Indian J. Plant Physiol., Vol. 15, No. 2, (N.S.) pp. 144-149 (April-June, 2010)

REGENERATION OF SOUR CHERRY (*PRUNUS CERASEUS*) THROUGH *IN-VITRO* PROPAGATION

H.A. DAR, S.R. SINGH*, K.K. SRIVASTAVA, M.K. SHARMA AND A.S. SUNDOURI

Division of Pomology, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar-191 121, Srinagar, India

Received on 23 Nov., 2009, Revised on 05 June, 2010

SUMMARY

Dormant cutting procured from mature plants, were subjected to forcing treatment for *in-vitro* propagation. The culture media i.e., Murashige and Skoog medium (MS) with three strength levels viz., full, half and three fourth were used. Plant growth regulators viz. BAP (0.25 mg1^{-1}, 0.50 mg1^{-1}, 0.75 mg1^{-1}) and IBA (0.00 mg1^{-1} and 0.01 mg1^{-1}) were supplemented with MS media. The design of experiment was CRD with three replications. MS half strength basal medium supplemented with 0.50 mg l^{-1} BAP plus 0.01 mg l^{-1} IBA resulted in the highest survival percentage of *in vitro* cultures. MS medium supplemented with 1 mg l^{-1} BAP + 0.10 mg l^{-1} IBA fortified with 2 mg l^{-1} BAP + 0.10 mg l^{-1} IBA recorded the maximum proliferation efficiency. MS medium supplemented with BAP @ 0.1 mg l^{-1} and devoid of auxin recorded the optimum elongation of micro shoots. MS medium fortified with 2 mg l^{-1} IBA gave the highest rooting percentage and average number of roots per explant. The studies culminated in standardization of protocol for *in vitro* propagation of sour cherry rootstock.

Key words: **Sour cherry, explant, i*n vitro* propagation, hormones**

INTRODUCTION

Cherry is a commercial stone fruit of the hilly regions and praised for its attractive and delicious fruit, rich in proteins, sugars, carotenes, folic acid and minerals. Modern fruit trees have dual genetic system with a combination of rootstock and scion. The production of cherry is based on the quality of rootstock. Under Indian conditions, Paja (*Prunus cerasoides*) is used commercially root stock for cultivation which shows delayed incompatibility and inferior commercial production. Clones of sour cherry (*Prunus ceraseus*) manifested the better response for compatibility and fruit production coupled with character of cold hardy and wet heavy soil survival. Conventional stool bed layering techniques for propagation of sour cherry are season bound, irregular, slow and prone to various diseases and can not meet the ever increasing demand of rootstock. Micro-propagation provides an effective and alternative way for the production of quality propagules for cherry production.

In woody plants more emphasis is given on repeated proliferation by sustaining subcultures (Webster and Jones, 1989). This situation can lead to over dependence of sustained subcultures of proliferating material of plantlet production with either no or occasional resource to imitating culture originating from primary explant (Dalal *et al.* 1992). The success of micro propagation depends upon the type of explant and combination of different growing media. The aim of present study was to standardize the media composition for establishment of culture and proliferation efficiency and rooting percentage of established culture for sour cherry.

*Corresponding author, E-mail: drsingh_sr@rediffmail.com

MATERIALS AND METHODS

The mature trees of sour cherry established in the orchard at experimental field of pomology were selected for collection of cuttings. Dormant cuttings from selected plants were pruned and procured during winter season. The terminal and sub-terminal dormant cuttings measuring 18-22 cm in length and 12-15 mm in diameter were brought to the laboratory and washed thoroughly in running tap water. These were subjected to forcing treatment (Dalal *et al.* 2006). In forcing, dormant cuttings were kept in jar having sufficient distilled water to cover 5 cm of the basal portion of the cutting. The distilled water in jar was changed every 3 to 4 days. The cuttings were incubated in growth chamber at 24±1ºC under 16/8 h photoperiod with 40±3 µmol $m^{-2}s^{-1}$ light intensity. The forced dormant cuttings resulted in sprouting of buds which served as explant source and were harvested in 20±4 days. Sprouting buds from the forced cuttings were collected into wide mouthed flask containing distilled water and washed under running tap water for 10 minutes. Washed explants were surface sterilized with 0.1 per cent $HgCl_{2-}$ for 10 min, after surface sterilizing the explants were thoroughly washed with double distilled sterile water for 10 min under aseptic conditions.

