LECTINS: ARE THEY INVOLVED IN RECOGNITION OF PARASITES BY PLANTS?

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ABSTRACT

Lectins, sugar binding proteins, present in a large number of plants and in a few microorganisms, have been implicated in the recognition of plant parasites. Evidences on *Rhizobium*-lectin binding clearly reveal the non-specificity of plant lectins in binding *Rhizobium*. Many observations are not compatible with the lectin theory on recognition. It fails to explain the gene-for-gene relationship of host-parasite interaction. Absence of lectins in a few leguminous plants but having the ability to be nodulated by rhizobia does not support the lectin theory. Recent investigations on molecular basis of host specificity and nodulation in rhizobia indicate the involvement of extrachromosomal genetic elements. The role of toxic substances—prohibitins and phytoalexins—present in plants and produced by them in recognition is emphasized.

PLANTS harbour a rich reserve of resident microflora including potential pathogenic microorganisms. They discriminate the infectious and non-infectious stimuli and respond immediately and rigorously to ward off the infection. It is this recognition that has generated fascinating and impressive research activity in the last few years. In this review we have examined the recent developments on the question of recognition.

Much research has been done on the lectins of plants and their capacity to nodulate rhizobia. Lectin is a sugar binding protein of non-immune origin[1]; it is widely distributed in plants[2-4] and in microorganisms[5-7].

According to Callow[8], lectins are involved in the recognition of plant parasites. Sequeira[9] could not reach any conclusion but emphasized the lectin-polysaccharide interaction as an avenue for experimentation. Much of the factual data on lectin is derived from *Rhizobium*-host interaction[10-12]. A careful perusal of the literature on *Rhizobium*-lectin binding as a basis of recognition of specific *Rhizobium* sp., by leguminous plants raises several gasping questions.

Concanavalin A (ConA) from jack bean *(Conavalia ensiformis)* and lectin from soybean combine strongly with both nodulating and non-nodulating strains of *Rhizobium* (table 1). The lectin is bound to cells of all the *rhizobium* strains regardless of their nodulating ability on jackbean[13]. Failure of binding by strains of *R. phaseoli* and *R. japonicum* to the lectins from their hosts revealed the absence of specific reaction in *Rhizobium*-lectin interaction[14].

Soybean nodulating strains of *R. japonicum* 11OY, 61A76 failed to bind detectable lectin but these nodulated Harosoy 63 soybean[15]. A few soybean nodulating strains of *R. japonicum* nodulated peanut plants but did not bind peanut lectin. Similarly peanut nodulating strains of *Rhizobium* which nodulated soybeans did not bind soybean lectin. Further, the strains which were bound to soybean did not nodulate soybean[16]. Chen and Phillips[17] by using fluorescein isothiocyanate labelled lectins, found that nonspecific pattern of binding existed

TABLE 1

Binding of fluorescein isothiocyanate-Con A to strains of species of Rhizobium[13]

Rhizobium sp.,	Strain	Host	Reaction*
R. trifolii	2S	*Trifolium*	4^+
	2L(nn)		4^+
	0403		4^+
	Bart A(nn)		4^+
	0435		4^+
	0435-2(nn)		4^+
	WU 290-I		4^+
	WU 290-N(nn)		4^+
	T37		4^+
	Bio-9(nn)		4^+
R. leguminosarum	3HOQ1	*Pisum*	3^+
	3HOQ51		3^+
R. japonicum	311661	*Glycine*	4^+
Rhizobium sp.	1	*Aesehynomenal*	3^+
	227	*Vigna*	3^+
	229		3^+
	35	*Lotus*	1^+
	127E10	*Phaseolus limensis*	3^+
	22A1	*Canavalis ensiformis*	3^+

*1 to 4^+.....increasing intensities of fluorescence; 127E10 and 22A1 : nodulate jack bean; nn : non-nodulating.

between host plant lectin and bacteria which normally infect and nodulate legume. The rhizobia bound to pea roots although only *R. leguminosarum* 128 C53 did successfully infect the plant.

Soybean is a promiscuous nodulating legume that produces nodules when inoculated with *Rhizobium* spp., isolated from *Cajanus cajan, Centrosema pubescens, Crotalaria intermedia, Macrophilium uniflorum* and *Macrotyloma africanum*[18]. If one believes in lectins as a recognition factor, one can deduce that soybean lectin has a common factor that can recognize and bind all the strains of *Rhizobia*. Alternatively all the strains may contain a common factor that recognizes soybean lectin specifically and their respective host plant lectins. But soybean lectin displayed very limited agglutinating capacity; it precipitated exclusively exopolysaccharides of *R. japonicum*, o-antigen containing lipopolysaccharides of *R. leguminosarum, R. lupini, R. meliloti, R. phaseoli* and cow pea *Rhizobium*[18]. But ConA precipitated the exopolysaccharides of all fast growing *Rhizobium* spp., and lipopolysaccharides. Certainly there is no correlation between polysaccharide binding and symbiotic specificity. This was true with pea and lentil lectins which precipitated exopolysaccharides of *R. japonicum, R. leguminosarum, R. lupini, R. meliloti, R. phaseoli* and *Rhizobium* sp., from cowpea[19].

