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Micropropagation of sour cherry rootstocks by employing forced and unforced explants

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ABSTRACT

For micropropagation of sour cherry (*Prunus cerasus* L.) rootstock, explants were harvested from dormant cuttings during start of winter season and were subjected to forcing. Explants obtained after forcing of dormant cuttings of stock plant yielded maximum percentage of survival and aseptic cultures than those from field grown unforced stock plants. Woody plant half strength medium supplemented with 0.50 mg l^{-1} BAP plus 0.10 mg l^{-1} IBA gave the highest survival percentage and took minimum number of days to growth initiation for establishment. Growth regulator IBA at 0.10 mg l^{-1} resulted in highest proliferation percentage. Maximum average length of shoot (14.93) was recorded at 0.05 mg l^{-1} IBA, whereas medium fortified with 0.3 mg l^{-1} BAP recorded minimum shoot elongation (28.31 mm). Minimum days taken for initiation of proliferation and maximum shoot multiplication was recorded on WP medium supplemented with 2 mg l^{-1} BAP plus 0.10 mg l^{-1} IBA. Medium fortified with IBA at 2 mg l^{-1} recorded the highest rooting percentage and average number of roots per explant.

Key words: Micropropagation, sour cherry, explant, media, Prunus, growth regulators

INTRODUCTION

Cherries are highly attractive and delicious fruits and occupy an important place among temperate fruits of the world. Its cultivation is mainly restricted to temperate Himalayan regions of Jammu and Kashmir, Himachal Pradesh and Uttarakhand. Among these states, Jammu and Kashmir has emerged as the major cherry growing state with the productivity of 3.2 MT/ha. (Anonymous, 2) which is much lower compared to horticulturally advanced countries like Switzerland. Although there are sizeable potential areas well suited for cherry cultivation in India but this fruit could not pick-up due to non-availability of quality planting material and desirable rootstock. The main bottleneck is the limited availability of clonally propagated dwarfing rootstocks. Historically, dwarfing rootstocks of clonal origin have received much attention in European countries with regards to their development and use (Zeiger and Tukey, 20). Today, much of commercial fruit production in US and Europe is on dwarfing rootstocks, because high density allows greater production and sustained high yield of good quality (Falahi *et al*., 6 and Wartheim *et al*., 18).

Micropropagation technique provides an effective and efficient mean for manifold multiplication of quality planting material which has been extensively exploited for many horticultural crops. Among the various applied techniques of biotechnology, tissue culture has opened new avenues for rapid and mass propagation of superior genotypes all the year round independent of seasonal limitations. Plant tissue culture has become a valuable technique for producing uniform, true to the type, disease free plants with distinctive characteristics. Besides this, *in vitro* technologies offer tremendous opportunities for improvement in fruit crops through genetic engineering resulting in the production of superior genotypes to cope with environmental stresses. The potential uses of tissue culture include the micropropagation of rootstock or scion cultivars in short supply resulting from breeding or virus elimination programmes, genetic improvement through *in vitro* mutant selection and the regeneration of plants from protoplasts or callus via somatic embryogenesis or androgenesis (Ramming, 13; Zimmerman, 21) and finally to provide material for physiological and biochemical studies and insight into the processes examined in such studies. However, reports regarding the mass propagation of cherry are scanty and the literature are sporadic especially pertaining to sour cherry. The present investigation is an attempt for multiplication of sour cherry through micropropagation and to overcome the problem associated with heterozygosity. Sour cherry being limited

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in distribution and less preferred for table purposes than sweet cherry, very little work on tissue culture technique of sour cherry has been carried out in India and abroad. The present investigation is thus an attempt in the direction and this work will definitely help in clonal propagation and mass production of sour cherry plantlets irrespective of time and season on commercial level.

