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Effect of plant genotype and nitrogen fertilizer on symbiotic nitrogen fixation by soybean cultivars

G. HARDARSON, F. ZAPATA
FAO/IAEA Agricultural Biotechnology Laboratory, A-2444, Seibersdorf, Austria –

and S. K. A. DANSO
Joint FAO/IAEA Division, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria

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Summary Isotopic as well as non-isotopic methods were used to assess symbiotic nitrogen fixation within eight soybean [*Glycine max* (L.) Merr.] cultivars grown at 20 and 100 kg N/ha levels of nitrogen fertilizer under field conditions.

The ^{15}N methodology revealed large differences between soybean cultivars in their abilities to support nitrogen fixation. In almost all cases, the application of 100 kg N/ha resulted in lower N_2 fixed in soybean than at 20 kg N/ha in the first year of the study. However, N_2 fixed in one cultivar, Dunadja, was not significantly affected by the higher rate of N fertilizer application. These results were confirmed by measurements of acetylene reduction activity, nodule dry weight and N_2 fixed as measured by the difference method. Further proof of differences in N_2 fixed within soybean cultivars and the ability of Dunadja to fix similar amounts of N_2 at 20 and 100 kg N/ha was obtained during a second year experiment. Dunadja yield was affected by N fertilizer and produced larger yield at 100 kg N/ha than at 20 kg N/ha. This type of cultivar could be particularly useful in situations where soil N levels are high or where there is need to apply high amounts of N fertilizer.

The present study reveals the great variability between legume germplasms in the ability to fix N_2 at different inorganic N levels, and also the potential that exists in breeding for nitrogen fixation associative traits. The ^{15}N methodology offers a unique tool to evaluate germplasms directly in the field for their N_2 fixation abilities at different N fertilizer levels.

Introduction

Nitrogen fertilization is normally not recommended for cultivation of legumes, since under favourable conditions they are able to grow well on soil N plus N_2 derived from symbiotic fixation. There are, however, instances in which N fertilization has been recommended to ensure maximum crop yields. During the early period of plant growth when nodules have not fully developed, the young plant relies on soil N and N stored within the cotyledons for normal growth. Where the soil N supply is inadequate, young legume plants can often be retarded in growth. For this reason, the application of a small starter dose of N has been found to stimulate not only legume growth, but N_2 fixation as well[7]. On the other hand, the adverse effect of large amounts of N fertilizer on symbiotic N_2 fixation is well documented[7,9]. Under multiple cropping systems, including legumes and cereals, the need to apply N fertilizers to achieve optimum yields of the non N_2-fixing cereal crop becomes crucial, especially in N-deficient soils. The legume in such a system may on the other hand thrive well on soil N and the additional N it derives from symbiotic N_2 fixation.

It is therefore necessary to judiciously assess what levels of N could be applied under the above-stated conditions to achieve optimum N_2 fixation while ensuring high crop yields. An approach which has not been sufficiently explored is the inherent genetic variability within species of plants to fix N_2 under different levels of inorganic N. The main objective of this study was, therefore, to compare the capabilities of different soybean cultivars to fix N_2 in the presence of low and high levels of supplemental N fertilizer.

Materials and methods

Two experiments were carried out at the experimental field of the FAO/IAEA Agricultural Biotechnology Laboratory, Seibersdorf, in 1981 and 1982. The soil is classified as a Typic Eutocrepts and is a coarse loam[10].

Symbiotic N_2 fixation in eight soybean cultivars [*Glycine max.* (L.) Merr.] was determined at 20 and 100 kg N/ha levels of nitrogen fertilizer application (Table 1). In experiment I each treatment was replicated four times in a split plot design with the two N levels as main plot treatments and the eight nodulating and one non-nodulating soybean cultivars as the subplot treatments. In experiment II each treatment was replicated five times in a randomized block design. Experiments I and II were sown on 14 May 1981 and 28 May 1982, respectively. Individual subplots (Exp. I) or plots (Exp. II) measured 1.6 by 1 m with a row spacing of 40 cm and a planting distance of 5 cm.

Soybean seeds in both experiments were inoculated with a composite mixture of effective strains of *Rhizobium japonicum* obtained from Nitragin Co., Milwaukee, Wisconsin, USA.

