Introduction

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1.1 Rice.

Rice is one of the most important economic plant in Thailand. The cultivation of rice takes up about 9.4 million hectares of land, amounting to 6.6% of the world rice arable land (Swaminathan, 1984).

Rice is an annual grass belonging to the genus of *Oryza* and Gramineae family. The two cultivated rices are i) *Oryza sativa* of Asia, and ii) *Oryza glaberrina* of West Africa. There are many varieties of these cultivated rice.

Over the millienniums, the Asian cultivated species (*Oryza sativa*) differentiated into three subspecies based on geographic conditions. They are indica, japonica (also called sinica) and javanica. A further classification, which emphasizes the habitat in terms of soil and water is rain-fed lowland and upland rice, and irrigated and deep-water rice. Indica rices were originally confined to the humid regions of the Asian tropics and subtropics. Japonicas were cultivated in subtropical temperate zone regions. Javanicas flourished in the equatorial region of Indonesia.

In addition to their adaptation to climate, the three races differ in characteristics of the grain, including the content of amylose (a derivative of starch), the elongation of the grain, the temperature at which the grain become gelatinous and the aroma in cooking.

Rice grows in a diversity of environments almost unparalleled in the plant kingdom. Rice is cultivated in cool climates high in the mountains of Nepal and India and in the hot deserts of Pakistan, Iran and Egypt. It is grown as a dry-land crop in parts of Asia, Africa and Latin America. At the other extreme of cultivation are floating rices, which thrive in floodwaters 1.5 to 5 meters deep in parts of Bangladesh, Burma, eastern India, Thailand and Vietnam. Rice has an efficient system of air passage from shoot to root that makes it adaptable to a wide range of environmental conditions. This system enables rice to grow in waterlogged soils. Air enters the plant through the stomata of leaf blades and leaf sheaths and moves to nodes at the base of the plant. As the air moves from the shoot to the root, oxygen is supplied to the tissues, where it is utilized for respiration. At the

roots, the air diffuses into the surrounding soil. In rice, the efficiency of oxygen transport from the shoot to the root is ten times greater than it is in barley and four times greater than it is in maize (Raalte,1940).

1.2 Environmental condition in flooded rice soils.

Waterlogging occurs in flooded rice fields, and may cause several environmental changes. However, the most important factor affecting the microflora is probably the influence of water on the state of aeration of the soil. As gas moves over 10 thousand times faster through a gas phase than through a liquid one, the capacity of soil to exchange gases with the atmosphere will decrease as the soil becomes water saturated. Waterlogging of soils will, therefore, result in a deficit of oxygen, and if prolonged, it could lead to strongly reducing condition. Water in flooded rice field is kept moving over the soil and away into ditches at the sides of the fields. In such a manner, a full anaerobic condition will not be obtained and two different layers of soils will be finally established (Fig. 1). In the uppermost layer, which is a few