MATERIALS AND METHODS

The mature and bearing sour cherry trees growing at Experimental Farm of Division of Pomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J&K) were used as stock plants in the present study. The investigation involved different types of explants viz., forced and unforced. For forcing, dormant cuttings measuring 15-20 cm in length and 3-5 cm in diameter of terminal and sub terminal portion of cuttings were collected from source trees during the month of December. Collected dormant cuttings were washed in running water, pretreated with 0.2% Capton (Captan 50 WP) stored in cold store at 4±3ÚC in polythene bags until used. After meeting the requisite chilling units of atleast 45 days, cutting were incubated in growth chamber at 23±2ÚC for under 16/8 h photoperiod with 40±3 µmol m^{-2} s^{-1} light intensity. In course of the incubation, the cuttings began to sprout and these sprouted buds served as explant source and become ready for harvesting in 20±3 days. For unforced, fresh and actively growing shoot tips measuring 2-3 cm in length were procured from mature trees of sour cherry growing in the open field. The unforced explants were surface sterilized with 0.1 per cent $HgCl_2$ for 10 minutes. After surface sterilization, the explants were thoroughly washed with double distilled sterile water (3-4 times) under the aseptic conditions of laminar flow chamber. The excised shoots from both forced and unforced explants were subsequently cultured on various medias for studying different establishment parameters.

The explants were cultured in Woody Plant (WP) medium (Lloyd and McCown, 1981) in three different strength viz., full strength medium (W_1), half-strength medium (W_2) and three fourth ($^3/_4{}^{th}$) medium strength (W_3). The growth regulators Benzylaminopurine (BAP) supplemented with WP medium at concentrations of 0.25 mg l^{-1}, 0.5mg l^{-1}, 0.75mg l^{-1} and Indole–3-butyric acid (IBA) at concentrations of 0.00 mg l^{-1} and 0.01 mg l^{-1}, respectively. Each treatment combination was assigned 20 culture tubes with one explant per tube and each replicated thrice. After 4 ± 1 weeks of inoculation observations were recorded on survival percentage, per cent aseptic cultures and days taken to growth initiation for establishment.

For shoot multiplication (proliferation) growth regulators Benzylaminopurine (BAP) and Indole–3-butyric acid (IBA) each with four different concentration levels viz., 0, 1, 2 and 3 mgl^{-1} and 0.00, 0.05, 0.10, 0.15 mgl^{-1} were used respectively. After 5±1 weeks in the proliferation medium observations like days taken for initiation of proliferation, number of shoots per explant, average length of shoots and percentage of shoots with desired length for rooting were recorded. Within 4 ± 1 weeks of shoot proliferation, the cultures was transferred to the shoot elongation medium supplemented with Benzylaminopurine at concentration levels of 0.1, 0.2 and 0.3 mg/l. Maximum shoot length (mm) was recorded within 4±1 weeks of subculture and was finally cultured with different concentrations of IBA at 1, 1.5, 2.0 and 2.5 mg l^{-1}, respectively for rooting. In this each treatment comprised of 20 explants with one explant per test tube. Observations on number of rooted explants i.e., rooting percentage and number of roots/rooted explant were recorded 4 ± 1 weeks after inoculation in rooting media. However, average root length (mm) was recorded 10 days after transfer of root initiated microcuttings to hormone free basal media. Data obtained from the various treatments were analysis in Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

The effect of septic culture and survival per cent of explants was found to be statistically highly significant. The unforced explants registered the lowest aseptic culture percentage (36.29%) and survival percentage (27.01%) as compared with the forced explants registering highest aseptic culture percentage 65.99 and survival percentage 38.47 (Fig. 1). Maximum aseptic culture of 50% was observed when explants were culture on WP with full strength medium followed by 37.50 on WP with half strength medium (Table 1). The lowest percentage of 37.02 per cent aseptic cultures was recorded on WP medium with ¾ strength. Highest mean percentage of explant survival to the tune of 26.47 per cent was observed when explants were cultured on WP half strength medium followed by 25.02 per cent on WP medium with ¾th strength (Table 1) and the lowest survival percentage of 23.72 per cent was recorded on full strength WP medium. Various levels of WP medium were found to be statistically at par for this trait. These findings are in close conformity with Dalal *et al*. (5) who observed that explants derived from forced sources had highest *in vitro* survival and growth of explants in case of grape. Similar findings were observed by Dalal *et al*. (4) in apple who reported that protected incubation of cuttings under controlled conditions ensures sanitary situation that resulted in improved survival and culture asepsis. The

