ANATOMICAL STUDIES ON ROOTSTOCK/SCION COMPATIBILITY IN KINNOW

Waqar Ahmed*, Muhammad Asim** And Mian Iqbal Muhammad**
* *Assistant Prof. Institute of Horticultural Sciences*
** *M.Sc. Students Institute of Horticultural Sciences*

Abstract

Shoot tip grafting was carried out for Kinnow on five rootstocks viz-a-viz. Rough Lemon, Sour Orange, Troyer Citrange, Carrizo Citrange and Brazilian Sour Orange.The selected rootstocks were sown on two media i.e. sand and MS media (Murashige and Skoog 1962). Germination percentage was more on M S medium than on sand. Graft success frequency of 20 to 40% was obtained on these seedlings using 0.17mm shoot tip from field grown Kinnow plants. The grafted plants were allowed to develop *in vitro* under 16 hour daily exposure to 1000-lux illumination and were provided with nutrient solution containing a high concentration (8%) of sucrose. Grafted plants were transplanted on pots, 5 to 8 weeks after grafting and transferred to glasshouse for hardening. Internal structures of scion/stock union of Kinnow with the rootstock were studied by microscopic observation having Crosectional, Longitudinal and Tangential section prepared through microtomy technique.

INTRODUCTION

There is no dispute on the importance of citrus cultivars and rootstock to desert citrus production In Pakistan citrus holds first position with an area of 197.7 (thousand hectares) and annual production of 1943.2 thousand tonnes (Anonymous, 2000-2001).

Successful citrus cultivars should be adaptable to the harsh climate, must be vigorous and able to produce high yield of good quality fruit of marketable size. The ideal citrus rootstock must be compatible with the scion.

Shoot apices from Citrus cultivar of known virus content were successfully grafted in vitro on to disease–free rootstock seedling and some virus–free plants were obtained. The prolong ed juvenile phase which characterizes disease–fee nucellar lines was by passed by this procedure Murashige and Bitter (1994) Shoot tip grafting is a technique based on the fact that the apical meristem contains little or no virus. It has been used successfully to produce virus-free citrus plants in the world. Success in establishing a permanent graft union between rootstock and scion depends upon intimate relationship or affinity often termed as compatibility between the subjects joined together and the other contact between their cambia.

Most of the citrus orchards are affected by in congeniality between scion and stock. Either no union is formed when the components are grafted together or they fail to grow in a healthy manner or premature death occurs, too such failure can be attributed as compatibility differences between stock and scion.

Interactions between rootstock and scion occurs, both through the exchange of resources like water, nutrients, carbohydrate and also via hormonal messengers. The physical union between rootstock and scion, or its degree of compatibility,

influences the exchange of these materials and to a some extent affects the relationship between scion and stock.

The Pakistan citrus industry has traditionally used only Rough lemon (*Citrus jambhiri*) in Punjab province while in other province no existence of citrus industry. This rootstock has important advantages like tolerance to Citrus Tristeza Virus (CTV), Citrus Xyloporosis viroid (CXV) and well adapted to deep sandy soil. But it has fallen out of favour in recent years because of its susceptibility to citrus blight, yet an unresolved decline problem.

Edriss and Burger (1984) stated a simple technique and growth regulators treatments to improve micro grafting success of 'Mexican' lime, Valencia orange and 'Star Ruby' grapefruit. Scion grafted on 'Carrizo' Citrange [*Poncirus trifoliata*) (L.) Raf Check X *Citrus sinensis* (L.) Osbeck] have better compatibility than to other rootstocks. Dipping the shoot tip in 2,4-D or Kinetin before grafting doubled the success percentage occurred when the shoot tip was placed in an inverted-T incision on the epicotyle of the rootstock.

Jonard et al. (1989) reported the use of micrografting, internodes association and callus fusion as tests for distinguishing between homograft (e.g. Myrobalan or Troyer citrange with themselves) which showed complete callus interpenetration, compatible heterograft (e.g. apricot cv. Luizet/myr of lemon cv. Villafrancea/CT) and incompatible heterograft (e.g. apricot cv. Canino /myr or lemon cv. Eureka/CT) where necrosis occur at the junction. In cell culture incompatibility was manifested by the inhibitory effect of Eureka on growth of CT stock cells.

Starrantino (1992) described that micro grafting technique used to obtain citrus clones free from virus and virus-like diseases. It includes shoot-tip grafting (0.1-0.3 mm) onto the apical or lateral part of decapitated epicotyle seedling rootstocks cultured *in vitro*. The apical portion of the rootstock in lateral micrografting was decapitated after germination of the grafted apex. The germination percentage was 48% for apical grafting and 63% for lateral grafting. The percentage rate was increased by dipping the apex and seedling in a solution of 6-BAP [benzylaminopurine, benzyladenine] at 0.5 ppm.

