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Standardization of *in-vitro* media for culture establishment in sour cherry (*Prunus cerasus*)*

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Clones of sour cherry (*Prunus cerasus* L.) are being less preferred for the table purpose than other commercial species of *Prunus* and used as rootstock for other species. Therefore, the seeds of *Prunus cerasus* are procured and commercially used as rootstock and interstock. The *Prunus cerasus* is more cold hardy tolerant and performs better in wet and heavy soils than species, like *avium* and *mahaleb*. The rootstock from *Prunus cerasus* is perpetuated by mound layering in field but rate of its multiplication is low. For augmenting the supply to meet the upsurge in demand, rapid and mass multiplication of cherry rootstock becomes essential. Therefore, attention must be given to all details of procedures requisite to successful *in-vitro* culture especially when mature trees of woody species are to be brought within its ambit. Therefore, an attempt was made to standardize media for survivability and cultural establishment of sour cherry through micropropagation. As very little work on tissue culture technique of sour cherry has been carried out in India and abroad, an attempt was made in this direction will definitely help in future for establishment of protocol for clonal propagation and mass production of sour cherry plantlets irrespective of time and season on commercial level.

The investigation involved 2 types of explants, namely forced and unforced. Forced explants were obtained from dormant cuttings of 15–20 cm length and 1.0–1.5 cm diameter from mature sour cherry trees, during the early months of dormany, ie November–December. These cuttings were treated with 0.2% captan and stored in the refrigerator wrapped with polyethylene packs. After removing these cuttings from the refrigerator, they were placed in glass jars containing distilled water up to the height of 5 cm. The number of dormant buds on these cuttings ranged from 5 to 8. The buds sprout within 15–20 days after transferring cuttings to the incubation chamber. Shoots emerging out of sprouted buds served as forced explant and unforced explant were obtained from fresh and actively growing shoots tips from mature trees of sour cherry (SSC–1) in March–April measuring 2–3 cm in length were procured for the isolation of explant served as unforced stock plant. Shoot tips were collected on-spot in conical flasks containing double distilled water. These were brought to the laboratory and thoroughly washed under gushing tap water. Two different media, i e Murashige and Skoog (1962) medium and Woody Plant (Lloyd and McCown 1981) medium, each with 3 strength levels, viz MS full strength, MS half strength, MS ¾ strength and WP full, WP half and WP ¾ were used. Six different types of basal media with full, half, and ¾ strength each of The MS and WP medium were supplemented with vitamins, amino acids and plant growth regulators (PGRs), like Benzylaminopurine (BAP) and Indole-butyric-acid (IBA). MS and WP medium were supplemented with growth regulators like BAP having 3 levels of concerntration (0.25, 0.50 and 0.75 mg/litre) and IBA having 2 levels of concentrations (0.00 and 0.01 mg/litre) respectively.

The excised shoots were subsequently cultured on establishment media for studying various establishment parameters. Both forced and unforced explants were surface sterilized with 0.1% $HgCl_2$ for 10 min under the aseptic conditions of laminar flow chamber. After surface sterilization, the explants were thoroughly washed with double distilled sterile water (3–4 times). The explants were then placed on to the medium maintaining their original polarity with the help of sterilized surgicals. Observations on survival percentage, per cent aseptic cultures and days taken to growth initiation for establishment were made within 4±1 weeks of inoculation. Each treatment combination was assigned 20 culture tubes with 1 explant/tube and each replicated thrice.

The individual effect of explant (Forced-S_1 and Unforced-S_2), media and growth regulator regime on survival

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Table 1 Individual effect of explant, media and growth regulators on aseptic culture and survival percentage of sour cherry explants

Treatment	Type	Survival (%)
Explants	Forced	38.47
	Unforced	27.01
	LSD (*P*=0.05)	6.67
	SEm±	3.23
Media	MS	37.12
	½ MS	44.29
	¾ MS	39.69
	WP	23.72
	½ WP	26.47
	¾ WP	25.02
	LSD (*P*=0.05)	3.85
	SEm±	1.86
Growth regulators (mg/litre)	BAP (0.25)	24.84
	BAP (0.50)	36.65
	BAP (0.75)	25.38
	BAP (0.25) + IBA (0.01)	45.91
	BAP (0.50) + IBA (0.01)	69.09
	BAP (0.75) + IBA (0.01)	29.70
	LSD (*P*=0.05)	4.54
	SEm±	2.28

