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Micropropagation studies on guava

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ABSTRACT

Micropropagation of guava cv. Allahabad Safeda was achieved on nodal explant from field grown mature trees. The nodal explants excised during April-June period were found better for culture initiation. Multiple surface sterilizing agents (H_2O_2, $AgNO_3$ and $HgCl_2$) were used for getting pure cultures. The problem of phenolic browning was minimized to a great extent by agitation of explants in PVPP (0.5%) and sucrose (2%) for 40 min. followed by quick dip in antioxidant solution (citric acid and ascorbic acid) prior to inoculation. The explant bud sprouting was not significantly influenced by various basal media supplemented with 1 mg l^{-1} BAP. The shoot multiplication was better on WPM with 1 mg l^{-1} BAP and incorporation of IBA could not increase the shoot proliferation rate. The best rooting response was obtained on WPM supplemented with 0.2 mg l^{-1} each of IBA & NAA and 200 mg l^{-1} activated charcoal. The regenerated plantlets were established on peat and soilrite based rooting mixture for hardening in the glass house. Finally 50% of the plantlets survived on field transfer.

Key words: Guava, micropropagation, nodal explant, phenolic browning.

INTRODUCTION

Guava is one of the important fruit crops for tropical and subtropical climatic regions. Its fruits are good source of vitamin C and pectin. Owing to wide adaptability and higher production potential, its cultivation can be extended to light, sandy and gravelly soils. Urgent need is being felt to extend the area under guava in arid and semi arid region to increase the production. However, the availability of quality planting materials is major bottleneck for its cultivation in newer areas. Besides, the conventional propagation methods, micropropagation can be an effective tool to supplement the availability of planting materials. Although, some, efforts have been made in the past to propagate guava by *in vitro* methods (Amin and Jaiswal, 3; Prakash, 11; Fuemayor and Montero, 5) but still efforts are required to make guava micropropagation technology a commercially viable one. Attempts were therefore, made to develop an effective protocol for microprogation of guava var. Allahabad Safeda with the explants derived from field-grown mature plants through enhanced auxiliary bud multiplication.

MATERIALS AND METHODS

The studies were carried out in the division of Fruits and Horticultural Technology and Central Tissue Culture Laboratory, NRC on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi. The explants were collected from coppiced growth of 15-year-old plants of Allahabad Safeda. The nodal segments and shoot tip explants (2-3 cm) were collected in three seasons, i.e. spring (March-April), summer (May-June) and autumn (September-October). The explants were surface sterilized with H_2O_2 (hydrogen peroxide, 10% v/v) for 5 min., $AgNO_3$ (0.25%) for 5 min. followed by $HgCl_2$ (0.05%) for 3 min. in sequence. The phenolic browning was reduced by agitation of explant in solution having 0.5% polyvinyl polypyrrolidone (PVPP) and 2% sucrose for 40 min. followed by quick dip in a solution of ascorbic acid solution (50 mg l^{-1}) and citric acid (75 mg l^{-1}) prior to inoculation.

Four basal media, *viz*. MS (Murashige and Skoog, 10), half MS (half major salts and full micro & organics), WPM (Woody Plant Medium; Lloyd and McCown, 8) and B5 medium (Gamborg *et al.*, 6) were tried for culture initiation. Each basal medium was supplemented with four levels of 6-Benzylaminopurine (BAP) @ 0 to 3 mg l^{-1} as per treatment combinations. Sucrose and agar-agar were added @ 3% and 0.7% respectively, while pH of the media was adjusted to 5.7 before autoclaving. The cultures were initiated in test tubes while shoot multiplication was carried out in conical flasks. The shoot proliferation experiments were conducted on five different media, *viz*. MS, half-MS, WPM, MBM (Modified Blue berry Medium, Zimmerman and Broome, 16 as modified by Amin, 1) and OM (Olive Medium; Rugini, 12). The

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studies on rooting of micro-shoots were undertaken on WPM with 2% sucrose and various concentrations of IBA, NAA and AC (Activated charcoal). The cultures were maintained at 25± 2^{0}C with 16/8 h photoperiod at a photon flux intensity of 50-70 E m^2s^{-1}. For hardening, rooted plantlets were transferred to sterile jam bottles containing autoclaved peat and soilrite (1:1) moistened with 1/10 MS (macro-calcium) and Fe-Na EDTA. The bottles were then incubated in culture room for four weeks. The plantlets were next transferred on to earthen pots containing normal potting mixture for shifting to glasshouse. The experiments were laid out in CRD (completely randomized design) with three replications. Each experiment was repeated for three times to confirm the results. The data were analyzed statistically.

