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Lectin biosynthesis in callus culture established from seeds of *Canavalia virosa*

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Abstract

Plant tissue cultures established from isolated embryos or cotyledons were used to investigate the lectin biosynthesis in *Canavalia virosa*. The lectin (CVL) was detected by double immunodiffusion and hemagglutination tests. CVL was present in all callus cultures. The stem and leaf of the plantlet generated from the embryo also contain CVL, but no CVL was detected in the roots. As compared to mature seeds, callus derived from cotyledon of immature seeds exhibited the largest CVL content.

Additional key words: hemagglutination, immunodiffusion.

Introduction

Lectins, carbohydrate binding proteins, are commonly found in seeds of *Leguminosae* and accumulate during seed maturation. Concanavalin A (Con A) from the seeds of *Canavalia ensiformis* is one of the most investigated lectin (Cunningham *et al.* 1986). Analogous lectin have been isolated from other *Canavalia* species (Hague 1975, Terada *et al.* 1987, Yamauchi *et al.* 1989, Kojima *et al.* 1991). Recently, we have purified a new lectin (CVL) from the seeds of *Canavalia virosa* and the amino acid sequence was elucidated (Fujimura *et al.* 1993, Terada *et al.* 1993). CVL is highly homologous to Con A with the same circular permutation and amino acid substitution occurring at residues other than those for the binding site of carbohydrates and metal ions (Terada *et al.* 1993). There have been few reports in the literature of efforts to produce lectin in *in vitro* cultures. Del Campillo *et al.* (1981) detected soybean agglutinin (SBA) like materials on the surface of soybean callus cells. Meimeth *et al.* (1982) have reported the production of lectin in differentiated callus tissue of *Psophocarpus tetragonolobus* roots. Using

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Abbreviations: ConA - concavalin A, CRM - cross reaction material; CVL - *Canavalia virosa* lectin; SBA - soybean agglutinin.

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radioimmunoassay James *et al.* (1985) have localized a lectin like cross reactive material (CRM) in the cell wall and cytoplasm of callus cells of *Dolichos biflorus*.

In the present study we report the synthesis and distribution of lectin in *Canavalia virosa* cultivated *in vitro*.

Materials and methods

Plants: *Canavalia virosa* plants were grown in the experimental farm of our institution and the seeds were grown heterogeneous batches collected in the field.

Culture media: Murashige and Skoog (1962, MS) medium was used as the basal medium. The pH was adjusted to 5.8, sterilized in an autoclave for 15 min at 120 °C and then the filter sterilized hormones were added: 2 mg dm^{-3} naphthalene acetic acid (NAA) or 2 mg dm^{-3} 2,4-dichlorophenoxyacetic acid (2,4-D), along with 0.1 mg dm^{-3} kinetin. The solid media were prepared by incorporating 0.8 % agar.

Callus culture: The seeds were surface sterilized by alternate 3 min exposure to 70 % ethanol, sterile deionized water and 1 % NaOCl and washed three times in sterile deionized water. The cotyledons and embryos were separated aseptically in a laminar flow chamber. Explants (1 mm) taken from hypocotyl and embryo were placed on a *Whatman No.1* filter paper in a tube with culture media. The resulting calli were grown at 25 °C using 24 h photoperiod of low fluorescent light (25 - 50 μmol m^{-2} s^{-1}) and subcultured every 4th week.

Tissue extraction: Calli obtained 4 weeks after proliferation were frozen in liquid N_2, ground to a powder with a mortar and pestle and then incubated for 2 h at 4 °C, in the presence of extraction medium consisting of 0.1 M potassium phosphate (pH 7.2), 0.1 M isoascorbic acid, 2 mM reduced thioglycolic acid, and 0.1 mM phenylmethylsulfonyl fluoride. 3 cm^3 of medium was used per 1 g of tissue. The extract was centrifuged at 27 000 *g* for 20 min at 4 °C. The pellet was washed two times with the same amount of the extraction medium. The supernatant and the two washes were dialyzed overnight against phosphate buffered saline (PBS) containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$.

