Novel nodule formation and development in a supernodulating soybean mutant SS2-2

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Objective

To investigate the micro-structural events associated with the formation and development of nodules of supernodulating soybean mutant SS2-2 and its wild type.

Materials and Methods

Soybean genotype: SS2-2 (supernodulating soybean mutant) and Sinpaldalkong 2 (the wild type).

Inoculum: *Bradyrhizobium japonicum* USDA 110.

Treatment: Surface-sterilized-soybean seeds were inoculated with *B. japonicum* and grown in greenhouse in the absence of exogenous nitrogen supply. Nodules and primary root segments, harvested at various days after emergence/inoculation were fixed in modified Karnovsky's fixative solution for 2-4 hour. After fixation, the samples were *en bloc* stained for overnight and dehydrated, and then embedded in Spurr's resin. Microtome sections were placed on a glass slide coated with poly-lysine and stained with Toluidine Blue and then observed with light microscope.

Results and Discussion

SS2-2 produced substantially higher curled root hairs than its wild type especially at 14 days after inoculation (Fig. 1 & 2). Beside through root hairs, *B. japonicum* also entered mutant host, SS2-2 through "cracks" created by the emerging adventitious root primordial (Fig. 3). Early steps of nodule ontogeny were faster in SS2-2, more frequent and a higher proportion of initiated nodules continued to develop than its wild type (Fig. 4, Table 1). Both in the wild type and supernodulating mutant, vascular bundles bifurcate from root stele and branch off in the nodule cortex to surround the central infected zone (Fig. 5). This findings indicate that supernodulating soybean has complete endosymbiosis and forms complete developed nodule vascular bundles as the wild type, even though both they have different speed on nodule ontogeny.

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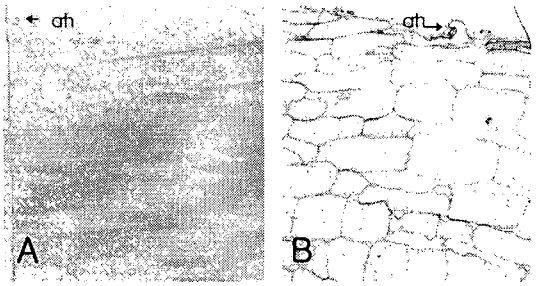

Fig. 1. Longitudinal section of primary root harvested 14 DAE/DAI. (A) Wild type, (B) Supernodulating mutant, Abbreviations: crh: curled root hair.

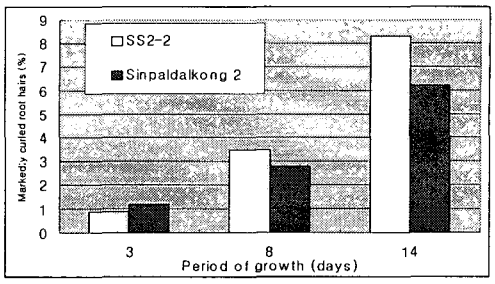

Fig. 2. Proportion of markedly curled root hairs of the primary root from total number observed between symbiotic supernodulating mutant and its wild type.

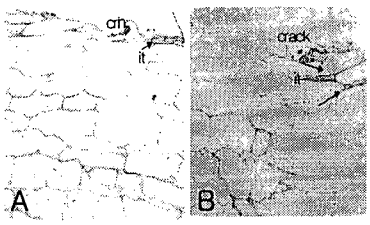

Fig. 3. Toluidine-stained primary root harvested 14 DAE/DAI (A) Infection of *B. japonicum* through root hair caused curled root hair; (B) Infection of *B. japonicum* through "crack" in the surface.

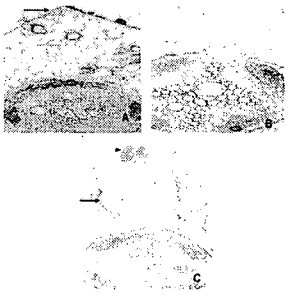

Fig. 4. Different stages of nodule development in soybean shown in transverse sections. A, Arrow indicates the developing primary, B, secondary meristem and C, the connections of them (arrow) and nodule differentiation.

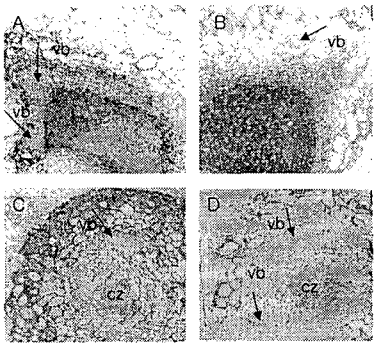

Fig 5. Serial sections of nodules harvested 30 (A-B) and 50DAE/DAI (C-D); c: cortex, cz: central zone, vb: vascular bundles.

Table 1. Frequencies of centers of cortical cell division in various developmental stages on primary roots of soybean 8 days after emergence and inoculation of *B. japonicum.*

Genotype	Density cells/ml	No. of sections	Sections observed	Stage[1]		
				A	B	C
SS2-2	0	60	25	0	0	0
	10^8	61	27	8	5	14
Sinpaldalkong2	0	59	24	0	0	0
	10^8	63	25	7	4	4

[1]The different stages correspond to the classification of Calvert et al. (1984)