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BIOCHEMICAL PROFILE OF EMBRYONIC AND NON-EMBRYONIC CALLUS OF CLERODENDRUM PHLOMIDIS L.

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ABSTRACT

Clerodendrum phlomidis L. (Arani) is a medicinal plant used in the treatment of Diabetes mellitus. Enzymatic studies helps to differentiate between embryonic and non-embryonic callus at very early stage. In vitro experiments were conducted using leaf, stem, node, internode, apical bud as an explant. Callus 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 mg/l 2,4-D : Kinetin). Large variations were observed in the callus cultures owing to hormonal variations. Higher hormonal concentration 5 mg/l 2, 4-D + 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. Enzymatic activities of peroxidase, IAA- oxidase, invertase, protease, □ and □ amylase, polyphenol oxidase (PPO), catalase and enzyme protein were estimated from cytoplasmic as well as wall-bound fractions of 2, 4, 6and 8 week old callus using standard methods. In two week old culture peroxidase and polyphenol oxidase (both wall bound and cytoplasmic) activity increases in non-embryonic callus and decreases in embryonic callus while IAA oxidase and Protease (both wall bound and cytoplasmic) activity decreases in non-embryonic callus and increases in embryonic callus. Total amylase and a-amylase activity of wall bound enzymes increases in non-embryonic callus and decreases in embryonic callus while cytoplasmic activity decreases in non-embryonic callus and increases in embryonic callus. On the basis of the above study enzymes could serve as a biochemical marker to determine the embryonic and non-embryonic callus at very early stage.

Keywords: *Clerodendrum Phlomidis, Diabetes mellitus, Enzymatic activities*

INTRODUCTION

Clerodendrum phlomidis L. (Arani) is a medicinal plant used in the treatment of *D. mellitus*.Itis 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 anappetite stimulant (Kirtikar and Basu, 1933; Sheba Rani et al. 1999). There are many reports on the presence of flavonone and their glycosides in *C. phlomidies*(Anam, 1999) and sterols by (Joshi et al. 1999).

Biochemical analysis of embryonic and non-embryonic callus can enhance our basic understanding in the development of stage-specific biochemical markers that can be used to optimize somatic embryogenesis protocols. It also gives support to our basic understanding ofthe biochemical changes underlying the formation of somatic and zygotic embryos (Misra, 1994). Storage proteins were the first compounds used as markers in comparing the developmental programs of two types of embryogenesis (Hakman et al. 1990; Hakman, 1993). The presence of homologous proteins in mature somatic embryos together with their triglyceride content was suggested to indicate embryo quality (Cyret al. 1991). Still other data indicate the potential of some enzymes to function as stage-specific markers of somatic embryogenesis. The same role was also postulated for peroxidase and esterase, whose

isoenzyme patterns were shown to reflect the embryogenic potential of *Medicago sativa* and *Dactylisglomerata*suspensioncultures (Hrubcovaet al. 1994).

MATERIALS AND METHODS

Plant material of*C. phlomidis* required for tissue culture studies wascollected 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 $HgCl_2$ and sodium hypochlorite.

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 signof contamination, swelling and initiation of results. The callus obtained was harvested at the end of 2, 4, 6 and 8 weeks. Influenced by the hormonal combination, the explant differentiated either into embryogenic or non-embryogenic callus.

Biochemicals present in the embryogenic and non-embryogenic callus were measured. Enzymatic activities of peroxidase, IAA-oxidase, protease, α and amylase and polyphenol oxidase (PPO), were estimated from cytoplasmic as well as wall-bound fractions of the fresh materials using standard methods: Enzyme activities of peroxidase (George 1952), IAA-oxidase (Mahadevan 1964), protease (Penner and Ashton 1967, modified by Cruz *et al.* 1970), α and
amylase (Sumner and Howell 1935), polyphenol oxidase (PPO) (Kar and Mishra 1976).

