SCREENING WITH NUCLEAR TECHNIQUES FOR YIELD AND N_2 FIXATION IN MUNG BEAN IN THAILAND

N. BOONKERD
Suranaree University of Technology
Nakhon Ratchasima

P. WADISIRISUK
Department of Agriculture
Bangkok

S. SIRIPIN
Maejo University
Chiang Mai

Thailand

T. MURAKAMI
Nagano Chushin Agricultural Experiment Station
Soga Nagano, Japan

S.K.A. DANSO[1]
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agricullture
Vienna, Austria

Abstract

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For a farmer to reap benefit from mung bean's (*Vigna radiata*) capacity to fix N_2, the crop's requirement for N must come mainly from the atmosphere through symbiotic fixation in the root nodules. The aim of this study was to evaluate recommended mung-bean cultivars and advanced breeding lines, and identify high fixers. Preliminary investigations with the ^{15}N natural-abundance method indicated its utility for measuring N_2 fixation, and the examination of five recommended cultivars and two advanced breeding lines of mung using the ^{15}N-dilution method showed diversity in N_2 fixation and yield.

More than 400 lines of mung bean were screened in soil in cement containers for growth, nodulation, N accumulation and N_2 fixation at 35 days after planting, with the natural-abundance method used to determine N_2 fixation. Genetic variability was observed for all characteristics. Estimates of fixed N ranged form 0-300 mg N/plant. Whereas some lines obtained N mainly from fixation, recommended cultivars apparently obtained their N mainly from soil. The data are discussed in terms of reliability of the ^{15}N natural-abundance method.

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[1] Present address: University of Ghana, Legon, Accra, Ghana.

1. INTRODUCTION

Mung bean (*Vigna radiata*) is an economically important crop, and, as a legume, it is capable of fixing atmospheric N_2 in symbiosis with *Bradyrhizobium*. Rhizobial strains vary in their N_2-fixing effectiveness [1, 2], and the genotype of the legume host also influences the symbiosis. In peanut (*Arachis hypogaea*) for example, certain host-genotype traits were observed to be indicative of N_2 fixation [3]. Genotypic variation in host-plant control of nodulation and N_2 fixation has also been demonstrated with soybean (*Glycine max*); in high-nitrate soil, lines of Korean origin produced more nodules and fixed more N than did commercial lines of US origin [4]. And "supernodulating" soybean genotypes produced by mutation-breeding formed up to five times more nodules than did the parent cultivar [5], indicating the opportunity to improve nodulation in other legume species In this study, we were interested in identifying mung-bean genotypes with superior N_2-fixation ability.

There are several methods for measuring N_2 fixation by legumes, and it is generally accepted that the ^{15}N-dilution technique is the most reliable and accurate [6]. However, isotope dilution requires expensive ^{15}N-labelled fertilizers, whereas the ^{15}N natural-abundance technique does not [7]. This paper describes preliminary mung-bean work with the natural-abundance and isotope-dilution methods to investigate genetic diversity for N_2 fixation.

2. MATERIALS AND METHODS

2.1. Natural-abundance experiment

The experiment was conducted in the field in 1991 at Chainat Field Crop Research Center, Thailand, on a low humic glay of sandy clay loam texture, with two mung-bean lines, VC 2768A and VC 4000-7, the seeds of which were treated with a peat-based inoculant containing rhizobial strains THA302, THA305, and THA100. The non-N_2-fixing reference crops were sorghum (*Sorghum bicolor* cv. Chainat 60), maize (*Zea mays* cv. Suwan-1), upland rice (*Oryza sativa* cv. DOA) and non-nodulating soybean (*Glycine max* cv. D68-0099). Three seeds were planted per hill, spaced at 10 cm with 50 cm between rows, in plots of 4 x 6 m; the seedlings were thinned to one per hill at 7-10 days. The experiment had a randomized complete-block design with four replications. Phosphorus and potassium were applied at 55 kg/ha P_2O_5 and 37 kg/ha K_2O, respectively. Four plants per replicate were harvested at 12, 20, 30, 40, 50, 61, 71, and 81 days after planting. Plants were dried at 70°C and ground for analysis of natural ^{15}N abundance (at the National Institute of Agro-Resources, Tsukuba Japan, with a Finigan MAT251 mass-spectrometer). Estimates of percent N derived from fixation (i.e. from the atmosphere, %Ndfa), were made from $\delta^{15}N$ values as follows [7]:

