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Genetic Mapping of Hypernodulation in Soybean Mutant SS2-2

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ABSTRACT: Hypernodulation soybean mutant, SS2-2, is characterized with greater nodulation and nitrogen fixing ability in the root nodule than its wild type, Shinpaldalkong 2. The present study was performed to identify a genetic locus conferring hypernodulation in soybean mutant SS2-2 and to determine whether the gene controlling the hypernodulation of SS2-2 is allelic to that controlling the supernodulation of nts382 mutant. Hybridization studies between SS2-2 and Taekwangkong revealed that the recessive gene was responsible for the hypernodulation character in soybean mutant SS2-2. Allelism was also tested by crossing supernodulating mutant nts382 and hypernodulating mutant SS2-2 that both hypernodulation and supernodulation genes were likely controlled by an identical locus. Molecular marker mapping of hypernodulation gene in SS2-2 using SSR markers confirmed that the gene conferring hypernodulation was located at the same loci with the gene conferring supernodulation. It is interesting to note that the same gene controlled the super- and hyper-nodulation characters, although SS2-2 and nts 382 exhibited differences in the amount of nodulation in the root system. Further genetic studies should be needed to clarify the genetic regulation of super- and hyper-nodulation in soybean.

Keywords **:** soybean, hypernodulation, genetic map, SSR marker

Soybean is the most important legume crop, contributing the biological fixation of nitrogen in the root nodule. Since nitrogen-fixing bacteria provide a significant source of ammonia for host plant growth, biological nitrogen fixation is of major agronomic importance. Recent isolation of super-/hyper-nodulating soybean mutants has stimulated the research on the enhancement of biological nitrogen fixation for agricultural purposes.

A number of soybean nodulating mutants have been isolated that are characterized with greater nodulation and continue to nodulate even in the presence of exogenous nitrogen supply (Gresshoff and Delves, 1986). These are called supernodulating and hypernodulating soybean mutants. Supernodulating soybean mutant produces nodule number far in excess of wild types, and hypernodulating soybean mutant exhibits nodulation between supernodulationg and wild type nodulation.

Carroll *et al.* (1985) selected several supernodulating *nts* lines which were defined as having from six- to ten-fold increase in nodule number relative to their parent. When the supernodulating mutants were crossed with the wild-type cultivar Bragg, all F_1 plants exhibited wild type nodulation, indicating that a recessive gene controlled supernodulation in this mutant (Delves *et al.* 1988). Also, the *nts* locus in the F_2 population was segregated as a single Mendelian gene (three wild-types/one supernodulating). Using a similar mutagenesis approach, Gremaud and Harper (1989) isolated hypernodulating mutants (NOD1-3, NOD2-4, NOD3-7, and NOD4) which expressed a two- to four-fold increase in nodule number relative to their parent, cv. Williams. Pracht et al. (1993) reported that a single recessive gene was also responsible for the nodulation phenotype of NOD4. Also, Akao and Kouch (1992) selected a supernodulating mutant En6500 which showed a six-fold increase in nodule number in the presence of combined nitrogen relative to the Enrei parent. Kokubun and Akao (1994) reported that a single recessive allele was involved in supernodulation of En6500. This gene was allelic to that in a supernodulating mutant nts382 (Carroll *et al.*, 1985).

Using clones from the RFLP map generated at Iowa State University (Keim *et al.*, 1990), Landau-Ellis *et al.* (1991) reported that nts382 allele was linked to the pA-132 marker on what is now known as Molecular Linkage Group H. However, the pA-132 clone turned out to be a chimera comprising three unrelated and non-contiguous *Pst*I fragments (Landau-Ellis and Gresshoff., 1994). One of the subclones from pA-132, labeled pUTG-132a, 1.72 kb in length, was the only one linked to *nts*.

Recent soybean public linkage map (USDA/ISU) was constructed using about 1004 polymorphic markers containing SSR, RFLP, RAPD, isozyme and classical genetic markers (Cregan *et al.*, 1999). This map consisted of 486 SSR markers that exhibited high levels of length polymorphism in soybean (Akkaya *et al.* 1992; Maughan *et al.*, 1995; Powell *et al.*, 1996; Diwan and Cregan, 1997). On the basis of public USDA soybean genetic map, supernodulation gene mapped to pA132 on linkage group (LG) H.