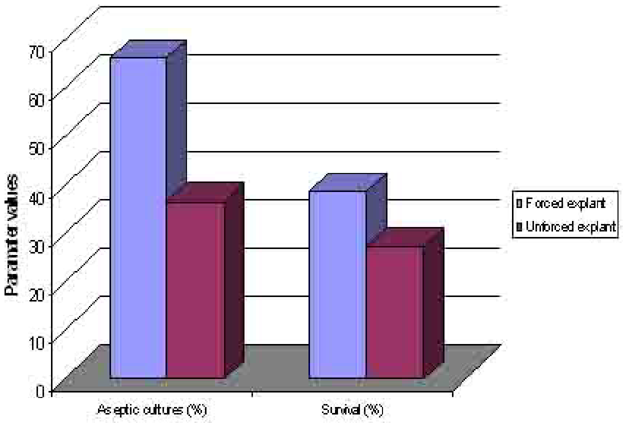

Fig. 1. Forced and unforced explants for aseptic culture (%) and survival (%).

Table 1. Effect of media on aseptic culture and survival of sour cherry explants.

Media	Response	
	Aseptic culture (%)	Survival (%)
WP	50.00	23.72
½ WP	37.50	26.47
¾ WP	37.02	25.02
LSD at 5%	3.28	3.85

results are also in agreement with the findings of Hammerschlag (7) who observed reduction in contamination from 100 to 3.3 per cent by culturing surface sterilized forced lateral shoots of peach than similarly sterilized actively growing shoots.

The main effects of growth regulator regime on explant survival percentage and days taken to growth initiation for establishment were found to be statistically significant. Highest mean survival percentage of 69.09 per cent was recorded when BAP was used at 0.50 mg l^{-1} plus 0.01 mg l^{-1} IBA, followed by 45.91 per cent with hormonal regime of 0.25 mg l^{-1} BAP plus 0.01 mg l^{-1} IBA (Table 2). The lowest survival percentage of forced explants to the tune of 24.84 per cent was recorded when BAP was used at 0.25 mg l^{-1} strength whereas, survival percentage recorded at 0.25 mg l^{-1} BAP and 0.75 mg l^{-1} BAP were statistically at par. The minimum number of days taken to growth initiation for establishment (20.53) were recorded with growth regulator regime of 0.50 mg l^{-1} BAP + 0.01 mg l^{-1} IBA followed by 24.29 days when BAP used at 0.50 mg l^{-1}. BAP @ 0.25 mg l^{-1} recorded the maximum number of days (29.22) to growth initiation for establishment. Half-strength levels proved to be superior than their

Table 2. Effect of growth regulator regime and media strength on establishment of explants.

Growth regulators (mg l^{-1})	Response	
	Survival (%)	Days taken to growth initiation for establishment
BAP (0.25)	24.84	29.22
BAP (0.50)	36.65	24.28
BAP (0.75)	25.38	27.53
BAP (0.25) + IBA (0.01)	45.91	28.25
BAP (0.50) + IBA (0.01)	69.09	20.53
BAP (0.75) + IBA (BA (0.01)	29.70	25.16
Media		
WP	27.81	25.77
½ WP	28.61	28.79
¾ WP	28.91	30.43
LSD at 5%	4.54	3.17

corresponding full strength levels which is thought to be due to reduced salt strength of these media as high salt strength does not suit the initial establishment of explants and results in decreased survival percentage. The survival percentages recorded on three strength levels of WP medium were found to be statistically at par. Full strength WP medium recorded the lower survival percentage of 27.81 per cent. The minimum number of days (21.87) taken to growth initiation for establishment were recorded on full strength WP medium followed by 28.79 days on half strength WP medium and maximum number of days (30.43) were recorded with ¾ WP medium (Table 2). Requirement of growth regulators varies considerably with the nature of the tissue because of endogenous hormonal levels (Bhojwani, 3). Days to growth initiation for establishment were found to follow a decreasing trend (quicker establishment) by increase in BAP concentration but increasing concentration of BAP from 0.50 mg l^{-1} resulted in significant increase in number of days taken to growth initiation for establishment. The superiority of reduced salt strength media in terms of survival may be attributed to the fact that high salt strength does not suit the initial establishment of explants (Amin, 1).

The results of different parameters Viz. days to initiation of proliferation, number of shoots per explant, average length of shoots and percentage of shoots with desired length for rooting were recorded after 5 ± 1 week of transfer to the proliferation medium (Table 3). The minimum number of days taken for initiation of proliferation (25.01) were recorded with 2 mg l^{-1} BAP followed by 27.86 days with 3 mg l^{-1}. The maximum

Table 3. Effect of BAP and IBA concentrations levels on cultural proliferation of sour cherry.

