

# OBSERVATION OF BINDING OF *BRADYRHIZOBIUM JAPONICUM* A1017 TO ROOT HAIR, CURLING OF ROOT HAIR AND INFECTION THREAD FORMATION BY BRIGHT AND PHASE CONTRAST MICROSCOPY

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The objective of this study is to analyse the morphological characteristics of the components responsible for the feature of the nodulation process to promote the introduction of more effective rhizobial strains into soils.

## Materials and Methods

*Bradyrhizobium japonicum* strain A1017 was cultured in a liquid medium composed of mannitol-yeast for 5 days at room temperature. Bacterial cells were harvested by centrifugation at 5,000 rpm for 20 minutes. The rhizobial pellets were washed two times with sterile saline and suspended in 20ml of the same solution. The suspension (original solution) was stirred for 20 minutes in order to release clusters. The numbers of rhizobia in the suspension were  $2 \times 10^8$  cells/ml.

Seeds of the soybean cultivar Doko were surface-sterilized for 20 min. in 70% ethanol, rinsed once in sterile distilled water, and then the surface-sterilized seeds were sown together with 1ml of rhizobial suspension in sterilized vermiculite beds.

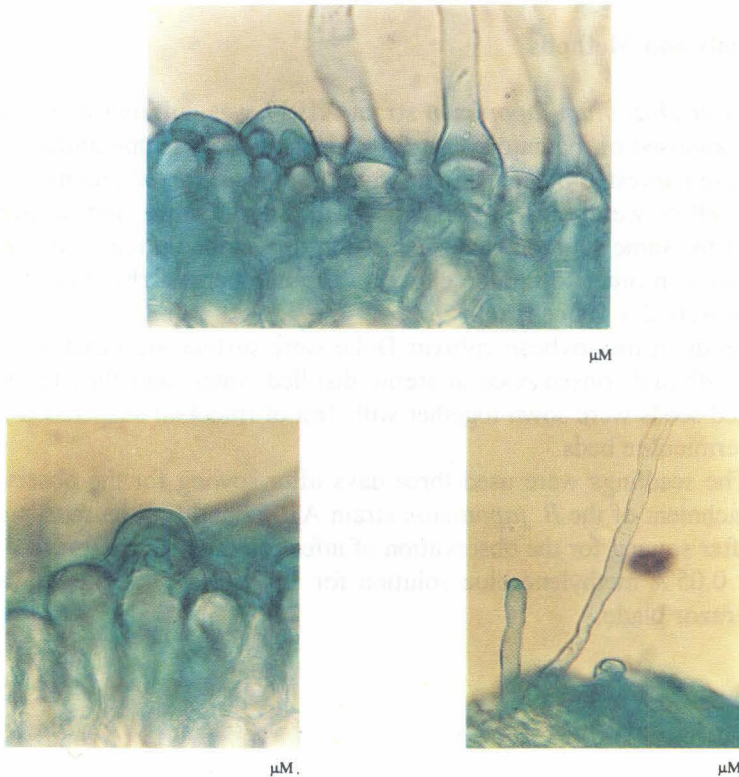
The seedlings were used three days after sowing for the observation of the attachment of the *B. japonicum* strain A1017 and root hair curling, and 7 days after sowing for the observation of infection threads. Roots were stained with a 0.05% methylene blue solution for 15 - 20 sec. Segments were cut with a razor blade.

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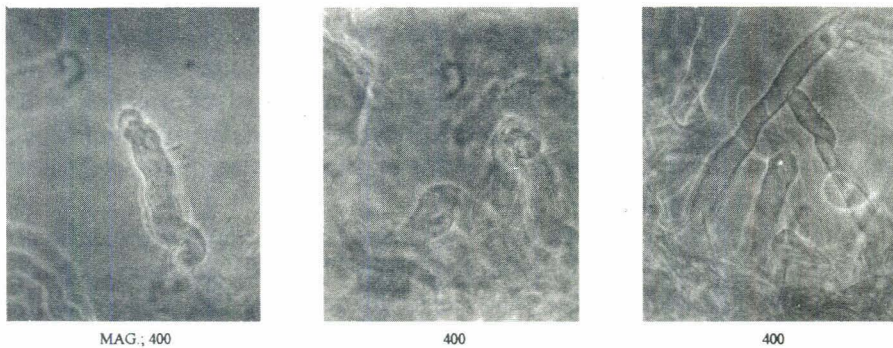
## Results and Discussion

Competition involves the following sequence: rhizobium multiplication in the rhizosphere, attachment of rhizobia to the root hair of host plant, formation of infection threads within the root hair, etc. - during nodule formation. Figure 1 (A) shows the attachment of inoculated rhizobia on the surface of a growing epidermal cell. In soybean, the rhizobia which become attached to epidermal cells on the last stage of growth can form infection threads and root nodules.

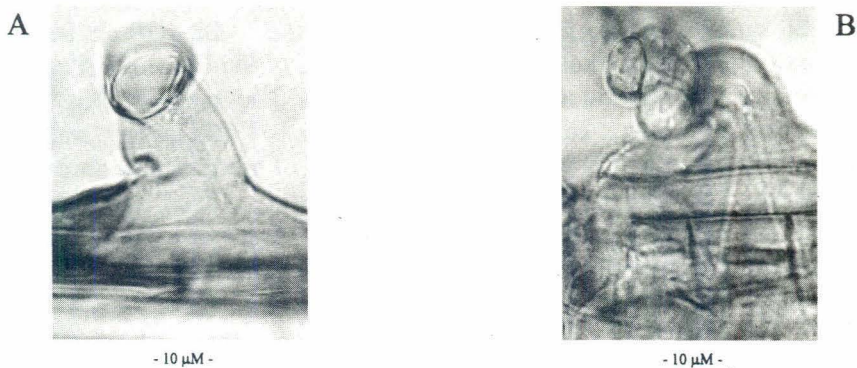


**FIG. 1 - Bright microscopic observation of the binding of *B. japonicum* strain A1017 to root hairs and root hair curling in the soybean cultivar Doko after staining with methylene blue (3 days after sowing together with inoculation).**

The microscopic photographs in Figures 1, 2 and 3 were obtained from segments cut with a razor blade at CPAC. The use of the fluorescent-antibody technique in conjunction with the observation of rhizobia on the surface of the epidermal cells by bright microscopy, enabled to differentiate the inoculated strains from the indigenous strains. As a result it was possible to distinguish the competitiveness ability between inoculated and indigenous strains.



**FIG. 2 - Phase contrast microscopic observation of the binding of *B. japonicum* strain A1017 to root hair and root hair curling in soybean cultivar Doko.**



**FIG. 3 - Bright microscopic observation of infection thread in soybean.**

A: Infection thread formed in root hair

B: Infection thread growing toward inner cortex cells