Discussion

4.1 Association between *Klebsiella spp.* R15, R17 and rice (cv. RD7) in hydroponic culture.

The association between diazotrophs and plant is usually evident by i) the changes in root morphology, ii) the adherence of bacteria individually, or as cluster or forming some special structure, and iii) the invasion of diazotrophs into root tissues.

4.1.1 Changes in root morphology. Our results show that inoculation of *Klebsiella spp.* R15 or R17 (10^8 cells) in 7-day-old rice seedlings grown in sterile water resulted in more branching, denser and longer root hairs as compared with the control of *E. coli* inoculated or non-inoculated seedlings (Fig. 3). This phenomenon has never been reported before in the association of rice plant and other diazotrophs, although root hair deformation, branching and elongation have been reported in the association of other Gramineae and *Azospirillum spp.*, namely wheat (Jain and Patriquin, 1984; Kapulnik *et al*, 1985 a, 1985 b), pearl millet (Tien *et al*, 1979; Umali-Garcia *et al*, 1980), foxtail millet (Kapulnik *et al*, 1981), and guinea grass (Umali-Garcia *et al*, 1980),

and very common in the interaction between *Rhizobia* and several legumes (Bauer, 1981; Bhuvaneswari and Solheim, 1985).

The morphological changes of pearl millet roots were observed when plants in solution culture were inoculated with 10^8 cells of *Azospirillum brasilense* (Tien *et al*, 1979). Later in 1980, Umali-Garcia *et al* reported similar result of the denser and longer roots in 2-day-old seedlings of *Pennisetum americanum* (pearl millet) and *Panicum maximum* (guinea grass) grown in nitrogen-free nutrient medium inoculated with 10^8 cells of *A. brasilense*.

Kapulnik *et al* (1981) also observed a marked development of roots of *Setaria italica* (foxtail millet) grown in sterilized, washed quartz sand and inoculated with 10^8 colony-forming unit (cfu) *A. brasilense* (ATCC 29279) for 5-6 weeks.

In wheat, root hair deformation of 48-h-old seedlings inoculated with 10^8 *Azospirillum brasilense cells* and the magnitude of root hair changes of the four different varieties of wheat inoculated with seven different strains of *A. brasilense* were reported by Jain and Patriquin (1984). It is concluded that in wheat

there is a strain-specific effect of *Azospirillum spp.* on root hair deformation. Inoculation with a mixture of *Azospirillum brasilense* strains Cd, Sp7, and Cd-1, the local strains at a final density of 2 x 10^7 cfu per plant grown in hydroponic system significantly enhanced root elongation and branching (Kapulnik *et al*, 1985a). The wheat roots respond differently to different density of *Azospirillum* inoculum, Kapulnik *et al* (1985b) reported that inoculation with 10^5 to 10^6 cfu in wheat seedlings grown in sterile tap water caused the increased in root elongation and total root surface of seedlings whereas 10^8 to 10^9 cfu caused inhibition of root development.

In conclusion, the association of *Klebsiella spp.* R15 and R17 on rice (cv. RD7) in hydroponic culture results in a marked development of rice roots in more or less the same way as that observed in the association between *Azospirillum spp.* and several other Gramineae.

The root development occurs in these plants either by *Azospirillum* or *Klebsiella* inoculation should be beneficial to the plants, as more nutrients can be absorbed, and increasing root mass should also stabilize the plant. Lin *et al* (1983), reported that *Azospirillum*

inoculation enhanced wheat growth during early stages through effects other than nitrogen fixation, such as by root elongation, increases in root surface area, and density of root hairs, which lead to more minerals uptake into root segments of *Zea mays* and *Sorghum bicolor*.

