	Apical triangle cut	17.25	1.80	3.00	46.66
M2	Window cut	16.87	1.77	3.00	34.66
M3	Top cut surface	13.25	1.88	3.75	27.50
	CD at 5%	1.68	–	–	

* Unreplicated data; •Observations recorded six weeks after grafting

1975. Improvement of shoot tip grafting *in vitro* for virus-free citrus. *J. Amer. Soc. Hort. Sci.* **100**: 471-79.

6. Panse, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers.* 4th Ed. ICAR Pub., New Delhi, pp. 131-143.
7. Starrantino, A. and Caruso, A. 1988. The shoot tip grafting technique applied in citriculture. *Acta Hortic.* **227**: 101-103.
8. Vijayakumari, N. and Singh, S. 1999. Effect of size, source of shoot tip and growth regulators on success of *in vitro* shoot tip grafting in *Citrus reticulata* Blanco cv. Nagpur mandarin. *Proceedings Int. Symposium Citriculture,* NRCC, Nagpur, pp. 245-48.
9. Vijayakumari, N., Dass, H.C. and Singh A. 1994. Standardization of *in vitro* shoot tip grafting technique for eliminating virus and virus-like diseases from Nagpur mandarin. *Indian J. Hort.* **5**: 311-15.

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