^{15}N labelled ammonium sulphate fertilizer was sprayed as an aqueous solution 1 week after planting. Experiments I and II were harvested on 1 September 1981 and 8 September 1982, respectively. All cultivars were harvested at the same time, when plants were still immature to minimize loss of leaves due to senescence. Early cultivars were in R6-7 (Table 1) and late ones in R4 stages of growth[2]. Harvesting was done by removing all above ground plant material in the three central rows per plot. The length of each row harvested was 80 cm. In addition, all roots from the harvested area were removed in Experiment I to determine root and nodule yields. Plant samples were separated into pods, leaves and stems and fresh weight measured. Each of these individual plant parts were chopped into small fragments with a forage chopper. Fresh weight and dry weight measurements were made on appropriate sub samples. Drying was done at 80°C, total nitrogen analysis in ground samples by the Kjeldahl procedure[1] and $^{15}N/^{14}N$ ratio measurements using a mass spectrometer[3].

N_2 fixed was calculated according to the equations used by Fried and Broeshart[4], Fried and Middelboe[5], and Hardarson *et al.*[6]. $^{15}N/^{14}N$ ratio in harvested plants was used to calculate the percentage of N derived from fertilizer (% Ndff), percentage of N derived from atmosphere (% Ndfa) and the total amount of N_2 fixed in each of the eight nodulating soybean cultivars.

Acetylene reduction activity was measured in Experiment I only, and at the time of harvest. Two root systems per subplot were placed in 500 ml plastic containers sealed with rubber stoppers. Ten percent acetylene by volume was added to the container and the roots were incubated in this atmosphere for 1 h, after which gas samples were withdrawn and ethylene produced measured by a gas chromatograph.

Table 1. Soybean cultivars and N treatments used in Exp. I and Exp. II

	Exp. I			Exp. II
Treatments	Cultivar	Type	Origin	Cultivar
Cultivars	Ada	Early	Austria	Chippewa
	Altona	Early	Austria	Dunadja
	Chippewa	Late	USA	Evans
	Dunadja	Medium early	Romania	
	Evans	Medium early	USA	
	ISZ-10	Medium early	Hungary	
	Kalitur	*	India	
	Pannonia	Medium early	Austria	
Ref. crop	Chippewa (non-nod. isoline)	Late	USA	Chippewa (non-nodulating isoline)
N treatment	20 kg N/ha, 4.810% ^{15}N at. excess; 100 kg N/ha, 0.877% ^{15}N at. excess			20 kg N/ha, 4.970% ^{15}N a.e.; 100 kg N/ha, 0.777% ^{15}N a.e.

* Did not flower under the experimental conditions.

According to the following equation nitrogen difference (N difference method) between fixing and non-fixing crops was also used to quantify N_2 fixation:

$$\text{Fixed N} = \text{total N}_{(\text{fixing crop})} - \text{total N}_{(\text{non-fixing crop})}$$

Results and discussion

Significant ($P < 0.05$) differences were observed in % Ndfa between the eight N_2 fixing soybean cultivars (Table 2). Of all the cultivars Chippewa and Evans derived the greatest proportion of their N from atmosphere when the fertilizer level was 20 kg N/ha, with each obtaining 26% of its N from atmosphere, while cultivars like Kalitur, ISZ-10 and Dunadja fixed only 11% of their N. Similar large differences in actual amount of N_2 fixed (^{15}N method) were also observed between cultivars (Table 2). Values as high as 50 kg N/ha for Evans and as low as 13 kg N/ha for the cultivar Kalitur were recorded in the soil amended with 20 kg N/ha.

The application of 100 kg N/ha when compared to the 20 kg N/ha application was found to reduce on the average the % Ndfa within the eight N_2 fixing soybean cultivars (Table 2). The mean value of % Ndfa for the eight cultivars was 16% at 20 kg N/ha level, but was reduced to 5% at the 100 kg N/ha level. Similarly, the amount of N_2 fixed (^{15}N method) was reduced from 26 to 8 kg N/ha by raising the N fertilizer level from 20 to 100 kg N/ha. It was again observed at 100 kg N/ha level that % Ndfa and total N_2 fixed varied between varieties. However, an important observation was the extent to which the N_2 fixed within each variety was reduced by applying 100 kg N/ha

Table 2. Percentage of nitrogen derived from fertilizer*, percentage of nitrogen derived from atmosphere and amount of fixed nitrogen (estimated by the ^{15}N methodology) of eight nodulating and one non-nodulating soybean cultivars at 20 and 100 kg N/ha fertilizer application (Exp. I)