MS, Murashige and Skoog Medium; BAP, benzylamino-purine; IBA, indole-butyric acid; WP, woody plant

percentage of explants was found highly significant (Table 1). The highest explant survival of 38.47% was observed with forced, whereas unforced explant resulted in minimum survival of 27.01. The highest mean survival of 44.29% was observed when explants were cultured on MS half strength media and lowest of 23.72% was recorded on full strength WP medium; however, highest mean survival of 69.09% was recorded when BAP was used at 0.50 mg/litre + 0.01 mg/litre IBA. The forced explants had superiority the unforced explants in terms of survival percentage which may be due to the fact that forced explants have a lesser problem of microbial contamination than those derived from unforced sources that have every apprehension of harbouring micro-organisms that discourage the culture asepsis during establishment of culture (Dalal *et al*. 2000).

Forced explants cultured on half-strength MS medium (interaction) recorded the highest survival percentage (50.38), followed by 47.91% recorded on full-strength MS medium cultured with forced explants (Table 2). The lowest survival percentage (19.64) was recorded for unforced explants cultured on full-strength WP medium. The survival percentage recorded on full-strength WP medium inoculated with forced explants was at par with full strength MS medium inoculated with unforced explants. Moreover, half-strength levels proved superior to their 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 protected incubation of cuttings under controlled conditions ensured sanitary situation that resulted in improved survival and culture asepsis. These findings are in close conformity with Dalal *et al*. (1992), who observed that explants derived from forced sources have a high *in-vitro* survival and growth of explants in grape.

The main effects of media on survival percentage and days taken to growth initiation for establishment were found to be statistically significant (Table 3). Half-strength MS medium recorded the highest survival of 50.38%, followed by 47.91% recorded on full strength MS medium. Full strength WP medium recorded the lower survival percentage of 27.81%. The minimum number of days (21.87) taken to growth initiation for establishment standing at 21.87 days were recorded with half-strength MS medium followed by 23.78 days recorded on full strength MS medium. The maximum numbers of days to growth initiation for establishment (30.43) were recorded with ¾ WP medium. Requirement of growth regulators varies considerably with the nature of the tissue because of endogenous hormonal

Table 2 Interaction effect of explant origin and media on aseptic culture and survival percentage of sour cherry explants

Explant × Media	Response	
	Aseptic culture (%)	Survival (%)
S_1 × MS	79.16	47.91
S_1× ½ MS	87.50	50.38
S_1×¾ MS	77.78	46.94
S_1× WP	54.17	27.81
S_1×½ WP	50.00	28.61
S_1×¾ WP	47.37	28.91
S_2 ×MS	58.33	26.32
S_2×½ MS	33.33	38.21
S_2×¾ MS	28.57	32.44
S_2 ×WP	45.83	19.64
S_2×½ WP	25.00	24.34
S_2×¾ WP	26.67	21.13
LSD (*P*=0.05)	2.32	2.72
SEm±	1.12	1.32

MS, Murashige and Skoog Medium; BAP, benzylamino-purine; IBA, indole-butyric acid; WP, woody plant

Table 3 Effect of media on explant survival and days taken to growth initiation for establishment

Media	Response	
	Survival (%)	Days taken to growth initiation for establishment
MS	47.91	23.78
½ MS	50.38	21.87
¾ MS	46.94	24.33
WP	27.81	25.77
½ WP	28.61	28.79
¾ WP	28.91	30.43
LSD (*P*=0.05)	4.54	3.17
SEm±	2.28	1.59s

MS, Murashige and Skoog Medium; BAP, benzylamino-purine; IBA, indole-butyric acid; WP, woody plant

Table 4 Interaction effect of growth regulator regime (mg/litre) and media on survival and days taken to growth initiation for establishment

