〔韓作誌(Korean J. Crop Sci.) 42(2) : 247～253(1997)〕

Introduction, Development, and Characterization of Supernodulating Soybean Mutant.
1. Mutagenesis of Soybean and Selection of Supernodulating Soybean Mutant

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다량 뿌리혹 형성 콩 계통의 도입 개발 및 생육특성구명
1. 돌연변이유기에 의한 콩 초다뿌리혹형성 계통선발

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ABSTRACT : Development of soybean cultivars with great nodulation and high nitrogen fixation activity, derived mostly from mutagenesis, may decrease inputs of chemical fertilizer nitrogen into the soil-plant system. Soybean seeds(cv. Jangyupkong, Hwanggeumkong, and Geomjungkong 1) were treated with three different levels of EMS(ethyl methanesulfonate) concentration(30, 50, and 70mM). Increasing the doses of EMS resulted in decreased field emergence rate of seeds, whereas it did not increase M_2 mutation frequencies. This indicated that the most efficient concentration of EMS was 30mM for generating mutants. Extensive mutagenesis of Sinpaldalkong 2 with 30mM EMS was undertaken to isolate soybean mutants with greater nodulation. Approximately 8,200 M_2 families were screened for greater nodulation on 5 mM nitrate after inoculation with *Bradyrhizobium japonicum* strain YCK213-KFCC-10728. Mutant SS-2 nodulated more than the wild type. Comparison of supernodulation between SS-2 and two nts mutants(nts 1007 and nts 1116) revealed that SS-2 showed the supernodulation character at an earlier growth stage than the two nts mutants. Further studies should be needed to characterize the difference in timing of nodulation between SS-2 and nts mutants.

Key words : Soybean, Nitrogen fixation, Nodulation, Mutagenesis.

Nitrogen is generally the major limiting factor in crop production. However, if run off, abundant N fertilized in the field can result in ground water pollution and river eutrophicaton(Lee and Hwang, 1993). In a soybean crop, enhancing N_2 fixation by the symbiotic interaction between the plant host and *Bradyrhizobium japonicum* is an attract-

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본 연구는 1996년도 교육학술연구조성비(식물환경과학:농95-7)에 의하여 연구되었음.

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ive approach to reducing the nitrogen fertilizer requirements and protecting the environments. As a result, much research in legume crop breeding has focused on the creation of genetic variation in nodulation and nitrogen fixation.

In soybean, nodulation mutants have been isolated either from normal soybean populations or by chemical or physical mutagenesis (Carroll et al., 1985a, 1985b, 1986; Gremaud and Harper, 1989; Gresshoff and Delves, 1986; Vance et al., 1988). The isolated mutants showed four major phenotypes, including non-nodulation, ineffective nodulation, hypernodulation, and supernodulation.

Recently, three groups of scientists have independently isolated supernodulating soybean mutants from 'Bragg' cultivar in Australia(Carroll et al. 1985a, 1985b), 'Williams' cultivar in USA (Gremaud and Harper, 1989), and 'Enrei' cultivar in Japan(Akao and Kouchi, 1992; Kokubun and Akao, 1994) of soybeans. Several supernodulating mutants have been isolated in another legume crops, including *Pisum sativum*(Jacobsen and Feenstra, 1984) and *Phaseolus vulgaris*(Park and Buttery, 1988; Buttery and Park, 1989). These supernodulating mutants may not only be unique biological materials for study of host plant factors which could be involved in the process of nodulation, but also be an useful germplasm for breeding program to improve nodulation and nitrogen fixation ability.

The present study was performed to survey the variability of M_2 plants caused by EMS mutagenesis on three soybean genotypes. In addition, the isolation of supernodulating soybean mutant, derived from Sinpaldalkong 2, was described here, and comparison of this mutant was made with with supernodulating nts(nitrate tolerant symbiotic) mutants derived previously from Bragg by EMS mutagenesis.

MATERIALS AND METHODS

Soybean seeds of three varieties, 'Geomjungkong 1', 'Hwanggeumkong', and 'Jangyupkong', were mutagenized with 30, 50, 70 mM EMS(ethyl methanesulfonate) concentrations. Seeds of soybean were presoaked for 16hrs in water aerated vigorously with electric air pump, then treated with 30, 50, and 70mM EMS for 6 hrs. After postwashing for 10hrs in running tap water, seeds were planted immediately in the greenhouse. Irrigation was made enough to reduce the dry-back damage. On the basis of the seed emergence and the frequency of morphological variants, the most effective EMS concentration was shown to be 30mM for generating mutants. Extensive screening for soybean mutants altered in nodulation was carried out on 13,000 M_2 families derived from mutagenesis of 'Sinpaldal 2' with 30 mM EMS(pH 7.0). Seeds of soybean were mutagenized as described above. M_1 plants were harvested individually to give M_2 families for screening nodulation mutants.

