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Studies on *in vitro* propagation of sweet cherry cv. Bigarreau Noir Grossa

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ABSTRACT

Present investigation on sweet cherry (*Prunus avium* L.) cv. Bigarreau Noir Grossa was carried out on its micropropagation. Surface sterilisation of shoot tip explant with 0.1% $HgCl_2$ for 10 min. yielded maximum aseptic cultures and explant survival. Using explants from forced stock stem plant cuttings significantly improved culture initiation. Maximum explant establishment (50.57%) was observed on Murashige and Skoog (1962) medium supplemented with BAP + kinetin (0.25 + 0.25 mg l^{-1}). Treatment BAP + kinetin (0.25 + 0.25 mg l^{-1}) accounted for maximum proliferating cultures (99.96%) with highest multiplication efficiency in terms of proliferation grade (4.0) and shoots/explant (17.28). Rooting was carried out by incubation of micro-shoots in IBA supplemented MS medium for 10 days under darkness followed by their transfer on to hormone-free MS medium with incubation under light conditions. Micro-shoots equal to or greater than 10 mm in length gave good rooting. Auxin IBA (2.50 mg l^{-1}) was found most effective, which not only gave the highest rooting (86.33%) but also maximised root number/shoot (4.90) and root length (44.33 mm).

Key words: Axillary shoot proliferation, *in vitro* propagation, sweet cherry.

INTRODUCTION

Cherry is one of the important stone fruits of Jammu & Kashmir state and considered a novelty fruit in the country. Kashmiri cherries are always in high demand and enjoy virtual monopoly as the fruits are preferred across the country for their taste and quality. The increased demand for the cherry fruit has led to a substantial increase in the area in the state, which, has touched 2,752 ha with an annual production of 8,732 metric tonnes (Anon, 1). There is still a lot of potential for extending its cultivation in the hilly areas of the state having temperate climatic conditions. Further, the demand for plant material is likely to increase with the adoption of high density orcharding.

Conventional propagation of cherry by grafting buds onto rootstock plants is slow and time consuming. Micropropagation techniques are becoming available for rapid and reliable clonal propagation different genotypes of stone fruits. Rapid multiplication of newly developed or identified elite cherry cultivars through micropropagation would boost the production in the state by increasing the availability of planting material. The present study was undertaken to develop appropriate technique of stock-plant manipulation for obtaining explants suitable for *in vitro* culture leading to standardization of commercial micropropagation protocol of sweet cherry rootstock cv. Bigarreau Noir Grossa (*Misri*) - a commercial cherry cultivar grown in Kashmir.

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MATERIALS AND METHODS

Present studies were carried out for developing an *in vitro* propagation protocol of sweet cherry rootstock cv. Bigarreau Noir Grossa in the Biotechnology Laboratory, Division of Pomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar. Dormant stem cuttings were collected from the field grown stock plants and forced to sprout and grow in growth chamber (Fig. 1). The forced explants developed under controlled conditions in the growth chamber were excised for culture establishment. The shoot tip explants were cultured on four different types of basal media, *viz*., MS medium (Murashige and Skoog, 11), DK medium (Driver and Kuniyuki, 4), Knop's macro and MS organics (Snir, 16) and Woody Plant Medium (Lloyd and McCown, 9) supplemented with different concentrations of growth regulators. The pH of the medium was adjusted to 5.7 with 0.1 N HCl or 0.1 N NaOH prior to the addition of agar (7 g l^{-1}). The media were autoclaved at 15 psi and 121°C for 15 min.