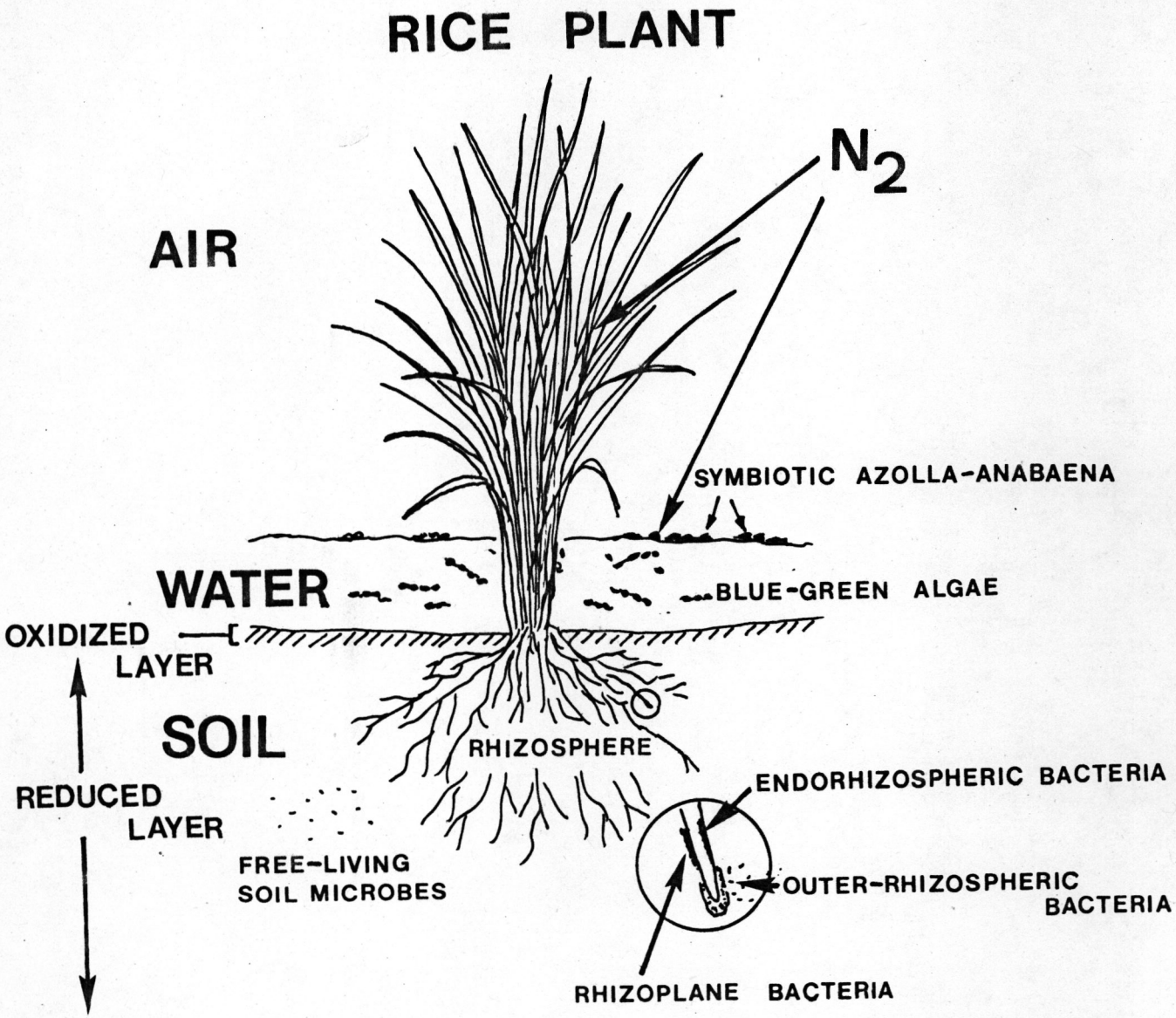

Figure 1 Biological nitrogen fixation in wetland rice ecosystem

centimeters in thickness and deep brown in color, an aerobic and oxidative processes predominate. Below this oxidized layer, a bluish-grey or greyish-black horizon is found, in which an anaerobic condition and consequently reduction processes prevail.

The amount of soil water also affects the temperature of the soil, since the specific heat of water (1 cal/g/^{o}C) is higher than dry soil (0.2 cal/g/^{o}C). Waterlogged soil warms up slowly and never attains a temperature as high as that of dry soil. The lower soil temperature will consequently cause changes in the rate and even the type of metabolism of soil microflora.

The presence of rice plants in flooded soil creates special environmental conditions for the microflora. Whereas at a depth of a flooded soil, the condition will generally be anaerobic, the penetration of rice roots into such a soil will alleviate the anaerobiosis partially, since along every root oxidation occurs. The blue ferrous iron is oxidised to ferric one, which is precipitated around the roots, giving them a brown color commonly observed in rice fields (Yoshida and Ancajas, 1973).

From the preceeding description, it can be concluded that flooded rice fields are very complex habitats for microflora. In the upper layer aerobic conditions predominate, whereas in the lower layer the conditions are predominantly anaerobic, intercepted with micro-habitats of aerobic environment surrounding the roots.

Flooding the soil has many advantages for rice production : i)it provides a continuous water supply to the crop, ii)it changes the pH of alkaline and acidic soils towards neutrality or slight acidity which is favorable for rice growth, iii)it diminishes the incidence of soil pathogenesis and outbreak of soilborne diseases usually observed under continuous monocropping in upland soils, iv)it depresses weed growth, v)it favors biological N_2-fixation, giving flooded soils a higher spontaneous fertility than upland soils (Barraquio *et al*, 1982), vi)irrigation water supplies nutrients such as Ca, Si, and K, and vii)bounded rice fields act as water reservoirs and prevent soil erosion.

1.3 Biological nitrogen fixation in lowland rice.

Nitrogen is a key element of proteins and nucleic acids upon which all life is based on, thus it is an indispensable component of simple organisms such as bacteria as well as complex organisms like higher plants and animals. The available form of nitrogen for plants are ammonium-nitrogen and nitrate-nitrogen. Plant gains the available nitrogen either from nitrate, ammonium and urea fertilizers, or from several natural processes so-called biological nitrogen fixation (BNF) via enzyme nitrogenase which catalyzes reduction of N_2 to ammonia.