RESULTS AND DISCUSSION

The cultures were initiated from nodal segments and shoot tip explants collected in three seasons. The data on per cent sprouting as influenced by explant type and explanting season are given in Table 1. The nodal segments proved much better as an explant for culture initiation for micropropagation in guava in all the seasons as compared to shoot tip explants. This was evidenced by recording significantly higher per cent sprouting on nodal explant. Among different seasons of explanting, spring and summer season exhibited significantly higher per cent sprouting irrespective of type of explant. The differences were however, non-significant in per cent sprouting was noted between spring and summer seasons. Amin and Jaiswal (3) reported April to June period to be the best period for culture establishment of guava. The lesser per cent sprouting recorded in shoot tip explant might be associated with relatively greater injury to the tender tissues of growing points by sterilizing agents coupled with more inhibitory effects of higher phenolic exudates from shoot tip explants as compared to nodal ones.

The effect of different media and BAP levels on explant bud sprouting was also recorded (Table 2). The differences in bud sprouting percentage were non significant among various media, however, maximum bud sprouting was recorded on ½ MS medium (66.66%). This is supported by earlier findings of Fuenmayor and Montero (5) who also obtained highest bud sprouting on half-MS medium as compared to full and ¼th strength MS medium. As regards to the role of BAP in bud sprouting was concerned, the highest bud sprouting was obtained with 1 mg 1^{-1} BAP. The highest bud sprouting at low level of BAP (1 mgl^{-1}) is in agreement with the findings of many previous workers (Jaiswal and Amin, 7; Loh and Rao, 9; Prakash, 11). In this connection Blomstdt *et al.* (4) reported that though BAP has been known for breaking apical dominance and enhancement of axillary branching, but higher level of the same may sometimes suppress growth or even it may be toxic to the tissues.

The effects of different media were investigated by

Table 1. Effect of explant and season on *in vitro* bud sprouting (%) in guava cv. Allahabad Safeda.

Explant	Season			
	Spring	Summer	Autumn	Mean
Nodal	87.66 (70.08)	88.33 (70.69)	15.00 (22.66)	63.66
Shoot tip	5.83 (13.48)	7.66 (15.22)	3.66 (10.93)	6.74
Mean	46.75	47.99	9.33	

$CD_{0.01}$ Season = 3; Explant = 3.01; Season x explant = 5.22

Table 2. Effect of media and BAP on bud sprouting(%) of guava var. Allahabad Safeda.

Medium	BAP (mg l^{-1})				
	0	1	2	3	Mean
½ MS	61.66 (51.81)*	85.00 (67.40)	63.33 (52.66)	56.66 (48.84)	66.66
MS	55.66 (48.26)	81.66 (65.76)	71.66 (58.06)	41.66 (40.16)	62.66
WPM	46.66 (43.07)	71.66 (48.06)	65.00 (53.76)	60.00 (50.88)	60.83)
B5	50.00 (45.00)	80.00 (63.54)	78.33 (62.47)	53.33 (46.93)	65.41
Mean	53.49	79.58	69.58	50.91	

$CD_{0.01}$ BAP = 637; Medium = NS; BAP x Medium = NS

keeping BAP level (1 mg l^{-1}) constant on shoot proliferation on original explants (Table 3). It was revealed that medium giving best results at culture establishment stage in terms of maximum bud sprouting need not essentially be best for subsequent shoot proliferation, since, initially the explant might utilize reserve food for growth. The shoot proliferation rate can be adjudged in terms of maximum number of usable shoots of sufficient length for further multiplication. The highest number as well as length of shoots was obtained on WPM on original explant (first cycle) as well on secondary explant. However, these results are not in confirmation with the findings of Yasseen *et al.* (15), and Loh and Rao (9) reported shoot multiplication on MS medium, while Amin and Jaiswal (2) found modified Blueberry medium better than MS medium in guava. Superman and Blake (13) reported WPM to be more effective for shoot multiplication than half-MS in clove belonging to myrtale family. The MS medium also showed the symptoms of leaf chlorosis and shoot tip necrosis in this study. Amin and Jaiswal (2) also reported non satisfactory shoot multiplication rate and incidence of shoot tip necrosis in guava.