The stimulating effect of nitrogen-fixing bacteria on root development in Gramineae plants led to the question of its mechanism. Tien *et al* (1979) proposed that the production of plant growth hormones by *Azospirillum brasilense* might be the mechanism for enhancement of root development in pearl millet, because indole acetic acid (IAA), gibberellic acid (GA) and at least three cytokinin-like substances were found in concentrated cell-free culture broth of *A. brasilense*. The change in root morphology of pearl millet induced by inoculation with *A. brasilense* can also be mimicked by the addition of the mixture of IAA, GA and cytokinin to seedlings of pearl millet grown in hydroponic condition at the concentration of each hormone in the mixture very close to the amount found in culture broth of *A. brasilense*.

Generallly, IAA is known to be synthesized in the root apex and polarly transported back to the elongation zone of the root where it determined the rate of root elongation (Street *et al*, 1978), therefore, it is possible that IAA produced by *Klebsiella spp.* R15 and R17 (Choonhaʼ.ran 1986) might be absorbed by the root cells and resulting in root hair elongation, and other development.

4.1.2 Colonization and spherical micronodule formation. Besides, the changes in root morphology, the inoculation of *Klebsiella spp.* R15 or R17 leads to the adherance of *Klebsiella spp.* individally and gradual formation of special structure in spherical shape of 10-15 μ in diameter via small cluster formation (Fig. 2-3). A few clusters of invading bacteria can be observed in the epidermal and cortical layer of root tissue although at low frequency (Fig. 6). At least 2 h is required for strong adherance of these bacteria that withstand PBS washing, and not less than 36 h for the appearance of spherical micronodules of 10-15 μ in diameter in bag-like structure (Fig. 3). These phenomena are consistently observed under the condition that at least 10^8 cells were added per 3 rice seedlings grown in ~5 ml

sterile distilled water, and the incubation period lasts longer than 36 h. This result confirms the supposition that *Klebsiella spp.* R15 and R17 are rhizoplane bacteria since they were isolated from the non-sterile washed rice roots (Harinasut, 1981). Similar bag-like structure or apparently membrane bound structure was also reported to occur in the association betweeen the *Azospirillum brasilense* 245 and wheat (Patriquin *et al*, 1983), where *Azospirillum*-filled spherical structures were observed on the root surfaces of 3-week-old wheat grown in sand-vermiculite. Recently, Reinhold *et al* (1987) have reported the presence of nitrogen-fixing bacteria with enveloped, round bodies of 14 μ in diameter residing in the aerenchyma tissue of Kallar grass. The spherical micronodules of *Klebsiella spp.* R15 and R17 on rice root, and of *A. brasilense* on wheat root, both observed on the outer periphery of root epidermis, are approximately the same size as the enveloped, round bodies of diazotrophic rods in the root interior of Kallar grasses. The formation of this structure in the process of association between N_2-fixing bacteria and plants might protect these diazotrophs from oxygen on the exterior part of root or

in the aerenchyma tissue, and hence increased the efficiency of N_2-fixation. This speculation seems to correspond with our result in Fig. 9 that there is a sharp increase of the nitrogen fixing activity induced by associative diazotrophs of *Klebsiella oxytoca* NG13, *Klebsiella spp.* R15 and R17 on day 3 after the extensive formation of micronodules. Further field inoculation trials are needed to confirm the potential of nitrogen-fixing activity by the association between *Klebsiella spp.* R15 and R17 and rice plants.

4.1.3 The invasion of diazotrophs into root tissue. From our result shown in Fig. 6G, there are some *Klebsiella spp.* R15 and R17 localized in the epidermal and cortical layer of root tissue without any disruption of these cells. Patriquin and Dobereiner (1978) demonstrated that roots of field-grown tropical maize, *Panicum maximum* Jacq. and *Digitaria decumbens* Stent., and of sorghum and wheat grown in monoxenic culture with associative diazotroph *Azospirillum lipoferum* are filled with this bacteria between and inside the cells of the cortex, in intercellular spaces between the cortex and endodermis, in xylem cells, and in and between pith cells without disrupting endodermis. The colonization