For culture establishment, forced explants were cultured on three different strenghts of media viz., full strength Murashige and Skoog's (MS) basal medium (M_1), half-strength MS basal medium (M_2) and ¾th strength MS basal medium (M_3). The media were supplemented with different concentrations of growth regulators benzylaminopurine (BAP) (0.25 mgl^{-1}, 0.50 mgl^{-1} and 0.75 $mgl^{-1)}$ and indole–3-butyric acid (IBA; (0.00 mgl^{-1} (Control) and 0.01 mg l^{-1}). Observations on survival percentage, per cent aseptic cultures and days taken to growth initiation for establishment were made within 4±1 weeks of inoculation. The established explants were transferred to proliferation media within 4 ± 1 weeks of culture establishment. The MS medium supplemented with BAP at four concentrations (0.00 (control), 0.50 mgl^{-1}, 1.00 mgl^{-1} and 1.50 mgl^{-1}) and IBA at four concentrations (0.00 mg $^{-1}$ (control), 0.05 mg l^{-1}, 0.10 mgl^{-1}, 0.15 mgl^{-1}) were used for shoot multiplication. Observations on days taken for initiation to 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 in the proliferation medium. Treatment combinations were assigned 20 culture tubes (one culture/test tube) and was replicated thrice. The proliferated cultures were then transferred to the elongation medium within 4±1 weeks of proliferation. The elongation media consisted of MS with various levels of BAP (0.1 mgl^{-1}, 0.2 mgl^{-1} and 0.3 mgl^{-1}). The maximum shoot length (mm) was recorded within 4±1 weeks of subculture. Here, each treatment was assigned 10 culture tubes with one culture per test tube and each treatment replicated thrice. For rooting, shootlets (micro-shoots) were cultured on MS medium with different concentrations of IBA (1.0 mgl^{-1}, 1.5 mgl^{-1}, 2.0 mgl^{-1} and 2.5 mgl^{-1}). Each treatment comprised of 20 explants with one explant per test tube. Observations on rooting percentage and number of roots/ rooted explant were recorded 4±1 week 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. The data generated from the various parameters were put to statistical analysis in Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

The effect of different strength of media on aseptic culture percentage, days taken to growth initiation for establishment and survival percentage of explants was found to be highly significant (Table 1). Forced explants culture on full strength MS basal media registered the highest aseptic culture percentage of 68.74 followed by half-strength MS basal media (60.42 %) and ¾th strength MS basal media (53.18 %). Lowest survival percentage of forced explants of (46.94 %) was recorded with **¾th** strength MS basal media (M_3). The survival percentage recorded at full (M_1) and half-strength MS basal medium (M_2) were not significantly different. The minimum number of days taken to growth initiation for establishment (21.87) were recorded with half-strength MS basal medium (M_2) followed by full (M_1) and ¾th strength MS basal media (M_3), respectively. Half-strength media was superior than full strength media. High salt strength resulted in decreased survival percentage (Dalal *et al.* 1992, Dalal *et al.* 2000 and Hammerschlag 1982).

Table 1. Effect of different strength of MS media on aseptic culture, survival and days taken to growth initiation for establishment of sour cherry explants

Media strength	Aseptic culture (%)	Survival (%)	Days taken to growth initiation for establishment
Full MS	68.74	47.91	23.78
½ MS	60.42	50.38	21.87
¾ MS	53.18	46.94	24.33
LSD at 5%	2.98	2.13	3.17
S.E.	1.59	1.86	1.61