$$\delta^{15}N\,(\text{‰}) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

$$\%Ndfa = \frac{\delta^{15}N_{reference} - \delta^{15}N_{legume}}{\delta^{15}N_{reference} - \delta^{15}N_{a}} \times 100$$

where $\delta^{15}N_a$ is the natural-abundance value for plants deriving N solely from fixation, determined by culturing inoculated plants with N-free nutrition.

2.2. Isotope-dilution experiment

This experiment was carried out in an irrigated farmer's field in Pakthongchai District, Nakhon Ratchasima. This soil also had a sandy clay loam texture (0.07% N). Three recommended mung-bean cultivars, CN36, PSU1 and KPS2, and two advanced breeding lines, 9-5 (CNM-I-8709-5) and 9-8 (CNM-I-8709-8) were used. Maize and sorghum were again used as non-fixing reference crops. Seed inoculation, P and K applications, plot size, planting distances, thinning, and experimental design were as described in **2.1.**

Labelled fertilizer, $(^{15}NH_4)_2SO_4$ at 10% atom excess, was applied at 20 kg N/ha to an area of 1 m^2 within each plot, by syringe-injection of 50-mL aliquots of solution into the soil near each plant. Unlabelled $(NH_4)_2SO_4$ was applied around each microplot at 20 kg N/ha. For the reference crops, $(^{15}NH_4)_2SO_4$ at 3.3% atom excess was applied at 60 kg N/ha.

At 45 days, mung-bean plants were sampled to determine nodulation and plant dry weight. Seed yield was determined at maturity. In the microplots, all plants were harvested at maturity, and dried, weighed, and ground vegetative components were mixed with ground seed from the respective microplot. Fifty-g aliquots were sent to the FAO/IAEA Soil Science Unit at Seibersdorf, Austria, for ^{15}N analysis.

The proportions of N derived from fixation and the amounts on N fixed by the mung-bean plants were determined as follows:

$$\%Ndfa = \left(1 - \frac{\%^{15}N\ atom\ excess_{legume}}{\%^{15}N\ atom\ excess_{reference}}\right) \times 100$$

$$N\,fixed\ (kg/ha) = \frac{\%Ndfa}{100} \times Total\ N\,yield$$

2.3. Screening experiment

Diverse lines of mung bean, 423 in total, were obtained from the Asian Vegetable Research and Development Centre, Taiwan, the Chainat Field Crop Research Center, Thailand, and Kasetsart University, Thailand. The screening was done in a single batch using cement tanks of 75x75x50 cm containing a low humic glay soil (0.07% N), with 2% by weight of ground corn cobs added to immobilize mineral N. One mung-bean line, fourteen plants, was planted per tank, with spacings of 10 cm intra-row and 50 cm inter-row. Maize (*Zea mays* cv. Suwan-1) was similarly grown as the non-fixing reference crop.

The soil was inoculated with a mixture of broth cultures (10^8 cells/mL) of ten strains of rhizobia that had previously been determined, in a trial of thirty-two strains, to be effective on four test cultivars of mung bean. The strains were: TAL420, TAL1000, TAL441, THA201, THA302, THA305, THA308, CB756, NC92, NC146. Aliquots of approximately 500 mL of the inoculum mixture were added per tank and incorporated into the surface 5 cm of soil. The shoots of four plants per tank were harvested at 35 days after planting to determine total dry weight, nodule number and weight, %N, and amount of N fixed using the ^{15}N natural-abundance method (see **2.1.**)