Table 3. Effect of different media supplemented with 1 mg l^{-1} BAP on number and length of shoots on original explant of guava var. Allahabad Safeda (after 10 weeks of inoculation).

Medium	No. of shoots	Shoot length (cm)
MS	3.25	1.32
½ MS	1.87	1.75
WPM	4.87	2.22
MBM	3.50	1.12
OM	2.75	1.20
Mean	3.24	1.52
$CD_{0.05}$	0.53	0.33

The shoot proliferation on original explants in all media exhibited arrested growth beyond a certain period (8-10 weeks) after which there was no further growth. The micro-shoots also showed typical adult morphological characteristics on leaf and internodes. This behaviour of shoots produced in first cycle on original explants may be related to ontogeny of the explants. Vieitez *et al.* (14) observed that number of shoots and segments per shoot depended more on position of explant on stock plant than on the medium. After identifying the best medium for shoot proliferation, the effect of different BAP levels in combination with low concentration of IBA was tried for multiplication of *in vitro* developed shoots (Table 4). It was again confirmed that increasing the concentration of BAP.

Table 4. *In vitro* shoot proliferation from secondary explant of guava on WPM as influenced by BAP and IBA after 10 weeks of culture.

IBA + BA (mg l^{-1})	Mean number of shoots* per explants
0 + 1	4.83
0 + 2	3.83
0.1 + 1	4.16
0.1 + 2	4.0
0.2 + 1	4.5
0.2 + 2	3.5
SEm±	0.29

*Shoots of 5 mm or more were counted.

The effect of IBA, NAA and activated charcoal on rooting response of micro-shoots were studied (Table 5). The highest percentage of rooted shoots (73.91%) was recorded on the medium supplemented with 0.2 mg l^{-1} each of IBA and NAA. However, this was at par with the per cent rooting obtained on IBA+NAA (0.2+0.5 mg l^{-1}) and IBA + NAA (0.5 to 0.2 mg l^{-1}). The addition of activated charcoal also improved the rooting per cent over control and 200 mg l^{-1} AC recorded maximum per cent rooting. The higher per cent rooting in dual auxin at low concentration may be attributed to synergistic effect of both the auxins (Prakash, 11; Amin and Jaiswal, 3). The interaction of auxin and AC was also found highly significant with maximum per cent (83.50%) rooting with 0.2 mg l^{-1} each of IBA and NAA and 200 mg l^{-1} AC. (from 1 to 3 mg $^{-1}$ and incorporation of IBA (0.1 & 0.2 mg l^{-1}) did not increase the number of shoots over 1 mg l^{-1} BAP alone. Similar results were also reported earlier in guava (Yasseen *et al.*, 13; Loh and Rao, 9). It is possible that guava shoot might contain sufficient endogenous auxin levels and thus addition of auxin disturbed the balance causing decreased shoot proliferation. In this regard, Jaiswal and Amin (7) reported that addition of 0.1 mg l^{-1} IBA and GA_3 to BAP containing medium did not improve shoot proliferation, rather the shoot growth retarded and leaves turn yellowish.

The hardening of rooted shootlets was achieved by transferring them in sterile jam bottles containing autoclaved peat and soilrite (1:1) moistened with 1/10th MS (Macro-C_aCl_2) and Fe EDTA. The bottle covers were gradually loosened after about 20-25 days. Finally the plantlets were transferred in earthen pots containing normal potting mixture for hardening in glass house. About 50% plantlets survived on field transfer.

Table 5. Effect of auxin(s) and activated charcoal (AC) on *in vitro* rooting (%) in guava.

