LECTIN CONCANAVALIN A DISTRIBUTION AT DIFFERENT STAGES IN THE TISSUES OF *CANAVALIA GLADIATA*

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ABSTRACT

The distribution of Lectin Concanavalin A (Con A) at different ages in the tissues was studied in the plant *Canavalia gladiata*. The distribution of Con A was investigated in the root, stem, leaf, cotyledonary leaf, cotyledon, embryo, seed coat, fruit coat, flower, immature fruit, callus tissues etc with the help of double diffusion immunoprecipitation assay. It was found that Con A was present in relatively large quantities in the tissues of cotyledon and embryo. Small amount of Con A was detected in the epicotyl and hypocotyl at the early stages. The quantity gradually declined in cotyledon as the growth of seedlings advanced.

The lower content of the lectin in several tissues, by normal buffer extraction procedure might be due to its immobilisation in the membranes and enzymatic fragmentation. The fragmented parts may escape conventional assay systems.

INTRODUCTION

THE distribution of lectins within the same plant and its interrelationship in respect to site of origin may provide some meaningful information about their *in vivo* physiological functions. It was believed that lectins were restricted to the seeds and cotyledons alone as they were the main storage sites for proteins. But the available reports confirmed that lectins are present to smaller extent in root, stem, leaf, tuber, fruit and bark[1–4].

It was also shown that lectins with various sugar-binding specificities were present in mitochondria, golgi bodies, endoplasmic reticulum and plasma membrane fractions of mung bean hypocotyls[5]. The presence of β-lectins (with a specificity directed towards β-D-glycopyranosyl linkages) in all above ground parts of some plants and their absence in the underground part, excepting *Rumex obtusifolia*, was reported[6]. No lectin was detected by radio-immunoassay in roots of *Dolichos biflorus* at any stage of development but it was found in leaves, stems, pods, and mature seeds[7]. Pistil of *Primula* contains phytohemagglutinin[8]. Soybean lectin (*Glycine max*) was found in all the tissues of young seedlings, but decreased as the plants matured and could not be detected in plants after 2 to 3 weeks[9]. Hemagglutinating lectins have been reported from the sieve tube sap of *Robinia pseudoacacia*[10] and in the latex of *Synadenium grantii*[11].

The distribution and quantification of Con A have been investigated from morphogenetically recognized tissues, starting from germination through organogenesis upto the age of seed maturation.

MATERIALS AND METHODS

The chemicals used for these experiments were of analytical grade. The antisera against the purified Con A were prepared with Freund's adjuvant in rabbits separately by intermuscular injections specially near the lymph nodes. Before immunisation with lectin normal serum was collected and preserved as the control. Con A was obtained from V. P. Chest Institute, New Delhi, India.

Collection and Sampling: The seed surface was sterilized with 0.5% mercuric chloride and then washed thoroughly. The seeds were allowed to germinate and then grown in earthen pots in green house at 28° to 32°.

The samples of various stages of growth were serially collected from about 5 to 20 seedlings. The 24 hr soaked seeds in glass distilled water were dissected into embryo, cotyledon and seedcoat (testa). Similarly 7, 15, 30 and 90-day old plants were dissected into cotyledonary leaf, leaf, stem, root, mature fruit coat (pericarp and mesocarp), fruits and seeds of different ages were collected. The roots were washed with distilled water and blotted and weighed immediately. The callus tissues from *C. gladiata* using 30-day old hypocotyl as explant were grown in Murashige and Skoog medium[12].

Extraction: The respective tissue samples were homogenized with mortar and pestle. The extraction was made with tris-buffered saline (0.05 M Tris-HCl, 1 M NaCl, pH 7.4, containing 1 mM each of Ca^{2+}, Mg^{2+}, Mn^{2+} ions). The homogenates were centrifuged at

13 000 g for 30 min and the process repeated again with the residue. The final volume of the extraction was kept in an approximate ratio of 1:3 (fr wt/vol) with the same buffer. Then the supernatant solutions were dialysed thoroughly against TBS (0.02 M Tris-HCl, 0.15 NaCl, pH 7.4 containing 1 mM each of Ca^{2+}, Mg^{2+}, Mn^{2+} ions) with atleast 6 changes of buffer.

