Efficient regeneration of *Psidium spp*. For *in vitro* screening of wilt resistant rootstock

Pallavi srivastava, Pravesh Chandra srivastava

Abstract- Guava wilt is a serious problem in its cultivation. Wilting of young and adult trees has caused enormous damage to the standing crop and orchards. The source of resistance to the particular disease is not reported within the commercial varieties. However, some of its wild relatives like *P. molle*, P. *chinensis*, *P. friedrichshthalianum*, *P. cattlianum and P. guineense* have been recorded escaping the disease. Thus, an easy strategy was evolved to screen and develop wilt resistant plants, which can be used as efficient rootstocks. The possibility to perform such a selection strategy under *in vitro* conditions depends largely upon availability of efficient regeneration and clonal propagation system. No earlier reports are available on the micropropagation technique for these *Psidium* spp. Our study has indicated the importance of seasonal influence and media constituents on *in vitro* proliferation of the nodal buds. Rooting of the micro shoots was attempted and good success was achieved.

Index Terms—. *guava species, wilt, micropropagation, growth regulators, BA, NAA*

1 INTRODUCTION

Guava (Psidium guajava L.) is the fourth major fruit crop of the India and popularly known as poor man's fruit or apple of the tropics (Yadava, 1996). Guava wilt disease (GWD) is causing heavy damage, depending upon various agro-climatic and soil conditions. Large numbers of pathogens have been reported to be associated with the disease. Efforts have been made over past few decades to widen its narrow genetic base for wilt resistance/ tolerance by conventional breeding methods. However, control measures other than the eradication of disease tree do not exist so far (Vos et al., 2000). Identification and development of wilt resistant rootstock, is the only strategy to combat the disease. Some of the wild guava species viz. Psidium molle, Psidium chinensis, Psidium guineense and Psidium cattleianum were found promising as wilt resistant / tolerant rootstock for cultivable guava (Edward, 1960; Edward and Gaurishankar, 1964; Singh etal. 1977). These species are propagated by conventional method of stooling as well as through seedling raised from open pollinated seed but the success rate is quite low. Micropropagation of these guava species would be useful in mass multiplication of genetically uniform plants and ensure availability of plant material for conventional methods of grafting. Shoot bud culture of these species would also aid in in vitro screening of shootlets against fungal toxins, so as to ascertain the exact nature of the species as far as reaction to the disease pathogen is concern.

- *Pallavi Srivastava is currently pursuing Ph.D. in Bharathiar University, Coimbatore,India, PH-+918750850769. E-mail: lashu2005@gmail.com*
- *Pravesh Chandra Srivastava is currently pursuing Ph.D. in Dravidian University,Kuppam, PH-+919415485286. E-mail: praveshchandra78@gmail.com*

2 MATERIALS AND METHODS

The trees of guava species viz. Psidium molle, Psidium chinensis, Psidium guineense and Psidium cattleianum were severely headed back. The new sprouts were sprayed regularly with fungicide and antibiotics (0.1%) regularly. Nodal buds were collected in October and April to study the seasonal response. Nodal buds (1-2 cm) from the new vegetative growth were excised and brought to the laboratory in Polyvinylpyrrolidone (PVP) 100mg/l + Ascorbic acid (100 mg/l), Gentamycine sulphate (50 mg/l), Bavistin (100 mg/l) solution. The buds were washed thoroughly under tap water. The washed material was agitated for one hour at 120 rpm in antioxidant solution (PVP + Ascorbic acid 100 mg/l) for removal and control of excessive phenol leaching. Surface sterilization was carried out with 10 % H2O2 for all the species followed by 0.1% Hgcl2 for 6 minutes. Explants were washed with autoclaved water at least five times. Explants were inoculated on Murashige and Skoog (MS) media (Murashige and Skoog, 1962) fortified with plant growth regulators. The MS media was prepared as per standard procedure. Explants were incubated vertically in culture tube. The cultures were incubated in the dark for 8 hours initially and allowed to grow umnder 5000 lux at 25°C + 2°C with 16 hour photoperiod. Observations were taken on percent bud induction and number of microshoots / explant in each media. All the treatments were replicated three times and data was subjected to statistical analysis in CRD.

3 RESULTS AND DISCUSSION

Explants of all the species of guava under study were collected throughout the year to study the most favorable season. October- November was found most suitable season for culture. Good proliferation and growth was recorded in explants cultured during this period. Thus the seasonal response was not in confirmation with Amin and Jaiswal's (1987) reports,

where April – June was reported most suitable season for culture initiation. Results revealed (Table –1) that shoots of P. molle. and P. chinensis (Fig. 1)responded well for in vitro bud induction and subsequent growth of microshoots. Observation were taken on various traits and found highly significant when subjected to CRD analysis. Critical differences were given at 1% and 5% level of significance. The perusal of data clearly indicate that BA at 3.0 mg/l along with 0.1 mg/l NAA registered early and maximum bud induction in P. molle. and P. chinensis whereas GA3 in the same combination gave early and maximum bud induction in P.cattleianum and P.guineense. Role of BA and NAA in micropropagation has already been discussed in Psidium guajava L. (Papadatou et. al. 1990). A proliferation rate of 1-5 microshoots (Table – 1) was achieved within 8 weeks of incubation. The shoots were kept in state of active growth by serial subcultures. The multiplication via nodal buds was the favoured strategy because of absence of basal callus and fairly uniform shoot proliferation. Generally genotype dependent multiplication via buds is the preference so as to maintaining the genetic stability (George, 1993).