of *Azospirillum brasilense* in the intercellular spaces of the outer root cortex of 2-day-old pearl millet was also demonstrated by SEM (Umali-Garcia *et al*, 1980). In their study, pearl-millet seedlings were grown in Fahraeus assemblies without agar and inoculated with 10^8 cells *Azospirillum brasilense*. Recently, Wang *et al* (1987) reported that the inoculation of 10^8 cells of either *Azospirillum lipoferum* FS, or *Klebsiella oxytoca* NG-13, or *Klebsiella pneumoniae* K-12, or *Enterobacter sp.* E-25 in rice seedling cultured on C- and N-free medium for 2 weeks, resulted in the invasion of these bacteria into root cortex, and propagation within the cells can be examined by SEM, and confirmed by re-isolation experiments. It is difficult to explain how the invasion of these diazotrophs occur without disrupting the epidermal, cortical or even endodermal cells. Anyhow, Patriquin and Dobereiner (1978) and Umali-Garcia *et al* (1980) suggested that *Azospirillum* eventually enter the root through lysed root hairs and void spaces of epidermis created by epithelial desquamation and lateral root emergence in regions of branches. In addition, our results in Fig. 6F also suggest that along the process of root hair curling and

branching, some diazotrophs are evaginated and trap into the epidermal layer.

In conclusion, the association of *Klebsiella spp.* R15 and R17 on rice root resulted in changes in root morphology, micronodule formation on the outer periphery of epidermis and invasion of some bacteria in the epidermal and outer cortical layers of root.

4.2 Micronodule formation and N_2-fixation.

The new finding of micronodule formation as the result of association between *Klebsiella spp.* R15 or R17 and rice (cv. RD7) roots led us to question whether this phenomenon occurs specifically only between *Klebsiella spp.* R15 or R17 and the rice RD7, and whether the micronodule formation promotes nitrogen fixation.

Our results (Table 5) shows that micronodules are formed on roots of other rice variety such as HCCMM when inoculated with associative nitrogen-fixing bacteria such as *Klesiella oxytoca* NG13 and *Azospirillum lipoferum* FS, but not with *Pseudomonas* H8, or free-living nitrogen fixing bacteria such as *Klebsiella pneumoniae* M5a1, and non-N_2-fixer such as *E. coli* K12. This result also suggests that there is a complementary

matched pair between diazotrophs and rice root for micronodules formation, but not specific only pairing between *Klebsiella spp.* R15 or R17 and rice cv. RD7.

The relationship between micronodule formation and nitrogen-fixing activity can not be concluded from the result in Table 5 because ARA was measured in the whole system of hydroponic culture tube. The nitrogenase activity in the whole system therefore resulted from the total combination of free living and associative diazotrophs whether forming micronodules or not. Anyhow, the tendency of correlation between micronodule formation and N_2-fixing activity is shown in Fig. 9 where there is a sharp increase in the nitrogen-fixing activity induced by the associative *Klebsiella spp.* strains NG13, R15 and R17 on day 7 which correspond to the period which excessive micronodules were formed.

4.3 The role of rice lectin as associative factor.

Our fluorescence micrographs and scanning electron micrographs on enzymatic treatments of micronodules for 20 h indicate extensive damage of the enveloped structures after treatment with glucans-digesting

enzymes, and trypsin which support the hypothesis in two important aspects. First, the adhesive factor could be glycoproteins and second, these enveloped structures once formed are fairly stable. This finding increases the possibility of rice lectin as an adhesive factor because of its agglutinating nature via N-acetylglucosamine binding and the following results ; i) rice lectin is detected in free form in the root exudate and in bound form on root epidermis, ii) rice lectin receptors are demonstrated on the outer surface of both associative bacteria, *Klebsiella spp.* R15 or R17, and rice (cv. RD7) root, and iii) purified rice lectin enhances free bacterial agglutination and attachment of free *Klebsiella spp.* R15 and R17 on PBS-washed rice root.

