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SOMATIC EMBRYOGENESIS IN *PERGULARIA DAEMIA* (FORSK.) CHIOV. – A MEDICINAL PLANT

C. KIRANMAI[1] V. ARUNA[2], UDAY SANKAR ALLAM[1], P. VIJAYA BHASKAR REDDY[3] AND T. PULLAIAH[2]

1 Department of Biotechnology, Vikrama Simhapuri University, Nellore-524003, Andhra Pradesh, India

2 Department of Biotechnology and Botany, Sri Krishnadevaraya University, Anantapur- 515003, Andhra Pradesh, India

3 Department of Life Science and Bio-Informatics, Assam Central University, Diphu Campus, Diphu-782462, Assam, India

E-mail: pullaiah.thammineni@gmail.com ; cdpkiranmai@yahoo.co.in

Somatic embryogenesis and whole plant regeneration were achieved from leaf, cotyledon, internode and root explants excised from seedlings of medicinally important shrubby twiner *Pergularia daemia*. Somatic embryogenesis depends on the type of plant growth regulators. Nodular callus developed from the explants taken from the seedlings grown on Murashige and Skoog (MS) medium supplemented with 3mg/l Picloram underwent somatic embryogenesis. Globular embryos dominated during somatic embryogenesis. MS medium supplemented with 2iP (0.1mg/l) was found to be effective for the germination of these somatic embryos. The plants regenerated via somatic embryogenesis were transferred to MS basal medium and the plants with well developed roots and shoots were successfully transferred to field with more than 50% survival.

Key words: *Pergularia daemia*, Somatic embryogenesis, Plant regeneration.

Abbreviations: BA: 6-Benzyladenine, 2iP: 2-Isopentyladenine, KN: Kinetin, 2, 4-D: 2, 4-Dichlorophenoxyacetic acid, SE: Somatic embryo.

Pergularia daemia (Forsk.) Chiov. (Asclepiadaceae) is a shrubby twiner distributed in Africa and tropical Asia. The leaves of the plant have long been used for the treatment of helminthiasis, haemorrhoids and leprosy. This plant is useful in urethororrhoea, strangury, metropathy, inflammations, cough, asthma, amenorrhoea, intermittent fevers and leucoderma. The plant extract is useful in uterine and menstrual disorders and in facilitating parturition (Prajapathi *et al.* 2003). There is no vegetative propagation of this plant. Moreover this particular plant has considerable seed dormancy.

We reported earlier micropropagation and direct organogenesis from callus in *Pergularia daemia* (Kiranmai *et al.* 2007, 2008). There are no reports so far on somatic embryogenesis in this species. Hence we undertook the present investigation.

MATERIALS AND METHODS

Mature follicles of *Pergularia daemia* were collected from the campus of Sri Krishnadevaraya University, Anantapur, India. Mature follicles were shade dried and seeds were collected for raising aseptic seedling. The seeds of *Pergularia daemia* were taken in 250 ml clean Erlenmeyer flask and washed with detergent for 30 mins. Further operations were carried under aseptic conditions inside laminar chamber. Seeds were first washed with sterilized distilled water and then subjected to 70% ethyl alcohol treatment for 60 seconds and again washed with sterilized distilled water. It is followed by 20% v/v hydrogen peroxide treatment for 4 mins and later rinsed 4-5 times with sterile water. Seedlings of 40 day incubation are used as an explant source.

Aseptic seedling explants such as cotyledon, leaf, internode and root were excised aseptically and cut into appropriate sizes (leaf and cotyledon-1 cm^2, root and internode- 1cm) and placed singly in test tube containing 20 ml of agarified MS medium with various concentrations of plant growth regulators alone. The pH of the medium was adjusted to

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5.8 before addition of agar. The media were autoclaved at 121^{0}C for 20 mins under 15 lbs pressure. All the cultures were incubated at 25^{o}C under 16 hrs photoperiod with light intensity of 2000 lux.

Callus growth and number of somatic embryos were evaluated after 30 days of culture. The experiments were conducted in a completely randomized design. Fifteen replicates were used for each treatment, and all the treatments were repeated thrice. The data were statistically analyzed using one way analysis of variance and means were compared using the Tukey test at the 0.05% level of significance.