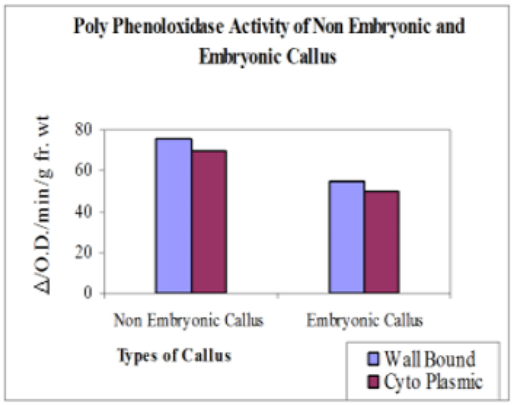
RESULT AND DISCUSSION

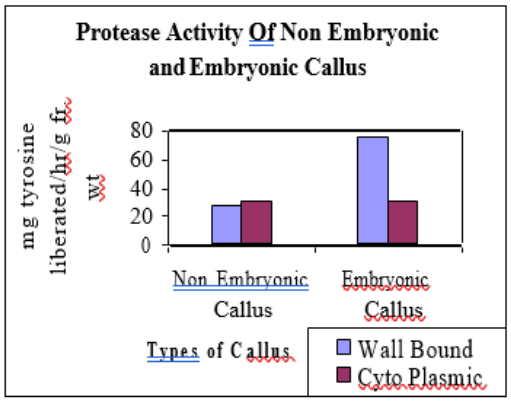
Large variations were observed in the callus cultures owing to hormonal variations. Callus obtained on the medium with higher hormonal concentration 4 and 5 mg/l 2, 4-D +4 and 5 mg/l kinetin resulted in somatic embryoids while lower concentrations 1:1, 2:2, 3:3 mg/l, 2,4-D : kinetin resulted in non-embryonic cells. In two week old culture peroxidase and polyphenol oxidase (both wall bound and cytoplasmic) activity increases in non-embryonic callus anddecreases in embryonic callus while IAA oxidase and Protease (both wall bound and cytoplasmic) activity decreases in non-embryonic callus and increases in embryonic callus. Totalamylase and α-amylase activity of wall bound enzymes increases in non-embryonic callus anddecreases in embryonic callus while cytoplasmic activity decreases in non-embryonic callus and increases in embryonic callus.

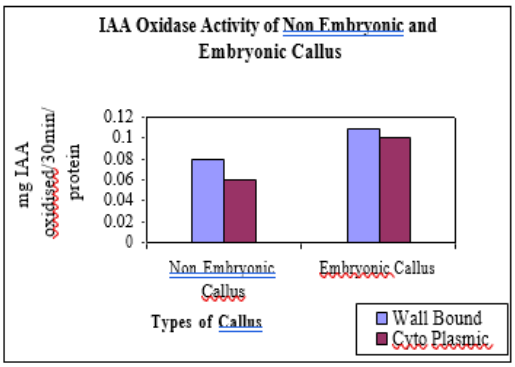
Egertsdotter, 1998 reported difference in the amount of peroxidase between developmental stages of *Piceaabies*somatic embryogenesis. According to Bagnoli et al. (1998) the antioxidant enzymes superoxide dismutase and catalase could be convenient markers to define the developmental stages in *Aesculushippocastanum*somatic and zygotic embryogenesis. Isoenzymepatterns of peroxidase and esterase were shown to reflect the embryogenic potential of*Medicago sativa* and *Dactylisglomerata*suspension cultures (Hrubcovaet al. 1994). Thus, the present study indicates that the process of somatic embryogenesis is characterized by some biochemical changes induced by plant growth regulators.

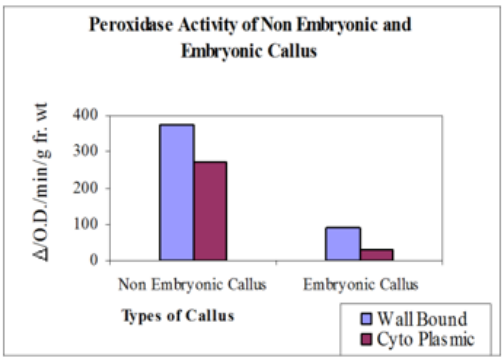
Table 1: Effect of 2,4-D and kinetin on callus induction

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

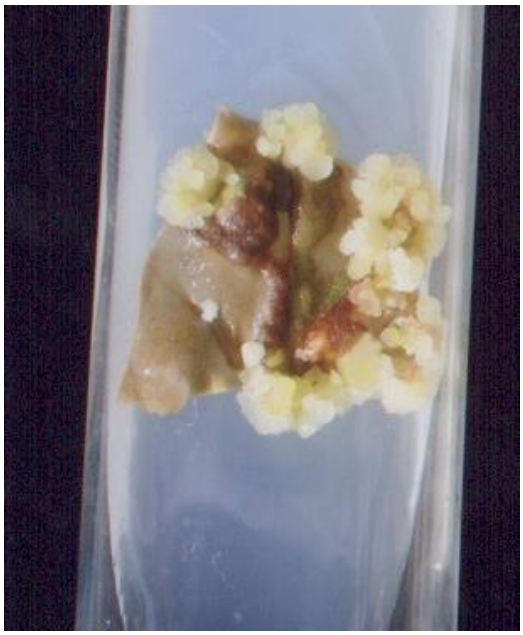








1:1 Kin:2,4-D 2:2 Kin:2,4-D



4:4 Kin:2,4-D 5:5 Kin:2,4-D

CONCLUSION

Somatic embryoids of *C. phlomidis* were obtained at higher concentration of 2,4-D and kinetin (4-5 mg/l 2,4-D : Kinetin).Biochemical (Enzymes) are very effective to determine embryonic and non-embryonic callus even after just first week of cultures physiological conditions of embryonic and non-embryonic callus is different so the enzymes and its activity rate is also vary in the system. Peroxidase and polyphenol oxidase (both wall bound and cytoplasmic)

activity increases in non embryonic callus and decreases in embryonic callus. IAA oxidase and Protease (both wall bound and cytoplasmic) activity decreases in non-embryonic callus and increases in embryonic callus. On the basis of the above study enzymes could serve as a biochemical marker to determine the embryonic and non-embryonic callus at very early stage.

SUMMARY

The results obtained in this experiment shows that the latex of *Calotropis procera* (Aiton) Dryand. is a good source of many phytochemicals. It can be inferred that the various medicinal and pharmacological properties of this plant is due to the presence of latex throughout the plant [15]. Some of the phytochemical groups showed their presence in the methanolic extract whereassome displayed their presence in the aqueous extract. To confirm the presence of these phytochemicals further evaluation is needed. Methods like HPLC and HPTLC can further help inverification of the occurrence and in finding the exact amount of the phytochemicals present in the extracts [15].

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