The established explants were sub-cultured within 3 to 5 weeks of culture initiation on MS medium supplemented with different concentrations of BAP and kinetin (0.00, 0.25, 0.50, and 1.0 mg l^{-1}) for proliferation through stimulation of axillary buds. Micro-shoots from the proliferated cultures were aseptically separated and divided into four groups on the basis of shoot length. Each group was cultured on MS medium supplemented with different concentrations of IBA (1.0, 2.0 and 3.0 mg l^{-1}) for standardisation of shoot

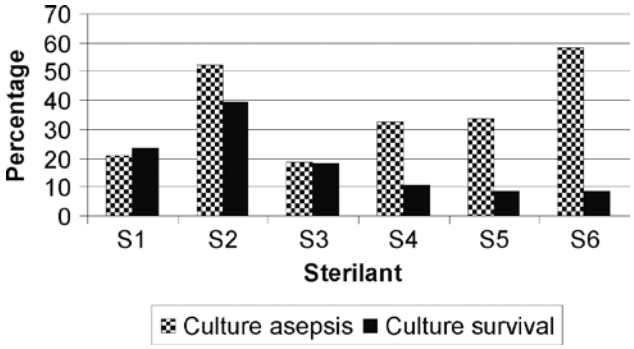

Fig. 1. Influence of sterilants on asepsis culture and survival of forced explants of sweet cherry cv. Bigarreau Noir Grossa [S1 (10% NaOCl for 10 min.), S2 ($HgCl_2$ (0.1%) for 10 min.), S3 (70% ethyl alcohol for 10 s), S4 (S_1 + S_3), S5 (S_2 + S_3), S6 (S_1 + 0.01% Carbendazim + 0.25% Cefataxime for 10 min.)].

length for rooting. Each treatment comprised of 10 explants with one explant per tube. For rooting, micro-shoots were first cultured on root induction medium (MS supplemented with different concentrations of IBA) under dark conditions for 10 days. These shoots were then transferred on to root development medium (hormone-free MS medium) and incubated under light conditions. Standard culture room conditions (16/8 h photoperiod, 24±1°C temperature, 40±3 mmol $m^{-2}s^{-1}$ light intensity using 40 W fluorescent tubes) were maintained during incubation of cultures.

Plantlets with well developed roots were removed from 6-week-old cultures and washed thoroughly with double-distilled sterile water to remove traces of agar jelled medium. Hardening of the rooted plantlets was done by planting them in glass jars containing a mixture of vermiculite-perlite-coco peat (1:1:1). Lid of the bottles was closed and plants were initially kept under high humidity for 10 days under culture room conditions. The cap of the bottle was gradually removed to reduce the humidity and plants were transferred to poly bags containing soil, later vermiculite, coco peat and sand in the ratio of 8:2:2:1 for acclimatization under glasshouse.

RESULTS AND DISCUSSION

Forced and unforced explants derived from the donor stock plants were subjected to six different sterilization regimes using MS basal medium. Effect of sterilization regimes on culture asepsis and survival of forced explants was significant (Fig. 1). Highest mean aseptic cultures (58.08%) were obtained under S_6 sterilization regime (10% sodium hypochlorite + 0.01% carbendazim + 0.25% cefataxime for 10 min.) followed by 52.31% under S_2 sterilization regime (0.1% $HgCl_2$ for 10 min.). However, the explant survival was maximum (39.58%) with S_2 compared to S_6, which resulted in more necrosis and injury to explants leading to lowest survival (8.53%).

Many workers have found that a single sterilant is more effective than the combinations for explant survival. These results are in close conformity with Muna *et al.* (10) and Erbenova *et al.* (5). Forcing had a significant influence upon culture asepsis as well as on explant survival (Fig. 2). Maximum cultural asepsis (36.49%) and explant survival (24.43%) were recorded when forced explants were used. This may be due to relatively lesser inoculum load on the forced explants developed from the cuttings incubated in the growth chamber in comparison to unforced explants taken from field-grown stock plants.