Mukhopadhyay *et al.* (1997) raised seedling of the local rootstocks viz rough lemon, sour orange and Rangpur lime (*Citrus limonia*) on MS medium in standard conditions of temperature, light duration and plant age. Microbud (0.2 mm long), aseptically excised from Darjeeling orange (Darjeeling mandarin), were grafted onto the decapitated rootstock seedling. Rootstock seedlings showed optimum growth at 25-30^{o}C. Optimum growth of the micrografts resulted from treatment in complete darkness for 4 days after grafting followed by exposure to 750 W for 16 hr for 15 days and 1500-2000 W for 16 hr for 39 days. The success of micrografting depended on the age of the sour orange, rough lemon and Rangur lime, respectively. The grafts were then indexed for trestiza virus and greening diseases after 6 months, grown in an insect proof screen house and found to be free from these graft transmissible diseases.

MATERIALS AND METHODS

The proposed experiments were conducted at the tissue culture laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad and Biological Chemistry

Division (BCD), Nuclear Institute for Agriculture and Biology (NIAB). Two types of media (Sand and MS) were used for growing of citrus rootstocks with 100 seeds of each rootstock (Rough Lemon, Sour Orange, Troyer Citrange, Carrizo Citrange and Brazilian Sour Orange).

Seed treatment

Seeds were dipped into 70% ethylene alcohol + 1% Tween- 20 (wetting agent) for 10 minutes, washed with distilled water then treated with 0.1% Hg Cl_3 for 1-2 minutes, later on the treated seed were washed with double distilled water for 2 - 3 times.

Media Preparation

Sand

Sand was sterilized in autoclave at 121 ^{o}C for 15 minutes. Sterilized sand filled in pots and seeds were sown.

MS Media

Seeds were peeled (both seed coats were removed). Simple MS media (Murashige and Skoog, 1962) solidified with 8g Agar having pH adjusted between 5.7 to 5.8 was distributed in culture tubes and the tubes were capped with polypropylene closures.

The medium was sterilized by autoclaving at 121 ^{o}C for 15 minutes. One seed per tube was cultured after peeling and tubes were kept in growth room at 27 ^{o}C for germination.

Scion Collection

The actively growing shoot tips of healthy Kinnow plants were obtained from Experimental Fruit Garden, Sq # 9, Institute of Horticultural Sciences, University of Agriculture, Faisalabad.

Size of shoot tip

The shoot tips of (1-2mm in length and 0.3-0.5mm) leaf primordial length were prepared with surgical knife and sterilized with 95% Ethanol for 30 seconds, followed by 3 washing with double distilled water.

Shoot tip Grafting procedure

Shoot tips were sterilized by soaking in 0.25 % sodium hypochlorite solution for five minutes. The disinfected tissues were rinsed 3 times with autoclaved distilled water and their shoot tips were excised and used as scions. Two weeks old rootstock seedlings were decapitated, leaving 1-1.5 cm of epicotyls. The root was also shortened to 4-5 cm and the cotyledons were removed. Shoot tip composed of apical meristem tissue to include three leaf primordia isolated, with surgical blade. Shoot tip was placed at the top of decapitated epicotyls on the cortex surface in an inverted T- incision, under aseptic conditions. The grafted cultures kept at constant temperature of 27^{o}C in growth room. The shoot tip was placed on the top cut surface of the decapitated epicotyls, and make contact with the cortex (Fig-1).

The grafted plants were kept upright in test tube and exposed for 16 hour/day to 1000-lux illumination; at constant temperature of 27^{o}C was maintained *in vitro*. The grafted plants were observed periodically using a magnifying glass, and adventitious shoots arising from the rootstocks were removed aseptically with the help of surgical knife.

Transfer of plants to pots

After two weeks, plants transplanted into 10 cm pots containing a soil mixture, previously developed for citrus. They were fertilized once with the Murashige and Skoog salt solution at the transplant time. The pots were kept in green house for the hardening of plants.

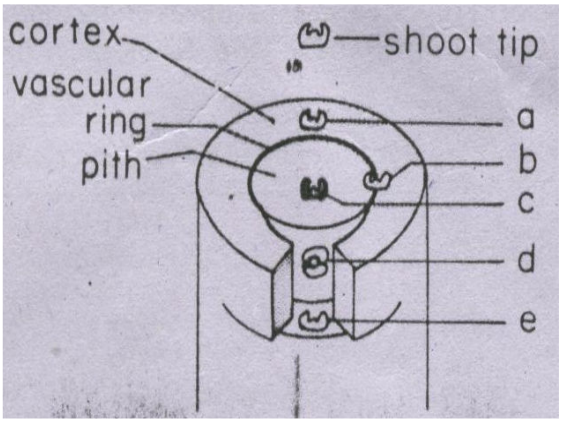

Fig. 1: Care of shoot tip grafted plants *in vitro*

RESULT AND DISCUSSION

The present research work was carried out to ascertain the effect of simple media on the development of seeds and micrografting of Kinnow scion on Rough Lemon, Sour Orange, Troyer Citrange, Carrizo Citrange and Brazilian Sour Orange rootstocks. Effect of various rootstocks on the stionic compatibility growth and development of scion was also recorded.

