Indian J. Plant Physiol., Vol. 12, No. 1, (N.S.) pp. 23-27 (Jan.-Mar., 2007)

ESTABLISHMENT OF SHOOT CULTURES AND PLANT REGENERATION OF *PASSIFLORA EDULIS* SIMS

G. SHARMILA BANU, *G. KUMAR[1], M. RAJASEKARA PANDIAN

Centre for Biotechnology, Muthayammal College of Arts & Science, Rasipuram, Namakkal 637 408, Tamilnadu
[1]Faculty in Biochemistry, Selvamm Arts and Science College, Namakkal 637 003, Tamilnadu

Received on 30 Dec., 2006, Revised on 14 March, 2007

SUMMARY

***Passiflora edulis Sims,* a woody climber belonging to the family passifloraceae has been micropropagated successfully by culturing nodal and internodal segments. The explants were cultured in Murashige and Skoog (MS) basal medium augmented with 6-benzylaminopurine. Maximum percentage of shoot proliferation was achieved in MS basal medium fortified with 6-benzylaminopurine (4.44 mM). The maximum percentage of callus formation was achieved on MS medium augmented with 2, 4-dichlorophenoxy acetic acid (4.52 mM). Maximum percentage of shoot proliferation from the internodal derived calli was achieved on MS medium supplemented with 6-benzylaminopurine (2.22 µM and 4.44 mM). The *in vitro* raised shootlets were transferred to MS medium fortified with indole butyric acid (IBA) and highest percentage of rooting was observed in IBA-4.90 mM.**

Key words*: Micropropagation, nodal explants, *Passiflora edulis.

INTRODUCTION

Passiflora edulis Sims, belonging to the family passifloraceae, is a perennial vine. The stem is hispid with tendrils and leaves deeply 3-lobed. The common names of this plant include love-in-a-mist, wild passion fruit, passionflower, stinking passionflower etc. The fruits are usually eaten fresh or used to flavour drinks. The fruit, flowers and the leaves have a variety of medicinal properties (Chopra *et al.*1956). It significantly affects the nervous system and finds a wide application in spasmodic disorders and as a rest-producing agent. It proves especially useful in insomnia of infants and old people. It is known for its control over the spasms of childhood, whether from dentition, worms or indigestion-related ailments. It is used for flavouring candy, ice-cream, cake fillings, frostings, carbonated beverages and cordials (Prajapathi *et al.* 2003).

Owing to its medicinal importance there is a need for mass propagation because conventional method of propagation through stem cuttings has not been satisfactory. The *in vitro* multiplication techniques are used as an alternative tool for the large scale multiplication. A number of reports are available for the large scale multiplication of medicinally important plants like *Ceropegia candelabrum* (Beena *et al* 2003), *Rotula aquatic* (Martin 2003), *Pelargonium graveolens* (Gupta *et al.* 2003), *Fragaria x ananassa* Duch (Kanika Kaushal *et al.* 2006) using the plant tissue culture techniques. The present study was initiated under this background. The main objective of the present study

* Corresponding author, E-mail: pgsvedhakumar@yahoo.com (G. Kumar); gsharmikumar@yahoo.co.in (G. Sharmila Banu)

is to standardize a protocol for the large-scale multiplication of *Passiflora edulis* using nodal explants.

MATERIALS AND METHODS

Passiflora edulis plants were collected from the wild and were established in the green house of Muthayammal College of Arts & Science as the explant source. Young shoots were defoliated, cut into 3-5 cms length and washed first with tween 20 for 5 minutes and then washed with distilled water. Then, they were surface decontaminated with an aqueous solution of 0.1% (w/v) mercuric chloride for 30 seconds and rinsed thoroughly with sterile distilled water. The nodes were then cut into 1-5 cm in length and leaves, into 1x1 cm size. The explants were placed vertically as well as horizontally on to the MS basal medium (Murashige and Skoog, 1962) (3% sucrose, 0.6% (w/v) agar (Himedia, Mumbai)) and supplemented with different concentrations and combinations of plant growth regulators BAP, NAA, IAA & 2, 4-D respectively. The pH of the medium was adjusted to 5.8 before adding agar and autoclaved at 121°C for 15 minutes. After inoculation the cultures were incubated at 25 ± 2°C under cool fluorescent light (2000 Lux, 14 hr photoperiod).

The *in vitro* raised shootlets of 4-5 cm length were transferred onto different concentrations of IBA and 2, 4-D for root formation. After 3-5 weeks the plantlets were transferred to the polycups containing sterilized soil and sand in 1:1 ratio for hardening. After 6 weeks they were transferred to greenhouse. The internodal derived callus was subcultured on MS medium augmented with different concentrations of BAP, IAA and NAA for shoot regeneration.