Different media have been tried earlier for *in vitro* culture of cherry (Jones and Hopgood, 8; Snir, 16; Erbenova *et al.*, 5; Dai-Hong *et al*., 3; Sedlak *et al.*, 14). In the present study four media were evaluated and MS followed by DK media were found superior to others, yielding maximum explant establishment (Table 1). Exogenous application of cytokinins released lateral buds from apical dominance and promotes cyto-differentiation, while BAP has been widely used for *in vitro* multiplication of temperate fruit and nut tree species (Peer *et al*., 13). BAP proved superior to kinetin in improving culture establishment. Combination of cytokinins further improved the explant establishment. Maximum explant establishment (38.05%) was recorded when medium was supplemented with BAP + kinetin (0.25 + 0.25 mg l^{-1}). The interaction effect of medium and growth regulator on the establishment of explants was also significant. Explants cultured on MS medium containing BAP + kinetin (0.25 + 0.25 mg l^{-1}) recorded the highest explant establishment (50.57%).

Similarly, cytokinins were found essential for axillary shoot proliferation as lowest proliferating

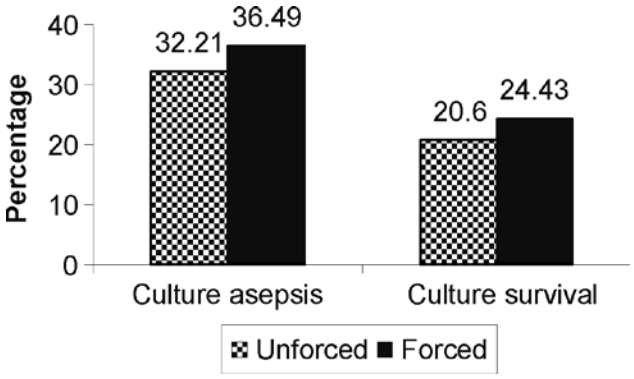

Fig. 2. Influence of stem forcing on culture asepsis and explant survival in sweet cherry.

Table 1. Influence of medium and growth regulators on explant establishment (%) with forced explants of sweet cherry cv. Bigarreau Noir Grossa.

Growth regulator(s)	Conc. (mgl^{-1})	Medium				
		M_1	M_2	M_3	M_4	Mean
BAP	0.25	18.30 (25.33)	12.44 (20.66)	11.68 (19.99)	10.95 (19.33)	13.34 (21.32)
	0.50	39.02 (38.66)	29.64 (32.99)	28.60 (32.33)	25.50 (30.33)	30.69 (33.58)
	1.00	7.90 (16.33)	6.98 (15.33)	6.69 (14.99)	5.57 (13.66)	6.78 (15.08)
Kinetin	0.25	11.31 (19.66)	9.89 (18.33)	9.53 (17.99)	7.58 (15.99)	9.57 (17.99)
	0.50	25.50 (30.33)	23.48 (28.99)	23.00 (28.66)	19.67 (26.33)	22.91 (28.58)
	1.00	4.80 (12.66)	4.08 (11.67)	3.85 (11.33)	3.21 (10.33)	3.98 (11.49)
BAP + Kinetin	0.25+0.25	50.57 (45.33)	38.46 (38.33)	35.64 (36.66)	27.54 (31.66)	38.05 (37.99)
	0.50+0.50	30.72 (33.66)	26.00 (30.66)	23.99 (29.33)	16.97 (24.33)	24.42 (29.49)
	1.00+1.00	7.28 (15.66)	5.84 (13.99)	5.57 (13.66)	4.56 (12.33)	5.81 (13.91)
BAP + IBA	0.25+0.02	26.00 (30.66)	17.84 (24.99)	16.97 (24.33)	10.95 (19.33)	17.94 (24.83)
	0.50+0.02	35.09 (36.33)	23.99 (29.33)	23.00 (28.66)	13.62 (21.66)	23.92 (28.99)
	1.00+0.02	10.23 (18.66)	8.87 (17.33)	8.21 (16.66)	5.57 (13.66)	8.22 (16.57)
Kinetin + IBA	0.25+0.02	12.07 (20.33)	10.23 (18.66)	9.89 (18.33)	6.12 (14.33)	9.58 (17.91)
	0.50+0.02	14.84 (22.66)	12.07 (20.33)	11.31 (19.66)	9.20 (17.66)	11.85 (20.07)
	1.00+0.02	8.53 (16.99)	7.28 (15.66)	6.98 (15.33)	5.84 (13.99)	7.15 (15.49)
Mean		20.14 (25.55)	15.80 (22.48)	14.99 (21.86)	11.52 (18.99)	
CD (P = 0.05)						
Growth regulator	1.95					
Medium	1.00					
Growth regulator × Medium	3.90					