4.3.1 Localization of rice lectin in the vicinity of rice rhizosphere. This study reports the finding of rice lectin activity in seedling root exudate of rice cv. RD7 to be ~65 HU. plant^{-1} (Table 6) which is about 10 times higher than the amount present in the root tissue (~4.5 HU. plant^{-1}) and two times higher than the amount stored in embryo (~34 HU. embryo^{-1}) (Table 9), the significant amount of rice lectin in root exudate

should resulted from *de novo* synthesis of lectin in seedling roots which is supported by previous study of Stinissen (1985) that rice lectin is *de novo* synthesized in 2-day-old seedling roots. Since our results using purified lectin isolated from either bran or embryo or seedling root exhibits similar effect on bacterial agglutination (Fig. 22), micronodule formation (Table 13) and competitive binding activity for lectin-binding site on bacterial cell (Fig. 24), therefore, one can not exclude the possibility that the embryo lectin and seed coat lectin are transported to epidermal cells and secreted into root exudate too.

Other supporting evidences for the role of rice lectin as associative factor come from the comparative study of lectin activity in root exudate of rice IR 42 and IR 58 which are hundreds-fold lower than RD7, (Table 6), and no association or micronodule was observed on rice root samples of either IR 42 or IR58 inoculated with *Klebsiella spp.* R15 and R17 (Table 5).

Lectin in the vicinity of rice rhizosphere is not only existed as free form in root exudate but also in bound form on the outer surface of rice root too (Table 7), therefore it is possible that inoculated diazotrophs

firstly interact with bound lectin on the root surface, and then piled up as clusters, and finally form enveloped micronodule structure bridging by the free lectin in surrounding root exudate.

4.3.2 Localization of lectin receptors on *Klebsiella spp.* R15, R17 and rice (cv. RD7) root.

Several markers can be used for localization of lectin receptors such as lectin-horseradish peroxidase, lectin-ferritin and lectin-gold complexes, but in this study only lectin-gold complex was chosen as the specific markers. The superiority of gold-protein complexes as markers has been well documented (Roth and Binder 1978; Horisberger 1979, 1985). The most important properties of their superiority are i) the gold method requires only small amount of pure lectin, ii) no reduction of the biological activity of the lectin occurs in the presence of gold, iii) gold marker shows little nonspecific adsorption, and iv) gold particle is electron dense, so it is easily detected by TEM. The disadvantage of this method is due to the large size of gold-protein complex which might not be able to reach the receptors, thus both direct and indirect labelling techniques were used. For indirect labelling lectin

receptors were first occupied by lectin. The protuding bound lectin can then be localized by gold-ovomucoid complex. Another consideration for gold-labelling technique is the loss of bound particles during the embedding process. The binding constants of lectin-labelled gold particles to cell surface glycoconjugates (10^9 - 10^{10} M^{-1}) is several order of magnitude higher than that of lectins to monosaccharide (Horisberger, 1985). The higher affinity results from multivalent interactions and possible from secondary interactions such as hydrophobic bonds which increase the strength of binding (Horisberger, 1985). Therefore, this property enables marked specimens to be processed for embedding with minimal loss of bound particles. In addition, the approach by using intact bacteria and intact root segment in this gold-labelling study is certainly specific for only surface labelling, because gold complex can not penetrate the slime and cell wall of these samples as reported previously by Geoghegan and Ackerman (1977); Horisberger (1977); Sinowartz and Friess (1983); and Piche *et al* (1985).

Both direct and indirect colloidal gold labelling techniques used for localization of lectin receptors on the bacterial surface and root epidermis show no significant discrepancies and indicate the presence of lectin receptors on the outer periphery without any structural hindrance effect occurs in the binding between RL or RL-Au complex and the lectin receptor.

