Indian J. Hort. 71(3), September 2014: 385-389

In vitro propagation of herbaceous peony (*Paeonia lactiflora* Pall.) cv. Sara Bernhardt using shoot tips

Z.A. Rather*, I.T. Nazki, Z.A. Qadri, M.A. Mir, K.M. Bhat** and G. Hussain****

Division of Floriculture, Medicinal and Aromatic Plants, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar 191121, J & K

ABSTRACT

Present investigation was carried out to establish an effective micropropagation protocol for herbaceous peony (*Paeonia lactiflora* Pall.) cv. Sara Bernhardt using shoot tips of vegetative shoots. Explants were collected when the plants were in full bloom. Combined surface sterilization of explants with carbendazim (0.01%) + mercuric chloride (0.1%) + ethyl alcohol (70%) yielded maximum aseptic cultures (73.33%) and good explant survival (70.00%). Highest explant establishment (43.33%) was recorded with BAP (1.00 mg/l) but maximum shoot length (2.36 cm) and leaf number/shoot (7.31) was recorded with BAP + GA_3 (0.50 + 0.10 mg/l). Maximum proliferating cultures (72.0%) with good shoot multiplication (5.60) and shoot length (1.64 cm) were obtained with BAP + kinetin (0.50 + 0.50 mg/l). BAP (1.0 mg/l) yielded maximum shoots/explant (6.20) but minimum shoot length (1.22 cm). Maximum rooting (53.33%) and roots/shoot (2.62) were obtained with IBA (0.25 mg/l). IBA proved superior to NAA in improving rooting percentage and root number. *Ex vitro* survival of the rooted plantlets was 46.67% under glasshouse.

Key words: Herbaceous peony, *in vitro* propagation, *Paeonia lactiflora*.

INTRODUCTION

Herbaceous peony is a long living herbaceous perennial belonging to family Paeoniaceae. There are more than 3,000 cultivars and 30 species of peonies. This aesthetically pleasing flowering plant has been known and admired for more than 10,000 years (Rogers, 9). These plants are prized for their attractive large blooms, which are deliciously fragrant and hence wonderful for landscape gardening and indoor arrangements. Herbaceous peonies are excellent cut flowers and have a good vase-life. These are vegetatively propagated by crown division which is necessary to maintain the desirable characteristics of a particular cultivar. However, the propagation coefficient is very low and hardly 5-8 new plants can be obtained following the division of a mother plant after a period of 4-5 years. Traditional propagation methods are slow and cannot meet the increasing demand in the market, especially for a quick release of a new cultivar and massive production of a favorite variety. The development of micropropagation protocol for peonies is necessary not to only overcome this problem but also accelerate peony breeding progress.

Micropropagation of peony began in the middle of 1960s but commercial micropropagation is still a distant goal. Planteck Biotechnologies Inc., Quebec (Canada) commercially produced tissue cultured herbaceous Itoh peonies for the first time in 2006 (Whysall, 10). There is still much work to be done in order to make *in vitro* propagation of peony commercially successful.

Herbaceous peony grows from axillary buds located under the bud scales (cataphylls) on large vegetative buds that develop underground on the root clump (Buchheim and Meyer, 4). These vegetative buds develop into shoots with one to three lateral flower buds which are typically visible in the axils of the upper leaves below the terminal flower bud. Nodes below the flower buds typically do not develop vegetative or reproductive shoots (Wilkins and Halevy, 11). Generally, main shoot tips and axillary shoot tips on crown of herbaceous peony have been used for *in vitro* propagation. Multiplication rate from these explants is not encouraging and also the rate of microbial infection is high as the buds grow subsoil. For the first time, we describe the protocol on *in vitro* propagation of herbaceous peony using shoot tip vegetative shoots.

MATERIALS AND METHODS

Studies were carried out for the micropropagation of herbaceous peony (*Paeonia lactiflora* Pall.) cv. Sara Bernhardt in Biotechnology Laboratory, Division of Fruit Science, SKUAST-K, Shalimar during 2006-2010. Terminal buds of herbaceous peony are generally reproductive and produce large attractive bloom. However, some shoots remain vegetative and do not terminate into a flowering bud. These vegetative

*Corresponding author's E-mail: zahoornishat@yahoo.com
** Division of Fruit Science, SKUAS&T-K, Salimar

shoots develop from small underground buds present on the crown. Vegetative shoots were collected when the plants were in full bloom as it was not possible earlier to distinguish between vegetative and reproductive shoots. Shoot tips (1-2 cm) were cut and surface-sterilized with different sterilants individually or in combination for different time duration. Following surface sterilization, explants were rinsed 3-5 times with sterile double-distilled water. Few drops of Tween 20® was added to sterilant solutions to improve their efficiency. Explants about 1.0 cm in length were prepared and inoculated on ½ MS medium supplemented with 0.5% polyvinyl pyrrolidone and incubated in a refrigerator for 48 h followed by 48 h incubation under cultural room conditions to prevent explant browning as suggested by Rather *et al*. (8). All the healthy explants without any browning damage were removed from the pre-establishment medium and inoculated on MS medium supplemented with different growth regulator combinations. Explants were sub-cultured after every 4 week. All the explants were incubated under culture room conditions at a temperature of 24 ± 1°C with photoperiod of 16:8 h light and dark cycles.