For mutant selection altered in nodulation, 4 to 5 seeds from each family were planted in a paper pot (approximately 7cm in diameter and 7.5cm in height) filled with river sand, and after emergence inoculated with *Bradyrhizobium japonicum* strain YCK213-KFCC-1 0728 which was supplied by Wee-Keum Kang (Yeongnam Agricultural Experiment Station). Screening procedure was done in the vinyl house during winter, and outside during spring and summer. Plants were fed two times per week with modified Munn's solution containing the high level of nitrate (5mM). At the V4 to V5 growth stage, each M_2 plant was screened for nodulation characters. Two M_2 families segregated for the greater nodulation phenotype were selected, and transplanted into the Wagner pot.

One of these two putative mutants was again verified on greater nodulation in M_3 generation. Growth and nodulation of this soybean mutant derived from sinpaldal 2, named as 'SS-2', were compared with its wild type and previously isolated supernodulating mutant(nts 1007) and hypernodulating mutant(nts 1116) by Carroll et al.(1985a, 1985b). Two nodulating nts mutants were provided by P. M. Gresshoff(Univ. of Tennessee).

RESULTS AND DISCUSSION

To assess plant injury caused by EMS mutagenesis, emergence rate of M_1 seeds was determined at 14 days after planting (Table 1). Within the same EMS concentration, field emergence of EMS-treated seeds of Geomjungkong 1 was higher than that of Jangyupkong and Hwanggeumkong. Regardless of soybean genotypes, increasing the doses of EMS resulted in decreased germination frequencies. The same result that decreasing seed emergence rate is associated with the increase in the concentration of mutagens was also observed using MME (methanesulfonic acid methyl ester) or sodium azide as a mutagen (data not shown). This is consistent with most earlier studies that examine the association of field germination with mutagen concentration(Carroll et al., 1986; Ryan et al., 1983). Contrary to this, our previous study revealed that field germination was not affected by EMS concentration in the range of 30 to 50mM (Lee et al., 1993; 1995). This inconsistency of the sensitivity to mutagenic treatment may be related to the difference in plant genotypes as well as mutagenic procedure including presoaking and postwashing conditions.

Table 1. Genotypic differences in seed emergence rate as affected by EMS concentration

Genotypes	Concentration (mM)		
	30	50	70
	……………%	……………	
Jangyupkong	25.2	20.0	7.5
Hwanggeumkong	19.7	13.7	4.7
Geomjungkong 1	59.7	28.5	11.8

Of 1,144 families in M_2 generation, 66 families showed morphological mutants within family(Table 2). Several types of mutant could be distinguished : 1) chlorophyll-deficient, 2) necrotic, 3) abnormal leaf, 4) dwarf and stunted growth, 5) four to five leaflets, and 6) other morphological variants. Of the several mutation types, chlorophyll-deficiency was the major type of mutation in this study. Regardless of soybean genotypes, increasing the EMS concentration did not result in the increased mutation frequencies, indicating that mutagenesis with 30mM EMS was fairly effective in generating mutants when compared to that 50 or 70mM EMS.

In view of the field emergence rate(Table 1) as well as the frequency of the morphological variants(Table 2), the optimum EMS concentration was thought to be 30mM for generating mutants and maintaining the field emergence rate of M_1 seeds. To isolate supernodulating soybean mutant, approximately 18,000 seeds of Sinpaldalkong 2 were treated with 30 mM EMS. About 46 % of M_1 seeds were germinated in the field. Of

Table 2. Frequency of morphological variants in M_2 soybean plants mutagenized with EMS

Genotypes	Dose (mM)	Morphological variants						
		Chlorophyll-deficient	Necrotic	Abnormal leaf	Dwarf	Abnormal leaflet number	Others	Tatal
Jangyupkong	30	3	1	0	1	0	1	6
	50	4	1	0	1	0	1	7
	70	4	1	0	1	2	1	9
Hwanggeumkong	30	3	0	3	1	0	0	7
	50	1	0	2	2	0	0	5
	70	4	0	1	3	0	1	9
Geomjungkong 1	30	5	0	2	0	0	0	7
	50	6	0	2	0	0	1	9
	70	4	1	1	1	0	0	7
Total		34	4	11	10	2	5	66

Table 3. Comparison of nodulation and growth characters of nodulating mutants and wild-type soybean plant

Genotypes	Days after seeding			
	30			72
	Plant height	Nodule dry weight	Nodule number	Nodule number
	cm	mg /plant	number /plant	number /plant
Sinpaldal 2	$27.4^{a\dagger}$	37.5^{b}	35.9^{b}	20.7^{c}
SS-2	13.4^{c}	76.2^{a}	141.8^{a}	169.3^{a}
nts 1007	19.5^{b}	22.9^{b}	28.7^{b}	113.3^{b}
nts 1116	18.5^{b}	23.4^{b}	25.2^{b}	73.3^{bc}

† Within trait, means not followed by the same letter are significantly different at $P \leq 0.05$ based on LSD.

