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Proliferation and rooting efficiency studies in sour cherry (*Prunus cerasus*) using *in vitro* techniques

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ABSTRACT

Murashige and Skoog's medium (MS) and Woody plant (WP) medium supplemented with IBA and BAP at different concentrations were tested for culture proliferation in sour cherry. MS medium supplemented with 1 mg L^{-1} BAP + 0.1 mg L^{-1} IBA, and, Woody plant medium fortified with 2 mg L^{-1} BAP + 0.1 mg L^{-1} IBA recorded maximum proliferation efficiency. MS medium supplemented with BAP @ 0.1 mg L^{-1} and devoid of auxin recorded optimum elongation of micro-shoots. MS medium fortified with 2 mg L^{-1} IBA gave highest rooting percentage and average number of roots per explant. BAP and IBA in MS medium were ideal for proliferation indices like number of shoots per explant, average length of shoots (mm) and percentage of shoots with desired length for rooting, and, can be used in a multiplication programme.

Key words: Cherry, MS medium, Woody Plant Medium (WPM), indole–3-butyric acid, benzylaminopurine

INTRODUCTION

Cherry is known to be native of South-East Europe and Asia Minor and, as far east as Northern India and China. Diverse forms of sour cherry exist in Eastern Europe and western region of Russia and the Himalayas. In India, cultivation of cherry is confined to Jammu and Kashmir, Himachal Pradesh and Uttarakhand. The state of Jammu and Kashmir is a major cherry-growing state, with productivity of 3.2 $MTha^{-1}$. (Anonymous, 2006) which is for less than the highest global productivity. Although there are sizeable, potentially well-suited areas for cherry cultivation in India, this fruit has not made much progress due to non-availability of quality planting material and desirable rootstocks. Traditionally, 'Paja' (*Prunus cerasoides*) is the only rootstock used in India. However, delayed incompatibility has rendered this rootstock fairly inferior in commercial production. Clones of sour cherry (*Prunus cerasus),* though, have been selected, propagated and used commercially as rootstocks. Generally, these rootstocks are cold-hardy and perform better in wet and heavy soils than do *P. avium* and *P. mahaleb*. The rootstocks can be propagated by mound-layering, but, the rate of multiplication is low. For augmenting supplies to meet an upsurge in demand, rapid and mass multiplication of cherry rootstock is essential.

Micropropagation represents the greatest use of tissue culture, especially for multiplying rootstocks. Many temperate fruit and nut species orchards are planted with trees that are propagated by budding or grafting the desired scion cultivar onto a suitable rootstock. With experimental systems moving towards ultra-high density plantings, budded or grafted trees may be too expensive, and the use of these planting systems may have to depend on alternative means of propagation. Potential advantages of tissue culture include micro-propagation of the rootstock/scion in a short period of time, yielding true-to-type propagules and aiding rapid mass multiplication. However, reports of mass multiplication in cherry are scanty and the literature sporadic, especially so in sour cherry. The present study addresses mass-multiplication of sour cherry with an objective of studying proliferation efficiency and rooting percentage in cultures.

MATERIAL AND METHODS

During the year 2006-07, shoot tips measuring 2-3 cm in length, procured from mature trees of sour cherry growing in the main Campus of Sher-e-Kashmir University Agricultural Sciences and Technology of Kashmrr, Shalimar (J&K), were used as the source of explants in the present investigation. New and actively-growing shoots were selected for isolation of explants. The investigation involved

two different media, i.e., Murashige and Skoog medium (MS) and Woody plant medium (WP), along with two growth regulators, i.e., an auxin-Indole–3-butyric acid (IBA) and a cytokinin-Benzylaminopurine (BAP). For shoot proliferation, IBA and BAP each at four different concentrations viz., 0.00, 0.05, 0.10, 0.15 mgL^{-1} and 0, 1, 2 and 3 mg/l, were used respectively. Observations like days taken to initiation of proliferation, number of shoots per explant, average length of shoots and percentage of shoots with desired length for rooting, were recorded at 5 ± 1 week in the proliferation medium. Cultures were transferred to shoot elongation medium supplemented with Benzylaminopurine at concentration levels of 0.1, 0.2 and 0.3 mgL^{-1} within 4 ± 1 weeks of shoot proliferation. Maximum shoot length (mm) was recorded within 4 ± 1 weeks of sub-culture. These shoots were finally cultured on different concentrations of IBA, viz., 1, 1.5, 2.0 and 2.5 mgL^{-1} for rooting. Each treatment comprised of 20 explants with one explant per test tube. Rooting percentage and number of roots/rooted explants were recorded 4 ± 1 week from inoculation in the rooting media, whereas, average root length (mm) was recorded 10 days after transfer of micro-cuttings (showing root initiation) to hormone-free basal media. Data obtained from various treatments were analyzed in Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

