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MICROPROPAGATION OF WILT RESISTANT, INTER-SPECIFIC (*PSIDIUM MOLLE* X *PSIDIUM GUAJAVA*) ROOTSTOCK OF GUAVA

Manasi Rai Sharma*, Swati Kumari[1] and Maneesh Mishra[2]

*Junior Research Assistant, ICAR- Central Institute of Subtropical Horticulture, Rehmankhera, P. O. Kakori, Lucknow-226101 (U.P.) India
[1]Ph. D. Scholar, ICAR- Central Institute of Subtropical Horticulture, Rehmankhera, P. O. Kakori, Lucknow-226101 (U.P.) India
[2]Principal Scientist, ICAR- Central Institute of Subtropical Horticulture, Rehmankhera, P. O. Kakori, Lucknow-226101 (U.P.) India

Abstract

The present study describes a protocol for *in vitro* micropropagation of wilt resistant, hybrid rootstock of guava, developed at ICAR- Central Institute for Subtropical Horticulture, Lucknow April to June was found to be the most suitable season for collection of explants. A combination of 0.1% Carbendazim and 0.05% Metalaxyl for pre-washing followed by surface sterilization with 0.1% Mercuric chloride for 7 minutes was found effective in controlling contamination. In order to control oxidative browning, applying wax to the cut ends of the explants yielded better results than the use of antioxidants. Out of all the different media preparations used, MS + 0.1mg/L IAA+ 4mg/L BAP+ 30g sucrose gave maximum proliferability (2.6 shoots/explant) and maximum leaf number/shoot (5.6) in the shortest duration *viz.* 7 days. The regenerated micro-shoots were transferred on rooting media containing MS +2mg/L IBA. The rooted plants were acclimatized on sterilized coco-peat supplemented with MS salt solution.

***Keywords**:* Guava Wilt Disease, Hybrid rootstock, Guava, mass multiplication

Introduction

Guava (*Psidium guajava* L.) is an important commercial crop. However, it suffers colossal losses due to the prevalence of Guava Wilt Disease (GWD) which is present in India, South Africa and other guava growing countries of the world. This disease is a sudden, catastrophic killer of guava trees. Das Gupta and Rai (1947) were the first to report this disease. It is a soil borne disease and no chemicals proved to be an effective control. Early symptoms of GWD include yellowing accompanied by slight curling of the leaves of the terminal branches. In later stages, plants show unthirftyness with yellow to reddish discoloration of leaves. Fruits on the affected branches remain underdeveloped, hard and stony. Eventually, the entire plant defoliates and dies. Two types of symptoms have been identified- slow wilt and sudden wilt (Chakraborty and Singh, 1989). Though the exact cause for GWD is not understood completely, it has often been attributed to the pathogens *Fusarium oxysporum, Fusarium solani, Macrophomina phaeseoli, Rhizoctonia bataticola, Cephalosporium sp. and Gliocladium roseum* and many others. Wilt was associated with *Fusarium* sp. for the first time by Das Gupta and Rai (1947) and later on the name *Fusarium oxysporum* was proposed by Prasad *et al*. (1952). Comprehensive review of work has been done by Prakash and Misra (1993), Misra and Pandey (1996), Mishra and Pathak (2001) and Negi *et al*. (2001). There is a need to develop wilt resistant guava varieties.

In order to combat this disease, ICAR-Central Institute for Subtropical Horticulture, Lucknow has developed an interspecific hybrid rootstock of guava by crossing *Psidium guajava* × *Psidium molle*. The rootstock has been found to be resistant to GWD. However, this rootstock is shy to rooting. Conventionally, it is being propagated through two leaf cutting. However, multiplication rate through this method is very slow and it is season dependant. The rootsock has been grafted with commercial guava varieties successfully. The rootstock

***Author for correspondence :** E-mail : manasiibsindia@gmail.com

has been advanced to multilocational trials under All India Coordinated Research Project on Fruits (AICRP). Commercial guava varieties need to be grafted on this hybrid rootstock in order to contain the spread of *Fusarium* wilt. This warrants large scale production of this rootstock.

Micropropagation can be gainfully utilized for mass multiplication of elite plant varieties. Micropropagation in guava is quite successful. Rapid clonal multiplication of guava through *in vitro* shoot proliferation from nodal explant of mature tree was reported by several authors (Amin, 1986; Amin and Jaiswal 1987; Amin and Jaiswal, 1988). However, micropropagation protocol for this hybrid guava rootstock is lacking. The present paper describes the steps involved in micropropagation of rootstock through shoot bud.

