Triazole Mediated Somatic Embryogenesis in Guava (*P. guajava* L.) cv. Allahabad Safeda

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Abstract

Induction and proliferation of somatic embryos from immature fruit mesocarp under the influence of 4 mg/L 1,2,4-1-H Triazole has been obtained. 1,2,4-1-H Triazole triggered the production of globular embryoids to the tune of 8.66 embryoids per culture. Around 85% somatic embryos converted into plantlets.

INTRODUCTION

Many new improved varieties of various horticultural crops particularly of fruit crops have been developed for various locations and uses. However, the inputs could not reach the end users owing to practice of old conventional propagation methods. Micropropagation technique, particularly somatic embryogenesis, is a very useful tool not only for rapid mass production of elite fruit crop varieties but also for production of transgenic fruit crops.

Guava (*Psidium guajava* L.), the apple of tropics, is one of the most common fruits in India and it belongs to the Myrtaceae family. Asexually propagated guava using air layering, stooling, inarching, grafting and budding of scions from desirable guava cultivars into superior guava root stock type is preferable and some of the vegetative methods are in practice, however, these methods are not adapted to mass multiplication of elite planting material in a short span of time due to dependence on season, slow multiplication rates and labour intensive practice. Micropropagation methods could assist in rapid and mass production of clonal stock of newly released improved cultivars of guava. Somatic embryogenesis in particular is a useful tool for large scale cloning, transgenesis and could be used for cryo-preservation. Few workers have reported micropropagation of guava using shoot bud culture (Jaiswal and Amin, 1986; Amin, 1986; Amin and Jaiswal, 1988) However, not much information has been generated on somatic embryogenesis of guava which can help in bulking up the planting material in large quantities. Recently, Chandra et al. (2004) has devised somatic embryogenesis system in guava using mesocarp as explant and auxin as stimulant. Auxins and cytokinins are the leading hormones that are being employed to obtain embryogenesis in majority of crops. Recently, Triazole compounds have shown to influence in vitro growth and somatic embryogenesis in few fruit crops like *Citrus*. Therefore, the present study was undertaken to examine the role of certain triazole compounds (Paclobutrazole, 1,2,4 Triazole, 2,4-1-H Triazole and 4L(1H-1,2,4 Triazole-1-Y) along with commonly employed hormones (2,4-D, BA, NAA) on somatic embryogenesis of guava.

MATERIALS AND METHODS

The present study was carried out at Biotechnology laboratory, CISH, Rehmankhera, Lucknow. Allahabad Safeda being the most popular and commercial cultivar was chosen as an experimental material. Murashige and Skoog (1962) formulation was used as basal media. The pH of medium was adjusted to 5.7 prior to autoclaving. The medium was gelled with agar at 8 g/L. Medium was autoclaved at 121°C for 25 minutes in test tubes and bottles containing 20 and 50 ml of medium respectively. Three types of explants viz, shoot, leaf and fruit were taken for the study. The explants were excised from guava plants maintained in the germplasm block of institute. The branches of above-mentioned variety of guava were taken from bearing tree. After removing the leaves from the bases of these branches, the nodal explants of 1-

1.5 cm size with at least two nodes per explant were obtained. Young leaves were taken from the juvenile branches and cut into small pieces of 0.5-1 cm along with mid rib. Small, immature fruits of same cultivar were also taken. Shoots, leaves and fruits were washed under running tap water for half an hour. These were then dipped in solution containing 0.1% Carbendazime (Bavestin), Tween - 20 along with 0.2% PVP for 1 hour. These explants were again washed under running tap water; the explants were further sterilized with 0.1% $HgCl_2$ for three minutes followed by washing in sterile double distilled water. The fruits were dipped in 70% alcohol for a few seconds and flamed thrice under laminar air flow and the mesocarp of fruits was scooped out with the help of sterilized knife. The processed leaf, shoot and mesocarp tissues were inoculated on agrified MS medium. The cultures were incubated in culture rooms maintained at 25 ±2°C, 50-55% RH and 4000 lux of fluorescent tube light illumination with 16/18 hours of light and dark cycling. The cultures were observed periodically and subculturing was done as and when needed. Ten culture tubes formed one treatment replicated thrice. The data obtained was subjected to statistical analysis using CRD factorial.