Auxin (mg l^{-1})	Activated charcoal (mg l^{-1})			
IBA + NAA	0	100	200	Mean
0 + 0	16.50 (23.94) *	20.1 (27.27)	24.0 (29.33)	20.20
0 + 0.2	32.50 (34.75)	37.20 (37.58)	43.50 (41.26)	37.73
0 + 05	35.00 (36.27)	39.5 (38.93)	42.0 (40.39)	38.83
0.2 + 0	47.00 (43.28)	58.30 (49.71)	65.50 (54.03)	56.93
0.2 + 0.2	65.00 (53.73)	73.25 (58.85)	83.50 (66.05)	73.91
0.2 + 0.5	68.00 (55.55)	75.70 (60.46)	77.00 (61.36)	73.56
0.5 + 0	66.00 (54.33)	67.30 (55.12)	72.50 (58.38)	68.60
0.5 + 0.2	68.50 (56.48)	73.35 (59.00)	79.00 (62.77)	73.61
0.5 + 0.5	52.50 (46.43)	59.20 (50.18)	62.50 (52.25)	58.06
Mean	50.11	55.98	61.05	55.71

*Angular transformed values; $CD_{0.01}$ Auxin = 2.11, AC = 1.22, Auxin x AC = 3.66

REFERENCES

1. Amin, M.N. 1987. *In vitro* clonal propagation of guava (*Psidium guajava* L.) and Jack fruit (*Arotocarpus heterophyllus* Lam.). Ph.D thesis submitted to Banaras Hindu University, Varanasi.
2. Amin, M.N. and Jaiswal, V.S. 1987. Rapid clonal propagation of guava through *in vitro* shoot proliferation on nodal explants of mature trees. *Plant Cell Tissue organ Cult*. **9**: 235-43.
3. Amin, M.N. and Jaiswal, V.S. 1988. Micropropagation as an aid to rapid cloning of guava cultivars. *Scientia Hort*. **36**: 89-95.
4. Blomstdt, C. Cameron, J. Whiteman, P. and Chandler, S.F. 1991. Micropropagation of *Eucalyptus regnans*. *Australian J. Bot*. **39**: 179-86.
5. Fuenmayor, M.E.P. and Montero, N.J.M. 1997. *In vitro* clonal propagation of guava (*Psidium guajava L*) from stem shoots of cv. Mara-7. *Acta Hort*. **452**: 47-51.
6. Gamborg, O.L, Miller, R.A. and Ojima, K. 1968. Nutrient requirement of suspension culture of soybean root cells. *Exp. Cell Res*. **50**: 151-58.
7. Jaiswal, V.S. and Amin, M.N. 1986. *In vitro* propagation of guava from shoot cultures of mature trees. *J. Plant. Physiol*. **130**: 7-12.
8. Lloyd, G. and McCown, B. 1981. Commercially feasible micropropagation of mountain laurel (*Kalmia latifolia*) by use of shoot tip culture. *Combined Proc. Int. Prop.Soc.* **30**: 421-427.
9. Loh, C.S. and Rao, A.N. 1989. Clonal propagation of guava from seedlings and grafted plants and adventitious shoot formation *in vitro*. *Scientia Hort.* **39**: 31-39.
10. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant*. **15**: 473-97.
11. Prakash, H. 1992. Micropropagation of guava. Ph.D. thesis, submitted to G.B. Pant University of Agriculture and Technology, Pant Nagar.
12. Rugini, E. *In* vitro propagation of some olive (*Olea europea sativa L*.) cultivars with different rootability and medium development using analytical data from developing shoots and embryos. *Scientia Hort*. **24**: 123-34.
13. Superman, U. and Blake, J. 1990. Studies on tissue culture of clove tree plant. *Indonesian J. Crop. Sci.* **5**: 67-75.
14. Vieitez, M.A. Barciela, J. and Ballester, A. 1989. Propagation of *Camellia japonica* cv Alba plena by tissue culture. *J. Hort. Sci.* **64**: 177-82.
15. Yasseen, M.Y., Barrenger, S.A., Schnell, R.J. and Splittstoesser, W.E. 1995. *In vitro* shoot proliferation and propagation of guava (*Psidium guajava* L.) from germinated seedlings. *Plant Cell Rep.* **14**: 425-28.
16. Zimmerman, R.H. and Broome, O.C. 1980. Blue barry micropagation. In: Proceedings conference on nursery production of fruit plants through tissue culture application and feasibility. *U.S. Dept. Agr. Sci. Educ*. *Adm.* ARR-RE-11, pp. 44-47.

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