In vitro developed plantlets (containing 4-5 nodes) were taken out of culture tubes, washed under running tap water to remove agar and transplanted into small cups containing autoclaved peatmoss and sand (3:1). The plants were covered with polythene covers and kept in a culture room for 2 weeks. The plants were irrigated with quarter strength MS basal salts weekly twice for 2 weeks. Later on plantlets were transferred to field conditions.

The explants (leaf, cotyledon, internode and root) were cultured on MS medium supplemented with Picloram (0.1 mg/l to 7 mg/l) for 9 weeks, with subculture at 3 week intervals, to study the induction of embryogenic callus. For the conversion of somatic embryos to complete plants, somatic embryos were isolated from embryogenic callus and cultured on MS medium supplemented with or without cytokinins. For each treatment 10-15 somatic embryos were cultured on 20 ml of medium.

RESULTS AND DISCUSSION

Cotyledon, leaf, internode and root explants cultured on MS medium supplemented with Picloram (0.1mg/l-7mg/l) developed nodular callus at the cut surface during the first 2 weeks of culture. The explants cultured on growth regulator free medium did not show any response during the first 2 weeks and become necrotic in 5-6 weeks. Nodular callus developed on MS medium supplemented with Picloram turned to embryogenic upon subculture on to the same medium. The number of SE's formed from nodular callus increased with the age of the culture. Globular SEs were produced in nodular embryogenic callus 7 weeks following culture initiation.

The calli which were obtained from leaf and cotyledons with Picloram supplemented to MS medium are pinkish and nodular (Fig 1 & 2) and they were bearing globular embryos on the surface. The frequency of embryogenic callus formation from cotyledon, leaf, internode and root explants varied depending upon the concentrations of Picloram used. Of all the treatments MS medium containing 3mg/l Picloram induced somatic embryo formation at the highest frequency. At lower and higher concentrations of Picloram 3mg/l lower number of somatic embryos was noticed.

Among the various explants of *Pergularia daemia* used for somatic embryogenesis leaf explants were more responsive on Picloram following cotyledons, root and internode. This was also observed in other Asclepiadaceae member such as *Tylophora indica* (Manjula *et al.* 2000).

In general somatic embryos are induced when cultures are exposed initially to medium containing higher levels of auxins followed by subsequent transfer to a medium with either lower levels of auxins or optimal levels of cytokinins (Mithila *et al*., 2003). Formation of somatic embryos depends on the balance between auxins and cytokinins. But in the present study it was found that auxin alone was sufficient in inducing embryogenic callus. Induction of somatic embryogenesis by Picloram alone is very unusual in Asclepiadaceae. Picloram was also found to be effective in somatic embryogenesis in *Caralluma stalagmifera* (Sreelatha and Pullaiah, 2010). In contrast, promoting effect of 2, 4-D on somatic embryo induction has been

reported in several members of Asclepiadaceae such as *Tylophora indica* (Rao and Narayanaswamy 1972), *Ceropegia* spp. (Patil 1998), *Holostemma ada-kodein* (Martin 2003) and *Ceropegia candelabrum* (Beena and Martin 2003).

In the present study a 100 mg callus induced more than 50 somatic embryos (table-1). The cultures showed globular to early cotyledonary stage embryos, but the globular stage embryos dominated in culture.

The conversion of somatic embryos to complete plantlets was significantly influenced by the stage of somatic embryo development and type of cytokinin used (Table 2). If the somatic embryos were transferred to MS medium without growth regulators complete plantlets could not be obtained. In the presence of lower cytokinins mature somatic embryos developed into complete plantlets. A higher concentration of cytokinin was detrimental to somatic embryo conversion to complete plantlets. Immature somatic embryos showed partial development without complete plantlet formation even at lower concentration of cytokinin. MS medium supplemented with 0.1 mg/l 2iP was found to be effective for the germination of these somatic embryos. Most somatic embryos became abnormal when KN was used for somatic embryo conversion as KN induced shoot elongation in mature somatic embryos without root formation.

Plantlets developed via somatic embryogenesis were transferred to MS basal medium, grew into plantlets with well developed shoots and roots with in 4 weeks. 52% of plants survived from hardened potted plants derived from somatic embryos.

Table 1: Somatic embryogenesis from different explants grown on MS medium with Picloram after 9 weeks of culture.