Rice lectin receptors on both compatible strains, *Klebsiella spp.* R15,R17 and rice (cv. RD7) roots are first demonstrated in this study. The previous study of rice lectin receptors on other nitrogen-fixing bacteria isolated from rice rhizosphere, was recently performed in *Beijerinckia* V. by Tabary *et al* (1984). Our quantitative study of lectin receptors on *Klebsiella spp.* R15 and R17 by using ^{14}C-embryo lectin gives non-linear Scatchard plot (Fig. 23) which differs from that obtained with *Beijerinckia* V, of homogeneous lectin receptors. The lowest affinity constant, K_a is reported for *Klebsiella sp.* R17 which is in the range of 0.58-4.80 μM^{-1} (Table 12) which is lower than the K_a of R15 (1.02-8.82 μM^{-1}) and significantly lower than *Beijerinckia* V. (10.4 μM^{-1}). The total concentration of lectin binding sites of R15 and R17 (0.13 and 0.14 μM)

are ~5-times higher than of *Beijerinckia* V. (0.025 μM). These different kinetic parameters indicate that *Klebsiella spp.* R15 and R17 have more lectin receptors and bind lectin with higher affinity than *Beijerinckia*, which might be due to bacterial species and possibly the different nature of rice embryo lectins, since our lectin binds to whatman GF/C and millipore filter which is not so for Tabary's rice embryo lectin. The apparent non-linear Scatchard plot and the scattering of points from the curve observed in Fig. 23 might result from the mixture of four different molecular forms of rice embryo lectin and the whole bacteria used in this binding study.

The binding of ^{14}C-embryo lectin on *Klebsiella spp.* R15 and R17 can be competed by cold embryo lectin, root lectin and GlcNAc in the same pattern (Fig. 24), which indicates that either root lectin or embryo lectin occupies the same receptor on bacterial surface, and can similarly be inhibited by the same sugar hapten, GlcNAc, with the same degree of specificity.

The distribution of rice lectin receptors on *Klebsiella spp.* R15 and R17 in this study are mainly on glycocalyx (or extrapolysaccharide, EPS) especially when

cells are grown in NF medium. Some receptors are located on the cell wall, where lipopolysaccharide (LPS) is the target components (Fig. 27-30). The increase in production of glycocalyx and in number of lectin receptors of *Klebsiella spp.* R15 and R17 cultivated in NF medium reported in this study, and the previous report that *Klebsiella spp.* R15 and R17 do fix nitrogen only in NF medium, whether they have been cultivated in either RM or NF medium (Harinasut 1981 and Choonhahiran, 1986), suggest that *Klebsiella spp.* R15 and R17 cultivated in either RM or NF medium have the potential to associate with rice root via lectin binding, but those grown in NF medium can associate better and hence have greater potential for nitrogen fixation.

Regarding that the nature of lectin receptors on *Klebsiella* R15 and R17 are on both cell wall and glycocalyx, so it is possible that the lectin receptors on cell wall and glycocalyx are different in binding kinetic parameters which resulted in non-linear Scatchard plot of heterogeneous lectin receptors (Fig. 23).

Most of the analogous studies on the nature of lectin receptors on diazotrophs were performed in *Rhizobia*. Wolpert and Albersheim (1976) reported that

LPS extracts from *R. japonicum* bound specifically to lectin, whereas Tsien and Schmidt (1977) observed that EPS material was responsible for lectin receptor in *R. japonicum*. Subsequently in 1978, Calvert *et al* were able to obtain electron micrographs showing the ferritin-labelled lectin clearly bound to the lectin receptors or the EPS of *R. japonicum*. Lectin receptors on *R. trifolii* were also shown to be on the EPS (Dazzo and Hubbell, 1975; Dazzo, 1978).

It is noticable that the nature of lectin receptors on rice root is also demonstrated to be on glycocalyx or EPS or mucigel at the outer periphery of root epidermis (Fig. 31-34) which is similar to the *Klebsiella spp.* R15 and R17. Therefore, the origin of globular or fibrillar polysaccharide in the micronodule structure in Fig. 6 whether derived from bacteria or plant cannot be elucidated by this study. Anyhow, the demonstration of lectin receptors on glycocalyx of both parties (*Klebsiella spp.* R15 , R17 and rice RD7 root) in the association is a supporting evidence for the role of rice lectin as associative factor.