3. RESULTS

3.1. Natural-abundance experiment

The natural-abundance values for ^{15}N were higher for the 12-day seedlings than for the planted seeds (Fig. 1a). The values peaked at the 12- or 20-day samplings, and thereafter steadily decreased, with rates of decline highest for the mung beans. Nodules were present on the non-nodulating soybean, therefore N_2 fixation possibly contributed to the low $\delta^{15}N$ of 2.5‰ at 80 days. Similar low levels of $\delta^{15}N$ were found in corn and rice at the end of the experiment, possibly due to associative or endophytic N_2-fixing bacteria. The value of $\delta^{15}N$ for sorghum changed less, from 7‰ at 11 days to 5.5‰ at 80 days after sowing (Fig. 1a), therefore it may judged to be the best of the four reference crops. On the other hand, the fact that the $\delta^{15}N$ values increased during the first few days of growth shows that factors other than N_2 fixation influence natural-abundance levels.

In view of the presence of nodules and relatively low $\delta^{15}N$ values at 11 and 20 days, it seemed prudent to exclude the non-nodulating soybean data from the calculations of %Ndfa. Therefore, average values for the sorghum, maize and rice (Fig. 1b) were used to establish the trends of increasing inputs of fixed N for the two mung genotypes (Fig. 1c). The %Ndfa values for pods were closely similar to those for shoots, lending confidence in the method - on the other hand, the trend of decline in %Ndfa for mung cv. VC 2768 A between 50 and 71 days is difficult to explain other than in terms of experimental error. At 81 days, 60-70% of the N in both mung cultivars was derived from fixation.

3.2. Isotope-dilution experiment

At 45 days after sowing, there were no statistically significant differences among the seven mung-bean genotypes in shoot dry weight, nor in root-nodule number and dry weight (Table I). In contrast, at maturity there were significant differences in total dry weight and in stover and seed dry weights (Table II). Plant %N values did not vary significantly nor did values for %Ndfa, therefore the broad ranges in total N (25 - 67 kg N/ha) and amount of N fixed (16 - 42 kg N/ha) occurred as a result of genetic variability in total dry matter.