Picloram mg/l	Number of somatic embryos per 100 mg callus(Mean ± S.D)			
	Cotyledons	Leaf	Internode	Root
0.1	4.10 ± 1.00^{c}	4.80 ± 0.94^{c}	NR	NR
1.0	11.7 ± 1.04^{c}	14.5 ± 1.63^{c}	7.10 ± 1.26^{c}	9.60 ± 1.20^{c}
2.0	31.0 ± 2.86^{bc}	34.1 ± 3.58^{b}	14.1 ± 2.86^{c}	25.6 ± 3.31^{bc}
3.0	45.2 ± 3.20^{ab}	53.3 ± 6.38^{a}	23.3 ± 3.41^{bc}	41.2 ± 4.78^{ab}
5.0	38.3 ± 2.83^{ab}	47.2 ± 5.58^{ab}	19.6 ± 3.03^{bc}	34.8 ± 2.48^{b}
7.0	23.6 ± 2.74^{bc}	26.1 ± 3.53^{bc}	12.2 ± 1.39^{c}	15.1 ± 1.90^{c}

Means followed by the same letter not significantly different by the Tukey test at 0.05% probability level; NR-No Response

Table 2: Conversion of somatic embryos (SEs) at different stages of development to complete plantlets.

Cytokinin(mg/l)		% of SEs converted to complete plantlet	% of SEs converted to shoot development	% of SEs converted to root development
BA	0.1	34.1 ±3.54^{a}	18.0 ±1.09^{b}	11.3 ±2.16^{bc}
	0.5	15.2 ±1.51^{bc}	7.00 ±1.94^{c}	8.10 ±1.67^{bc}
	1.0	7.30 ±1.09^{c}	NR	NR

	2.0	2.00 ±0.57^{c}	NR	NR
2iP	0.1	34.9 ±3.31^{a}	16.8 ±3.03^{bc}	14.2 ±2.52^{bc}
	0.5	28.1 ±3.25^{a}	9.20 ±1.62^{bc}	11.4 ±2.17^{bc}
	1.0	10.6 ±1.15^{bc}	2.00 ±0.71^{c}	NR
	2.0	5.20 ±0.91^{c}	NR	NR
KN	0.1	8.40 ±1.19^{bc}	4.70 ±1.01^{c}	13.3 ±2.22^{bc}
	0.5	1.90 ±0.64^{c}	NR	9.70 ±1.39^{bc}
	1.0	NR	NR	1.80 ±0.62^{c}
	2.0	NR	NR	NR

Means followed by the same letter not significantly different by the Tukey test at 0.05% probability level; NR-No Response