Young micro-shoots were cut after 4-5 weeks and placed in the same medium for proliferation. Proliferating micro-shoots were cut and placed in rooting medium supplemented with IBA and NAA at different concentrations (0.25, 0.50 0.75 mg/l). Cultures were kept in dark for 2 weeks and then transferred to light in the culture room. Rooted plantlets were removed from the rooting medium and washed with sterile double-distilled water to remove traces of agar solidified media and planted in polypropylene glasses containing vermiculite-perlite-coco peat (1:1:1), watered with a nutrient solution and covered with another polypropylene glass. The glasses were made air tight to maintain high humidity inside. After 10 days small holes were made in covering polypropylene glasses to reduced humidity slowly and finally upper covering was removed completely. After four weeks, the plantlets were transferred to greenhouse conditions in potting media containing sand, soil and manure (1:1:1). Experiments were laid out in completely randomized design with 3 replications. Data was subjected to analysis of variance using Minitab statistical package. Mean comparison was performed using Least Significant Difference (LSD) test. Percent data were Arcsine transformed before analysis.

RESULTS AND DISCUSSION

Among the individual sterilization treatments, 0.1% mercuric chloride resulted in maximum explant asepsis. Combined treatment of sterilants gave better results. Sterilization of explants with 0.01% carbendazim for 30 min. + 0.1% mercuric chloride for 10 min.+ 70% ethyl alcohol for 10 sec. improved the culture asepsis significantly and yielded 73.33% aseptic cultures with 70.00% explant survival (Table 1).

Table 1. Influence of various sterilants on culture asepsis (%) and explant survival (%) of shoot tips of herbaceous peony cv. Sara Bernhardt.

Treatment	Culture asepsis (%)	Explant survival (%)
$HgCl_2$ (0.05%) for 10 min.	33.33 (35.21)*	80.00 (63.43)
$HgCl_2$ (0.1%) for 10 min.	60.00 (50.76)	70.00 (56.99)
Ethyl alcohol (70%) for 10 sec.	23.33 (28.78)	66.66 (54.78)
NaOCl (10%) for 10 min.	46.66 (43.07)	50.00 (45.00)
NaOCl (10%) for 10 min.+ ethyl alcohol (70%) for 10 sec	56.66 (48.84)	43.33 (41.15)
$HgCl_2$ (0.1%) for 10 min.+ ethyl alcohol (70%) for 10 sec	63.33 (52.77)	63.33 (52.77)
Bavistin (0.01%) for 30 min. + $HgCl_2$ (0.1%) for 10 min. + ethyl alcohol (70%) for 10 sec.	73.33 (59.00)	70.00 (56.99)
CD (P = 0.05)	6.91	5.74

*Data in parenthesis are Arcsine transformed values of the original percentage data.

Establishment of shoot tips was low and ranged between 33.33-43.33% but the differences were statistically non-significant (Table 2). Low establishment is attributed to the fact that many shoot tips had become reproductive and developed minute flower buds which were not visible to naked eye at the time of explant collection. There was no shoot growth from such explants. Shoot growth was observed only in vegetative shoot tips (Fig. 1). Growth regulator treatments had a significant influence upon the shoot growth and leaf number/shoot which was observed maximum with BAP + GA_3 (0.50 + 0.10 mg/l) followed by BAP + kinetin (0.50 + 0.50 mg/l). Higher concentration of BAP reduced the shoot length and leaf number. Katarina *et al*. (7) also reported negative impact of BAP on shoot length in tree peony.

Elongated shoots from established cultures were excised from the mother explant and placed in the same medium for inducing axillary shoot proliferation. Axillary shoot proliferation was observed superior with BAP (1.0 mg/l) and BAP + Kin (0.50 + 0.50 mg/l) where 6.20 and 5.60 axillary shoots/explant were observed (Fig. 2; Table 3). Many researchers have developed propagation protocol for herbaceous peony but the proliferation rate was low. Hosoki *et al.* (6) recorded 2.4 axillary shoots/explant with shoot tips from underground buds of herbaceous peony. Proliferation rate obtained in the present studies with vegetative shoot tip explants is very high compared to other types of explant used so far by other researchers in herbaceous peony except Daike *et al*. (5), who reported higher proliferation rate with nodal segments.