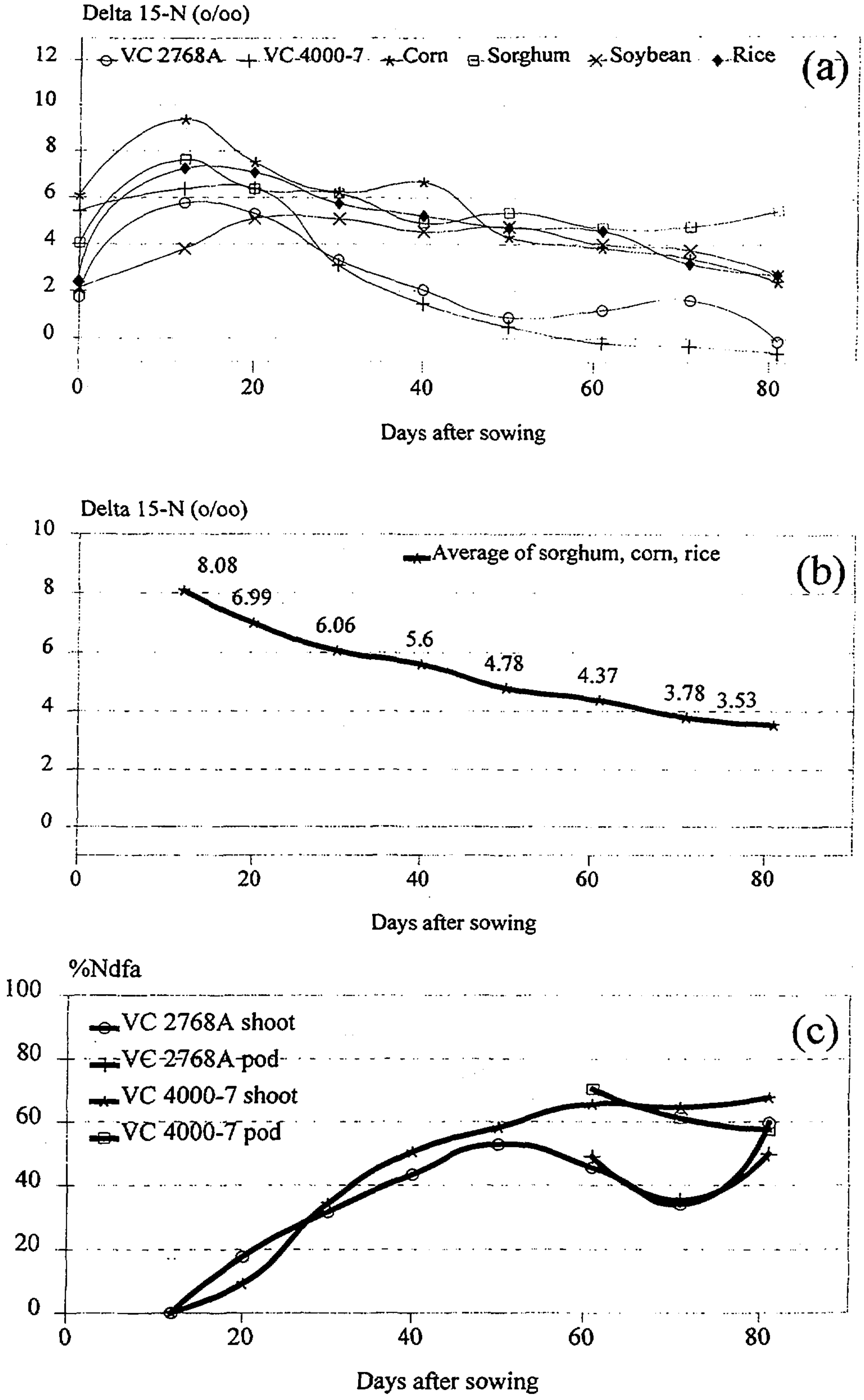

FIG. 1. Seasonal changes in $\delta^{15}N$ of two mung cultivars and four reference species (a), average $\delta^{15}N$ values of the three cereal reference crops (b), and %Ndfa values for the two mung-bean genotypes.

It is notewothy that cvv. KPS2 and 9-5, which fixed the highest amounts of N, are genetically similar: 9-5 (CNM-I-8709-5) was obtained from radiation of KPS2. This confirms the finding with cowpea (*Vigna unguiculata*) that N_2-fixation characteristics are transferable with yield potential [8].

3.3. Screening experiment

Among the 423 mung-bean lines examined, there was great diversity in terms of the total amount of N accumulated, and the degree to which that N was obtained from fixation. The amounts of N obtained from fixation and as mineral N from the soil are shown in Fig. 2 for 78 genotypes at 33 days after planting. The large majority of lines that accumulated less than 230 mg N/plant obtained the large proportion of that N from fixation, whereas the majority of lines that accumulated more than 230 mg N/plant obtained relatively more N from the soil. Three lines apparently did not utilize soil N, and had %Ndfa values of 100%.

Detailed data (Table III), for fifteen of the 423 gentoypes, show the following broad ranges at 33 days after planting: dry weight 4.2-10.1 g/plant, nodule number 17-168/plant, nodule dry weight 6-186 mg/plant, total N 152-467 mg/plant, and N from fixation 97-300 mg/plant. Also noteworthy are the determinations of amount of N from soil, giving the range 0-337 mg/plant.