Nitrogen fixation in the root zone is possible only if nitrogen gas is present. Rice plants have long been known to contain a transporting system whereby atmospheric nitrogen is supplied to the roots. Atmospheric nitrogen fixation in the rice rhizosphere was determined *in situ* conditions by growing flooded rice in the atmosphere of $^{15}N_2$, and fixed nitrogen was found to be translocated into the rice plant tissues (Yoshida and Yoneyama, 1980; Ito *et al* , 1980; Eskew *et al*, 1981). Rice plants at the heading and flowering stages have the maximum rate of nitrogen gas

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transportation which corresponds to the greatest nitrogen fixation of the rhizosphere in excised roots (Yoshida and Ancajas, 1973; Yoshida and Broadbent, 1975). The ability of rice to transport atmospheric nitrogen gas to the root zone is also suggested by the observation that planted rice soils contained more nitrogen gas than unplanted soil (Yoshida *et al*, 1975).

1.3.1 Evidence and quantification of BNF in lowland rice. Nitrogen is usually the limiting nutrient in both flooded and upland soils. Rice yields are lower in nonfertilized than in fertilized fields, yet through the years consistent yields have been obtained in successive rice crops without the benefit of nitrogen fertilizer and with no apparent decreases in the nitrogen content of the soil. The maintenance of nitrogen fertility in these soils has been attributed to nitrogen fixation (App *et al*, 1980). The long term nitrogen balance experiments in Japan and the Philippines have been determined. In non-fertilized plot, net gains of soil nitrogen ranged from 20 to 70 kg N. ha^{-1} per year (in Japan) or per crop (in the Philippines) except in peat soil at Sorachi (Watanabe and Roger, 1984).

The plain of Thailand is one of the area where lowland rice has been grown for 50 centuries with little or no fertilizer (Swaminathan, 1984). Firth *et al* (1973) estimated that inputs of at least 40 kg $N.ha^{-1}.year^{-1}$ are required to balance the amount of nitrogen removed by each crop, of which 6 kg $N.ha^{-1}$ should be accounted from nitrogen in irrigation water and 5-6 kg N from rain water. They assumed that the remaining 28 kg $N.ha^{-1}$ was supplied by biological nitrogen fixation. Matsuguchi *et al* (1975) investigated for the N_2-fixing potential in 40 rice fields of Thailand by acetylene reduction method, and reported that the average amount of annual nitrogen fixed in the whole country is 6.9 kg $N.ha^{-1}.year^{-1}$. Most of the rice fields in Thailand are revealed to fix less than 10 kg $N.ha^{-1}$, although some of the Marine Alluvial Soil and Fresh Water Alluvial Soils fixed about 20 kg $N.ha^{-1}$.

1.3.2 <u>N_2-fixing microorganisms in wetland-rice field</u>. The wetland-rice ecosystem is consisted of aerobic and anaerobic zone, and photic and nonphotic conditions, where all major N_2-fixing micororganisms can grow (Fig. 1). These are free living and symbiotic autotrophs, symbiotic heterotrophs, and aerobic,

facultative anaerobic, and anaerobic free-living heterotrophs. Anaerobic metabolites such as H_2, CH_4 and sulphide can also support chemolithotrophic N_2-fixation at the aerobic/anaerobic interface. Floodwater, submerged plants, and the surface of aerobic soil are sites for photodependent N_2-fixation. Heterotrophic N_2-fixation occurs preferentially in nonphotic environments such as the soil aggregates that contain organic debris, and the rhizosphere (Watanabe and Roger, 1984).

From the ecological point of view, the major N_2-fixing organisms in wetland rice ecosystem can be classified into two groups (Table 1): i)photoautotrophs, namely photosynthetic bacteria, free living blue-green algae (BGA) and symbiotic BGA in azolla, and ii)heterotrophs comprising of free-living, associative and symbiotic N_2-fixing bacteria. N_2-fixing bacteria associated with rice in 3 regions: i)outer rhizosphere, ii)rhizoplane, and iii)endorhizosphere or histosphere. Nitrogen fixing activity by acetylene reduction assay of washed roots is much higher than in the adjacent soil (Yoshida and Ancajas, 1973). The nitrogen fixation by organisms close to the root surface is termed "associative N_2-