Parameters	Days taken for initiation of proliferation	No. of shoots per explant	Average length of shoots (mm)	Percentage of shoots with desired length for rooting
BAP concentration levels (mg l^{-1})				
0.00	37.53	3.99	13.06	20.46
1.00	29.15	9.11	16.31	50.51
2.00	25.01	10.99	12.73	65.45
3.00	27.86	9.05	10.52	53.01
IBA concentration levels (mg l^{-1})				
0.00	31.96	7.05	12.62	36.74
0.05	29.13	8.57	14.93	46.71
0.10	28.73	10.09	13.11	57.07
0.15	29.73	7.42	11.96	48.91
LSD at 5%	2.35	0.79	0.32	5.46

number of days (37.53) were recorded when no IBA was used. The highest number of shoots per explant (10.99) were recorded at 2 mg l^{-1} BAP followed by 9.11 recorded at 1 mg l^{-1} BAP. The lowest number of shoots per explant (3.99) were recorded when no BAP was used. Number of shoots per explant recorded at 1 mg l^{-1} BAP and 2 mg l^{-1} were statistically at par. The highest average length of shoots (mm) to the tune of 16.31 mm was recorded at 1 mg l^{-1} followed by 13.06 mm at 0.00 mg l^{-1} BAP. The lowest average length (10.52 mm) was recorded at 3 mg l^{-1} BAP. Percentage of shoots with desired length for rooting were recorded highest (65.45) at 2 mg l^{-1} BAP followed by (53.01%) at 3 mg l^{-1} BAP. The lowest percentage of shoots with desired length to the tune of 20.46 per cent were recorded when no BAP was used. Percentage of shoots with desired length recorded at 1 mg l^{-1} BAP and 3 mg l^{-1} BAP were found at par. BAP at 2 mg l^{-1} was observed to be an ideal concentration for proliferation and multiplication as it recorded the lowest number of days for initiation of proliferation (25.01), highest number of shoots per explant (10.99), and highest percentage of shoots (65.45%) with desired length for rooting. The highest average length of shoots (16.31 mm) was, however, recorded at 1 mg l^{-1} BAP. Increase in concentration of BAP to 3 mg l^{-1} significantly reduced proliferation and multiplication showing the inhibitory role of excessive cytokinin for proliferation and multiplication. Higher cytokinins (BAP) results in the production of stunted shoots (Sharma *et al*., 14). Tornero and Burgos (17) obtained better proliferation on WP medium than other media while studying media requirements for micropropagation of apricot cultivars which is thought to be due to lower ammonium content of WP medium (Snir, 15; Murai *et al*., 11) and common for *Prunus.*

The effects of IBA on days to initiation of proliferation, number of shoots per explant, average length of shoots and percentage of shoots with desired length for rooting were statistically significant. IBA at 0.10 mg l^{-1} took the minimum number of days for initiation of proliferation (28.73) followed by 29.13 days by 0.05 mg l^{-1} IBA. The maximum numbers of days (31.96) were taken for this trait when no IBA was used (Table 3). Days taken for initiation of proliferation at 0.05, 0.10, and 0.15 mg l^{-1} of IBA were statistically at par. The highest numbers of shoots per explant (10.09) were recorded with 0.10 mg l^{-1} IBA whereas no IBA (control) resulted in the lowest number of shoots (7.05) per explant. The highest length of shoots (14.93 mm) was recorded with 0.5 mg l^{-1} IBA whereas IBA at 0.15 mg l^{-1} resulted in the lowest average length (11.96 mm) of shoots. The highest percentage of shoots with desired length (57.07%) was recorded at 0.10 mg l^{-1} IBA followed by 48.91 per cent recorded at 0.15 mg l^{-1} IBA. The lowest percentage (36.74%) of shoots with desired length was recorded with exclusion of IBA. IBA at 0.05 mg l^{-1} and 0.15 mg l^{-1} recorded the percentage of shoots with desired length that were statistically at par. The effects of IBA on various proliferation parameters were also found significant. IBA at 0.10 mg l^{-1} was found to be an optimum concentration for multiplication and proliferation of shoots as it recorded faster proliferation and took 28.73 days for initiation of proliferation recorded highest number of shoots (10.09) per explant, and highest percentage (57.07) of shoots with desired length for rooting. However, the maximum average length of shoots (14.93) was recorded at 0.05 mg l^{-1}. Use of excessive auxin (0.15 mg l^{-1}) significantly reduced number of shoots per explants, average length of shoots and percentage of shoots with desired length for rooting besides delaying initiation of proliferation. This may be due to inhibitory action of excessive auxin as it causes apical dominance and prevents multiplication of lateral shoots. The results are in conformity with the Hammerschlag *et al*. (8) who observed highest shoot proliferation in peach with 2 mg l^{-1} BA and 0.01 mg l^{-1} IBA. The effect of BAP on elongation of microshoots was found significant. BAP at 0.1 mg l^{-1} recorded the highest elongation of 41.49 mm followed by 34.24 mm at 0.2 mg l^{-1} BAP. The lowest elongation of microshoots (28.31 mm) was recorded (Fig. 2) at 0.3 mg l^{-1} BAP. The effects of media on rooting percentage, average number of roots per explant and root length were found to be statistically significant (Table 4). The results on shoot elongation are in agreement with that of Norton and Norton (12) who also reported maximum elongation at 0.10 mg l^{-1} BAP in *Prunus* species.