Effects of BAP and IBA on various proliferation parameters like days to initiation of proliferation, number of shoots per explant, average length of shoots (mm) and percentage of shoots with desired length for rooting, in MS medium, were found to be statistically significant (Table 1). Ideal hormonal combination was found to be 1 mg L^{-1} BAP + 0.10 mg L^{-1} IBA, as it recorded the lowest number of days (21.23) taken for initiation of proliferation and highest average number of shoots (14.62) per explant. The highest number of days taken for initiation of proliferation (35.25) recorded in a medium with no growth regulator (BAP or IBA). Days taken for initiation of proliferation with 1 mg L^{-1} BAP + 0.05 IBA and 1 mg L^{-1} BAP + 0.10 mg L^{-1} IBA, were at par. The present findings are in close agreement with findings of Banno *et al* (1989) who recommended supplementation of 1 mg L^{-1} BAP with 0.10-0.50 mg L^{-1} IBA for obtaining maximum shoot proliferation in Japanese pear explants.

Highest (21.24 mm) average length of shoots was recorded with 1 mg L^{-1} BAP + 0.05 mg L^{-1} IBA, followed by (19.50 mm) that with 1 mg L^{-1} BAP + 0.10 mg L^{-1} IBA. The lowest average length of shoots (10.88 mm) was observed at 1.50 mg L^{-1} BAP + 0.15 mg L^{-1} IBA. The highest percentage (82.35%) of shoots with desired length for rooting was recorded in 1 mg L^{-1} BAP + 0.10 mg L^{-1} IBA, followed by 71.58% in 1 mg L^{-1} BAP + 0.15 mg L^{-1} IBA. The lowest percentage of shoots with desired length (30.36%) was recorded in medium with no growth regulators (BAP or IBA). Similar results were reported by Kumar and Bist (2002) who recorded maximum average length of

Table 1. Effect of BAP and IBA concentration on proliferation of sour cherry on Murashige & Skoog (MS) medium

BAP concentration (mg l^{-1})	IBA concentration (mg l^{-1})	Days taken to initiation of proliferation	Number of shoots per explant	Average length of shoot (mm)	Shoots with optimum length for rooting (%)
0.00	0.00	35.25	2.18	12.34	30.36
0.00	0.05	32.29	4.13	14.36	42.86
0.00	0.10	33.62	6.46	17.22	46.91
0.00	0.15	34.52	4.52	13.26	37.54
0.50	0.00	29.14	7.81	14.62	39.75
0.50	0.05	27.32	10.64	13.86	58.58
0.50	0.10	25.16	12.38	15.26	69.73
0.50	0.15	26.20	11.35	15.13	53.16
1.00	0.00	24.51	9.27	14.81	48.21
1.00	0.05	22.17	11.96	21.24	64.18
1.00	0.10	21.23	14.62	19.50	82.35
1.00	0.15	25.31	12.06	16.25	71.58
1.50	0.00	26.21	8.19	11.38	41.43
1.50	0.05	24.42	9.83	16.22	59.31
1.50	0.10	23.16	10.29	12.16	76.41
1.50	0.15	31.37	8.23	10.88	57.29
CD *(P=0.05)*		1.05	1.19	0.46	2.12
SEm (±)		0.52	0.58	0.22	1.04

Table 2. Effect of BAP and IBA concentration on proliferation of sour cherry on Woody Plant Medium (WPM)