Table 1. Major groups of N_2-fixing microorganisms in lowland rice fields (Watanabe and Roger 1984)

Photoautotrophs				
	Free living			
		Photosynthetic bacteria		*Rhodopseudomonas*
		Blue-green algae (cyanobacteria)		*Nostoc, Anabaena*
	Symbiotic			*Anabaena azollae* in Azolla sp.
Heterotrophs				
	Free living			
		In the soil		
		Oxidized soil:	obligate aerobes	*Azotobacter, Beijerinckia*
			microaerobes**	*Methylomonas*
		Reduced soil:	obligate anaerobes	*Clostridium, Desulfovibrio*
		In association with rice		
		On submerged parts:	facultative anaerobes*	*Klebsiella*
		In the rhizosphere:	facultative anaerobes	*Enterobacter*
			microaerobes**	*Flavobacterium, Pseudomonas*
				Azospirillum
	Symbiotic			*Rhizobium* in legumes

*Growth in presence of O_2 but fix N only in absence of O_2
**Fix only in the presence of low O_2 concentrations

fixation" and may be a major source of nitrogen in nonfertilized rice fields.

Nitrogen-fixing activity in rhizosphere varies by the following factors : i)varieties of rice (Lee *et al*, 1977 ; Lee and Yoshida, 1977 ; Barraquio *et al*, 1986 ; Ladha *et al*, 1986), ii)growth stages of rice, the maximum nitrogen fixing activity was reported at the flowering stage (Yoshida and Ancajas, 1973 ; Yoshida and Broadbent, 1975 ; Panichsakpatana *et al*, 1979 ; Eskew *et al*, 1981), iii)seasonal and diurnal fluctuations (Balandreau *et al*, 1974 ; Sims and Dunigan, 1984).

A considerable number of different genera of nitrogen-fixing heterotrophs have been isolated from roots and rhizosphere of various Gramineae especially of rice. These several genera are *Klebsiella, Enterobacter, Erwinia, Azotobacter, Clostridium, Bacillus* (Rennie, 1980), *Beijerinckia* (Dobereiner *et al*, 1972), *Campylobacter* (McClung and Patriquin, 1980) and *Pseudomonas* (Barraquio *et al*, 1983). In genaral, 2-4 genera predominant nitrogen-fixing heterotrophs are isolated from each Gramineae (Nur *et al*, 1980 ; Haahtela *et al*, 1981). In the Philippines, about 80 per cent of the isolates from rice root give positive nitrogen

fixing activity (Watanabe and Barraquio, 1979), the majority of these nitrogenase positive bacteria are identified as *Pseudomonas* and *Azospirillum* (Barraquio *et al*, 1982, 1983). *Azospirillum* is also found at the density ten or hundred times lower than *Pseudomonas* (Barraquio *et al*, 1982). In France, Thomas *et al* (1982) used a "Spermosphere Model" to study the composition of the nitrogen fixing microflora of the rice rhizosphere (rhizospheric soil plus root). In this system N_2-fixing isolates were observed with a frequency of 65%. Thirty-two of the many isolates obtained are *Klebsiella oxytoca, Enterobacter cloacae, Pseudomonas paucimobilis* and *Azospirillum spp.* All these bacteria are present at the densities higher than 10^5 cells per gram of dry rhizospheric soil.

In Thailand, Harinasut (1983) estimated that the amount of total nitrogen fixed by associative bacteria in the rhizosphere is in the range of 20-72 kg N per hectare per crop. Screening by acetylene reduction assay of washed and surface sterilized roots, 259 bacterial isolates were collected from rice grown in acid soil and semi-arid soil of Thailand. All of them are gram-negative rod. Only 3 representative bacterial

cultures were selected from the top 8% according to their nitrogen fixing activity by ARA namely R17, R15 and R25, which were later classified to be *Klebsiella spp.* R17 and R15, and *Azospirillum sp.* R25 (Choonhahiran, 1986). The original information of these rhizospheric N_2-fixing bacteria are shown in Table 2.

1.4 The association between diazotrophs and Gramineae.

Preferential enrichment of diazotrophs in or attraction to the rhizosphere might be attributable to i)competitive advantage in a carbon rich, N-poor environment from the excretion by the roots, for instance, various amino acids and carbohydrates such as glucose, fructose, arabinose, xylose and sucrose (Mac Rae and Castro, 1967), ii)provision by the plant of essential vitamins such as biotin (Martin and Glatzle, 1982), iii)aerotactic attraction of diazotrophs to reduced pO_2 in the root region (Kimura *et al*, 1979; Okon *et al*, 1980), iv)associative factor such as lectin to attract diazotrophs, and v) catalase-like activity produced by *Beggiatoa* around the root tips of growing rice plants for decomposition of toxic peroxides (Pitt *et al*, 1972).