4.3.3 Effect of purified rice lectin on bacterial association with rice root. Three most direct lines of evidences for the involvement of rice lectin in the association between bacterium and bacterium or between bacterium and rice root are demonstrated by purified lectins. First, rice lectin can be detected in root exudate and purified from rice seedling roots. Purified rice lectins, no matter from bran, embryo or seedling roots have common molecular characteristics. Second, all forms of purified rice lectin agglutinate *Klebsiella spp.* R15 and R17, which are similarly inhibited by GlcNAc, the known sugar hapten of rice lectin. Third, PBS washed roots with diminished lectin are not colonized by *Klebsiella spp.* R15 and R17, unless purified rice lectin or root exudate is added.

1) Molecular characteristics of purified rice lectins. Our rice lectins purified from bran, embryos and seedling roots by affinity chromatography based on ligand of specific sugar (GlcNAc), exhibit one single band on PAGE (Fig. 14) and a symmetrical protein peak on Sephadex G-100 chromatography (Fig. 15), thus this purified lectin appears to be homogeneous and pure enough for further experiments.

The molecular characteristics of these purified rice lectins from bran, embryo and seedling root are more or less the same. In fact, rice bran consists of seed coat, embryo and broken grains (endosperm). According to our preliminary study, the PBS (pH 7.4) extraction of rice endosperm reveals no lectin activity (data not shown) which corresponds to the report of Newberg and Concon (1985) and Tsuda (1979), and since 90% agglutinating activity of rice seed is located in embryo (Tabary *et al*, 1984), so the lectin activity in rice bran represents mainly embryo lectin and a very small amount of seed coat lectin. Our purified rice lectins from any sources are i) single subunit polypeptide (Fig. 16) of molecular weight about 22-23 K (Fig. 15-16), ii) they have similar amino acids composition (Table 10), iii) they are glycoproteins but the amount of carbohydrats in the molecule are different (Table 11) among which RL contains the highest amount of 8-9% w/w carbohydrate, iv) they are slighly different in pI, for RL 4.5, 4.7, 5.0 and 5.05, for EL and BL (which are similar), the three low pI are the same as RL except another diffusable pI band from 5-5.2, and v) all polypeptides with different pI contain lectin activity.

(Fig. 19). Therefore, it is concluded that rice lectins isolated from bran, embryo or seedling root from the same variety (cv. RD7) are all glycoproteins with most likely the same polypeptide part, but difference in carbohydrate part which should affect the transportation of all these lectins to the site of its function as associative factor. These lectins contain multiple sugar-binding sites (Goldstein *et al*, 1982) and could have four equivalent saccharide-binding sites on one molecule of rice lectin as examined by Tabary and Frenoy (1985) in other rice variety.

In comparison, the molecular characteristics of our lectins from rice cv. RD7 and all other rice lectins previously reported by Takahashi *et al* (1973), Tsuda (1979), Peumans and Stinissen (1982), Kortanakul (1983), Shen *et al* (1983) and Indravathamma *et al* (1986) (Table 3 and 4) are different. It has been shown that rice lectins exist in several molecular forms. They are extracted with different pH buffer (Indravathamma *et al*, 1986). Isolation of lectin from different part of various rice cultivars could yield different molecular forms of rice lectin (Table 3). Despite these differences, there are some common properties in sugar

specificity (GlcNAc), and the dominant amino acids of cystein, glutamic acid, glycine and aspartic acid in the polypeptide of all rice lectins, except that reported by Takahashi (1973).

2) Bacterial agglutination by purified rice lectin. The agglutination of PBS-washed *Klebsiella spp.* R15 and R17 occurs in the presence of purified rice lectins, no matter from bran or embryo or seedling roots (Fig. 22) inferring to their homology of binding site. The effect of purified rice lectin in the agglutination of nitrogen-fixing bacteria from rice rhizosphere were previously reported by Kortanakul (1983) using rice bran lectin and Tabary *et al* (1984) using embryo lectin. The effect of rice lectin in the agglutination of rice callus cells was also reported by Shen *et al* (1983).