Protein estimation: The protein content of each of the samples was estimated following the method of Lowry *et al*[13].

Immunoprecipitation assay: The lectin was detected in bacto-agar gel plates in presence of antisera. The serial double dilution of both the samples and the antisera were done and sometimes the dilute protein samples were concentrated with aquacide IIA of Calbiochem to get proper titres for precipitation bands[14]. The qualitative precipitation assay was performed with all the samples following the method.

RESULTS

A time-bound sequential collection of tissues were made from the plant *C. gladiata* to detect lectin Con A. The detection and distribution of the lectin in the different tissues at different times were studied mainly by Ouchterlony immunoprecipitation method[14].

The mature seed soaked for 24 hr showed positive test for Con A in cotyledon and embryo. The lectin was absent in the seed coat and protein content was also low. The cotyledon and embryo showed good amount of extractable soluble protein *i.e.* 51.50 and 44.82 mg per g of fresh weight respectively (table 1).

The root showed the absence of Con A throughout the stages in 7- to 90-day old plants. The lectin Con A was detected marginally in the 7- and 15-day old stem (epicotyl and hypocotyl) but was found absent onwards in 30- to 90-day old plants. The cotyledonary leaf of 7- to 15-day old showed presence of Con A but at later stages when 30- and 90-day old showed absence of Con A. The leaf showed absence of Con A throughout these stages (plants of 7- to 90-day old).

After several times of subculturing from the tissue, the callus obtained from 30 day old hypocotyl, showed absence of Con A.

The tissues of root, stem, leaf of 90-day old showed absence of Con A. The protein content of the leaf was reasonably high but Con A was absent.

Con A could not be detected in the young flower bud, mature flower, fruit and tiny seed obtained after 90 days. During early stages of growth of the seed, lectin Con A's appearance in the tissues could not be noted. Lectin was detected in the cotyledon of the seed when pods were 22 cm in length (table 1).

Table 1 *The detection and distribution of lectin in the tissues of Canavalia gladiata at different ages of life cycle by agar gel immunoprecipitation method*

Tissues	Age after soaking	Assay by immuno-precipitation	Total soluble protein in mg/g fr. wt.
Seed coat	24 hr soaked	–	0.880
Cotyledon	"	+	51.500
Embryo	"	+	44.820
Root	7 day	–	4.610
Stem (Epicotyl & Hypocotyl)	"	±	5.520
Cotyledonary leaf	"	+	35.150
Leaf	"	–	8.955
Root	15 day	–	5.350
Stem (Epicotyl & Hypocotyl)	"	±	9.220
Cotyledonary leaf	"	+	23.500
Leaf	"	–	14.180
Root	30 day	–	4.600
Stem	"	–	10.440
Leaf	"	–	17.870
Hypocotyl callus tissue	"	–	4.720
Root	90 day	–	2.550
Stem	"	–	8.252
Leaf	"	–	22.225
Flower	Bud and mature	–	7.655
Fruit coat	Mature	–	2.620
Whole fruit	6 cm long	–	0.210
Tiny seeds	Of 14 cm long pod	–	0.185
Fruit coat	"	–	2.400
Seed coat	Of 22 cm long pod	–	3.120
Cotyledon	"	+	28.500
Fruit coat	"	–	2.550

(+) Positive, (–) Negative, (±) Ambiguious

DISCUSSION

The seeds are the rich source of reserve proteins. In contrast to other parts of a plant the proteins, confined primarily to the cotyledons, are present in large amount in most cases and gradually degrade with increase of age of the plant. The pattern of decline in the amounts of lectin in *C. gladiata* cotyledons (table 1) is identical to the observation of Rouge[15,16] in the

cotyledons of *Lens culinaris* and *Pisum sativum*.

As expected, large amounts of lectin and other proteins were confined mainly in the cotyledons, but they were also found in the embryo (table 1). In the seed coats of *C. gladiata* lectin Con A was absent. However, the presence of negligible amount of lectins in the stem of *C. gladiata* was observed at the initial stage which also disappeared after 15 days. It is interesting to note that total protein contents in the developing organs namely stem and leaf consistently increase with time but the lectin might not be produced adequately.