SS2-2 is a hypernodulating soybean mutant that was isolated from M_2 families of Shinpaldalkong 2 mutagenized

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with 30mM EMS (Lee *et al.*, 1997). Hypernodulating soybean mutants, SS2-2 showed three- to five-times more nodules and nodule mass, and greater C_2H_2 activity than the wild type, Shinpaldalkong 2, regardless of the level of exogeneous nitrogen supply (Lee *et al.*, 1998). However, the inheritance of the hypernodulation character has not yet been clarified.

The present study was performed to identify a genetic locus conferring hypernodulation in soybean mutant SS2-2 and to determine whether the gene controlling the hypernodulation of SS2-2 is allelic to that controlling the supernodulation of *nts* 382.

MATERIALS AND METHODS

Hybridization studies

Hybridization was made between hypernodulating SS2-2 and supernodulating nts 382 to test the allelism between hypernodulation and supernodulation. Nodulation characters for F_1 and F_2 were evaluated. Chi-square tests were used to determine the fitness of observed segregations to expected genetic ratios.

Genetic mapping population

A soybean population derived from a cross of SS2-2 × Taekwangkong was used to construct a genetic linkage map and to evaluate nodulation characters. Hypernodulating soybean mutant SS2-2 have white colored flower and green hypocotyl, while normal nodulating soybean Taekwangkong have purple colored flower and hyocotyl. From this cross, a total of 72 lines were developed with each line originating from a different F_2 plant.

SSR marker analysis

To obtain DNA for the SSR polymorphism survey of 72 F_2 derived lines from a cross between SS2-2 and Taekwangkong, seeds were sown at the University Farm of Seoul National University, Suwon, on 1999, and leaves were harvested from seedlings prior to full expansion. The genomic DNA was isolated according to the procedure of Keim et al. (1998). A total of 11 SSR markers in linkage group H were screened for amplicon length polymorphism. The SSR primer synthesis and fluorescent 5-end labeling of the forward primer of each primer pair was done by PE-ABI (Foster City, CA). The forward primers were labeled with either blue (6-Fam), green (Hex), or yellow (Ned) fluorescent tags (Ziegle *et al.* 1992). Amplication using PCR reactions was performed according to the protocol of Diwan and Cregan (1997). PCR was carried out in a total reaction volume of 10 μl, containing 0.5 U of AmpliTaq Gold polymerase (Perkin Elmer Biosystems, Foster City, CA), 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2.6 mM $MgCl_2$, 0.2 mM of each dATP, dCTP, dGTP, and dTTP (Perkin-Elmer Biosystems, Foster City, CA), including 50 ng of template DNA, and and 5uM of each primer. PCR was performed with a MJ Research PTC100 Programmable Thermal Controller (MJ Research, USA). The cycling consisted of 25s of denaturation at 94°C , 25 annealing at 46°C, and 25s of extension at 68°C for 32 cycles. Amplication products of a number of SSR markers (usually 3 to 5) with different fluorescent labels and/or with different allele sizes were pooled together, and the sample of combined PCR products was loaded and separated on an ABI Prism 377 DNA sequencer (AB-PEC, Foster City, CA). GeneScan software (AB-PEC, Foster City, CA) was used for gel image analysis, and Genotyper software (AB-PEC, Foster City, CA) was used for accurate characterization of the alleles and for automated data output.

Phenotypic data

Data were collected for nodulation characters in the root. Six to seven plants from F_2-dervied F_3 families were evaluated for nodulation characters at flowering stage in the field. Nodulation characters were classified as homozygous hypernodulating, homozygous normal nodulation, or heterozygous nodulation characters.

Genetic linkage map construction

SSR markers polymorphic with respect to the parents were used for mapping. A linkage map was constructed with SSR and nodulation data using the Kosambi map function of Mapmaker (Lander *et al.*, 1987). For grouping markers into linkage groups, a minimum LOD of 3.0 and a maximum distance of 40 cM were used.

RESULTS AND DISCUSSION

A hyernodulating soybean mutant SS2-2 was used as a female parent and crossed with a normal nodulating soybean Taekwangkong to establish a SSR linkage map and to map hypernodulating gene. From five F_1 plants for the cross were evaluated for nodulation pattern in the greenhouse. All F_1 progeny expressed normal nodulation. The result revealed a recessive gene was responsible for the hypernodulation character in mutant soybean SS2-2.