Data in parenthesis are transformed values ((Arc Sin^{-1} √%) of original percentage. M_1 = MS (Murashige and Skoog, 1962); M_2 = DK Medium (Driver and Kuniyuki, 1984); M_3 = Knop's macro and MS micro-organics (Snir, 1982); M_4 = WPM (Woody Plant Medium) (Lloyd and McCown, 1980)

cultures (2.26%) were recorded in medium devoid of any growth regulator (Table 2). BAP proved superior to kinetin in improving the percentage of proliferating cultures (93.29%) with BAP (0.50 mg l^{-1}). Combined application of the two cytokinins further improved the proliferating cultures, recording maximum value (99.96%) with BAP + kinetin (0.25 + 0.25 mg l^{-1}). Highest number of shoots/explant (17.28) with

Table 2. Influence of growth regulators on proliferation of forced explants of established cultures of sweet cherry cv. Bigarreau Noir Grossa.

Growth regulator(s)	Conc. (mgl^{-1})	Proliferation (%)	Shoots explant	*Proliferation grade
BAP	0.00	2.26 (8.66)	2.10	1.00
BAP	0.25	83.01 (65.66)	11.60	2.83
BAP	0.50	93.29 (74.99)	8.32	2.54
BAP	1.00	56.37 (48.66)	5.18	1.74
Kin	0.25	9.20 (17.66)	4.27	2.62
Kin	0.50	5.05 (12.99)	4.10	2.01
Kin	1.00	3.21 (10.33)	4.00	1.05
BAP + Kin	0.25 + 0.25	99.96 (88.99)	17.28	4.00
BAP + Kin	0.50 + 0.25	99.42 (85.66)	12.44	3.24
BAP + Kin	1.00 + 0.25	67.64 (55.33)	8.86	1.82
BAP + Kin	0.25 + 0.50	95.43 (77.66)	12.20	3.63
BAP + Kin	0.50 + 0.50	84.72 (66.99)	8.86	2.66
BAP + Kin	1.00 + 0.50	63.21 (52.66)	6.12	1.98
BAP + Kin	0.25 + 1.00	34.53 (35.990	9.09	2.78
BAP + Kin	0.50 + 1.00	64.88 (53.66)	7.72	2.56
BAP + Kin	1.00 + 1.00	31.25 (33.99)	5.32	1.66
CD (P = 0.05)		2.62	1.32	0.21

Data in parenthesis are transformed values (Arc Sin √%) of original percentage. Proliferation grade: 1 = Poor, 2 = Fair, 3 = Good, 4 = Excellent.

maximum proliferation grade (4.00) was obtained with BAP + kinetin (0.25 + 0.25 mg l^{-1}). Results related to culture proliferation in the present study on cheery are in close conformity to several workers (Sharma *et al.,* 15; Peer *et al*., 13).