Our experiments have indicated that pre treating with antioxidants alone was effective for combating problems associated with phenol leaching and its toxicity to growing cultures. Only P. molle presented some problems, which was overcome by pretreatment of antioxidant and subculturing. Thus the guava species under study differ from the culture and pre-conditioning requirements of P. guajava as reported earlier by Siddiqui and Farooqui (1997) and other workers.

Resistance to the soil born pathogens is the only permanent option for managing the wilt disease. In vitro screening is reliable and safe alternative to sick plot technique. Our experiments on shoot bud proliferation via nodal bud cultures are being used for developing efficient in vitro screening methods of guava wilt resistance. The reliable protocol for micropropagation of these wilt resistant/ tolerant species of guava and its utilization as rootstock for cultivable guava will go a long way in combating the menace of guava wilt disease. Alternatively it would be helpful in formulating *in vitro* micrografting strategies of commercial cultivar viz. Allahabad Safeda and Sardar on these rootstocks.

al., 1977). These species are propagated by conventional method of stooling as well as through seedling raised from open pollinated seed but the success rate is quite low. Micropropagation of these guava species would be useful in mass multiplication of genetically uniform plants and ensure availability of plant material for conventional methods of grafting. Shoot bud culture of these species would also aid in in vitro screening of shootlets against fungal toxins, so as to ascertain the exact nature of the species as far as reaction to the disease pathogen is concern.

ACKNOWLEDGMENT

The authors are grateful to Prof. R.K. Pathak, Director, CISH, Lucknow for providing facilities to carry out the work. The work was supported by the grant of UPDASP/ UPCAR-sponsored networking project on guava wilt (Biotechnology component).

REFERENCES

[1] Amin, M.N. & Jaiswal V.S. 1987. Rapid clonal propagation of guava through in vitro shoot proliferation on nodal explants of mature trees. Plant cell Tissue Org. Culture. 9: 235 – 44.

[2] Edward , J.C. and Gaurishankar. 1964. Rootstock trial for guava (Psidium guajava L.) . Allahabad Farmers. 38: 249-250.

[3] Edward, J.C. 1960. Wilt of Guava. Allahabad Farmers. 34: 289-293.

[4] George, E.F. 1993. Plant propagation by Tissue culture: Part-1 The Technology Exegetics, Baringstoke.

[5] Murashige, T. & F.Skoog .1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol plant. 15: Pp 473-497.

[6] Papadatou P., Pontikis CA, Ephtimiadau E. and Lydkt M.1990. Rapid multiplication of guajava seedlings by in vitro shoot tip culture. Scientia Hort. 45. 99-103.

[7] Siddiqui, Z.M. and Farooq, S.A. 1997. Tissue culture studies on nodal explants of guava. Indian J. Hort. 54 (4): 276-279.

[8] Singh, U.R., Dhar, L. and Singh, G. 1977. Note on performance of guava cultivars and Psidium species against wilt disease under natural field condition. Haryana J. Hort. Sci. 6: 149-150.

[9] Umedi L. Yadav. 1995. Guava (Psidium guajava L.): An exotic tree fruit with potential in south eastern united states. Hort Sci. 31 (5). 789-794.

[10] Vos JE, Schoeman MH, Berjak P., Watt MP and Toerin AJ. 2000. In vitro selection and commercial release of guava wilt resistant rootstock. Acta Hort, 51: 69-79

Table –(1) Initial response of nodal buds of different species of guava to different plant growth regulators.

Media		P. molle		P. chinensis		P. cattleianum		P. guineese	
		% Bud induc-tion	Number of microshoots	% Bud in-duction	Number of microshoots	% Bud in-duction	Number of micro-shoots	% Bud in-duction	Number of microshoots
MS+ BA 1.0 mg/l	S1	10.00	1.3	12.00	1.0	12.00	1.0	10.00	1.00
MS + BA 3.0 mg/l NAA 0.1 mg/l	S2	80.00	3.6	63.00	5.0	43.00	3.00	12.00	1.2
MS + BA 3.0 mg/l NAA 0.1 mg/l GA 1.0 mg/l	S4	55.00	2.1	30.00	2.1	55.00	4.00	60.00	3.8
MS + BA 3.0 mg/l GA 2.0 mg/l	S5	30.00	1.0	12.00	1.8	10.00	1.7	5.00	2.0
MS + BA 0.5 mg/l	S7	20.00	1.0	40.00	3.5	15.00	1.0	20.00	2.00
CD %	1 %	2.4	0.4	3.0	0.4	1.01	0.2	0.4	0.4
	5 %	1.6	0.3	2.1	0.3	0.8	0.1	0.2	0.3