Growth of axillary shoots was measured in terms of shoot length and leaf number. Maximum shoot length (1.98 cm) and leaf number (6.01) was observed with BAP + GA_3 (0.50 + 0.10 mg/l) but this treatment yielded minimum shoot proliferation rate (16.67%). Based on the statistical data and visual observation, BAP (1.00 mg/l) and BAP + kinetin (0.50 + 0.50 mg/l)

Table 2. Influence of growth regulators on the establishment of shoot tip explants of herbaceous peony cv. Sara Bernhardt.

Growth regulator(s)	Conc. (mg/l)	Explant establishment (%)	Shoot length (cm)	Leaf No./shoot
BAP	0.50	38.67	1.96 ± 0.2163	6.42 ±0.5923
BAP	1.00	43.33	1.92 ± 0.2107	6.16 ±0.3934
BAP+ Kin.	0.25 + 0.25	33.33	1.86 ± 0.1249	5.92 ±0.5892
BAP + Kin.	0.50 + 0.50	36.67	2.26 ± 0.1442	6.84 ±0.3607
BAP + GA_3	0.50 + 0.10	40.00	2.36 ± 0.2227	7.31 ±0.4411
CD (P = 0.05)		NS	0.34	0.88

Data (Mean ± SD) was recorded after 5 weeks of culture of explants on full strength Murashige and Skoog (1962) medium.

Fig. 1. Micropropagation stages in herbaceous peony cv. Sara Bernhardt. (a) Explant establishment in MS medium supplemented with BAP (0.5 mg/l), (b) Axillary shoot proliferation with BAP (1.0 mg/l), (c) Rooting of micro-shoots with IBA (0.25 mg/l), (d) Hardening of rooted plantlets, and (e) Hardened plant in greenhouse conditions.

Table 3. Influence of various growth regulators on axillary shoot proliferation of shoot tip explants in herbaceous peony cv. Sara Bernhardt.

Growth regulator(s)	Conc. (mg/l)	Proliferating cultures (%)	No. of axillary shoots explant	Shoot length (cm)	Leaf No. on main shoot
BAP	0.50	56.67 (48.83)*	4.30 ±0.45	1.80 ± 0.10	5.68 ± 0.38
BAP	1.00	63.33 (52.91)	6.20 ± 0.60	1.22 ± 0.0	5.23 ± 0.29
BAP + Kinetin	0.25 + 0.25	36.67 (37.24)	2.60 ± 0.300	1.76 ± 0.22	5.33 ± 0.55
BAP + Kinetin	0.50 + 0.50	72.00 (58.21)	5.60 ± 0.45	1.64 ± 0.30	5.46 ± 0.52
BAP + GA_3	0.50 + 0.10	16.67 (23.85)	1.16 ± 0.040	1.98 ± 0.18	6.01 ± 0.57
CD (P = 0.05)		8.51	0.75	0.35	NS

*Data in parenthesis are Arcsine transformed values of the original percentages.
Data (Mean ± SD) was recorded after 5 weeks of culture of explants on full-strength Murashige and Skoog (1962) medium.

were found superior to other treatments for various shoot proliferation parameters. Cytokinins have been found effective in breaking apical dominance and promoting axillary branching in peony (Bouza *et al*., 3). Following axillary shoot initiation, transferring the explants onto a medium supplemented with BAP (0.25 mg/l) + GA_3 (0.5 mg/l) helped in short elongation (data not shown). Diake *et al*. (5) also reported improvement of shoot length in herbaceous peony *in vitro* cultures following incorporation of gibberellic acid in the medium.

Healthy *in vitro* shoots (1.5-2.0 cm) of herbaceous peony were cultured in ½ MS medium supplemented with IBA (0.25, 0.50 & 0.75 mg/l) and NAA (0.25, 0.50 & 0.75 mg/l) for the first two weeks under complete darkness at 24 ± 1°C and then kept in the same medium under light in culture room conditions. Rooting percentage and root number/ shoot significantly decreased with the increase in auxin concentration (Table 4). Maximum rooting (53.33%) and roots number/shoot (2.62) were obtained with IBA (0.25 mg/l) followed by NAA

Table 4. Influence of various growth regulators on rooting of *in vitro* regenerated shoots of herbaceous peony cv. Sara Bernhardt.

Auxin	Conc (mg/l)	Rooting (%)	Root number/shoot (Mean ± SD)	Callusing (%)
IBA	0.25	53.33 (46.92)	2.62 ± 0.49	70.00 (56.99)
IBA	0.50	26.67 (30.78)	1.75 ± 0.32	83.33 (66.15)
IBA	0.75	13.33 (21.15)	1.00 ± 0.00	100.00 (89.09)
NAA	0.25	40.00 (39.23)	2.33 ± 0.57	63.33 (52.77)
NAA	0.50	20.00 (26.07)	1.33 ± 0.57	76.67 (61.22)
NAA	0.75	6.67 (12.59)	1.00 ± 0.00	100.00 (89.09)
CD (P = 0.05)		12.08	0.73	6.85

Data in parenthesis are Arcsine transformed values of the original percentage data.
Data recorded after 8 weeks of culture of shoots in half-strength Murashige and Skoog (1962) medium.