Table 2 The original information of nitrogen-fixing bacteria isolated from acid soil and semi-acid soil of Thailand (Harinasut,1981 and Choonhahiran,1986).

Bacterial code	Genus	Isolation site	N_2-fixing activity nmol $C_2H_4 \cdot OD_{420}^{-1} \cdot h^{-1}$
R17	Klebsiella sp.	Non-sterile root of rice cv.RD 6	52
R15	Klebsiella sp.	Non-sterile root of rice cv.RD 7	75
R25	Azospirillum sp.	Rhizospheric soil	non in aerobic-condition

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The mechanism of association between diazotrophs and Gramineae have been studied extensively in the last decade, but very little information can be drawn out. One of the major problem is the criteria indicating the presence of association between diazotrophs and Gramineae. In contrast to the *Rhizobium*-legume symbiosis, the diazotrophs-grasses interaction does not produce visible structures on roots which indicate successful infection. Studies with sugarcane (*Saccharum officinarum*) and bahia grass (*Paspalum notatum*) suggested that the majority of the N_2-fixing bacteria associated with roots probably resided within the mucilagenous sheath or mucigel layer on the root surface (Umali-Garcia *et al*, 1980). For these reasons, the interaction between grasses and this N_2-fixing microorganisms has been described as "associative". There are illustration of the *Azospirillum*-filled spherical structures on the root surfaces of sugarcane and the deformation of root hairs, and the evidence that *Azospirillum* breaches the root epidermal barrier and invades cortical and vascular tissues of the host (McClung and Patriquin, 1980).

These observations are also reported in other diazotroph-plant association such as *Azospirillum*-pearl millet and guinea grass (Umali-Garcia *et al*, 1980), and *Azospirillum*-kallar grass (Reinhold *et al*, 1987), but no report on diazotroph-rice system.

1.5 Recognition, Attachment and the Lectin Hypothesis.

In the study of bacterial colonization of pearl millet root and guinea grass root by *Azospirillum brasilense* SP7, Umali-Garcia *et al* (1980) also pointed out that there is a number of characteristics which are compatible with the lectin recognition model of legume-*Rhizobium* association (Dazzo, 1981; Dazzo and Truchet, 1983). These characteristics are i)the granular material associated with root hair surfaces and associated bacteria, and ii)the pearl millet roots released protease-sensitive nondialyzable substances which bound to *Azospirillum* and promoted their selective adherence to root hairs.

Cellular recognition has been defined as the initial event of cell-cell communication resulting in a defined biochemical, physiological or morphological response. Quite clearly, recognition qualifies as an

event that must be involved in the initiation of specific plant-microorganism interaction; lectin recognition model is one of the most probable model for the explanation of this specificity. There is now increasing evidences that plant lectins participate in such recognition processes. Most attention has been focused on the role played by lectins in the process whereby rhizobia and their respective hosts are mutually recognized, to account for the specificity inherent in many *Rhizobium*-legume symbiosis. Interest in lectin-mediated recognition has now spread to other diazotrophs-plant associative symbiosis and plant-pathogen interactions.

1.5.1 Lectins in *Rhizobium*-legume interactions. *Rhizobia*, despite their apparent capability on symbiosis with legume-rhizosphere environment, probably are always greatly outnumbered by other bacteria at the root surface. Just how recognition occurs between a host legume root and one specific kind of Rhizobium amid all the activity of the rhizosphere is a question of immense interest. A hypothesis that this specificity in legume-*Rhizobium* symbiotic interactions is mediated by lectins was advanced several years ago (Hamblin and Kent, 1973;

Bohlool and Schmidt, 1974).

Lectins are proteins or glycoproteins of non-immune origins which bind to cell surfaces via specific sugar residues and oligosaccharide determinants (Lis and Sharon, 1973; Barondes, 1981). Those best known with regard to structure and sugar-binding properties are derived from plants. They were formerly called phytohemagglutinins by virtue of their ability to agglutinate red blood cells. Lectins are particularly abundant in the seeds of many legumes, where they constitute up to 10% of the total protein (Liener, 1976). The hypothesis is that excreted lectins present a site on the legume root surface that interacts specifically with a distinctive saccharide on the surface of the appropriate *Rhizobium* cell as a prelude to nodulation.