Genetically, all supernodulating and hypernodulating mutants isolated previously were under recessive monogenic control, and supernodulation of nts 382 isolated from

Table 1. Polymorphism of SSR markers in linkage group H.

SSR markers	Genotypes	
	SS2-2	Taekwangkong
	bp	
Sat_ 127	277/279	283
Satt 192	244	250
Satt 253	149	149
Satt 279	195	192
Satt 314	239	236
Satt 353	178	169
Satt 434	347	347
Satt 442	236	245
Satt 469	148	148
Satt 541	174	171
Satt 568	242	235

Table 2. Segregation of molecular markers and nodulation character in F_3 families of SS2-2/Taekwangkong.

Marker/ phenotype	Genotype†			$X^2_{1:2:1}$‡
	S/S	S/T	T/T	
Hyp	3	42	27	18.0
Satt279	15	36	21	1.0
Satt314	19	29	24	3.4
Satt353	4	33	33	24.3
Satt442	13	32	27	6.3
Satt541	16	31	25	3.6
Satt568	12	37	23	3.4
Sat_127	17	32	21	1.0

†S/S : homozygous SS2-2/SS2-2
S/T : heterozygous SS2-2/Taekwangkong
T/T : homozygous Taekwangkong/Taekwangkong
‡Chi-square at the 5% level of significance and 2 degree of freedom is 5.99.

Bragg soybean variety using EMS mutagenesis was controlled by the same gene with that of En6500 from Enrei (Delves *et al.*, 1988; Kokubun and Akao, 1994; and Pracht *et al.*, 1993a). However, the relationship of our mutant SS2-2 with these genes was unknown. Thus, to investigate allelism, a hyernodulating soybean mutant SS2-2 was used as a female parent and crossed with a supernodulating soybean mutant *nts*382. Four F_1 progeny and all thirty F_2 progeny represented hyper-/super-nodulation characters. This indicated that both hypernodulation and supernodulation genes were likely controlled by an identical locus.

As the genetic locus controlling supernodulation in soybean was co-segregated tightly with the cloned molecular marker pUTG-132a in genetic LG H (Landau-Ellis *et al.*, 1991), eleven SSR primer pairs in LG H were selected to survey parental polymorphism against SS2-2 and Taekwangkong (Table 1). Seven out of eleven SSR primer pairs, detected polymorphism. SSR analysis was done on the F_2 derived populations of the hypernodulating mutant SS2-2 crossed with a normal nodulating soybean Taekwangkong using SSR marker shown polymorphic characteristic between parent.

The cosegregation of SSR markers and the nodulation phenotype were presented Table 2. However, Satt 353 marker and nodulation phenotypic data showed severe segregation distortion, the rest markers fit an expected ratio of 1 : 2 : 1. This may be partially explained by the fact that several genes such as gametophyte gene, low crossability gene, hybrid sterility gene, or gametic lethal may be closely linked to the genomic region exhibiting the segregation distortion (Tsunematsu, 1996).

Phenotypic data on hypernodulation from 72 F_3 families were combined with SSR marker segregating data, and genetic linkage map in LG H was constructed (Fig. 1). Eight

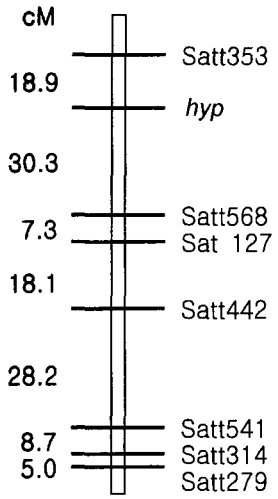

Fig. 1. Linkage map of linkage group H of soybean showing the location of the hypernodulation gene (*hyp*) and linked SSR markers.

markers including hypernodulation trait were genetically linked covering 116.7cM. Hypernodulation gene (*hyp*) was 18.9 cM away from Satt 353. Based on the USDA map (Cregan *et al.*, 1999), Satt 353 was near pA132 which was known to be closely linked to the supernodulating *nts* gene. From these results, it can be concluded that the gene conferring hypernodulation is located at the same loci with the gene conferring supernodulation. Further studies will be needed to know the inheritance mode for hypernodulation and supernodulation in soybean.

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