The rjl gene-in soybean prevents nodulation by *R. japonicum*. But several strains nodulate rjl plants in vermiculite and sand culture[20]. When a pure culture of one of the nodulating strains (61 Nal R) and a strain reproducing the typical non-nodulating response were mixed with rjl plants, 32% of the nodules contained both strains; 36% possessed only usually non-nodulating strain 1-110 ARS and 32% contained the 61 Nal R. Moreover, under

conditions of high inoculum density, roots of Clark rjl plants did not distinguish between *Rhizobium* strains 61 Nal R and 1-110 ARS.

Studies are lacking that demonstrate the competitive binding to root surfaces by nodulating and lectin binding bacterial strains versus non-nodulating and lectin binding strains when they are applied together. In fact, absence of lectin or haemagglutinin in some leguminous plants has been reported[21] (table 2). Further, out of 97 lines of soybeans screened for lectin, Columbia, Norredo, Sorty, T102 and Wilson-5 lacked detectable level, yet these cultivars were nodulated by *R. japonicum*[22].

TABLE 2
Legumes and lectin activity[21]

Legume species	Lectin activity
Amorpha fructicosa	−
Bandeirae simplicifolia	+
Bauhinia purpurea-alba	+
Caragana arborescens	+
Cercis siliquastrum	−
Colutea arborescens	−
Conavalia ensiformis	+
Cytisus multiflorus	+
Dolichos biflorus	+
Genista monosperma	−
Laburnum alpinum	+
Lathyrus latifolia	+
Lens culinaris	+
Lespedeza bicolor	−
Mimosa pudica	−
Phaseolus vulgaris	+
Sophora japonica	+
Spartium junceum	−
Ulex europaeus	+
Wistaria sinensis	+

− = absent; + = present

Lectins do not display any specificity in their reaction. Wheat germ agglutinin, soybean lectin and peanut lectin agglutinated 54 strains of *Neisseria gonorrhoeae*[23]. Agglutination of plant protoplasts by lectins from different plant species, also reveals the absence of specific reaction in lectin binding. Lectins from soybean, peanut, *Bandeitaea simplicifolia* and ConA showed difference in agglutinating corn, lettuce and tobacco protoplasts[24]. Ghosh *et al.*[25] reported that lectins from *Butea monosperma*, *Momordica charantia* var. *muricata*, *Trichosanthes anguina* and ConA agglutinated protoplasts non-specifically from the same plant tissues as well as others.

These apart, modulation in lectin concentration in legume cannot be correlated with nodulation. Old root tissues of soybean did not contain detectable levels of lectin but the highest rate of nodulation occurred in plants infected two to four weeks after sowing[26]. Nodulation occurred in plants with no significant levels of lectins in roots. In groundnut plants lectin level fluctuated; they disappeared in roots after 12 days of growth and had no relevance to nodulation[27].

Bhuvaneswari[11] stated that there is as yet little evidence from studies on many *Rhizobium*-legume systems to indicate that adsorption of rhizobia on host root hairs is quantitatively or qualitatively selective or that it constitutes recognition; and attempts in her laboratory to localize the soybean seed lectin that binds to *R. japonicum* cells on soybean roots using immunofluorescence techniques have been unsuccessful so far. She surmised that the lectin that is involved in recognition of *Rhizobium* is different from the lectin in the seeds. Extensive and critical investigations by Etzler and co-workers[28–32] on *Dolichos biflorus* failed to show the presence of lectin in the roots of the plant throughout its growth. But lectins were confined to cotyledons. In stems and leaves of *D. biflorus* occurred a glycoprotein that cross-reacted with antibodies (CRM) against seed lectin. This CRM associated with cell-wall, increased in concentration due to infection and wounding and was released by treating the cells with cellulase and pectinase. These observations tempted them to describe the

CRM as a functional lectin in plants; and seed lectin may be a degradation product of CRM which is involved in maintaining the integrity of cell-wall in response to stress. The role of seed lectin is attributed to storage reserve rather than to recognition.

Wong[33] pointed out that mere binding of *Rhizobium* strain to legume lectin is no proof of its nodulating ability. The examples cited above support this contention.

Obviously evidences in favour of lectin influencing the specificity of *Rhizobium* spp., in legumes are not only inadequate but confusing. We will agree with Stacey *et al*[34], who stated that the final proof of the specific role of lectin in *Rhizobium*-host interaction must await the isolation and reversion of non-nodulating non-lectin binding mutants.

With plant parasites, lectins bind but display no specificity. Mendgen[35] reported that bean rust *(Uromyces phaseoli)* hyphae bound to bean and non-host cells without any discrimination. Hyphal surfaces of both compatible and incompatible races of *Phytophthora infestans* bound non-specifically to potato lectin. Potato lectin agglutinated *Erwinia* sp., which seldom infects potato. Agglutination of *Erwinia* sp., by ConA and also by potato lectin indicates the non-specific reaction between lectins and microbial polysaccharides. Further, potato tubers exposed to anaerobic condition lost their resistance to *E. carotoyora* which could not be correlated with lectin concentration[36].