3) Enhancement of bacterial association with rice root. After diminishing of bound lectin from rice root by extensive washing in PBS, the firm attachment of *Klebsiella spp.* R15 and R17 and the formation of micronodules are not observed on rice root. Addition of purified rice lectins of any sources or root exudate can restore these association phenomena except when *E. coli* is used instead of R15 and R17. These results indicate clearly the requirement of lectin in this specific

clearly the requirement of lectin in this specific association.

In conclusion, purified rice lectins isolated from bran, embryo or seedling roots of rice cv. RD7 are more or less the same in molecular characteristics and exhibit the similar role on promoting the association between bacterium and bacterium, as well as between bacterium and plant. This specific binding is mediated through specific sugar hapten, GlcNAc.

4.4 Lectin-binding hypothesis.

The main results from this study which support the "Lectin-binding hypothesis" are summarized as follows ; i) the present of rice lectin activity in root exudate from hydroponic culture of rice cv. RD7 which corresponds with micronodule formation after bacterial inoculation, whereas negligible lectin activity found in hydroponic culture of rice IR 42 and IR 58 correspond with no micronodule formation after bacterial inoculation, ii) the detection of bound lectin on epidermis of rice root RD7, iii) the demonstration of rice lectin receptors on glycocalyx and cell wall of *Klebsiella spp.* R15 and R17, and on glycocalyx at the

outer periphery of rice RD7 root, and iv) purified rice lectins mimic root exudate in enhancement of bacterial agglutination and association between *Klebsiella spp.* R15, R17 on PBS washed rice root. According to these results, rice lectin is concluded to play role as the associative factor between *Klebsiella spp.* R15 and R17, and rice cv. RD7 root. The association between these two strains of bacteria on rice root via rice lectin demonstrated in this study leads to firm adherance of bacteria on the rhizoplane as found in natural condition, and induction of associative N_2-fixing activity which is beneficial to the plant. In addition, these bacterial inoculations should increase uptake of other nutrients by increasing root surface, and promote bacterial adhesion via the changes in root morphology. All the association phenomena based on the lectin binding hypothesis is summarized in Fig. 35

Since lectin from rice and many other plants in the family Gramineae bind specifically to the same sugar, GlcNAc, and the associative role of rice lectin in this hypothesis is mediated through this hapten binding specificity. It implies that any bacteria which have GlcNAc-lectin receptors can bind to other Gramineae