Hamblin and Kent (1973), working with a single strain of *Rhizobium phaseoli*, found that lectin derived from the seeds of the bean *Phaseolus vulgaris* bound to the rhizobia. Lectin binding is indicated by the ability of lectin-treated bacteria to agglutinate erythrocytes. The possible relationship of lectin binding to the specificity of legume-rhizobia

interactions was first explored by Bohlool and Schmidt (1974) for the soybean-*Rhizobium japonicum* system. They used fluorescence microscopy to examine binding of fluorescein isothiocyanate-labelled soybean lectin to rhizobia. Lectin binding is positive for 22 of the 25 strains of *R. japonicum* tested, whereas 23 other strains, all representative of rhizobia that do not nodulate soybeans, are negative. Further support for the possible implication of lectins in symbiotic nitrogen fixation was soon forthcoming with respect to the *R. japonicum* soybean association (Wolpert and Albersheim, 1976; Bhuvaneswari and Bauer,1978; Halverson and Stacey, 1984, 1985; Ho *et al*, 1986) and that of the *Rhizobium trifolii*-clover association (Dazzo and Hubbell, 1975; Dazzo and Brill, 1978 ; Sherwood *et al*, 1984).

Not all reports have concurred with the lectin binding specificity noted above in the soybean and clover systems. Handelsman *et al* (1984) demonstrated that *Rhizobium* strains with less ability to be agglutinated by the alfalfa agglutinin actually are better nodulators than those that are highly agglutinable. A lack of specificity was noted in the survey by Chen and Phillips (1976) who found that

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heterologous rhizobia attached to legume root sections just as well as did the homologous species capable of forming nodule. Such discrepancies call attention to the complexities of the lectin-rhizobia interactions.

Evidence of another sort contrary to the lectin recognition hypothesis was reported by Pull and Pueppke (1978). Seeds of 102 different cultivars of soybean were examined for the presence of lectin, and five of these, Columbia, Sooty, Norredo, Wilson 5 and T102, were reported as lacking lectin. These five cultivars nevertheless nodulated normally when inoculated with appropriate strains of *R. japonicum*, which they apparently recognized adequately.

Among the many important aspects still to be resolved is the nature of the lectin receptors at the *Rhizobium* cell. In the view of Wolpert and Albersheim (1976) the lectin binding material was considered to be lipopolysaccharide (LPS). They reported that LPS extracts from *R. japonicum* and three other rhizobia bound specifically to homologous lectins on agarose affinity columns, whereas their exopolysaccharide (EPS) fractions were devoid of lectin binding activity However, Tsien and Schmidt (1977) observed that EPS

material loosely associated with the rhizobial surface was responsible for lectin binding in *R. japonicum* strain 138. Calvert *et al* (1978) subsequently were able to obtain electron micrographs, also of *R. japonicum* strain 138, that show ferritin-labeled lectin clearly associated with the EPS capsular material around the cell rather than with the cell wall where LPS is localized. Evidence also is in favor of capsular EPS as the material that binds clover lectin specifically to *R. trifolii* (Dazzo and Hubbell, 1975; Dazzo and Brill, 1978). Specific binding of trifoliin only to *R. trifolii* was confirmed by indirect immunofluorescence. Trifoliin from seeds and roots binds to encapsulated cells of *R. trifolii* (Dazzo and Brill, 1978). The capsule of *R. trifolii* consists of a β-linked acidic heteropolysaccharide (Dazzo and Hubbell, 1975).

Overall, it appears that EPS comprising at least a part of the cell capsule probably is the lectin binding material in rhizobia. Thus far, the possible involvement of LPS has not been ruled out, since all EPS preparation noted here could have included LPS material (Rothfield and Pearlman-Kothencz, 1969).

Most experiments concerned with rhizobial recognition have made use of seed lectins rather than root lectin, because useful amount could be easily obtained from seeds. Occurrence of lectins are well known in root legume (Lis and Sharon, 1973a; Liener, 1976), and usually these root lectins are similar to their corresponding seed lectins. Dazzo and Brill, (1978) purified and partially characterized the clover lectin (they call trifoliin) from seed and seedling root. Both root and seed lectins are similar in their sugar specificity of agglutination with *R. trifolii* which is inhibited only by 2-deoxyglucose. The same electrophoretic mobilities, and immunoprecipitation with anti-trifoliin also support the identity of root and seed trifoliin. In soybean, Pueppke and Bauer (1978) reported that lectin in the roots of young soybean seedlings is the same as that of the seeds, but the presence of root lectin is no longer detectable in seedling roots older than 2-3 weeks.

