ABSTRACT

The present investigation involves a comparative study on the production, purification and characterization of some metabolites (lectins, sweetners and sterols) in *in vitro* and *in vivo* tissues of *Abrus precatorius* (Family: Fabaceae; common name: Jequirity bean). Special emphasis was given on the *in vitro* lectin synthesis.

Conditions for callus culture, cell suspension culture and regeneration of plantlets have been established using two variants of the plant having Cream/Brown and Red/Black seed coats. A modified MS medium (MMS) performed the best for callussing and side shoot regeneration from hypocotyl and stem explants in presence of 1.5 - 2.0 mg/L of 2,4-D and 2.0-3.0 mg/L BA respectively. Neo-bud from callus was obtained in MMS with BA. Rooting could be induced only in presence of 0.1 mg/L IBA. The plantlets exhibited 80% survival in the field. Suspension culture was possible with friable callusses derived from cotyledon, stem and hypocotyl. In suspended cells maximum lectin yield of 3395 μg/gm cells was obtained in 2,4-D containing medium (1.0 mg/L). About 11.7% lectin was released in the medium under this condition. Lectin release in the medium was maximum of 81% in BA grown cells, however, the yield was as low as 1660 μg/gm of cell. a comparison of lectin content per gm dry weight of callus and seed revealed that the amount is close to each other (for example AB_7 seed yields 33 mg and its hypocotyl callus produced 30 mg). Subculturing of *Abrus* callus into fresh media showed better result than prolong culture in the same medium, however it was so upto 3rd/4th passage and after that the growth rate as well as lectin content in callus decreased. This tissue culture study of *Abrus precatorius* possibly constitutes the first report on the subject.

The purification of isolated lectins were done by lactamyl-Sepharose affinity chromatography and a cell wall matrix (of carrot callus) specially developed during this investigation. The binding capacity with *Abrus* agglutinin

was calculated to be 0.14mg/mg of lactamyl-Sepharose and 0.084 mg/mg of the cell wall matrix. From 100 gm AB_5 callus tissue (fresh) about 270 mg total lectin was obtained after affinity chromatography. Addition of lactose in the extraction buffer resulted in 5 fold lectin recovery from callus tissue. HPSEC analysis and PAGE revealed the lectins from both *in vitro* and *in vivo* tissues have similar molecular weights close to 132 kDa. The HAU/mg of callus lectin in general was lower than that of the seed (eg. AB_5 seed: 2667 HAU/mg; AB_5 hypocotyl callus: 533 HAU/mg) where as the toxicity compared well with each other (IC_{50} of AB_7 seed against $SP_{2/o}$ cell line 4.0 ng/ml; AB_7 hypocotyl callus 4.2 ng/ml).

FITC-conjugated antilectin antisera has been utilized successfully in the localization and quatification of lectins in various tissues and organs of the plant by using immunofluorescence and image analysis techniques. The quantitative data did not differ significantly with those of ELISA. The method can be extended for rapid localization and quantification of various other metabolites in different tissues of plants.

Apart from the lectins, the *in vitro* grown tissues also produced other costly metabolites such as the sweetner-Abrusosides and sterols (β -sito; stigma and campesterol).