RESULTS AND DISCUSSION

Guava is one of the recalcitrant plant species for tissue culture. In vitro phenol exudation and in borne contamination are two major bottlenecks during in vitro culture of guava. Therefore, three types of explants viz., leaf, shoot and fruit mesocarp were chosen for the experiments (Table 1). It was found that tissue derived from immature fruits (mesocarp) was especially amenable to induce tissue proliferation as compared to mature explant (leaf and shoot). The reason for this may be attributed to exudation of phenol and in borne contamination of these two explants. Mesocarp from young and immature fruits was found most responsive explant for guava probably due to its developmental stage and may be because there is neither oxidative browning nor microbial contamination. The most important parameters that determine somatic embryogenesis are explant type, the developmental stage of explant and the interaction between explant and growth medium (Litz and Jaiswal, 1993). Maximum callusing frequency was reported in explants which were subjected to paclobutrazol followed by triazole. Callus was observed creamy yellow and compact in all the treatments of Paclobutrazol and Triazol whereas cytokinin + auxin (2,4-D + BA) and cytokinin + auxin + ADS (BA+NAA+ADS) produced whitish brown callus (Table 2) which was loosely arranged. The callus colour and texture largely depends on genotype, type of explant, culture condition and phytohormone in question. Primordial leaf explant of persimmon cv. Jiro produced yellowish white callus in the dark on MS medium. In general BA and NAA found to produce compact and dark green callus in passion fruit (Otoni et al., 1995) and carambola (Islam et al., 1996). It is evident from the data that 1, 2-4-1-H Triazole at 1 mg/L augmented the production of callus in just five days followed by another triazole (1, 2, 4 Triazole) at 1 mg/L in 5.66 days (Table 3). Callus induction was delayed in medium devoid of any hormone. Profound effect of Triazole(s) on early induction of guava callus has been observed. The similar effect of triazole has been reported by Chandra et al. (1992) who evaluated the efficacy of two triazols, triadine and uniconazole, compound to BAP for in vitro micro tuber production. Both triazols were effective in micro tuber production and its augmentation, and at the lowest concentration tested (0.01 mgL^{-1}) uniconazole was more effective in producing higher number and average weight of micro tubers than 10 mgL^{-1} of BAP, although the best results were obtained with 0.5 mgL^{-1} of uniconozale. The early induction of callus under the influence of triazole may be attributed to accelerated metabolism. It is evident from our results that triazole plays crucial role mostly in early induction of callus. However, callus induction also depends on the plant genotype, the source of origin of the explant and the physiological state of tissue at culture (Murashige, 1974). Linear growth trend in fresh callus weight was observed in all the treatments tested. Paclobutrazol at 4 mg/L registered maximum fresh weight of callus (3140 mg) after 45 days. The role of triazoles particularly paclobutrazole has been observed in augmentation of somatic embryogenesis in *Citrus*. It has been demonstrated that in *Citrus sinensis*, where GA_3

suppresses somatic embryogenesis, paclobutrazol stimulated the process in the cultures (Spiegel Roy and Saad, 1986). The first sign of differentiation of embryogenic structures was observed within 6-8 weeks in explant subjected to triazoles. The morphogenetic response of a tissue in cultures depends on the equilibrium between exogenously and endogenously applied hormones. Maximum fresh weight of callus was observed in explant subjected to paclobutrazole, however, formation of somatic embryos was pronounced in explants treated with triazoles. The transformation of an explant in to a proliferating callus mass under culture conditions is reflected in a change in the basic architectural pattern of the tissue, by cell division, loss of certain cell types, development of new cell types and quiescent cells becoming metabolically active. The data revealed that (Table 4) 1,2,4-1-H Triazole at 4 mg/L was found most effective and triggered the production of globular embryoids to the tune of 8.66 embryoids per culture followed by 4L (1,H,1,2,4) Triazole (8.33). It is clear from the data that triazoles have profound effect on induction of somatic embryogenesis in guava. Triazoles is a big group of bioregulants which are recently being used in plant tissue culture. Recently, Mishra et al. (1997) showed that Paclobutrazol augments somatic embryogenesis in few *Citrus* species. The induction and conversion of somatic embryos was observed simultaneously. Around 85% somatic embryos converted in to plantlets.

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Tables

Table 1. Effect of different explants on callus growth and survival.