BAP concentration (mg l^{-1})	IBA concentration (mg l^{-1})	Days taken to initiation of proliferation	Number of shoots per explant	Average length of shoot (mm)	Shoots with optimum length for rooting (%)
0.00	0.00	35.25	2.18	12.34	30.36
0.00	0.00	39.32	2.64	13.61	15.62
0.00	0.05	37.17	3.09	14.24	19.35
0.00	0.10	36.72	5.73	12.26	28.12
0.00	0.15	36.93	4.51	12.13	18.75
1.00	0.00	33.94	7.01	16.23	36.11
1.00	0.05	29.23	9.76	19.55	41.93
1.00	0.10	26.25	11.19	14.24	65.38
1.00	0.15	27.19	8.47	15.21	58.62
2.00	0.00	26.07	10.42	11.19	53.85
2.00	0.05	24.67	11.64	13.64	67.24
2.00	0.10	24.13	12.73	13.92	73.81
2.00	0.15	25.17	9.17	12.18	66.91
3.00	0.00	28.52	8.13	9.46	41.38
3.00	0.05	25.45	9.81	12.28	58.33
3.00	0.10	27.82	10.71	12.02	60.97
3.00	0.15	29.64	7.54	8.32	51.36
CD *(P=0.05)*		1.17	0.40	0.65	2.73
SEm (±)		0.57	0.19	0.32	1.34

shoots at a hormonal regime of 1 mg l^{-1} BAP + 0.05 mg l^{-1} IBA.

Effect of BAP and IBA in WP medium on days taken for initiation of proliferation, number of shoots per explant, average length of shoots (mm) and percentage of shoots with desired length for rooting, was found to be significant (Table 2). BAP at 2 mg L^{-1} + 0.1 mg L^{-1} IBA was found ideal for proliferation and multiplication as it recorded lesser number of days for initiation of proliferation (24.13 days), highest number of shoots (12.73) per explant, and, highest percentage of shoots with desired length for rooting (73.81%). The highest average length (mm) of shoots was, however, recorded with 1 mg L^{-1} BAP + 0.05 mg L^{-1} IBA. The highest number of days (39.32) taken for initiation of proliferation, lowest number of shoots (2.64) per explant, and, lowest percentage of shoots (15.62%) with desired length for rooting was achieved when no growth regulator (BAP or IBA) was used. The lowest average length (mm) of shoots (8.32 mm) was recorded with 3 mg L^{-1} BAP + 0.15 mg L^{-1} IBA. These findings are in close conformity with results of a number of workers who used either BAP or a combination of BAP and IBA for studying proliferation and multiplication. Dwivedi and Bist (1999) found that BAP and IBA interacted significantly for increase in the number of shoots per culture. Hammerschlag *et al* (1987) observed highest shoot proliferation in peach with 2 mg L^{-1} BA + 0.01 mg L^{-1} IBA. Requirement for higher concentration of IBA (0.10 mg L^{-1}) in the present study may be due to differential response of genotypes of *Prunus*. The highest average length (mm) of shoots was reported by Kumar and Bist (2002) at 1.0 mg L^{-1} BAP + 0.05 mg L^{-1} IBA, which further confirms these results and who obtained better proliferation on WP medium than on other media in studies on media requirement for micropropagation of apricot cultivars. This response was thought to be due to lower ammonium content in WP medium (Snir, 1984) and Murai *et al* (1997), seen commonly in *Prunus.* Similarly, better proliferation on WP medium was achieved by Dwivedi and Bist (1999) in studies on *in vitro* propagation of low-chill pear cv. Gola. The present results are contradictory to that of a number of workers who reported that only BAP resulted in shoot proliferation. Shibli *et al* (1996) reported highest rate of multiplication on MS medium with BA (1 mg L^{-1}) in almond. Similar results were also reported by Sharma *et al* (1992) in cherry rootstock Colt.