For statistical analysis, means were based on 12 replicates for each treatment. The percentage of response and average number of shoots per explant obtained were summarized in Table 1. The data was statistically examined by ANOVA.

RESULTS AND DISCUSSION

MS medium augmented with different concentrations of BAP was used for multiple shoots emergence from the nodal segments. The role of BAP in stimulating multiple shoots in many plants has been studied extensively by many workers. After 15-20 days axillary bud proliferation was observed. The effect of BAP on shoot multiplication is shown in Table 1. The percentage

Table 1. Effect of plant growth regulators on the multiple shootlets formation from the nodal segments of *Passiflora edulis* on MS medium

Plant growth regulators (μM)			Explant	No. of shootlets /
BAP	**NAA**	**IAA**	**responded (%)**	**explant**
0.0	0.0	0.0	41.6[b]	1.4
2.22	0.0	0.0	75.0[a]	2.2
4.44	0.0	0.0	83.3[a]	3.1
6.66	0.0	0.0	66.6[a]	2.5
8.90	0.0	0.0	58.3[a]	1.57
2.22	2.98	0.0	0.0[d]	0.0
4.44	2.98	0.0	16.6[c]	1.5
6.66	2.98	0.0	33.3[c]	2.25
8.90	2.98	0.0	16.6[c]	1.33
4.44	0.0	5.71	41.6[b]	2.2

Means followed by the same letter are not significantly different at P=0.05 according to ANOVA test.

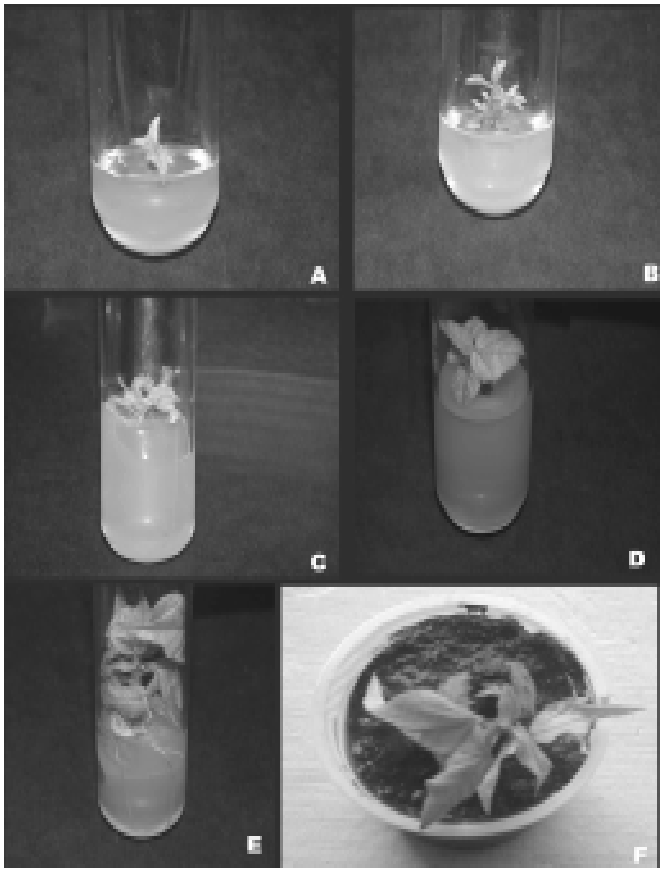

Plate 1: *In vitro* propagation of *Passiflora edulis* sims using nodal explants
A : Initial stage of the nodal explants; B, C, D: Multiple shoots on MS Medium with BAP (4.44 mM); E : Rootlets formation on *in vitro* raised shootlets; F : *In vitro* raised plantlets