The effect of concentration levels of BAP and 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 was found to be statistically significant (Table 2). Minimum number of days (23.30) taken for initiation of proliferation were recorded with 1.00 mg l^{-1} BAP (Table 2) followed by 25.79 days when IBA was used at 0.10 mg l^{-1}. Maximum number of days (33.92) for initiation of proliferation was recorded with exclusion of BAP followed by 29.35 days with 0.15 mg l^{-1} of IBA. The days recorded for initiation of proliferation at 0.50 mgl^{-1} BAP, 1.50 mgl^{-1} BAP and 0.05 mgl^{-1} IBA were statistically at par. Highest mean number of shoots per explant (11.98) were recorded with 1.00 mgl^{-1} BAP followed by 10.94 shoots per explant when IBA was used at 0.10 mgl^{-1}. The maximum average length of shoots (17.95 mm) was recorded with 1.00 mg l^{-1} BAP followed by 16.42 mm recorded with 0.05 mgl^{-1} IBA. Lowest average length of shoots (12.65 mm) was recorded with 1.50 mgl^{-1} BAP. Percentage of shoots with desired length for rooting stood highest at 68.85 per cent when IBA was used at 0.10 mgl^{-1} followed by 66.85 per cent recorded at 1.00 mgl^{-1} BAP. The lowest percentage of shoots with desired length for rooting (39.42 %) was recorded in control. The explant survival increased with increase in BAP concentration but beyond 0.50 mgl^{-1} BAP concentration, explant survival was found to decrease. 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 mgl^{-1} resulted in significant increase in number of days taken to growth initiation for establishment. Increasing concentration of IBA beyond 0.10 mgl^{-1} resulted in production of stunted shoots, thus bringing percentage of shoots with desired length for rooting down to 54.89 per cent. Use of excessive auxin (0.15 mgl^{-1} IBA) significantly reduced number of shoots per explants, average length of shoot and percentage of shoot with desired length for rooting (Ranjit and Kester 1988), besides delaying initiation of proliferation. This is may to be due to inhibitory action of excessive auxin as it causes apical dominance and prevents multiplication of lateral shoots (Sharma *et al*. 1992).

Table 2. Effect of growth regulators on days taken for initiation of proliferation, number of shoots per explant, average length of shoots and percentage of shoots with desired length for rooting

Growth regulators (mg l^{-1})	Days taken for initiation for proliferation	Number of shoots per explant	Average length of shoots (mm)	Percentage of shoots with desired length for rooting
0.00 BAP	33.92	4.32	14.29	39.42
0.50 BAP	26.95	10.54	14.81	55.30
1.00 BAP	23.30	11.98	17.95	66.58
1.50 BAP	26.29	9.14	12.65	58.61
0.00 IBA	28.78	6.86	13.29	39.94
0.05 IBA	26.55	9.14	16.42	56.23
0.10 IBA	25.79	10.94	16.03	68.85
0.15 IBA	29.35	9.04	13.88	54.89
LSD at 5%	2.09	2.39	1.23	4.23
S.E.	1.03	1.17	0.60	2.08

The interaction 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 highly significant (Table 3). 1 mgl^{-1} BAP + 0.10 mgl^{-1} IBA treatment recorded the lowest number of days (21.23) to initiation of proliferation and highest

Table 3. Interaction effect of growth regulators on days taken for initiation of proliferation, number of shoots per explant, average length of shoots and percentage of shoots with desired length for rooting.

Growth regulators (mg l^{-1})	Days taken for initiation for proliferation	Number of shoots per explant	Average length of shoots (mm)	Percentage of shoots with desired length for rooting
0.00 IBA + 0.00 BAP	35.25	2.18	12.34	30.36
0.00 IBA + 0.00 BAP	32.29	4.13	14.36	42.86
0.10 IBA + 0.00 BAP	33.62	6.46	17.22	46.91
0.15 IBA + 0.00 BAP	34.52	4.52	13.26	37.54
0.00 IBA + 0.50 BAP	29.14	7.81	14.62	39.75
0.05 IBA + 0.50 BAP	27.32	10.64	13.86	58.58
0.10 IBA + 0.50 BAP	25.16	12.38	15.26	69.73
0.15 IBA + 0.50 BAP	26.20	11.35	15.13	53.16
0.00 IBA + 1.00 BAP	24.51	9.27	14.81	48.21
0.05 IBA + 1.00 BAP	22.17	11.96	21.24	64.18
0.10 IBA + 1.00 BAP	21.23	14.62	19.50	82.35
0.15 IBA + 1.00 BAP	25.31	12.06	16.25	71.58
0.00 IBA + 1.50 BAP	26.21	8.19	11.38	41.43
0.05 IBA + 1.50 BAP	24.42	9.83	16.22	59.31
0.10 IBA + 1.50 BAP	23.16	10.29	12.16	76.41
0.15 IBA + 1.50 BAP	31.37	8.23	10.88	57.29
LSD at 5%	1.05	1.19	0.46	-
S.E.	0.52	0.58	0.22	-