These fifteen genotypes fell into two categories (Table III): five had %Ndfa values of <38 and ten had %Ndfa values of >89. The lines in the low %Ndfa category had average dry-weight and total-N values of 9.2 g/plant and 370 mg N/plant, respectively, whereas the high %Ndfa category had average values for dry weight and total N of only 6.4 and 235 mg N/plant. The group of five had a lower average nodule mass, 48 vs. 143 mg/plant, which inversely correlated with the amount of N derived from soil, averages of 251 vs. 17 mg N/plant. It is noteworthy that the hybrid 2768×1560, one of the group of ten, had plant dry weight and total N values similar to those of parent VC 2768 A, although its other characteristics were similar to those of parent VC 1560 D.

4. DISCUSSION

Preliminary work with the natural-abundance method for determining %Ndfa and amount of fixed N indicated that it provided meaningful data. Mung genotypes VC 2768 A and VC 4000-7 showed accumulation of fixed N with growth, with %Ndfa values of 60-70 at 80 days after sowing (Fig. 1c). On the other hand, there were three sources of concern: (i) increases in $\delta^{15}N$ values during early seedling growth (Fig. 1a), (ii) trends of decreasing $\delta^{15}N$ values in the non-fixing reference plants during growth (Fig. 1b), and (iii) a trend of increasing $\delta^{15}N$ values in VC 4000-7 from 50 to 71 days. Since items (i) and (ii) occurred across genotypes they appear to be real, and necessary subjects for further investigation; it is possible that item (iii) resulted from sampling an insufficient number of replicate plants.

The use of the istotope-dilution method revealed genotypic diversity for N_2 fixation among seven mung genotypes (Table III). This was a function of growth rather than of fixation per se; the genotypes that showed poorest growth, KPS 1 and CN 60, had %N values not signigicantly lower than those of the other genotypes, therefore their lack of vigour did not result from N deficiency, but from some other limiting factor.

TABLE I. MUNG-BEAN GROWTH AND NODULATION AT 45 DAYS

Genotype	Shoot dry wt. (g/plant)	Nodulation	
		Dry wt. (mg/plant)	Number (per plant)
CN 60	6.71	32	19
CN 36	5.95	34	20
PSU 1	7.05	24	17
KPS 1	8.42	37	36
KPS 2	6.28	32	25
9-5	7.42	42	34
9-8	8.56	45	43
CV (%)	27	49	60
F-test	ns[a]	ns	ns

[a] Not significant at $P = 0.05$.

TABLE II. YIELDS, N ACCUMULATION AND N_2 FIXATION AT MATURITY

Genotype	Dry weight			N	N yield	^{15}N excess	Ndfa	N fixed
	Total	Stover	Seed					
	(kg/ha)			(%)	(kg/ha)	(%)	(%)	(kg/ha)
CN 60	1126d[a]	758d	368c	2.22	25.0	0.271	65	16.2
CN 36	2858a	1425a	1034a	2.35	67.1	0.350	55	36.9
PSU 1	2239abc	1363bc	876ab	2.20	49.2	0.368	53	26.1
KPS 1	1702cd	1021cd	581bc	2.02	34.4	0.360	54	18.6
KPS 2	2747ab	1872ab	1174a	2.40	65.9	0.283	64	42.2
9-5	2487abc	1528abc	959a	2.25	55.9	0.254	68	38.0
9-8	1944bcd	1088cd	756ab	2.27	44.1	0.325	58	25.6
CV (%)	25	22	29	8.3	en[b]			en
F-test	5%	5%	5%	ns				

[a] Means within a column followed by the same letter are not significantly different at $P = 0.05$.

[b] Editor's note: These were not subjected to statistical analysis; the authors assume the same patterns of signficance shown by Total dry weight.

The screening of 423 strains revealed broad genotypic diversity for total N accumulated, and, using the natural-abundance technique, for the N-from-air/N-from-soil relationship (Fig. 2). The detailed data for growth, nodulation, N accumulation and N_2 fixation in Table III matched the N-accumulation/N source data in Fig. 2 in revealing that the genotypes fell largely into two broad groups: (i) those with high total N and low %Ndfa, and (ii) those with lower total N and high %Ndfa. The genotypes in category (i) utilized soil N efficiently, whereas those in category (ii) took up little N from the soil. A possible explanation for these two patterns is that high-yielding lines, such as PSU 1 and KPS (Table III) have been bred and selected for good performance with fertilizer N applied, as is the case in some breeding programmes. However, two of the genotypes listed in Table III and three in Fig. 2 were calculated to have assimilated no N from the soil, and other genotypes appeared to take up very little soil N. These genotypes did not show poor early growth that would be consistent with a lesion in ability to assimilate mineral N, indicating that the %Ndfa values were over-estimates.