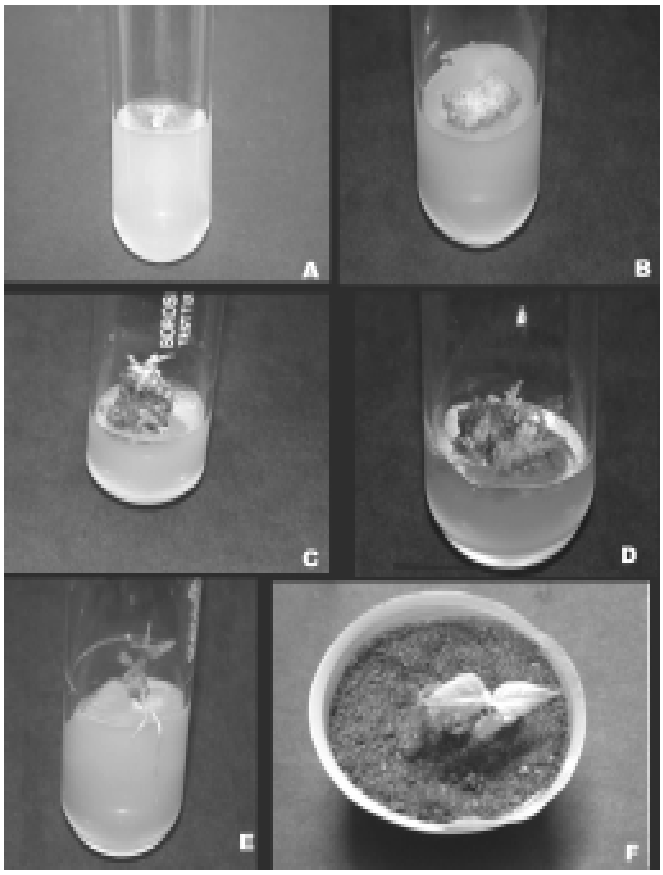

Plate 2: Organogenesis of *Passiflora edulis* Sims
A, B : Internodal derived calli; C, D : Callus regeneration; E : Rootlets formation on *in vitro* shootlet raised from callus; F : *In vitro* raised plantlets

of shootlets formation varied according to the concentration of BAP. The MS medium supplemented with BAP (4.44 mM) recorded maximum shooting response. In *Celestrus paniculatum* maximum multiple shoot formation was reported on the same concentration of BAP (Nair and Seeni 2001). BAP induced multiple shoot formation was reported by other workers in *Sapindus mukarossia* (Philomina and Rao 2000), *Solanum surattense* (Pawar *et al*. 2002), *Rauvolfia micrantha* and *R. tetraphylla* (Vishwananth *et al.* 1997), *Baliospermum montanum* (Johnson and Manickam 2003), *Momordica charantia* L, (Agarwal and Kamal 2004) and *Entada pursaetha* (Vidya *et al.* 2005). In the present study, the nodal segment inoculated on MS medium with BAP showed maximum number of shoots. The combination of BAP and NAA showed inhibitory

Table 2. Effect of plant growth regulators on callus formation of *Passiflora edulis* cultured on MS medium

Plant growth regulators (μM)		Explant	Amount of
2, 4-D	IAA	responded (%)	callus
2.26	0.0	73	++
4.52	0.0	88	+++
6.78	0.0	75	++
9.05	0.0	43	+
0.0	5.71	35	+
0.0	8.56	20	+

+ : Low ++ : Medium +++ : High

Table 3. Effect of plant growth regulators on the formation of rootlets from the *in vitro* raised shootlets of *Passiflora edulis* on MS medium

Plant growth regulators (μM)			Explants showing	No. of rootlets/
IBA	**NAA**	**2, 4-D**	**rooting (%)**	**per shootlet**
2.46	0.0	0.0	25.0	1.5
4.90	0.0	0.0	87.5	3.85
0.0	0.0	4.52	75.0	2.66
0.0	2.98	0.0	0.0	0.0

effect to the shoot initiation. The same result was obtained in *Pelargonium graveolens* (Gupta *et al*. 2002).

Callus was initiated from the leaves and internodes of *Passiflora edulis* on MS medium supplemented with different concentrations of 2, 4-D and IAA (Table 2). The maximum percentage (88%) of callus proliferation was observed on the MS medium supplemented with 2, 4-D (4.52 mM). Similar report was observed on *Withania somnifera* (Manickam *et al.* 2000).

Callus obtained from the internodal segments were subcultured on the MS medium supplemented with BAP at different concentrations for shoot regeneration. Maximum response was found in BAP 2.22 mM and 4.44 mM. Callus regeneration on BAP was reported in *Aegle marmelos* (Varghese *et al.* 1993).

The *in vitro* raised shootlets from nodal as well as callus were transferred to MS medium supplemented with IBA, NAA and 2, 4-D for rooting (Table 3). Maximum response was observed in MS medium with 2, 4-D (4.52 mM) and IBA (4.90 mM). The roots were obtained on the same concentration in *Baliospermum montanum* (Johnson and Manickam 2003) and *Vitis vinifera* (Nalwade and Shitole 2004). In most of the studies IBA was used for efficient rooting (Loreti *et al.* 1988). The effectiveness of IBA in rooting has been reported in the medicinal plants of asclepiadeceae in *Gymnema sylvestres* (Komalavalli and Rao 2000), *Hemidesmus indicus* (Sreekumar *et al*. 2001), *Datura metel* (Muthukumar *et al.* 2004) and these results were similar to the present study as IBA showed maximum rooting.

Organogenesis through callus of internode explant as well as multiple shoot induction from nodal explants (Plate 1 and 2) could be effectively used in the multiplication of *Passiflora edulis* Sims. Using this procedure, plants may be regenerated on a large scale in a short span of time. The protocol standardized here could be used to isolate the medicinally important secondary metabolites from the multiplied shoots and this protocol would also have importance in genetic transformation of this medicinally important plant.