Materials and Methods

Nodal explants (3-4cm) were collected from the hybrid rootstock of guava planted in the germplasm block of the institute.

The shoots were collected and defoliated. They were then cut into desirable size and washed thoroughly under running tap water for one hour. These nodal segments were then dipped in a cocktail containing 500 mg/l Cefotaxime + 100 mg/l PVP + 50 mg/l Citric acid + 2 drops Tween-20 and a combination of fungicides (Carbendazim 0.1%+Metalaxyl 0.05%). This solution containing the explants was kept in incubator shaker for 2 hours to ensure proper sterilization. Then the explants were washed properly with running water and taken to laminar hood which was pre-sterilized by UV treatment for 20 minutes. The explants were subsequently surface sterilized using 0.1% mercuric chloride ($HgCl_{2)}$ solution for 7 minutes and then washed with double distilled water 6-7 times. The washed explants were then allowed to blot dry. Various measures were taken to prevent oxidative browning which included fortifying growth media with antioxidants such as PVP, ascorbic acid, and activated charcoal. Another method employed was sealing the cut ends of the explant with paraffin wax. The explants were then inoculated in growth medium which was sterilized by autoclaving at 121^0C and 15 psi pressure for 20 minutes.

All the treatments were prepared using MS medium (Murashige and Skoog, 1962) along with 0.1mg/L Indole-3-acetic acid (IAA) 30g/L sucrose and 8g/L agar as gelling agent. Differential regime of cytokinins was employed to assess the shoot bud induction and proliferation. For induction of shoot bud, 6-Benzylaminopurine (6-BAP), Kinetin and Thiadiazuron (TDZ) were used. The microshoots that developed *in vitro* were transferred into a rooting medium containing IBA. The rooted plantlets were shifted to different sterilized carrier substrate (cocopeat, perlite, vermiculite) fortified with MS salt solution in bottles with aerated caps. The cultures were incubated at 25 ± 2^0C, 50-55% RH and 4000 lux light under 16/8 hours of light and dark regime. Each treatment was replicated thrice. Thereafter, the plants that were successfully acclimatized and started growing were taken forward. The caps were removed and bottles were covered with polythene bags to allow the plant to grow. Then these bags were punched to make holes for aeration. Finally, when the plants attained a suitable length, they were transferred into pots and kept in the polyhouse. The data obtained was subjected to Completely Randomized Design analysis and Critical Difference (C. D.) and Standard Error of Mean [S.E.(m)] was calculated.

Results and Discussion

The results obtained during the course of investigations are being discussed in the light of existing references.

Effect of Season on Regeneration

The physiological state of the mother plant at the time of explants collection has a direct influence on the response of buds. Subtropical zone experiences extreme cold and hot conditions. Therefore choosing the right season is a prerequisite for success of micropropagation. The explants were inoculated throughout the year and it was observed that explants collected during April to June were most responsive for enhanced axillary branching in hybrid rootstock (manasiibsindia@gmail.com (fig. 1). Explant survival as well as per cent explants that induced shoots were much higher during April to June *viz.* 43.33 per cent and 21 per cent respectively. This may be attributed to weather factors. During July to September, heavy inborn fungal contaminations lead to the death of most of the tissues (65.85 per cent). However, during October to December, most of the explants did not respond due to low temperature. The greater responsiveness of season could be attributed to new flushes during April to June. The best season for bud induction in most woody trees has been considered to be spring, coinciding bud break and late summers (Wealander, 1983; Bonga, 1987). In aonla, Mishra *et al.,* 1999 reported that maximum bud break was observed during August to November followed by April to July. Skirvin and Chu (1978) found that scion collected during spring season was an ideal explants in peach whereas in mulberry, early *in vitro* sprouting occurred in explants

collected during summer than in rainy and winter season (Vijaya Chitra and Padmaja, 2002).

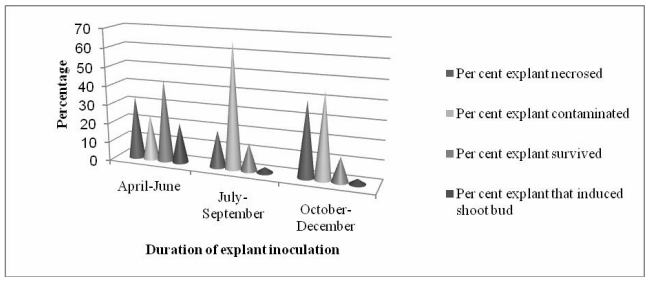

Fig.1: Impact of Season on shoot bud induction

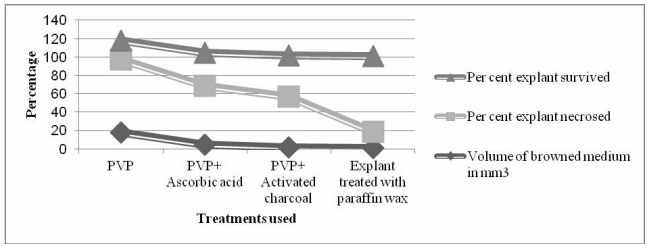

Fig. 2: Effect of antioxidants and explant waxing on control of oxidative browning