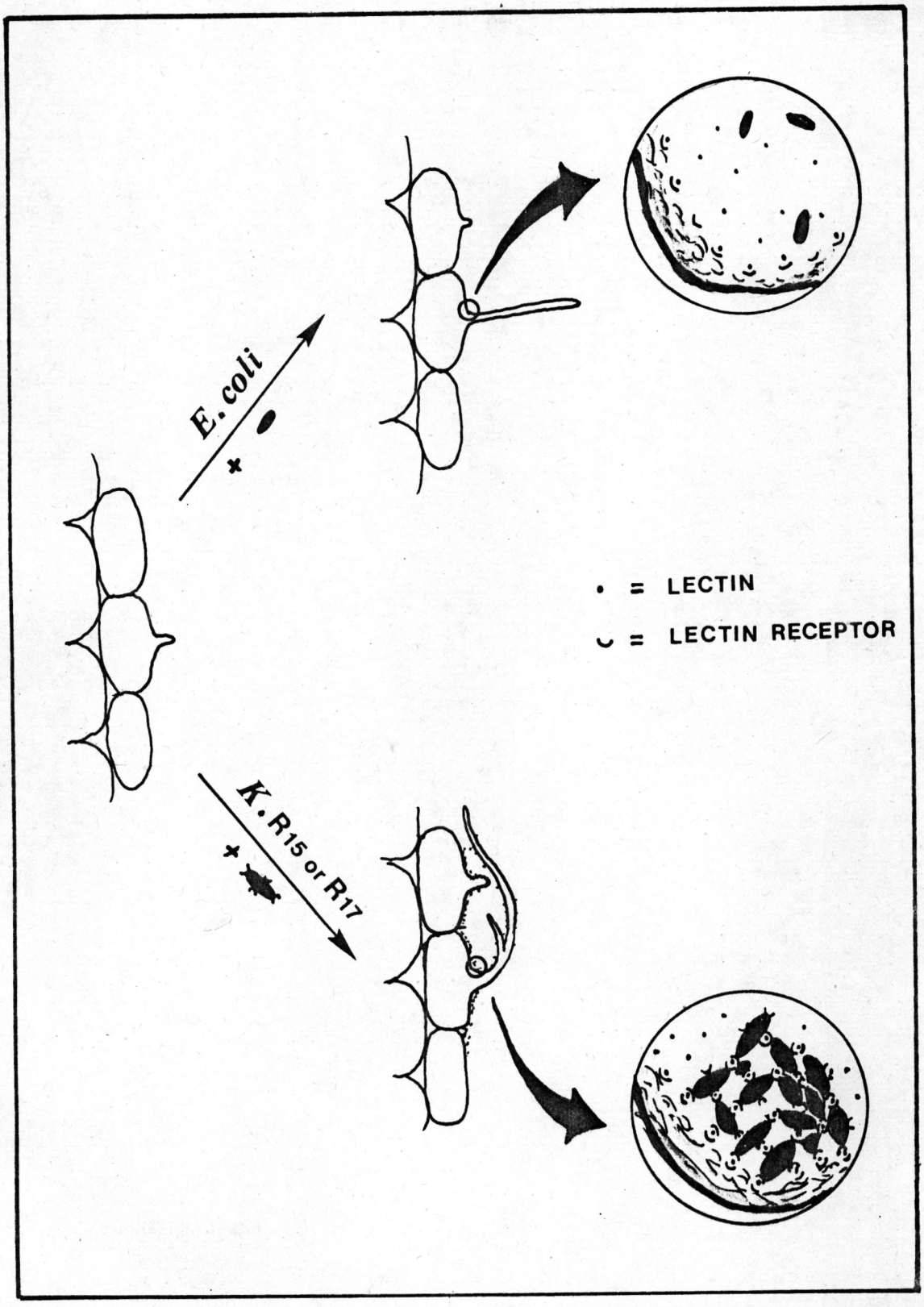

Figure 35 LECTIN BINDING HYPOTHESIS : Role of rice lectin

which excrete sufficiently the same sugar-specific lectin in their root exudate as well as possession of bound lectin. In comparison of this aspect to legume-*Rhizobium* association; such as between white clover-*R. trifolii*, pea-*R. leguminosarum* and soybean-*R. japonicum*, in which different legumes produce different sugar specific lectin (Table 15), the specificity between diazotrophs-Gramineae should have broader host-range than that of *Rhizobium*-Leguminosae. In fact, such example is known, *Azospirillum brasilense* has been reported to associate with pearl millet and Guinea grass (Umali-Garcia *et al*,1980), wheat (Tien *et al*,1979; Jain and Patriquin , 1984; Kapulnik,1985a, b), and foxtail millet (Kapulnik *et al*,1981), all in the Gramineae.

On the other side of this aspect, broad spectrum of diazotrophs are reported as the associative bacteria in the rhizosphere of rice via lectin binding, for example, *Beijerinckia* V. and *Azospirillum lipoferum* 4B (Tabary *et al*, 1984), and *Klebsiella* and *Azospirillum* (in this report).

Further research needs to be done in order to throw some light on the associative nitrogen fixation in those economic cereal crops.

Table 15 Plant lectins proposed to function in binding bacteria to plant surfaces (Pueppke, 1984).

Lectin	Binding specificity	Source
Pea lectin	Man, Glc	*Pisum sativum*
		Seeds
		Roots
Soybean lectin	GalNAc, Gal	*Glycine max*
		Seeds of most varieties
		Roots of one variety
		Glycine soja
		Seeds of some varieties
Trifoliin A	D-Glc, quinovosamine	*Trifolium repens*
	GalNAc?	Seeds of one variety
		Roots of one variety