REFERENCES

Beena, M.R., Martin, K.P., Kirti, P.B. and Hariharan, M. (2003). Rapid *in vitro* propagation of medicinally important *Ceropegia candelabrum*, *Plant Cell Tiss. Org. Cult.* **72:** 285-289.

Chopra, R.N., Nayar, S.L., Chopra, I.C. (1956). Glossary of Indian Medicinal Plants. CSIR, 1st edition, p. 186. New Delhi, India.

Gupta, R., Gupta, S.K., Banerjee, S., Mallavarapu, G.R. and Kumar, S. (2002). Micropropagation of elite cultivars of rose-scented Geranium (*Pelargonium graveolens* L Herit) for industrial production of propagules. *Indian J. Biotechnol.* **1:** 286-291.

Johnson, M. and Manickam, V.S. (2003). *In vitro* micropropagation of *Baliospermum montanum* (wild). Muell-Arg- a medicinal plant. *Indian. J. Exp. Biol.* **41:** 1349-1351.

Kanika Kaushal, Amarjit K. Nath. and Sharma, D.R. (2006). Establishment of callus cultures and plant regeneration in strawberry (*Fragaria x ananassa* Duch) CV. Chandler. *Indian J. Plant Physiol.* **11:** 136-144.

Komalavalli, N. and Rao, M.V. (2000). *In vitro* micropropagation of *Gymnema sylvestres* – a multipurpose medicinal plant, *Plant Cell Tiss. Org. Cult.* **61:** 97-105.

Nair and Seeni (2001). Rapid *in vitro* multiplication and restoration of *Celestrus paniculatum* wild sub Sp. Paniculatum (Celestraceae) a medicinal wood climber. *Indian. J. Exp. Biol.* **39:** 697-704.

Loreti, F., Morini, S. and Concetti, S. (1988). Effect of potassium and nitrogen concentration on growth of peach shoots cultured *in vitro, Acta Hort.* **227:** 311-317.

Agarwal, M. and Kamal, R. (2004). *In vitro* clonal propagation of *Momordica charantia* L. *Indian. J. Biotechnol.* **3:** 426-430.

Manickam, V.S., Elango Mathavan, R., and Antonisamy, R. (2000). Regeneration of Indian ginseng plantlets from stem callus. *Plant Cell Tiss.Org. Cult.* **62:** 181-185.

Martin, K.P. (2003). Rapid *in vitro* multiplication and *ex vitro* rooting of *Rotula aquatica* Lour, a rare rhoeophytic woody medicinal plant, *Plant Cell Rep.* **21:** 415-420.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture, *Physiol. Plant.* **15:** 473-497.

Muthukumar, B., Arockiasamy, D.I. and Natarajan, E. (2004). Direct organogenesis in *Datura metel* L. from *in vitro* and *in vivo* nodal explants, *Indian J. Biotechnol.* **3:** 449-451.

Nalwade, A. R. and Shitole, M. G. (2004). *In vitro* multiplication of grape (*Vitis vinifera* L.) CV TAS A Ganesh from nodal sector and axillary bud explants. *Indian J. Plant Physiol* **9:** 145-157.

Prajapathi, N.D., Purohit, S.S., Arun K. Sharmi, Tarun Kumar. (2003). A Handbook of Medicinal Plants: A Complete Source Book. Agrobios (India), pp 383. Shyam Printing Press, Jodhpur.

Pawar, P.K., Pawar, C.S., Narkheda, B.A., Teli, N.P., Bhalsing, S.R. and Maheswari, V.L. (2002). A technique for rapid micropropagation of *Solanum surattense brum*. F. *Indian. J. Biotechnol.* **1:** 201-204.

Philomina, N.S. and Rao, J.R.S. (2000). Micropropagation of *Sapindus mukorossi* (Gaertn). *Indian J. Exp. Biol.* **38:** 621-624.

Varghese, S.K., Inamdas, J.A., Kiran Kalia, Subramanian, R.B. and Nataraj, M. (1993). Micropropagation of *Aegle marmelos* (L) Corr., *Phytomorphology*, **43:** 87-92.

Sreekumar, S., Muthukumar, S. and Seeni, S. (2001). Morphogenetic responses of 6 philodendron cultivars *in vitro*, *Indian J. Exp. Biol.* **39:** 1280-1287.

Vidya, S. M., Krishna, V., Manjunatha, B. K. and Shankarmurthy, K. (2005). Micropropagation of *Entada pursaetha* DC –An endangered medicinal plant of Western Ghats. *Indian. J. Biotechnol.* **4:** 561-564.

Vishwananth M. Patil and Jayanthi, M. (1997). Micropropagation of two species of *Rauvolfia* (Apocynaceae), *Curr. Sci.* **72:** 961-965.