8,238 M_2 families, some of individual plants in 132 families were chlorophyll-deficient. Several types of mutant were also observed in M_2 generation, including abnormal leaf(12 families), dwarf(4 families), abnormal leaflets(8 families), and other mutants (21 families).

Two mutants with greater nodulation were selected in M_2 generation on the high exogeneous nitrate supply(10 mM). Greater nodulation character in M_3 generation was again found in one of two mutants, and this was named as SS-2. Mutant SS-2 plants were characterized with smaller plant height, greater nodule dry weight, and nodule number when compared to the wild type Sinpaldalkong 2(Table 3), which is consistent with the previous experiment by Carroll et al. (1985b) that supernodulating nts mutant showed smaller growth than parent cultivar Bragg. This is probably due to the greater demand of leaf photoassimilates for nodule growth and maintenance of supernodulating mutants when compared to the wild type. Of specific interest was the earlier nodulation of supernodulating SS-2 mutant than two nts mutants (Table 3). Greater nodulation was shown in SS-2 mutant at 30 days after planting, whereas two nts mutants were not different from normal soybean cultivar Sinpaldalkong 2. However, greater nodulation was also observed in nts mutants at 72 days after

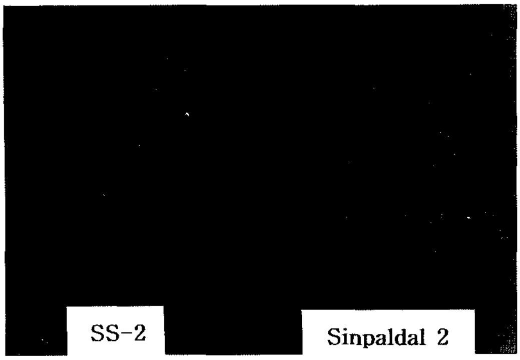

Fig. 1. Nodulation of Sinpaldalkong 2 and M_4 soybean mutant (SS-2) selected from segregating EMS-derived M_2 families. Plants were grown for 72 days in the greenhouse.

planting.

As shown in Fig. 1, nodulation of wild type Sinpaldal 2 were clustered near the root crown. This might be due to the autoregulatory response of nodulation(Bhuvaneswari et al., 1980; Pierce and Bauer, 1983), which was caused by suppressive nodulation in the younger regions of the root caused by prior inoculation of more matured regions of the root. However, this autoregulation of nodulation was not present in nts mutants (Day et al., 1989; Olsson et al., 1989). Also, our mutant SS-2 shows good nodulation in the younger regions of the root in the presence of his nitrate sappcy (Fig. 1).

The nodulation mutant isolated in this study should be an useful biological material to characterize the host plant role in the regulation of nodulation. Also, this supernodulating characters could be of highly agronomic value in improving nodulation and nitrogen fixation. Further genetic crosses and grafting studies among SS-2 mutant, nts mutants, and their wild types will be made to confirm precisely the mode of the inheritance of supernodulating characters.

ACKNOWLEDGEMENTS

The authors thank Prof. P. M. Gresshoff, Institute of Agriculture and Center for Legume Research in the Univ. of Tennessee for providing seeds of supernodulating nts soybean mutants.

적 요

콩에서 돌연변이를 효율적으로 유기시킬 수 있는 적정 EMS농도를 결정하기 위하여, 황금콩, 장엽콩, 검정콩 1호에 30, 50, 70mM EMS 세 수준으로 처리한 다음 M_1종자의 포장발아율과 M_2세대의 돌연변이체 출현빈도율을 조사하였으며 다량 뿌리혹형성 변이체 선발을 위하여 신팔달콩 2호 종자 약 18,000립에 30mM EMS를 처리하여 M_2세대에서 선발하고, 선발된 변이체의 뿌리혹 형성능력을 미국 초다뿌리혹형성 nts계통과 비교한 결과는 다음과 같다.

1. M_1종자의 포장출현율은 검정콩 1호가 황금콩과 장엽콩보다 높은 경향이었으며, 세 품종 모두 EMS처리 농도가 증가함에 따라 포장출현율이 낮았다.
2. M_2세대에서 돌연변이개체는 엽록소 결핍개체가 가장 많이 출현하였는데, 그외에도 생장점 괴사, 엽이상, 엽수변이체, 단경 등 다양한 변이양상을 보였다.
3. 30mM EMS처리가 포장발아율도 높고, 돌연변이개체 출현율도 50, 70mM에 비하여 양호한 편으로 판단되었다.
4. 신팔달콩 2호로부터 30mM EMS처리에 의하여 M_2세대에서 뿌리혹 형성 nts계통보다도 많은 뿌리혹이 형성되었으며, 뿌리혹형성도 일찍 시작되었다.

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