Effect of Treatments on Control of Oxidative Browning

In vitro oxidative browning remains a major problem in culturing shoot explants of hardy, woody perennials. Phenol is a natural defense mechanism of the plant system which gets triggered upon wounding to combat bacteria and fungus. In Tissue culture, phenol is released after excision of explants. It gets oxidized and turns into quinol which is phytotoxic. Different antioxidants such as PVP, ascorbic acid and citric acid have been used individually or in combinations by several authors in different crops. Chandra *et al*. (2004) used a combination of PVP and ascorbic acid in mango while Jaiswal and Amin (1987) used a combination of citric acid and ascorbic acid in guava. PVP alone was found effective in aonla (Mishra *et al.,* 2006) whereas activated charcoal was used in annona (Encina *et al.*, 1994). However, controlling oxidative browning through these antioxidants did not prove to be promising in hybrid rootstock. Therefore, we resorted to the use of melted paraffin wax to seal the cut ends of the explants. A perusal of data (fig. 2) revealed that the application of molted wax on the cut ends of the explants significantly reduced the browning of explants by 20% as compared to non-waxified explants. The reason may be attributed to complete blockage of outlet for phenol and thereby establishment of explants in the medium. Presumably, nutrients were absorbed by the side surface of the nodal shoots. Similar, results were obtained by Sharma (1984) in *Citrus sinensis* and *Citrus aurantifolium* and by Mishra *et al.* (1998) in aonla.

Table 1: Effect of cytokinins on bud induction and proliferation

Cytokinin	Conc. (mg/l)	Mean No. of days taken for bud induction	Mean No. of shoots	Mean shoot length in cm	Mean leaf number /shoot
BAP	2	10	1.0	1.8	4.6
	4	7	2.6	1.8	5.6
	6	7	1.3	2.6	2.6
TDZ	2	11	0.0	0.0	0.0
	4	10	1.6	0.5	2.0
	6	8	1.0	0.5	2.0
KINETIN	2	12	0.0	0.0	0.0
	4	10	1.0	0.5	2.0
	6	9	1.0	1.0	2.0
S.E. (m)		0.44	0.55	0.48	0.47
C.D.		1.30	1.64	1.42	1.41

Effect of Hormone on Shoot Proliferation

Early bud break and obtaining multiple microshoots from a single explant is the major goal of shoot bud culture. Plant growth regulators or plant hormones play an important role in achieving the above goal. It is evident from the data (table 1) that out of the three different cytokinins used, maximum number of shoots (2.6 mean shoots) could be induced under *in vitro* conditions by fortification of BAP at 4mg/L. The days taken for bud induction was reduced to 7 days compared to other treatments. Maximum number of leaf/shoot was also highest in this treatment. This result was in accordance with the findings of Cronauer and Krikorian (1984) in banana and Siddiqui and Farooq (1997), Perez *et al.* (2002), Ali *et al.* (2003) and Raziuddin *et al.* (2004) in guava. Various authors have used BAP in combination with other auxins like IBA and NAA in crops like citrus (Bhat *et al.* 1992; Kanjilal *et al.* 2006) and guava (Prakash 1992; Prakash and Tiwari 1993, 1996). Several authors have reported multiplication of shoots by microshoots and stem explants on MS medium containing BAP, Kinetin and NAA (Arya *et al.,*1981; Hossain *et al.,* 1993; Mishra *et al.,* 2006). Tissue culture plants show profuse cytodifferentiation of cambial region leading to development of multiple meristematic loci. The varied anatomical behavior of tissue culture plants may be due to high regime of cytokinin in the medium.

In vitro Rooting

Rooting plants *in vitro* has always been a very challenging proposition for researchers who deal with micropropagation throughout the world.