(0.25 mg/l). IBA proved superior to NAA in improving rooting percentage and root number (Fig. 3). There was an increase in callusing frequency at the base of shoots with the increase in auxin concentration which decreased the quality of roots and survival of rooted plantlets. These results are supported by the findings of Albers and Kunneman (1) who obtained better rooting with lower concentration of IBA (0.1 mg/l). They also observed that concentration of auxins (IBA & NAA) higher than 0.5 mg/l resulted in abundant callus formation and decreased the shoot quality. Hosoki *et al.* (5) and Albers and Kunneman (1) also reported superiority of IBA over NAA in improving rooting parameters in herbaceous peony. An (2) also reported higher rooting rate in tree peony with IBA than NAA.

Rooted plantlets were put in polypropylene glasses filled with autoclaved rooting medium containing vermiculite-perlite-coco peat (1:1:1). Plantlets were watered with nutrient solution and mouth of the glass closed and made air tight to maintain high humidity inside. Humidity was gradually lowered after 2 weeks. Plantlets were then transferred to pots containing soil, well decomposed farmyard manure and sand (1:1:1) for acclimatization. Some plantlets developed fungal infection during hardening process and failed to survive. *Ex vitro* survival of the rooted plantlets was 46.67%.

A successful micropropagation protocol was developed from the vegetative shoot tips of herbaceous peony.

ACKNOWLEDGEMENTS

The authors are highly thankful to Dr Julie Buchheim – Oklahoma State University, USA and Dr. Daike Tian-Auburn University, USA for providing literature on micropropagation of peony.

REFERENCES

1. Albers, M.R.J. and Kunneman, B.P.A.M. 1992. Micropropagation of *Paeonia. Acta Hort*. **314** : 85-92.

2. An, B.Y. 2005. Studies on the establishment of *in vitro* regeneration system of *Paeonia suffruticosa* Andr. M.Sc. thesis submitted to Northeast Forestry University, China.

3. Bouza, L., Jacques, M. and Miginiac, E. 1994. *In vitro* propagation of *Paeonia suffruticosa* Andr. cv. 'Mme de Vatry': Developmental effects of exogenous hormones during the multiplication phase. *Scientia Hort*. **57**: 241-51.

4. Buchheim, J.A.T. and Meyer, M.M.Jr. 1992. Micropropagation of peony (*Paeonia* spp.). **In**: *Biotechnology in Agriculture and Forestry*, Vol. 20. Y.P.S. Bajaj (Ed.), Springer-Verlag, Berlin Heidelberg, pp. 269-85.

5. Daike Tian, Tilt, K.M., Dane, F., Woods, F.M. and Sibley, J.L. 2010. Comparison of shoot induction ability of different explants in herbaceous peony (*Paeonia lactiflora* Pall.). *Scientia Hort*. **123**: 385-89.

6. Hosoki, T., Ando, M., Kubara, T., Hamada, M. and Itami, M. 1989. *In vitro* propagation of herbaceous peony (*Paeonia lactiflora* Pall.) by a longitudinal shoot-split method. *Plant Cell Rep.* **8**: 243-46.

7. Katarina, Eerna, Beata, Dedieova and Dana Borbelyova. 2008. Micropropagation of *Paeonia arborea* syn. *P. suffruticosa* Andr. Dept. of Plant Physiology, Faculty of Agronomy, Slovak University of Agriculture, Nitra. http://www.wlass.user. icpnet.pl/cerna.pdf

8. Rather, Z.A., Nazki, I.T., Peer, F.A., Mir, M.A. and Hussain, G. 2011. Minimizing medium browning during *in vitro* cultures of herbaceous peony (*Paeonia lactiflora* Pall.). *Indian J. Hort*. **68**: 529-34.

9. Rogers, A. 1995. *Peonies*, Timber Press, Inc. Portland.

10. Whysall, S. 2006. Pursuit of the perfect peony – New breed produces compact plants with full, deciduous foliage, large, colourful blooms. CanWest News Service. http://www.canada.com.

11. Wilkins, H.F. and Halevy, A.H. 1985. *Paeonia* species. **In**: *Handbook of Flowering* IV, H.A. Halevy (Ed.), CRC Press, Boca Raton, Finland, pp. 2-4.

Received: July, 2012; Revised: April, 2014;
Accepted: August, 2014