Effect of media and BAP on elongation of shoots was also found to be statistically significant (Table 3). MS medium supplemented with BAP 0.1 mg L^{-1} recorded maximum elongation (44.83 mm) of shoots, while, WP medium fortified with BAP 0.3 mg L^{-1} recorded minimum elongation (27.18 mm). MS medium and WP medium, each supplemented with 0.3 mg L^{-1} BAP, recorded elongation that was statistically on par. Elongation recorded on MS + 0.2 mg L^{-1} BAP, and, WP + 0.1 mg L^{-1} BAP was also statistically on par. Lower BAP levels generally result in reduced axillary shoot proliferation and cause elongation of

Table 3. Effect of culture medium and BAP concentration on elongation of micro-shoots in sour cherry

Medium	BAP concentration ($mg\ l^{-1}$)	Length of micro-shoots (mm)
MS	0.1	44.83
MS	0.2	36.27
MS	0.3	29.44
WP	0.1	38.16
WP	0.2	32.21
WP	0.3	27.18
CD *(P=0.05)*		2.38
SEm (±)		1.09

MS = Murashige and Skoog medium; WP = Woody plant medium

Table 4. Effect of IBA concentration and culture medium on rooting of explants in sour cherry

IBA concentration ($mg\ l^{-1}$)	Medium	Rooting percentage	Average no. of roots per explant	Root length (mm)
1.00	MS	41.66	4.43	37.46
1.00	WP	37.45	2.38	32.46
1.50	MS	61.11	6.09	68.73
1.50	WP	51.73	4.57	41.25
2.00	MS	76.47	7.75	53.92
2.00	WP	69.52	5.29	35.78
2.50	MS	59.24	5.21	32.58
2.50	WP	52.48	3.62	27.13
CD *(P=0.05)*	3.52	1.39	3.17	
SEm (±)	1.66	0.65	1.49	

MS = Murashige and Skoog medium; WP = Woody plant medium

shoots. These findings are in accordance with results of Tabachnik and Kester (1977) who reported maximum shoot elongation at 0.1 mg L^{-1} BAP during *in vitro* shoot culture of almond. Results on shoot elongation in the present study too are in agreement with those of Norton and Norton (1986) who reported maximum shoot elongation at 0.1 mg L^{-1} BAP in *Prunus* species.

Effects of IBA and media on rooting behaviour were highly significant (Table 4). Highest rooting percentage (76.47%) was observed on MS medium, followed by 69.52% on WP medium, both supplemented with 2 mg L^{-1} IBA. WP medium supplemented with 1 mg L^{-1} IBA recorded lowest rooting percentage (37.45). WP medium supplemented with 1.5 mg L^{-1} and 2.5 mg L^{-1} IBA recorded rooting percentages that were statistically on par. Supplementation of IBA @ 2 mg L^{-1} gave highest average number of roots per explant (7.75) on MS medium followed by (5.29) on WP medium. Average number of roots per explant of upto 2.38 was recorded on WP medium supplied with 1 mg L^{-1} IBA. Average number of roots per explant recorded with WP + 2 mg L^{-1} IBA, and, MS + 2.5 mg L^{-1} was statistically at par. Results pertaining to various rooting characteristics are in close agreement with that of a number of workers who studied *in vitro* rhizogenesis on various rooting media. Ranjit and Kester (1988) obtained 75% rooting in cherry rootstock Colt at 2 mg L^{-1} IBA in modified MS medium and recorded maximum number of roots per shoot at the same concentration of IBA, whereas, Poniedzialek *et al* (1986) reported higher rooting percentage and average number of roots per explant at 2 or 3 mg L^{-1} IBA in studies on *in vitro* rooting sour cherry cv. Schattenmorelle. These data are in accordance with findings of Ozzambak *et al* (1997), who reported maximum root length on 1.5 mg L^{-1} IAA during *in vitro* rhizogenesis of sour cherry cv. Heimanns Rubinweichsel. The results are also in contradiction with a number of workers who reported optimum rooting at lower levels of IBA than that observed in the present study. Sharma *et al* (1992) obtained 100% rooting in Colt on MS medium fortified with 1 mg L^{-1} IBA. Feliciano and Assis (1983) obtained 100% rooting in embryo-cultured peach seedlings of Cascata-163 on MS + 1 mg L^{-1} IBA and half-strength MS + 1 mg L^{-1} IBA. Incubation of microshoots on root induction media for 10-15 days in the dark are in close agreement with the work of Hammerschlag (1982) who reported that a week's treatment was necessary for 100% rooting in the plum rootstock 'Myrobalan' (*Prunus cerasifera*).

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