average number of shoots (14.62) per explant. The highest number of days to initiation of proliferation (35.25) were recorded when no growth regulator (BAP or IBA) was used. Days taken for proliferation recorded at 1 mgl^{-1} BAP + 0.05 IBA and 1 mgl^{-1} BAP + 0.10 mgl^{-1} IBA were found at par. The highest number (14.62) of shoots per explant to the tune of was recorded at 1 mgl^{-1} BAP plus 0.10 mg l^{-1} IBA. The present findings are in close. Similar with the findings of Banno *et al*. (1989) who recommended a combined supplementation of 1 mgl^{-1} BAP with 0.10-0.50 mgl^{-1} IBA for obtaining maximum shoot proliferation. When BAP and IBA were used together, the days required for establishment decreased significantly. The superiority of reduced salt strength media in terms of survival may be attributed to the fact that high salt strength did not suit the initial establishment of explants (Borkowska 1983, Ishida *et al.* 1989)

The main and interacted effect of media and various levels of BAP on shoot elongation of established explants were found to be statistically significant (Table 4). The highest elongation was recorded (36.83 mm) on MS media. Increasing concentration of BAP from 0.10 mgl^{-1} to 0.30 mgl^{-1} showed decreasing trend. The maximum shoot elongation of 41.49 mm was recorded at 0.10 mgl^{-1} BAP followed by 34.24 mm and 28.31 mm at 0.20 mgl^{-1} and 0.30 mgl^{-1} levels of BAP, respectively. The interaction effect of media and BAP also showed decreasing trend with increasing concerntration of BAP

Table 4. Main and interaction effect of MS media and BAP on elongation of microshoots

Treatments	Elongation of micro-shoots (mm)
MS media	36.85
0.1 mg l^{-1}BAP	41.49
0.2 mg l^{-1}BAP	34.24
0.3 mg l^{-1}BAP	28.31
MS × 0.1 mg l^{-1}BAP	44.83
MS × 0.2 mg l^{-1}BAP	36.27
MS × 0.3 mg l^{-1}BAP	29.44
LSD at 5%	2.87
S.E.	1.32

with maximum elongation of 44.83 mm when MS media supplemented with 0.10 mg l^{-1}BAP. This is because that lower BAP levels produce less axillary shoot proliferation (Tornero and Burgos 2000) and cause elongation of shoots (Norton and Norton 1986) who also reported maximum elongation at 0.10 mgl^{-1} BAP in *Prunus* species.

The effects of IBA on rooting percentage, average number of roots per explant and root length were found to be statistically significant. Highest rooting percentage (72.99 %) was recorded (Table 5) with 2 mgl^{-1} IBA. IBA at 1 mgl^{-1} resulted in lowest rooting percentage of 39.55. IBA at 1.50 mgl^{-1} and 2 mg l^{-1} resulted in average number of roots per explant which were statistically at par IBA at 1.5 mgl^{-1} (44.05 mm) resulted in the highest root length of 54.99 mm followed by at 2 mgl^{-1} IBA. The lowest root length (29.86 mm) was recorded with 2.50 mgl^{-1} IBA, due to inhibitory role of excessive auxin for rooting (Tornero and Burgos 2000). The highest root length (54.99 mm) was observed at 1.5 mgl^{-1} IBA which reduced drastically and significantly at elevated concentration levels of IBA which was due to inhibitory effect of IBA on root elongation.

Table 5. Effect of IBA concentrations on rooting characteristics of explants

IBA concentration (mg l^{-1})	Rooting percentage of roots per explant	Average number	Root length (mm)
1.00	39.55	3.40	34.96
1.50	56.42	5.33	54.99
2.00	72.99	6.52	44.85
2.50	55.86	4.42	29.86
LSD at 5%	4.98	1.97	4.48
S.E.	2.35	0.93	2.11

From the above study, it is concluded that the MS media with half strength proved to be better in terms of high survival percentage and lesser days taken for initial establishment. BAP in the media (1.00 mg l^{-1}) had maximum numbers of shoots per explant. Shoot length, desired length for rooting, media IBA with (2.00 mg l^{-1}) showed good results for various root characters of explants.