Shoot length is very important characteristics determining the capacity of shoots to produce roots during *in vitro* cultures. Shoot length of 10 mm or more resulted in maximum rooting (Fig. 3). After the standardization of the shoot length for rooting, micro-shoots of 10-15 mm length were cultured onto IBA supplemented MS medium for root induction and kept in dark for 10 days followed by their transfer to the hormone-free medium for root elongation in presence of light for 10-20 days. Two phase system for obtaining roots in micro-cuttings was followed which had been earlier used by Bouza (2) in rooting of *Prunus tenella.* Auxin IBA had a significant influence upon all rooting parameters (Table 3). Maximum rooting (86.33%), roots/shoot (4.90) and root length (44.33 mm) were recorded with 2.5 mg l^{-1} IBA. Concentration higher than this had a negative effect

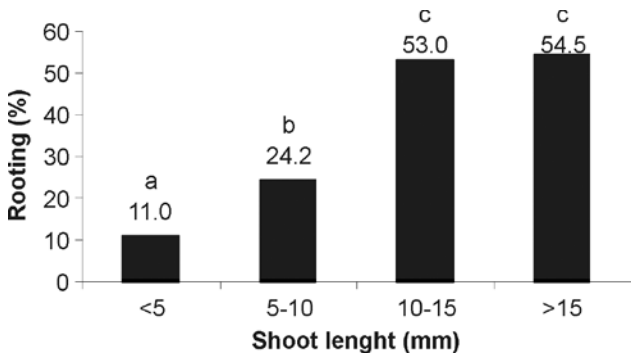

Fig. 3. Shoot length and rooting of micro-shoots in sweet cherry. Columns with different letters are significantly different at P = 0.01 with LSD test. Data in the columns is averaged over IBA concentrations.

Table 3. Influence of IBA concentrations on *in vitro* rooting of micro-shoots of sweet cherry cv. Bigarreau Noir Grossa.

IBA (mgl^{-1})	Rooting (%)	Roots shoot	Root length (mm)
0.50	5.67 (13.63)	3.22	25.66
1.00	43.33 (41.14)	3.48	28.66
1.5	55.33 (48.14)	3.38	37.33
2.00	64.67 (53.66)	4.00	37.66
2.50	86.33 (69.92)	4.90	44.33
3.00	44.67 (41.91)	2.90	18.33
CD (P = 0.05)	10.48	0.47	4.93

Data in parenthesis are transformed values (Arc Sin^{-1} $\sqrt{}$%) of original percentage.

upon rooting parameters. These results are in close conformity with Bouza (2) in *Prunus tenella*; Sharma *et al.* (15) in Colt; and Peer *et al*. (13) in sweet cherry. The rooted plants were successfully transferred for hardening. The different stages of *in vitro* propagation of sweet cherry in shown in Fig. 4.