The isotope-dilution experiment indicated that plant dry weight correlated positively with the amount of N fixed (Table II), whereas the detailed data from the screening experiment (Table III) revealed a negative correlation: the group of five had a dry weight average of 9.2 g/plant and an average amount fixed of 119 mg N/plant, whereas the group of ten had an average dry weight of 6.4 g/plant and an average amount fixed of 206 mg N/plant. This apparent discrepancy requires further investigation, but may be explainable also in terms of over-estimate of %Ndfa for the group of ten.

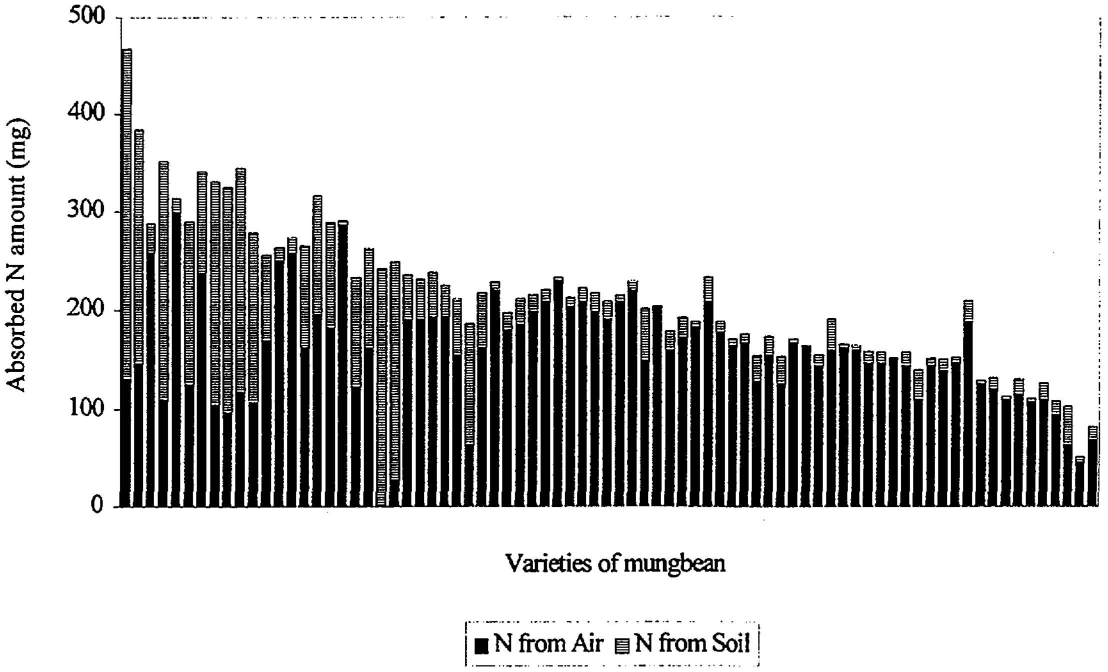

FIG. 2. Sources of N for selected mung-bean genotypes, at 33 days.

In conclusion, it is clear that genetic diversity exists in mung bean for N_2 fixation, as shown by the isotope-dilution experiment and the screening experiment. However, in neither trial was there evidence that inputs of fixed N were growth-limiting, as would be indicated by low %N values. The natural-abundance method holds promise as a convenient and relatively inexpensive method (provided that a four-decimal-place mass-spectrometer is available) for estimating %Ndfa in mung bean, but several questions must be addressed to provide assurance that the data are reliable.