1.5.2 Lectins in fungi-wheat interactions. One of the first well-defined hypotheses concerning the physiological role of plant lectins has been advanced for wheat germ agglutinin in its fungistatic properties

(Mirelman *et al*, 1975; Brambl and Gade, 1985). They found that purified WGA binds *in vitro* the hyphal tips of *Trichoderma viride* and *Fusarium solani* and inhibits sodium acetate incorporation, conidiation and hyphal elongation. Chitotriose, a potent hapten of wheat germ agglutinin, blocks all these effects. On the basis of these observations, they suggested that under natural conditions too, wheat germ agglutinin protects wheat against chitin containing phytopathogens during seed imbibition, germination and early seedling growth. More recently , the findings that wheat germ agglutinin as well as other cereal lectins (including rice) are i)located preferentially in cells of tissues that establish direct contact with the soil during germination and seedling development (i.e. at the periphery of embryonic roots and throughout the coleoptile) (Mishkind *et al*, 1983), and ii)located and synthesized *de novo* at seedling roots and adventitious roots (Stinissen *et al*, 1985), have been interpreted in favor of the earlier proposed defensive function of wheat germ agglutinin and these findings also have been interpreted in favor of the role of lectin in the beneficial association between the

Gramineae lectins and the nitrogen-fixing bacteria.

1.6 Rice lectin.

1.6.1 Molecular structure. Rice lectin, as well as all other Gramineae lectins exhibits the same carbohydrate binding specificity for N-acetylglucosamine (GlcNAc) and oligomers of this sugar (Peumans and Stinissen, 1982 ; Miller and Bowles, 1983 ; Stinissen and Peumans, 1985; Tabary and Frenoy, 1985). It has been purified and characterized from hulled rice seed (Takahashi *et al*, 1973), bran (Tsuda, 1979; Kortanakul, 1983), dehusked rice flour (Indravathamma and Seshadri, 1980,1984; and Indravathamma *et al*, 1986), rice embryo (Peumans and Stinissen, 1982 ; Peumans *et al*, 1983 ;Shen *et al*, 1984 ; Tabary *et al*, 1984), and rice endosperm (Newberg and Concon, 1985).

In all these previous reports (except of Newberg and Concon, 1985), rice lectins are isolated from bran, embryo and hulled seed, since 90% agglutinating activity of rice seed is located in embryo (Tabary *et al*, 1984); it implies that all these lectins are embryo lectins. The characteristics of rice lectin reported from various laboratories using different varieties of rice and

different procedures of isolation are summarized in Table 3 and 4. For example, rice lectin was reported to be monomer of MW 10,000 by Takahashi *et al*, 1973, and to be dimeric by Tsuda 1979; Shen *et al*, 1984; Indravathamma *et al*, 1986, with MW range from 23K to 40K. Some rice lectins are glycoproteins (Takahashi *et al*, 1973; Indravathamma and Seshadri,1980; Shen *et al*, 1984) and some are proteins (Tsuda, 1979 and Kortanakul, 1983). By comparing the mol% of amino acids composition (Table 4), it is likely that these lectins are different in some domains of their primary structure.

1.6.2 Cellular localization and physiological role of rice lectin. Rice water soluble lectin is confined to the embryo part of seed. More precisely, Mishkind *et al* (1983), by using light microscopic immunocytochemistry, reported the presence of embryo lectin only in the periphery of embryonic roots and throughout the coleoptile. The *de novo* synthesis of lectin in immature embryo as traced by ^{35}S-incorporation was observed in the primary axes (Stinissen *et al*, 1984). The absolute lectin content (estimated by agglutination assays) is about 0.2 $\mu g.$ embryo^{-1} which corresponds to 0.2% of the total embryo protein

Table 3 Some characteristics of rice lectins reported by different research groups.