Germination Percentage

Data concerning this parameter of study are presented in (Table-1). More germination percentage was high on MS media than sand was observed. On sand medium, highest germination percentage was observed in Rough Lemon on sand and MS media 52% while 81%, followed by Brazillian Sour Orange having 40% on sand and 79% on M.S media.

Carrizo citrange and Sour orange were satisfactory as rootstocks for Kinnow mandarin shoot tips. After two weeks systematic test disclosed that "Rough lemon" was superior to Sour orange and Carrizo citrange as the rootstock of the Kinnow mandarin shoot tips, Rough Lemon gave 27.5% graft success for Kinnow, while on Sour Orange and Carrizo graft success was 17.3% and 13.5% , respectively (Table-2). The

Table 1: Seed Germination Percentage of Citrus Rootstock on Two Growing Media

S. No	Rootstock	%age Successful Grafting
1	Rough lemon	27.5
2	Sour orange	17.3
3	Troyer citrange	5.0
4	Carrizo citrange	13.5
5	Brazilian Sour orange	5.3

Table 2: Influence of the rootstock on grafting success of Kinnow after two weeks

S. No	Rootstock	Sand (%)	M. S. Media (%)
1	Rough lemon	52	81
2	Sour orange	33	63
3	Troyer citrange	22	76
4	Carrizo citrange	10	63
5	Brazilian Sour orange	40	79

need to examine the cultivars influence when selecting rootstocks for certain species and cultivars (Kinnow mandarin) is emphasized. Troyer citrange have advantage of having trifoliate leaves, which can be easily identified in micrografting and removed. In order to avoid the adverse influence on the development of graft union. The absence of suitable morphological marker, however, should not preclude the use of *in vitro* grafting procedure.

The incidence of successful grafts is expected to rise with the increasing size of the scion shoot tip, but it is also probable that the proportion of virus-free plants will decline. The scion size that is ultimately chosen must enable a realistic degree of grafting success and result in a reasonable number of pathogen- free plants. Only an insignificant proportion 1.08% of the grafts of the shoot apical meristem alone as scion resulted in successes (Table-3). The degree of success increased progressively as larger shoot tips were used, by including subjacent tissue and 2, 4, 6 leaf primordia. The most successful case was with the Rough lemon 72.08% with apical meristem and 6 leaf primordia then followed by Sour orange, Troyer citrange and Brazillian Sour Orange 63.06, 47.03 and 0.7 respectively.

LITERATURE CITED

Navarro, L. 1981.Citus shoot tip grafting *in vitro* and its application .A Review. Proc. Int. Soc Citriculture, 1: 452-456.

Nauer E.M. et al .(1983) *In vitro* shoot tip grafting to eliminate citrus viruses and virus –like pathogens produces uniform budlines . Hort Science. Vol. 18(3): 308-309.

Edriss, M.H. and D.W. Burger. 1984. Micrografting shoot tip culture of citrus on three trifoliate rootstocks. Scientia Horticulture 23(3): 255-259 (Hort. Abst., 54 (9): 9518, 1984).

Jonard, R., D. Luckman, F. Schall and P.Villemur. 1989. Early test of grafting incompatibilities in two species apricot and lemon using several *in vitro* cultured techniques. Micrografting, internode association, callus fusion, cell suspension. Bordeaux port de la Maye, France, CTIFL-INRA.239-254.

Starantino, A. 1992. *In vitro* micrografting of citrus. Petria 2(1): 27-35.

Mukhopadhyay, S., J. Rai, B.C. Sharma, A. Gurung, R.K. Sengupta, P.S. Nath, J. Rai and A. Gurung. 1997. Micropropagation of Darjeeling orange (C*itrus reticulata* Blanco) by shoot tip grafting. J. Hort. Sci, 72(3): 493-499.

Table-3 Influence of Size of the excised shoot tip of Kinnow on the incidence of successful grafts on rootstocks

Scion Size	Graft Success percentage					Excised Primordia Size				
	Brazilian Sour Orange	Rough Lemon	Sour Orange	Troyer Citrange	Carrizo Citrange	Brazilian Sour Orange	Rough Lemon	Sour Orange	Troyer Citrange	Carrizo Citrange
Apical meristem alone	1.03	3.05	2.05	1.08	1.06	0.05-0.06	0.05-0.06	0.05-0.06	0.05-0.06	0.05-0.06
Apical meristem & 2 leaf primordia	11.09	20.01	17.05	14.06	12.03	0.1-0.15	0.1-0.15	0.1-0.15	0.1-0.15	0.1-0.15
Apical meristem & 4 leaf primordia	19.04	39.09	35.05	34.06	27.03	0.2-0.3	0.2-0.3	0.2-0.3	0.2-0.3	0.2-0.3
Apical meristem & 6 leaf primordia	29.07	72.08	63.06	47.03	39.01	0.4-0.7	0.4-0.7	0.4-0.7	0.4-0.7	0.4-0.7