TABLE III. PLANT DRY WEIGHT, NODULATION, AND SOURCES OF N FOR SELECTED MUNG-BEAN GENOTYPES AT 33 DAYS

Genotype	Dry wt.	Nodulation		N	Ndfa	Total N	Ndfa	Ndfs
		No.	Dry wt.					
	(g/plant)	(/plant)	(mg/plt)	(%)		(mg/plant)		
VC2768(PSU1)	10.1	130	50	4.6	27.9	467	130	337
UT 8101	9.9	122	130	3.9	37.9	384	146	239
VC1973A(KPS)	8.5	37	25	4.0	34.2	345	118	227
VC 2917 A	8.8	68	24	3.7	31.8	331	105	225
VC 2991 A	8.5	17	6.0	3.8	29.9	325	97	228
VC 2754 A	8.2	168	186	3.2	95.1	264	250	13
VC 2764 B	8.0	83	173	3.4	94.3	274	258	16
VC 2755 A	7.5	110	126	3.8	98.3	291	286	5
VC 2750 A	6.3	96	132	3.6	96.3	226	220	8
UT 8102-13	5.9	167	181	3.9	98.0	233	228	5
UT 8104-5	5.3	166	150	3.9	99.6	204	203	1
VC 1560 D	4.9	32	56	4.7	89.5	233	208	24
UT 8103-8	4.5	100	120	3.6	100	164	164	0
CV 2802 A	4.2	58	173	3.6	100	152	152	0
2768×1560	9.0	95	130	3.5	95.6	313	300	13

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REFERENCES

[1] BOONKERD, N., WEBER, D.F., BEZDICEK, D.F. Influence of *Rhizobium japonicum* strains and inoculation methods on soybeans grown in rhizobia populated soil. Agron. J. **70** (1987) 547-549.

[2] KUCEY, R.M.N., SNITWONGSE, P., BOONKERD, N., CHAIWANAKUPT, P., WADISIRISUK, P., SIRIPAIBOOL, C., ARAYANGKOOL, T., RENNIE, R.J. Nitrogen fixation (15-N dilution) with soybeans under Thai field conditions: I. Effect of *Bradyrhizobium japonicum* strain. Plant Soil **108** (1988) 33-41.

[3] ISLEIB, T.G., WYNNE, J.C., ELKAN, G.H., SCHNEEWEIS, T.J. Quantitative genetic aspects of nitrogen fixation in peanuts (Arachis hypogaea L.). Peanut Sci. **7** (1980) 101-105.

[4] HERRIDGE, D.F., BETTS, J.H. Field evaluation of soybean genetypes selected for enhanced capacity to nodulate and fix nitrogen in the presence of nitrate. Plant Soil **110** (1988) 129-135.

[5] CARROLL B.J., McNEIL D.L., GRESSHOFF P.M. Isolation and properties of soybean [*Glycine max* (L.) Merr.] mutants that nodulate in the presence of high nitrate concentrations. Proc. Natl. Acad. Sci. USA **82** (1985) 4162-4166.

[6] RENNIE, R.J., RENNIE, D.A., FRIED, M. "Concepts of ^{15}N usage in dinitrogen fixation studies," Isotopes in Biological Dinitrogen Fixation. IAEA, Vienna (1978) pp. 107-133.

[7] AMARGER, N., MARIOTTI, A., MARIOTTI, F., DORR, J.C., BOURGUIGNON, C., LAGACHERI, B. Estimates of symbiotically-fixed nitrogen in field-grown soybeans using variations in the nitrogen-15 natural abundance. Plant Soil **52** (1979) 269-280.

[8] ZARY, K.W., MILLER, J.C. Jr., WEAVER, R.W., BAMER, L.W. Interspecific variability of nitrogen fixation in southern pea (*Vigna unguiculata* (L.) Walp.). J. Amer. Soc. Hort. Sci. **103** (1978) 806-808.