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THE EFFECT OF HOMOLOGOUS AND HETEROLOGOUS STRAINS OF *RHIZOBIUM* ON ROOT HAIR INVASION OF *TRIFOLIUM ALEXANDRINUM*

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Effect of 10 heterologous and a homologous strains of *Rhizobium* was investigated with reference to root hair curling and infection thread formation in *Trifolium alexandrinum*. Root hairs were curled and deformed in both heterologous and homologous combinations. The maximum curling in root hairs occurred with homologous combination; while the minimum with *R. meliloti* strain 2. Infection thread formation in the root hairs occurred only with homologous strain. The curling and infection thread formation were not uniformly distributed on the root but in some localized zones.

INTRODUCTION

In certain leguminous plants, root hair curling is unspecific to *Rhizobium* spp. but infection thread formation is specific (Nutman, 1959, 65). Indoleacetic acid (IAA) was implicated in the curling reaction (Nutman *et al.*, 1945). Pure IAA is less active than the crude rhizobial filtrate in causing root hair curling and root hair branching (Sahlman & Fahraeus, 1962). Presumably some co-factor produced by legume root might be involved in root hair curling and branching (Fahraeus, 1963). Little is known regarding the effect of different strains of *Rhizobium* on root hair curling and infection thread formation in leguminous plants. In this study, we conducted investigations on the effect of 10 heterologous *Rhizobium* strains isolated from nodules of legumes other than the test plant and a homologous *Rhizobium* strain isolated from the nodules of the test plant on root hair curling and infection thread formation in *Trifolium alexandrinum*.

MATERIALS AND METHODS

Rhizobium phaseoli strain 1 from *Phaseolus mungo*, *R. phaseoli* strain 2 from *P. aureus*, *R. leguminosarum* strain 1 from *Pisum sativum*, *R. leguminosarum* strain 2 from *Cicer arietimum*, *R.* sp. from *Cajanus cajan*, *R.* sp. from *Lathyrus sativus*, *R.* sp. from *Vicia faba*, *R. japonicum* from *Vigna catjang*, *R. meliloti* strain 1 from *Melilotus alba* and *R. meliloti* strain 2 from *M. indica* were isolated from the respective nodules. Homologous effective strain, i. e. *R. trifolii* was isolated from a suitable healthy nodule of *Trifolium alexandrinum*. The rhizobia were maintained on yeast mannitol agar medium.

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For the study of root hair curling and infection thread formation, plant-slide culture technique devised by Fahraeus (1957) was employed. Seeds were surface sterilized with sulphuric acid for about half a minute. Thereafter, they were washed several times with sterile distilled water to remove the last traces of acid. The seeds were dipped in sterile distilled water for about 2 hr and thereafter placed in sterile Petri dishes containing moist cotton. The Petri dishes were incubated for two days at 26°C. Seedlings of about 1-3 cm were transferred in slide cells in culture tubes ($8 \times 1{\cdot}2''$) containing a medium of the following composition : $CaCl_2$. $2H_2O$, 0·1g; $MgSO_4$. $7H_2O$, 0·12 g; KH_2PO_4. 0·1 g; Na_2HPO_4, 0·15 g; ferric citrate, 0·005 g; Bacto-difco agar, 3 g; distilled water 1000 ml and 1 ml solution of trace elements (H_3BO_3, 2·86 g; $MnSO_4$, 2·03 g; $ZnSO_4$, 0·22 g; $CuSO_4$, 0·08 g; Na_2MoO_4, 0·11 g and distilled water 1000 ml.)

Half ml of different strains of rhizobial culture were added separately to culture tubes and three replicates prepared for each strain. Control received no inoculum. The culture tubes were incubated at 25°C and observations made after 10 days of inoculation under a microscope (450 magnification).

TABLE I

Effect of homologous and heterologous strains of Rhizobium *on root hair invasion of* Trifolium alexandrinum

Rhizobium sp.	Nodulating host	Percentage* of curled root hair	Percentage* of hairs with infection thread
R. trifolii	*Trifolium alexandrinum*	44	6
R. phaseoli strain 1	*Phaseolus mungo*	35	—
R. phaseoli strain 2	*Phaseolus aureus*	33	—
R. leguminosarum strain 1	*Pisum sativum*	27	—
R. leguminosarum strain 2	*Cicer arietinum*	27	—
Rhizobium sp.	*Cajanus cajan*	22	—
Rhizobium sp.	*Lathyrus sativus*	23	—
Rhizobium sp.	*Vicia faba*	20	—
R. japonicum	*Vigna catjang*	11	—
R. meliloti strain 1	*Melilotus alba*	10	—
R. meliloti strain 2	*Melilotus indica*	10	—
Control		—	—

*Mean of three replicates each with average of 5 microscopic fields.

Results And Discussion

Curling of root hairs occurred in plants inoculated with homologous as well as heterologous *Rhizobium* strains (Table I). With homologous strain, curling was maximum at 44% while it varied from 35% to 10% with different heterologous strains. Root hair infection in homologous and heterologous combination of rhizobia and legume is apparently common (Li & Hubbell, 1969; Burgin & Wolf, 1959). They considered a successful infection only when bacteria were present in root hairs. Fahraeus (1963) believed that a co-factor released from host root is

closely associated with curling of root hairs in legumes. Although nothing is known about the co-factor, the idea is attractive. We believe that the co-factor is responsible for curling induced by the strains and the homologous strains induced more production of the factor than the heterologous strains. Curling and deformation of root hairs were not uniformly distributed along the roots but at some localized area which are susceptible to infection and nodulation. This observation is in accord with that of Nutman (1959).

Infection thread developed only with the homologous strain which was specific. Clearly, infection thread formation is the basis for specificity in *Rhizobium* and *Trifolium alexandrinum* symbiosis. Similar observations have been observed in *Rhizobium* strawberry clover association (Li & Hubbell, 1969). Recently Bohlool and Schmidt (1974) postulated that lectins present on the legume root surface may interact specifically with the appropriate *Rhizobium* cell and influence specificity. However, Dazzo and Hubbell (1975) observed that concanavalin A, a lectin from jackbean *(Canavalia ensiformis)*, combined strongly with various nodulating and non-nodulating strains of *Rhizobium* regardless of host specificity. They further reported that the interactions between legume lectins and *Rhizobium* cells may not always account for the specificity expressed by nodule bacterie for their respective legume host. Admittedly, the specificity of *Rhizobium* to the respective legume host continues to remain moot question.

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