Analysis of extract: The lectin content was determined using hemagglutination and immunochemical methods. The hemagglutination assay was performed in 96 well microtitre plate using rabbit red blood cells as previously (Jayavardhanan and Panikkar 1991). The antisera to CVL was obtained by immunizing rabbits with CVL and double immunodiffusion was performed according to the method of Ouchterlony (1948) using 0.8 % agarose and 0.075 M sodium barbitone buffer, pH 8.6 containing 1 mM EDTA. After diffusion for 48 h in humidified chamber, the gels were washed in saline and distilled water, dried and stained with Coomassie brilliant blue R.

Results

The callus cultures used for this investigations were established using fragments of embryo and hypocotyl from mature and immature seeds. The callus initiation of *Canavalia virosa* was found to be the best in MS medium with 2 mg dm^{-3} 2,4-D and 0.1 mg dm^{-3} kinetin. After 4 weeks of culture, *Canavalia virosa* cotyledons developed as green compact calli (Fig. 1B, C). The embryo placed in MS medium containing kinetin and NAA, formed plantlets with well developed roots and leaves with clearly identificable epicotyl and hypocotyl regions (Fig. 1A). The hypocotyl placed in MS medium without growth hormones, formed calli smaller than those found in presence of hormones.

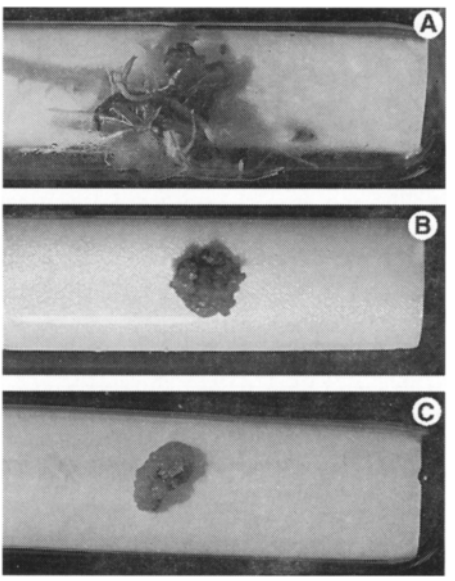

Fig. 1. *In vitro* culture of *Canavalia virosa*: (*A*) 30 d old *C. virosa* plantlet grown on MS medium containing 2 mg dm^{-3} NAA and 0.1 mg dm^{-3} kinetin from isolated embryo. Dense callus developed in MS medium containing 2 mg dm^{-3} 2,4-D and 0.1 mg dm^{-3} kinetin after 30 d from cotyledon of (*B*) mature seed and (*C*) immature seed.

The synthesis of CVL in the cultured tissues were analyzed using hemagglutination and double immunodiffusion assays (Table 1, Fig. 2). After 3 weeks of culture, lectin was detected in the extracts of calli obtained from cotyledons. This could be observed until 3 months of culture. The hemagglutination titre observed in the extract of callus obtained from immature cotyledons was higher than in the extract of callus from mature cotyledons. Small amount of lectin was also detected in the culture medium. Both callus extracts also showed positive reactivity in the immunodiffusion plate, forming total immunoidentity arcs with CVL toward anti-CVL antibodies (Fig. 2). Similar results were obtained with callus grown in absence of growth hormones. Positive immunoreactivity was also observed in

extracts of the stem and leaves of plantlets grown in MS medium in presence of kinetin and NAA for 30 d. But these extracts showed negative reaction in hemagglutination assay. No lectin was detected in roots of plantlets. Moreover, in the plantlets from embryo grown in absence of growth hormones no lectin was detected.