Type of explants	Days taken for callusing	Number of explants survived (%)	Callus morphology	Callus frequency
Mesocarp	6.0	90.3	Brown /compact	+++
Leaf	20.0	36.3	Whitish brown/loose	++
Shoot	--	0.0	-	-
SE.m±	1.06	2.02		
CD (p=0.05)	2.61	4.95		

* All the explants were inoculated on MS+BA(0.5)+NAA(0.1)+ADS (150 mg/L).
++++ =High callusing, ++ = Poor callusing

Table 2. Effect of bioregulants on callus morphology and frequency.

Treatment	Concentration (mg/L)	Colour of callus	Texture of callus	Frequency of callusing
MS	0.0+0.0	wb	L	***
MS+2,4-D+BA	0.5+0.1	wb	L	***
	1.0+0.1	wb	L	***
	5.0+0.1	wb	L	***
	0.1+0.2	wb	L	***
	0.1+0.05	wb	L	***
MS+BA+NAA+ADS	0.5+0.1+150	wb	L	****
	0.5+0.1+250	wb	L	***
MS+1,2,4Triazole	1.0	C.Y.	C	***
	2.0	C.Y.	C	***
	3.0	C.Y.	C	***
	4.0	C.Y.	C	***
MS+1,2,4-1-H Triazole	1.0	gw	C	****
	2.0	gw	C	***
	3.0	gw	C	***
	4.0	gw	C	***
MS+4L(1H-1,2,4Triazole-1-Y)	1.0	gw	C	***
	2.0	gw	C	***
	3.0	gw	C	***
	4.0	gw	C	***
MS+Paclobutrazole	1.0	C.Y.	C	***
	2.0	C.Y.	C	***
	3.0	C.Y.	C	***
	4.0	C.Y.	C	****

Colour
gw - Greenish white callus
CY - Creamy white callus
Wb - White brown callus

Texture of callus
C– Compact
L– Loose

Frequency
**** High
*** Moderate
** Low

Table 3. Influence of different treatments on induction and growth of callus.

Treatment	Concentration (mg/L)	Days taken for callusing	Fresh callus weight (After 45 days) (mg)
MS	0.0+0.0	13.66	2743
MS+ 2,4-D+BA	0.5+0.1	6.66	2615
	1.0+0.1	8.66	2537
	5.0+0.1	6.66	2182
	0.1+0.2	5.66	2222
	0.1+0.05	18.66	2134
MS+BA+NAA+ADS	0.5+0.1+150	6.00	3100
	0.5+0.1+250	18.00	2674
MS+1,2,4Triazole	1.0	5.66	2783
	2.0	6.66	2229
	3.0	7.00	2845
	4.0	6.00	2394
MS+1,2,4-1-H Triazole	1.0	5.00	2184
	2.0	7.33	3061
	3.0	8.66	1417
	4.0	6.66	1845
MS+4L(1H-1,2,4Triazole-1-Y)	1.0	8.66	1293
	2.0	8.00	2106
	3.0	10.00	1684
	4.0	7.66	1813
MS+ Paclobutrazole	1.0	6.66	2251
	2.0	11.00	1777
	3.0	11.66	1855
	4.0	6.00	3140
S.Em $\pm$		1.14	168.9
CD (p=0.05)		2.36	454.05

Table 4. Influence of different treatments on formation of somatic embryos.

Treatment	Concentration (mg/L)	Number of embryos
MS	0.0+0.0	0.00
MS+2,4-D+BA	0.5+0.1	0.00
	1.0+0.1	0.00
	5.0+0.1	0.00
	0.1+0.2	0.00
	0.1+0.05	5.66
MS+BA+NAA+ADS	0.5+0.1+150	6.66
	0.5+0.1+250	5.33
MS+1,2,4Triazole	1.0	2.33
	2.0	0.00
	3.0	0.00
	4.0	0.00
MS+1,2,4-1-H Triazole	1.0	7.00
	2.0	0.00
	3.0	5.00
	4.0	8.66
MS+4L(1H-1,2,4Triazole-1-Y)	1.0	8.33
	2.0	0.00
	3.0	0.00
	4.0	4.00
MS+Paclobutrozole	1.0	0.00
	2.0	0.00
	3.0	0.00
	4.0	0.00
S.Em±		0.68
CD(p=0.05)		1.42