What are the functions of lectins to the producers? At the cellular level, ConA and phytohaemagglutinin are restricted to cytoplasmic sites in jackbean and Red Kidney bean cotyledons, respectively[37]. The lectins are concentrated at the membrane binding starch grains, a fact suggesting a role in the organization of starch reserves. Excretion of lectins by roots of bean, corn and sunflower indicated a protecting role as these inhibited chitinous cell-walls of fungi[38].

Conveniently overlooked by enthusiastic lectin researchers are the reports on the presence of lectins in microorganisms. According to Fujita *et al*[5], 4 actinomycetes and 7 aspergilli produced lectin like substances, Takenaka *et al*.[6] screened 373 strains of bacteria for lectin production; 35 produced it. Lectins are produced by *Dictyostelium discoideum*[7]. Do common plant parasites and symbiotic bacteria contain lectin? What is the role of lectins in microorganisms?

Further, recognition can be negated, or manipulated by treatment of plants with inhibitors of protein synthesis[39–41], heat treatment[42,43] and prior inoculation with microorganisms[44–46]. Finally, the lectin theory fails to explain the gene-for-gene relationship of host-parasite interaction.

We believe that the available evidences are certainly inadequate to consider lectins in recognition and specificity of plant parasites, nor in legume-*Rhizobium* interaction.

In recent years, attention has been focused on genetic basis of recognition. Studies on *Rhizobium* sp., revealed that the genes responsible for host recognition, symbiosis, nitrogen fixation are located on extra chromosomal DNA fragments, plasmids. Nodulating ability was transferred among *Rhizobium* spp.[47]. In *R. leguminosarum* and *R. phaseoli,* host specificity was determined by plasmid borne genes[48]. Transfer of a plasmid from nodulating to non-nodulating *R. leguminosarum* strains enabled the latter to produce nodules on peas[49]. Hooykaas *et al*[50] isolated a plasmid from *R. trifolii* which when transferred to *R. leguminosarum* and *Agrobacterium tumefaciens* enabled them to nodulate clover plants. Host specificity, root hair curling, nitrogen fixing ability and some other characters which are necessary for effective nodulation were located on the plasmid, "sym" plasmid. Specific adsorption on *R. trifolii* cells to clover root hairs is plasmid determined[51].

Production of phaseotoxin by *Pseudomonas phaseolicola*[52] and

toxinogenesis in certain strains of *P. syringae*[53] are also plasmid borne. Several plasmids harbour the genes that control the degradation of toxic substances[54-56].

Plasmid borne traits such as host specificity, virulence, detoxification, toxinogenesis and resistant factors in different bacteria clearly suggest the active involvement of plasmids in infection and disease development. We believe that this approach offers exciting opportunities.

Almost three decades ago, Gaumann[57] aptly stated "The parasite does not *discriminates the hosts. It is the host that* discriminates the parasite". To this it can be added that the discrimination is effectively displayed by the biochemical shield, prohibitins[58] and phytoalexins[59].

Certainly the host recognizes the invading organism as foreign. It does not discriminate whether it is compatible or incompatible. It senses the stress and displays a variety of responses. Phytoalexin synthesis represents one of such responses. Oku *et al.*[60] aptly concluded "the determination of specificity depends solely upon the rate of phytoalexin production because phytoalexin synthesis appears to be host-specific".

Convincing evidences in the field of insect-plant relationship reveal the significance of toxic substances produced by plants in determining the specificity of *insects*[61]. *The brucid larvae readily* metabolize L-Dopa. The seeds of genotypes with lower concentration of L-Dopa are susceptible to attack by a wide range of insects and animals but those with high L-Dopa are seldom infected!

Analogous to host-parasite recognition is the interaction between style and pollen. Despite several theories, recently *convincing evidences on the participation of* inhibitory substances in the incompatible interaction have been recognized[62].

Another impressive evidence is the symbiotic relationship in sponges. According to Jakomska and Nigulli[63], antimicrobial substances produced by sponges were the determinants in symbiotic relationship between sponges and bacteria. Bacteria which tolerated the antibiotics survived and developed symbiotic *association!*

Recently Hadwiger and Loschke[64] reported a macromolecule, chitosan, a polymer of B-1, 4-linked glucosamine residue which is present in the walls of many fungi, possesses the potential to communicate regulatory changes between host and fungus. This interesting hypothesis cannot be accepted because several fungi *and bacteria do not contain chitosan*[65].

Admittedly the recognition phenomenon continues to be a Gordian knot and unwinding it poses several frustrating and challenging problems. We should keep in mind that no single mechanism decides the initial recognition. Lectins offered an impressive probe. Now plasmids have opened several exciting possibilities in resolving the recognition problem. And Plant Pathology has at last entered the portals of Molecular Biology.

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