Rescarch groups	Source of lectin isolation	pH of extracted soln	Some propertics of purified lectin			
			M.W.	No. of subunits	pI	Carbohydrate content
Takahashi et al 1973	Hulled rice seed - var. japonica	4	10,000	1	-	25% w/w[a]
Tsuda 1979	Bran - var. japonica	7.4	37,000	2 19K, 11K, 8.2K	8.8	0[b]
Peumans and Stinissen 1982	Rice embryo - japonica	3.8	-	- 23K, 12K, 10K	-	-
Kortanakul 1983	Bran - var. indica	7.4	40,000	4 13K,12K,9K,8K	-	0[c]
Shen et al 1983	Rice germ - japonica	4.5	23,000	2 19K,13.7K,11.3K	6.5-6.8	0.8% w/w[a]
Indrava-thamma and Seshadri 1980, 1984	Dehusked rice flour - var. indica	4.0	85,000	4 20K	-	0
			10,000	-	-	10% w/w
Indrava-thamma et al 1986	Dehusked rice flour - var. indica	7	36,000	2 20K, 12K, 9K	-	10% w/w[a]

(a) : phenol-sulfuric reaction

(b) : gas-chromatography

(c) : anthrone reaction

Table 4 Comparison of amino acids composition of rice lectin reported from Tsuda 1979, Shen 1984, Poola & Seshadri 1986 and Takahashi 1973.

	mol % recovered			
	Tsuda 1979	Shen 1984	Poola & Seshadri 1986	Takahashi 1973
Aspartic aicd	8.9	10.3	9.1	8.9
Threonine	1.6	2.2	3.5	4.0
Serine	7.3	9.3	8.3	6.2
Glutamic	9.6	10.8	12.3	9.4
Proline	3.0	3.3	-	6.8
Glycine	20.6	24.4	17.1	12.5
Alanine	4.4	3.8	7.8	11.2
Cysteic acid	24.3	15.7	12.8	6.1
Valine	-	0.6	3.5	6.5
Methionine	1.1	1.1	1.5	trace
Isoleucine	1.1	0.6	2.5	3.3
Leucine	2.8	2.9	5.5	6.7
Tyrosine	4.9	4.7	2.5	3.1
Phenylalanine	2.3	3.3	2.0	3.4
Lysine	3.7	0.6	5.0	4.8
Histidine	0.5	3.1	2.0	1.4
Arginine	3.0	3.3	4.3	5.7
Tryptophan	1.0	-	-	trace

(Stinissen *et al*, 1985). Both the absolute and relative amounts of lectins in rice embryos have been determined in a few varieties. It is possible, of course, that there is a considerable variation among different varieties.

Biosynthesis of rice lectin in embryo can be induced by plant growth substances (abscisic acid and gibberellic acid) to a 20-fold higher rate than control. Rice embryo grown in a medium with these plant growth substances do not germinate but still synthesize lectin. Therefore, Stinissen and Peumans (1985) have concluded that rice lectin is a dormancy-specific protein that is specifically synthesized upon the entry of these embryos into their natural or exogeneously induced developmental arrest. In addition, *de novo* synthesis and localization of rice lectin were reported in seedling roots and adventitious roots of six-month-old plants (Stinissen *et al*, 1985). Besides, rice root lectin cannot be distinguished from embryo lectin by their molecular weight, sugar binding-specificity and serological properties (Stinissen and Peumans, 1985). The finding of rice lectin in seedling root supports the physiological role of lectin as one of the defensive

agent against soil pathogenic microorganisms and as one of the associative factor in diazotrophs and plant interaction.

1.7 The aims of this thesis.

Manufacture of nitrogen fertilizer requires high fossil energy inputs and results in increasing fertilizer prices during energy crisis. Strategic research of nonconventional approaches should be developed to supply nitrogen to nonleguminous plants. One such approach is to evaluate and enhance association between microorganisms of high N_2-fixing potential in major food crops such as rice, wheat and corn which are known to be the associative heterotrophic diazotrophs in the rhizosphere. Exploitation of the full potential of these associations still depends on basic understanding of the nature of associative N_2-fixation especially in the indigeneous strains between *Klesiella spp.* (R15 and R17) and rice cv. RD7. The associative factor between these two partners shoud be emphasized. This understanding should not only advance our knowledge

of the development events in the association, but also indicate ways in which *Klebsiella spp.* R15 and R17 and rice RD7 may be manipulated physically or genetically to increase the efficiency of N_2-fixation and of rice production.

Therefore, the objectives of this thesis are :

1.7.1 to investigate the association between *Klebsiella spp.* R15 and R17 and rice (cv. RD7) in hydroponic culture , and the feasibility of plant exudate in enhancement of such association ;

1.7.2 to purify and characterize lectin from root, embryo and bran of rice (cv. RD7) ;

1.7.3 to study the role of purified rice lectin in the association between *Klebsiella spp.* R15 and R17 and rice root epidermal cells ; and

1.7.4 to establish a model of association between local diazotrophs and rice in order to promote the benefit of this system in the future.