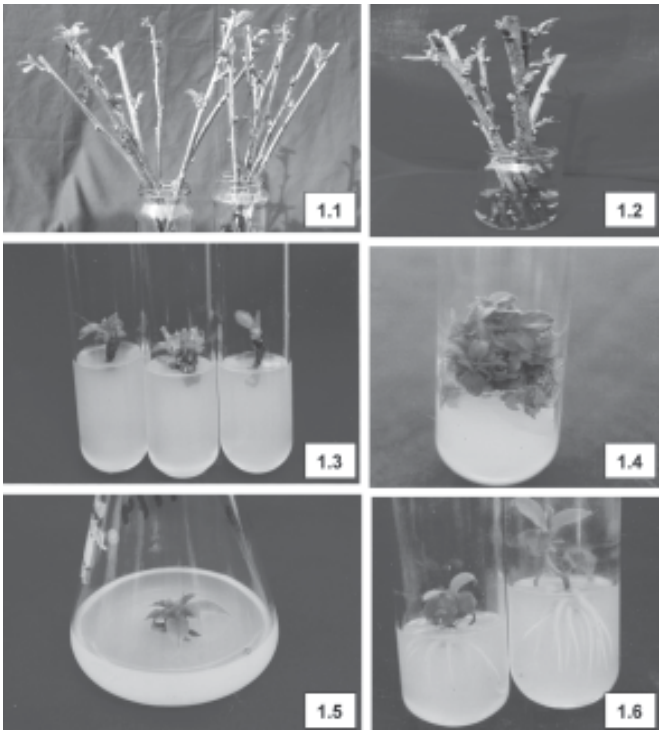

Fig. 1. Regeneration of Sour cherry rootstock: 1.1, Forced cuttings. 1.2, Unforced cuttings. 1.3, Aseptic cultures on M_1, M_2 and M_3. 1.4, Half MS medium supplemented with 0.50 mg l^{-1} BAP + 0.01 mg l^{-1} IBA. 1.5, MS medium (M_1) supplemented with 1 mg l^{-1} BAP + 0.10 mg l^{-1} IBA. 1.6, MS medium (M_1) supplemented with IBA @ 1 and 2 mg l^{-1}

REFERENCES

Banno, K., Yoshida, K., Hayashi, S. and Tanabe, K. (1989). *In vitro* propagation of Japanese pear cultivars. *J. Japanese Soc. Hort. Sci.* **58:** 37-42.

Borkowska, B. (1983). Micropropagation of sour cherry cv. Schattenmorelle, *Fruit Sci. Rep.* **10:** 59-63.

Dalal, M.A., Das, B., Sharma, A.K., Mir, M.A. and Sundouri, A.S. (2006). *In-vitro* cloning of apple employing forced shoot tip cultures of M9 rootstock. *Indian J. Biotech.* 5**:** 543-550.

Dalal, M.A., Kuchey, A.G. and Rather, M.A. (2000). Studies on stock plant treatment and culture initiation in control of browning and media exudation in *in vitro* cultures of apple (*Malus domestica* Borkh.). *SKUAST. J. Res.* **2:** 10-16.

Dalal, M.A., Sharma, B.B. and Rao, M.S. (1992). Studies on stock plant treatment and initiation culture mode in

control of oxidative browning in *in vitro* culture of grape vine. *Sci. Hort.* **51:** 35-41.

Hammerschlag, F. (1982). Factors affecting establishment and growth of peach shoots *in vitro. Hort. Sci.* **1:** 785-86.

Ishida M, Matsuyama H, Kitajima A and Sobajima Y. (1989). *In vitro* propagation of *Prunus japonica* Thumb, a dwarfing rootstock for peach tree. *J. Japanese Soc. Hort. Sci.* **58:** 49-54.

Norton, M.E. and Norton, C.R. (1986). Explant origin as determinant of *in vitro* shoot proliferation in *Prunus* and *Spiraea*. *J. Hort. Sci.* **61:** 43-48.

Ranjit, M. and Kester, D.E. (1988). Micropropagation of cherry rootstock, II. Investigation and enhanced rooting of 46-1 Mazzard by co-culture with "Colt". *J. Amer. Soc. Hort. Sci.* **113:** 150-154.

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:** 209-212.

Tornero, O.P. and Burgos, L. (2000). Different media requirements for micropropagation of apricot cultivars. *Plant Cell Tissue Org. Cult.* **63:** 133-141.

Webster, C.A. and Jones, O.P. (1989). Microporpagation of apple rootstock M_9: effect of subculture on apparent rejuvenation *in vitro. J. Hort. Sci.* **6:** 421-428.