The total protein in leaf tissue of *C. gladiata* is gradually increased in contrast to root and stem. The low levels of lectin was found in the stems and leaves of the plant *Dolichos biflorus* at all stages of its development that cross-reacts with antibodies against its seed lectin[7]. In table 1 the results of cross reactions of Con A with the extracts of roots, stems, leaves and flowers of *C. gladiata* are presented.

The absence or weak presence of lectin in the stem consisting of epicotyl and hypocotyl of 7- and 15-day old tissues might be due to strong immobilisation in the membranes and partial enzymatic breakdown. The fragmented parts are less effective to form matrix for immunoprecipitation reaction. The dialysed fragmented parts of lectin escape easy conventional method of detection. This assumption is supported when it was found that the lectin is undetected in roots, stems etc. No detectable activity was found in 2-week old soybean root and other tissues either in the inital soluble extracts or in the extracts after 0.1 M galactose extraction. When the extracted pellets (cell wall, membranes etc) were reextracted after sonication in presence of 0.5 % Triton x-100 and 0.5 M NaCl yielded good quantity of lectin[5,17] indicating strong immobilisation in the membranes.

The callus tissues of hypocotyl after several passages of subculturing showed the absence of Con A. From this initial finding, it showed that callus had no totipotency for the production of mRNA for Con A. It might happen that small amounts of Con A synthesised in the callus tissues are deeply embedded in the membrane and do not easily come in normal buffer extraction method without detergent[17].

The seat of synthesis of lectin is not yet clear. Lectins are found in sieve tube[10] and latex[11] indicating that they are transported from the seed to the developing plants. The presence of lectins in the newly developed tissues of the matured plants might be due to some fresh mRNA synthesis in tissues and subsequent translation. The study on cross-reactions of seed protein antibody and the leaf extract of *Phaseolus vulgaris* showed partial identity[18]. In the light of these it may be assumed that the lectin and fragmented lectin having one binding surface in these extracts, could not be detected due to limitations in the assay system.

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1. Toms, G. C. and Western, A., In: *Chemotaxonomy of Leguminosae*, (eds) J. B. Harborne, D. Boulter and B. L. Turner, Academic Press, New York, 1971, p. 367.
2. Callow, J. A., *Curr. Adv. Plant Sci.*, 1975, **7**, 181.
3. Ghosh, B. N., *Phytolectins*, Ph.D. thesis, Univ. of Calcutta, 1980.
4. Ghosh, B. N., Dasgupta, B. and Sircar, P. K., *Indian J. Biochem. Biophys.*, 1981, **18**, 166.
5. Bowles, D. J. and Kauss, H., *Plant Sci. Lett.*, 1975, **4**, 411.
6. Jermyn, M. A. and Yeow, Y. M., *Aust. J. Plant Physiol.*, 1975, **2**, 501.
7. Talbot, C. F. and Etzlor, M. E., *Plant Physiol.*, 1978, **61**, 847.
8. Golynskaya, E. L., Bashkirova, N. V. and Tomchuk, N. N., *Plant Physiol.*, 1976, **23**, 169.
9. Pueppke, S. G., Bauer, W. D., Keegstra, K. and Ferguson, A. L., *Plant Physiol.*, 1978, **61**, 779.
10. Kauss, H. and Ziegler, H., *Planta (Berl)*, 1974, **121**, 197.
11. Premaratna, A., Shadaksharaswamy, M. and Nanjappa, S., *Indian J. Biochem. Biophys.*, 1981, **19**, 32.
12. Murashige, T. and Skoog, F., *Physiol. Plant*, 1962, **15**, 473.
13. Lowry, D. H., Rosebrough, M. J., Farr, A. L. and Randal, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
14. Ouchterlony, O., *Acta Pathol. Microbiol. Scand.*, 1948, **25**, 186.
15. Rouge, P., *C. R. Acad. Sci. Paris. Ser. D.*, 1974, **278**, 3083.
16. Rouge, P., *C. R. Acad. Sci. Paris. Ser. D.*, 1975, **280**, 2105.
17. Barondes, S. H., *Ann. Rev. Biochem.*, 1981, **50**, 207.
18. Mialonier, G., Privat, J., Monsigny, M., Kahlem, G., Durand, R. and Welter, M. L., *Physiol. Veg.*, 1973, **11**, 519.