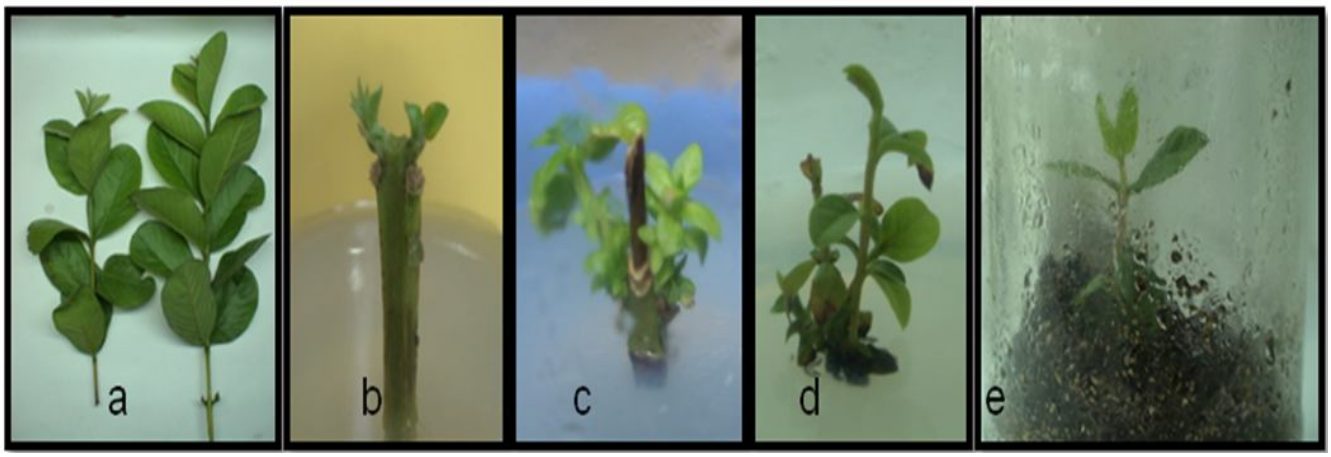

Fig. 3: Different stages of micropropagation of wilt resistant guava rootstock. a) Excised explant. b) Shoot bud induction. c) Shoot proliferation. d) Root initiation. e) Acclimatization of *in vitro* grown plants in cocopeat

Table 2: Effect of bioregulants on *in vitro* rooting.

Media (mg/L)	Mean No. of days taken for rooting	Mean No. of roots	Mean length of primary roots (cm)	Mean fresh weight of roots (mg)	Mean dry weight of roots (mg)
½ MS+ IBA 0.0	0.00	0.00	0.00	0.00	0.00
½ MS+ IBA 1.0	14.20	1.50	2.21	24.10	9.70
½ MS+ IBA 2.0	9.87	2.30	3.00	42.30	18.66
MS+ IBA 0.0	0.00	0.00	0.00	0.00	0.00
MS+ IBA 1.0	9.25	2.90	2.80	43.60	20.21
MS+ IBA 2.0	6.47	3.60	3.94	50.00	23.20
S. E. (m)	0.49	0.95	0.42	0.44	0.50
C. D.	1.54	2.94	1.32	1.37	1.57

In our study, 2 cm long microshoots were subjected to differential regime of IBA. Maximum *in vitro* rhizogenesis (3.6 roots/explants) was observed in MS+IBA 2mg/L (table 2). The length of primary root was 3.94 cm whereas the fresh weight of root was 50 mg. This treatment also reduced the time taken for root induction (6.47 days). Similar results were seen in banana (Vuylsteke and De Langhe, 1985) and guava (Ali *et al.* 2003; Mishra *et al.* 2007). However, many studies suggest the use of a combination of two auxins for rooting as in aonla (Mishra *et al.* 1999), jackfruit (Roy *et al.* 1993), guava (Amin and Jaiswal, 1987; Raziuddin *et al.* 2004) and peach (Kornova, 1995). The use of half strength MS instead of full MS for *in vitro* rooting has shown to bring out better results (Novak *et al.* 1986).

Acclimatization

Maximum mortality of micropropagated plants occurs during acclimatization phase because plantlets undergo rapid and extreme change in physiological functioning when they are removed from *in vitro* to *in vivo* conditions. The true worth of any micropropagation system can only be fully realized when plantlets are successfully transferred from culture vessels to *ex vitro* ambient conditions. Most species which are grown *in vitro* require an acclimatization phase to ensure that sufficient number of plants survive and grow vigorously when transferred to soil (Hazarika, 2003). Three different sterilized substrates, *viz.,* perlite, vermiculite and cocopeat fortified with MS salt solution were used. The survival of regenerated plantlets was highest in cocopeat substrate. These plants were shifted to polyhouse with 35±2^{0}C temperature and 80-85% relative humidity. Cocopeat was also effective substrate for acclimatization of guava (Mishra *et al.* 2007). Fig. 3 illustrates the entire protocol followed for the micropropagation of this hybrid rootstock.

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