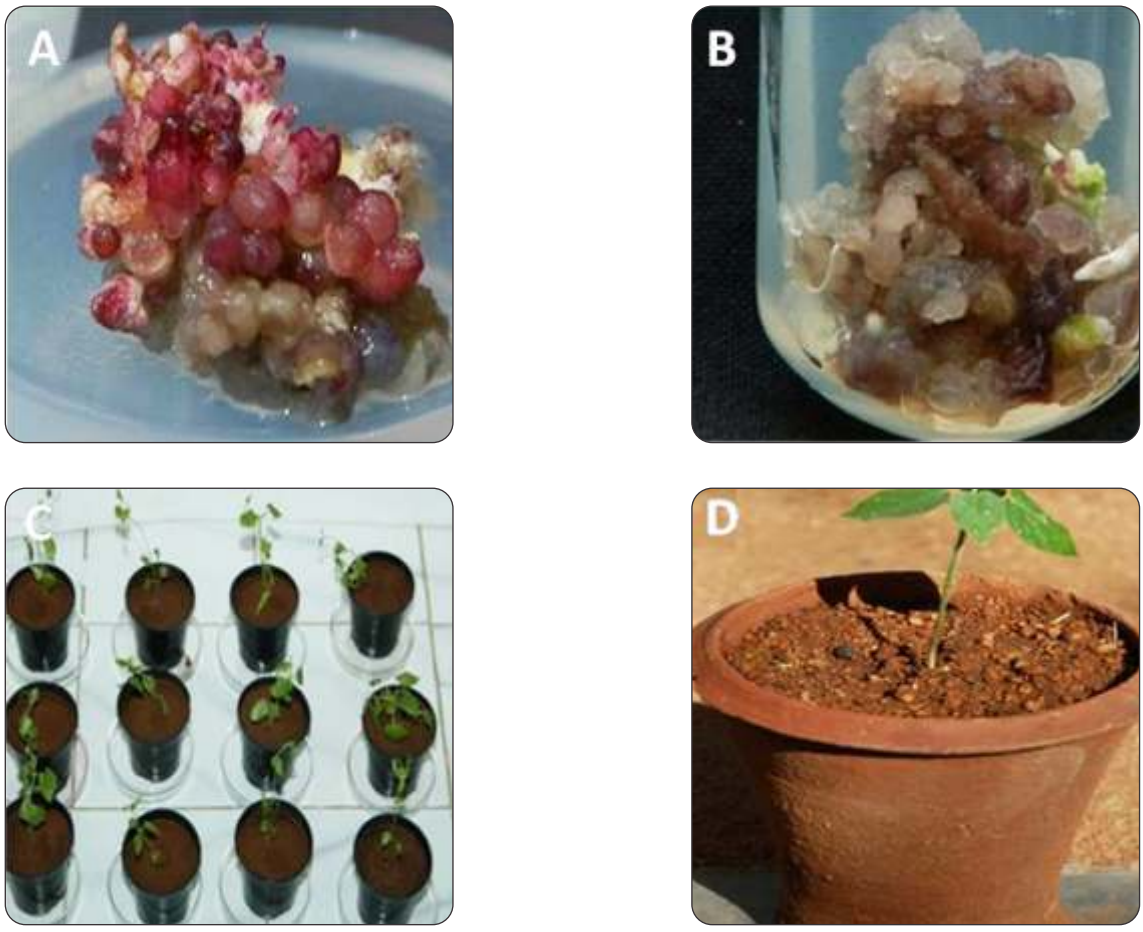

Figure1. A. Production of Somatic embryo's from leaf explant cultured on 3 mg/l Picloram. B. Germination of Somatic embryo's on 2iP 0.1 mg/l. C. Acclimatization of plantlets. D. Six weeks old acclimatized plant.

REFERENCES

Beena MR and Martin KP 2003 In vitro propagation of the rare medicinal plant Ceropegia candelabrum L. through somatic embryogenesis. *In vitro Cell Dev Biol Plant* **39** 510-513.

Kiranmai C, Aruna V, Karuppusamy S and Pullaiah T 2007 *In vitro* propagation of *Pergularia daemia* (Forsk.) Chiov. *Plant Cell Biotech. Mol Biol* **8** 67- 72.

Kiranmai C, Aruna V, Karuppusamy S and Pullaiah T 2008 Callus culture and regeneration from seedling explants of *Pergularia daemia* (Forsk) Chiov. *J Plant Biochem Biotechnol* **17** 99-101

Manjula S, Job A and Nair GM 2000 Somatic embryogenesis from leaf derived callus of *Tylophora indica* (Burm. f.) Merrill. *Indian J. Exp. Biol.* **38** 1069-10.

Martin KP 2003 Plant regeneration through somatic embryogenesis in *Holostemma ada-kodien*, a rare medicinal plant. *Plant Cell Tiss Org Cult* **72** 79-82

Mithila J, Hall JC, Victor JMR and Saxena PK 2003 Thiadiazuron induces shoot organogenesis at low concentrations and somatic embryogenesis at high concentrations on leaf and petiole explants of African violet (*Saintpaulia ionantha* Wendl.). *Plant Cell Rep* **21** 408-414.

Patil VM 1998. Micropropagation of *Ceropegia* spp. *In vitro Cell Dev Biol Plant* **34** 240-243.

Prajapathi ND, Purohit SS, Sharma AK and Kumar T 2003 *A hand book of Medicinal Plants.* Agrobios, India.

Rao PS and Narayanaswamy S 1972 Morphogenetic investigations in callus cultures of *Tylophora indica*. *Physiol Plant* **27** 271-276.

Sreelatha VR and Pullaiah T 2010 Induction of somatic embryogenesis and plant regeneration from internodal explants of *Caralluma stalagmifera*. *Botany Research International* **3** 17-20.