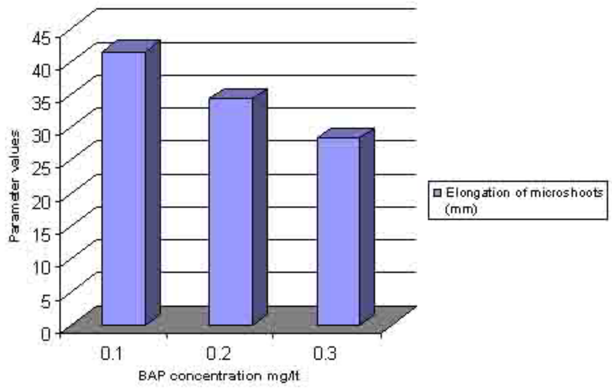

Fig. 2. Effect of BAP concentration on elongation of microshoots**.**

Table 4. Effect of IBA concentrations on rooting characteristics of explants.

IBA concentration ($mg\ l^{-1}$)	Rooting percentage	Average No. of roots per explant	Root length (mm)
1.00	37.45	2.38	32.46
1.50	51.73	4.57	41.25
2.00	69.52	5.29	35.78
2.50	52.48	3.62	27.13
LSD at 5%	3.52	1.39	3.17

WP medium recorded the highest rooting percentage of 52.79 per cent, highest average number of roots per explant of 3.96 and maximum root length of 34.15mm. The effects of IBA and media on rooting characteristics were found statistically significant. IBA at 2.00 mg l^{-1} supplied to WP medium recorded, the highest rooting percentage (69.52%) followed by 52.48% with 2.50 mg l^{-1} IBA. Medium supplemented with 1 mg l^{-1} IBA recorded the lowest rooting percentage (37.45%). The highest average number of roots (5.29) was recorded at 2.00 mg l^{-1} IBA and least (2.38) were recorded at 1.00 mg l^{-1} IBA. The highest root length (41.25 mm) was recorded with 1.50 mg l^{-1} IBA followed by 35.78 mm recorded on 2.00 mg l^{-1} IBA. The lowest root length (27.13 mm) was recorded with 2.50 mg l^{-1} IBA. Increase in concentration of IBA from 1 mg l^{-1} showed an increasing trend but increase in IBA concentration to 2.5 mg l^{-1} decreased rooting percentage and average number of roots per explant significantly, which was due to inhibitory role of excessive auxin for rooting. The highest root length (41.25 mm) was observed at 1.5 mg l^{-1} IBA which reduced drastically at elevated concentration levels of IBA which was due to inhibitory effect of IBA on root elongation (Went and Thimann, 19; Thimann, 16). The present findings are in accordance with the data of Harada and Murai (9) who reported lesser rooting percentage and average number of roots per explant on WP medium with the use of IBA.

The procedure describes strategy for large scale multiplication of dwarfing rootstocks which provides an opportunity to keep cherry production of Jammu and Kashmir State at par with the international scenario. The strategy encompasses, use of forced explants, half strength of medium along with different concentration levels of growth regulators for the development of successful protocol for micro propagation of sour cherry rootstock.

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