Plantlet Regeneration from Leaf Explant of *Clerodendrum Phlomidis v*ia Somatic Embryogenesis

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ABSTRACT

Plants have been an important source of medicine for thousands of years. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients from plant. It is important to select, multiply and conserve the critical genotypes of medicinal plants. *Clerodendrum phlomidis* L. (Arani) is a medicinal plant used in the treatment of *Diabetes mellitus*. *In vitro* experiments were conducted using leaf as an explant. Callus was cultured on MS basal medium supplemented with various combinations of 2, 4-D and Kinetin (1 : 1, 2 : 2, 3 : 3, 4 : 4, 5 : 5, 6: mg/l 2,4-D : Kinetin). Of various concentrations tried, the leaf explant of *Clerodendrum phlomidis* showed embryoids formation on medium containing 4:4 and 6:6 mg/l concentrations of Kin and 2,4-D each. The embryoids were further subcultured for plantlet regeneration on MS basal medium supplemented with combination of 0.25 mg/l NAA and 1, 2, 3, 4, 5, mg/l kinetin. Maximum numbers of embryoids germinated successfully on MS with 0.25 mg NAA + 3 mg/l kinetin.

Keywords: *Clerodendrum phlomidis,* plant tissue culture, somatic embryoids, plantlets

INTRODUCTION

Clerodendrum phlomidis L. (Arani) is a medicinal plant used in the treatment of *Diabetes mellitus*. It is used in the treatment of diabetes, gonorrhea, measles etc. This plant has aromatic, astringent, demulcent, anti-convulsion, anti-diarrheal activities. In India parts of the plant are used in post-natal conditions in women and in gastrointestinal disorders. The roots are employed as an appetite stimulant (Kirtikar and Basu, 1933; Sheba Rani et al. 1999). There are reports on the presence of flavonone and their glycosides (Anam, 1999) and sterols (Joshi et al. 1999) in *Clerodendrum phlomidies*.

Plant tissue culture technology may help to conserve rare and endangered medicinal plants. A variety of the plant species can be conveniently propagated through the techniques of cell, tissue or organ culture (Erdei et al. 1981, Hatano et al. 1986, Hiraoka and Oyanagi 1988, Nishioka 1988, Tsay et al. 1989, Huang et al. 2000, Chueh et al. 2001). For the regeneration of a whole plant from a cell or callus tissue, cytodifferentiation is not enough and there should be a differentiation leading to shoot bud or embryo formation. This may occur either through organogenesis or through

somatic embryogenesis. Somatic embryogenesis offers the possibility of rapid, easy production and selection of elite plant.

MATERIALS AND METHODS

Plant material of *C. phlomidis* required for tissue culture studies was collected from the Botanical Garden of Gujarat University Campus. Leaves were used as explants. Explants were sterilized using sterilizing reagents e.g. 2% Tween-20 solution, 5% Sodium hypochlorite, 0.1% $HgCl_2$, followed by washing with sterile double distilled water to remove the traces of chemicals. Sterilized explants were inoculated on basal media (Murashige and Skoog 1962) supplemented with different combinations of 2, 4-D and Kinetin (1 : 1, 2 : 2, 3 : 3, 4 : 4, 5 : 5 mg/l 2,4-D : Kinetin). The cultures were incubated in culture room. They were observed regularly for any sign of contamination, swelling and initiation of results. The explants swelled within three days of inoculation. At the end of three week depending of the hormonal concentrations the callus showed morphological variations in terms of colour, texture and mass and after growth of four weeks differentiated into embryogenic or nonembryogenic callus. The embryonic callus produced distinguishable somatic embryoids. These embryoids were sub-cultured on MS basal medium supplemented with BAP and NAA in combination ratio of 1:0.25, 2:0.25, 3:0.25, 4:0.25 and 5:0.25 mg/l for its germination and differentiation as root and shoot.

RESULT AND DISCUSSION

The leaf explants cultured on hormonal media swelled within three days of inoculation. At the end of three week depending of the hormonal concentrations the callus showed morphological variations in terms of colour, texture and mass and after growth of four weeks differentiated into embryogenic or non-embryogenic callus. The embryonic callus produced distinguishable somatic embryoids. Callus obtained on the medium with higher hormonal concentration 4 and 5 mg/l 2, 4-D + 4 and 5 mg/l kinetin resulted in somatic embryoids while lower concentrations 1:1, 2:2, 3:3 mg/l, 2,4-D : kinetin resulted in non-embryonic cells. The embryonic callus was cream, friable and globular in shape. These embryoids germinated on MS media with NAA 0.25/l and 1 to 5 mg/l kinetin. However embryoids germination was best achieved with 3mg/l of Kin. Plant regeneration via somatic embryogenesis from single cells, that can be induced to produce an embryo and then a complete plant, has been demonstrated in many medicinal plant species. Arumugam and Bhojwani noted the development of somatic embryos from zygotic embryos of *Podophyllum hexandrum* on MS medium containing 2 μM BA and 0.5 μM IAA (Arumugam and Bhojwani, 1990). Embryogenic calluses and germination of somatic embryos in nine varieties of *Medicago sativa* has been achieved (Fuentes, 1993).

Table-1 Effect of 2,4-D and kinetin on callus induction from leaf explant

Media	2,4-D (mg/l)	Kinetin (mg/l)	Callus Induction	Remarks
MS	1	1	++++	Non embryonic, green, friable
	2	2	+++	Non embryonic, whitish green, friable
	3	3	+++	Non embryonic whitish yellow, mucilaginous
	4	4	++	Embryonic whitish green compact
	5	5	++	Embryonic, compact, globular, green, with pink pigmentation

Table- 2 *In vitro* responses of embryoids germination

Media	NAA (mg/l)	Kinetin (mg/l)	% Germination of embryoids
MS Basal	0.25	1	50
	0.25	2	60
	0.25	3	65
	0.25	4	43
	0.25	5	25

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