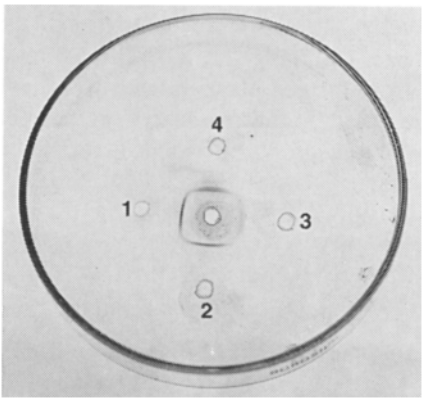

Fig. 2. Ouchterlony immunodiffusion pattern of lectin tested against anti-CVL antibodies. Centre well: anti-CVL IgG, and samples of 30 d old callus obtained from cotyledon of (*1*) immature seeds (*2*) mature seeds and the samples of stem (*3*) and leaf (*4*) of 30 d old plantlet obtained from isolated embryo.

Table 1. Distribution of CVL in the tissue extracts of *C. virosa* cultured *in vitro*.

Explant	Medium	Tissue extract of	Time [d]	Hemagglutination titre	Immunoreactivity
Cotyledon (immature)	MS + 2,4-D + kinetin	calli	20	8	+
			30	32	++
			60	32	++
			90	16	++
		medium	90	2	+
Cotyledon (mature)	MS + 2,4-D + kinetin	calli	20	4	+
			30	16	++
			60	16	++
			90	8	+
		medium	90	2	+
Embryo	MS + NAA + kinetin	plantlets	30	-	++
		stem	30	-	++
		leaves	30	-	+
		roots	30	-	-
Embryo	MS	plantlets	30	-	-
		stem	30	-	-
		leaves	30	-	-
		roots	30	-	-

The tissues were then transferred to fresh media and after 4 weeks of subculture, sufficient quantities of callus were produced enabling the assay of lectin in more free callus cells separate from the explant tissue. But the lectin was not detected in the subcultured tissues both by hemagglutination and immunochmical assays. Further successive subculture also resulted to the absence of lectin not only in the callus tissue but also in the medium.

Discussion

Plant tissue culture may provide a useful experimental system for studying the regulation and synthesis of lectins. The present investigation has shown that the CVL is produced in the primary callus cultures derived from hypocotyl region of mature and immature seeds. It was also present in the culture medium. These cultures were initiated on a medium supplemented with 2,4-D and kinetin. The direct extraction in PBS is unable to solubilize the lectin. Extraction after sonication in presence of 0.5 % Triton and 0.5 M NaCl yielded good quantity of lectin indicating strong immobilization of it in the membrane. In *C. obstucifolia* callus cultures, Gosh *et al.* (1985) failed to detect lectin even after several passages of subculturing. The CVL is also detected in the extract of whole plantlet generated in a medium contained kinetin and IAA, forming immunoreactivity with anti-CVL. The amount of lectin in the plantlet may be less and the lectin tightly bound to the membrane, which may be the reason for negative hemagglutination reaction. No lectin was detected in the roots of these plantlets; but CVL is present in the roots of the plant, developed from seeds in the field conditions. The plantlet grown in absence of growth hormones developed no lectin which suggests the involvement of hormone in lectin biosynthesis during organogenesis.

In the subsequent culture of callus derived from hypocotyl, no lectin was detected not only in the medium but also in the callus extracts. Borrebaeck and Linsefors (1985) and Raikhel *et al.* (1986) have observed that the production of lectin in callus culture is hormone dependent. The callus derived from immature wheat embryo grown in presence of 10 μM abscisic acid exhibited the largest increase in the lectin compared with material grown on other media (Raikhel *et al.* 1986). But Jones *et al.* (1967) detected no lectin in calli derived from *Maclura pomifera* and Owens and Northcote (1981) found no lectin in potato callus grown at different hormonal combinations. The failure to detect CVL after subsequent passages may indicate that these cultures do not contain the appropriate enzymes required for the formation of lectins. The presence of lectin in the primary culture may be due to the reason that the callus cells may utilize these limited enzymes from the explant itself for the production of lectin. These enzymes may be more abundant in immature seeds and so the callus generated from them may synthesize higher quantity of lectin than that from mature seeds. This indicate the involvement of lectin in cellular events during seed maturation. Detailed analyses of the subcellular localization and development should be of value for studying the regulation and biosynthesis of lectins during seed maturation.

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