Growth regulator regime (mg/litre) × Media	Survival (%)	Days to growth initiation for establishment
BAP (0.25) × MS	33.33	27.30
BAP (0.25) ×½ MS	41.66	24.40
BAP (0.25) ×¾ MS	25.00	26.30
BAP (0.25) × WP	21.43	29.50
BAP (0.25) ×½ WP	14.28	32.46
BAP (0.25) ×¾ WP	13.33	35.38
BAP (0.50) × MS	58.33	20.40
BAP (0.50) ×½ MS	50.00	19.40
BAP (0.50) ×¾ MS	42.86	21.60
BAP (0.50) × WP	23.52	26.71
BAP (0.50) ×½ WP	29.41	28.31
BAP (0.50) ×¾ WP	15.78	29.26
BAP (0.75) × MS	25.00	24.30
BAP (0.75) ×½ MS	46.66	23.40
BAP (0.75) ×¾ MS	25.29	26.70
BAP (0.75) × WP	11.76	27.83
BAP (0.75) ×½ WP	21.05	33.54
BAP (0.75) ×¾ WP	12.50	29.42
BAP (0.25) + IBA (0.01) × MS	58.33	25.20
BAP (0.25) + IBA (0.01) ×½ MS	52.94	23.70
BAP (0.25) + IBA (0.01) ×¾ MS	66.66	25.40
BAP (0.25) + IBA (0.01) × WP	27.27	28.43
BAP (0.25) + IBA (0.01) ×½ WP	28.57	34.51
BAP (0.25) + IBA (0.01) ×¾ WP	41.67	32.26
BAP (0.50) + IBA (0.01) × MS	70.83	19.10
BAP (0.50) + IBA (0.01) ×½ MS	87.50	16.10
BAP (0.50) + IBA (0.01) ×¾ MS	75.00	22.40
BAP (0.50) + IBA (0.01) × WP	69.56	19.61
BAP (0.50) + IBA (0.01) ×½ WP	58.33	21.32
BAP (0.50) + IBA (0.01) ×¾ WP	53.33	24.63
BAP (0.75) + IBA (0.01) × MS	41.66	26.40
BAP (0.75) + IBA (0.01) × ½ MS	23.52	24.20
BAP (0.75) + IBA (0.01) ×¾ MS	36.84	23.60
BAP (0.75) + IBA (0.01) × WP	13.33	22.53
BAP (0.75) + IBA (0.01) ×½ WP	20.00	26.71
BAP (0.75) + IBA (0.01) ×¾ WP	36.84	27.51
LSD (*P*=0.05)	1.85	1.29
SEm±	0.93	0.65

MS, Murashige and Skoog Medium; BAP, benzylamino-purine; IBA, indole-butyric acid; WP, woody plant

levels. Bhojwani (1990) reported that 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.

The interaction effects of growth regulator regime and media on survival percentage and days taken to growth initiation for establishment of forced explants were statistically significant (Table 4). The highest survival of 87.50% was recorded on-half strength MS medium impregnated with 0.50 mg/litre + 0.01 mg/litre IBA, followed by 75% with ¾ MS medium supplemented with similar growth regulator regime. The lowest survival percentage of 11.76 was recorded on full strength WP medium fortified with 0.75 mg/litre BAP. All other hormonal regimes recorded intermediate response for these traits. The minimum number of days taken to growth initiation for establishment of forced explants (16.10 days) were recorded on half-strength MS medium supplied with 0.50 mg/litre BAP + 0.10 mg/litre IBA, followed by 19.10 days on full-strength MS medium supplied with 0.50 mg/litre BAP + 0.01 mg/litre IBA. These findings are in agreement with those of Borkowska (1983), who reported successful establishment of shoot tip explants of sour cherry on half-strength MS medium. Ishida *et al*. (1989) successfully established *Prunus japonica*—a dwarfing rootstock for peach on one-half or one-third strength modified MS medium. Amin (2005) also found 0.50 mg/litre BAP + 0.01 mg/litre IBA as an ideal growth regulator regime for establishment of almond explants.

SUMMARY

Two types of culture media, namely Murashige and Skoog medium and Woody plant medium, each with 3 strength levels, viz full, ½ and ¾, explants involved forced and unforced and plant growth regulators, namely BAP with concentration level of 0.25, 0.50, 0.75 mg/litre and IBA with concentration levels of 0.00 and 0.01 mg/litre, were used for culture establishment under *in-vitro* condition. Forced explant registered higher *in-vitro* survival than unforced explants. Forced explants cultured on half-strength MS medium recorded the highest survival of 50.38%; however lowest survival of 19.64% was recorded for unforced explants cultured on full-strength WP medium. Murashige and Skoog's half strength basal medium supplemented with 0.50 mg/litre BAP + 0.01 mg/litre IBA resulted in the highest survival percentage of *in vitro* cultures.

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