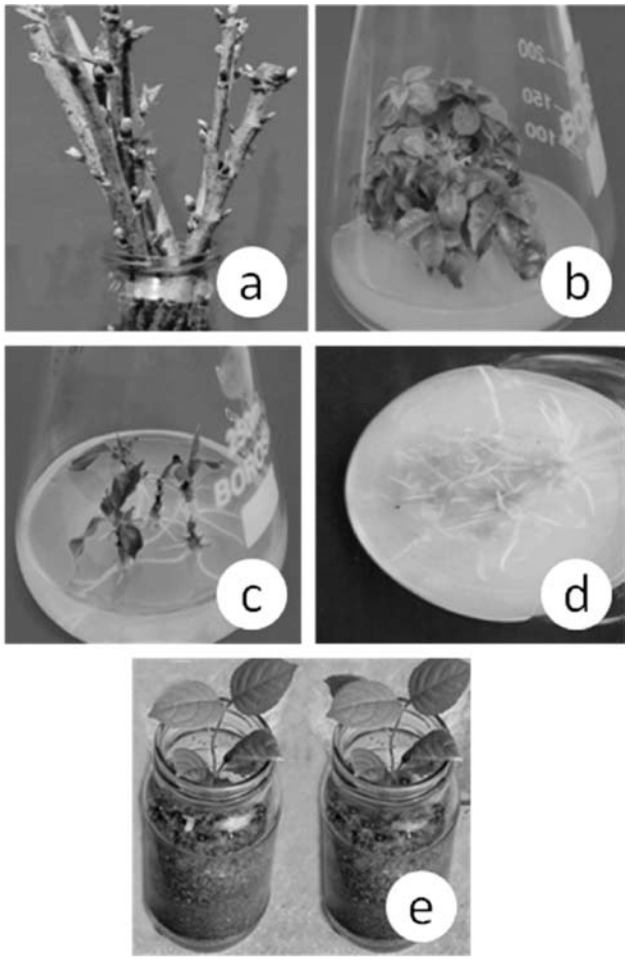

Fig. 4. Stages of micropropagation in sweet cherry. (a) Forcing of dormant cuttings,(b) Shoot proliferation with BAP + kinetin (0.25 + 0.25 mgl^{-1}), (c & d) Rooting in MS medium supplemented with IBA (2.5 mgl^{-1}), and (e) Hardening of plantlets.

REFERENCES

1. Anonymous, 2007. *Annual Progress Report*, Department of Horticulture, Govt. of Jammu & Kashmir, 2005-2006.

2. Bouza, L. 1997. Micropropagation of *Prunus tenella* (Dwarf Russian almond). *Biotechnology in Agriculture and Forestry*, Springer-Verlag, Berlin, Hiedelberg. Vol. VI, pp. 276-88.

3. Dai, H.Y., Zhang, Z., Gao, X.Y. and Wu, L.P. 2004. Establishment and optimization of micropropagation system of sweet cherry cultivars. *J. Fruit Sci.* **21**: 216-19.

4. Driver, J.A. and Kuniyuki. 1984. *In vitro* propagation of 'Paradox' walnut rootstock. *HortSci.* **19**: 507-9.

5. Erbenova, M., Paprstein, F. and Sedlak, J. 2001. *In vitro* propagation of dwarfed rootstocks for sweet cherry. *Acta Hort.* **560**: 477-80.

6. Hammat, N. and Grant, N.J. 1998. Shoot regeneration from leaves of *Prunus serotina*

Ehrh. (black cherry) and *P. avium* L. (wild cherry). *Plant Cell Rep.* **17**: 526-30.

7. Hammat, N. and Grant, N.J. 1997. Micropropagation of mature British wild cherry. *Plant Cell Tissue Organ Cult.* **47**: 103-10.
8. Jones, O.P. and Hopgood, M.E. 1979. The successful propagation *in vitro* of two rootstocks of *Prunus*: The plum rootstock Pixy (*P. institia*) and the cherry rootstock F12/1 (*P. avium*). *J. Hort. Sci.* **54**: 63-66.
9. Lloyd, G. and McCown, B. 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*. *Combined Proc. Int. Plant Prop. Soc.* **30**: 421-27.
10. Muna, A.S., Ahmad, A.K., Mahmoud, K. and Abdul Rahman, K. 1999. *In vitro* propagation of semi-dwarfing cherry rootstock. *Plant Cell Tissue Organ Cult.* **59**: 203-8.
11. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant.* **15**: 473-79.
12. Pandey, R.M. 1993. Biotechnology in horticulture. *Souvenir Golden Jubilee Symposium of Horticultural Society of India,* Bangalore, May 24-28, pp. 21-28.
13. Peer, F.A., Farooqui, K.D., Dar, K.R., Bhat, M.Y., Hussain, G. and Rather, Z.A. 2011. *In vitro* micropropagation of sweet cherry (*Prunus avium* L.) rootstock cv. Mazzard. *Appl. Biol. Res.* **13**: 10-16.
14. Sedlak, J., Paprstein, F. and Erbenova, M. 2008. *In vitro* propagation of the P-HL dwarfing sweet cherry rootstocks. *Acta Hort.* **795**: 395-400.
15. Sharma, D.R., Chauhan, P.S., Kaur, R. and Srivastava, D.K. 1992. Micropropagation of Colt-a semi-dwarf rootstock of cherry. *Indian J. Hort.* **49**: 209-12.
16. Snir, L.A. 1982. *In vitro* propagation of sweet cherry cultivars. *HortSci.* **17**: 192-93.

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