		Ndff (^{15}N method) (%)		Ndfa (^{15}N method) (%)		Fixed N (^{15}N method (kg/ha)	
Cultivar	kg N/ha	N_{20}	N_{100}	N_{20}	N_{100}	N_{20}	N_{100}
Ada		3.60	15.6	13	0	16	0
Altona		3.37	15.4	19	0	29	0
Chippewa		3.06	13.4	26	11	45	18
Dunadja		3.67	13.6	11	10	18	18
Evans		3.08	13.2	26	12	50	20
ISZ-10		3.69	14.5	11	4	14	6
Kalitur		3.69	15.7	11	0	13	0
Pannonia		3.52	14.4	15	4	23	6
Non-nod		4.14	15.1	–	–	–	–
Means				16	5	26	8
LSD 0.05 between cultivars		0.65	2.25				

* weighted averages of leaves, stems and pods.

instead of 20 kg N/ha. For example, while the % Ndfa was reduced from 26 to 11 or 19 to 0 for Chippewa and Altona, respectively, that for Dunadja was not reduced significantly. Similarly the amount of N_2 fixed was reduced from 50 to 20 kg N/ha or 29 to 0 kg N/ha for Evans and Altona respectively, whereas that for Dunadja still remained at 18 kg N/ha at both N fertilizer levels.

With the acetylene reduction technique significant differences ($P < 0.05$) were observed in nitrogenase activity among the eight N_2 fixing soybeans. At 20 kg N/ha level, Chippewa and Evans had the highest nitrogenase activity (Table 3). While each of these 2 cultivars produced 17 μm ethylene/plant/hour, the figure for Ada was only 2 μm/plant/hour. At the 100 kg N/ha fertilizer level, there were also large differences in nitrogenase activity of the various cultivars. The amount of ethylene produced at 20 kg N/ha level was higher for most cultivars than when the soil was fertilized with 100 kg N/ha. For example the ethylene/plant/hour figures were 13 and 2 for Altona at 20 and 100 kg N/ha levels respectively, while that for Dunadja showed a higher activity at the 100 kg N/ha level and remained almost unchanged for Chippewa and Evans.

N difference method was capable of detecting N_2 fixed in Chippewa, Evans and Dunadja, only (Table 4). Evans scored the highest of 40 kg N/ha fixed, followed by Chippewa and Dunadja, in which the N_2

Table 3. Nodule dry weight, acetylene reduction activity and specific nodule activity (estimated by the [15]N methodology) of eight nodulating soybean cultivars at 20 and 100 kg N/ha fertilizer application (Exp. I)

		Nodule dry weight (mg/plant)		Acetylene reduction activity (μm/plant/hour)		Specific nodule activity (mg N fixed/mg nodule dry weight)	
Cultivar	kg N/ha	N_{20}	N_{100}	N_{20}	N_{100}	N_{20}	N_{100}
Ada		97	26	2	0	0.33	0
Altona		192	50	13	2	0.30	0
Chippewa		205	218	17	16	0.44	0.16
Dunadja		90	94	6	20	0.40	0.38
Evans		460	126	17	18	0.22	0.32
ISZ-10		91	81	9	6	0.31	0.15
Kalitur		212	126	14	7	0.12	0
Pannonia		168	55	10	3	0.27	0.22
Means		189	97	11	9	0.30	0.15
LSD 0.05 between:							
N means		101		5.6			
Cultivar at same N		60		14.0			

Table 4. Nitrogen yield and fixed nitrogen (estimated by the difference method) of eight nodulating and one non-nodulating soybean cultivars at 20 and 100 kg N/ha fertilizer application (Exp. I)

		Nitrogen yield (kg/ha)		Fixed N (difference method) (kg/ha)	
Cultivar	kg N/ha	N_{20}	N_{100}	N_{20}	N_{100}
Ada		122	138	0	0
Altona		151	144	0	0
Chippewa		174	165	21	5
Dunadja		163	185	10	25
Evans		193	170	40	10
ISZ-10		130	142	0	0
Kalitur		121	126	0	0
Pannonia		152	154	0	0
Non-nod.		153	160	–	–
Means		151	154	9	5
LSD 0.05 between:					
N means		41			
Cultivar at same N		27			

fixed values were 21 and 10 kg N/ha, respectively, in the soil supplemented with 20 kg N/ha fertilizer. At 100 kg N/ha fertilizer application, Chippewa and Evans showed a reduction in the amounts of N_2 fixed compared with the 20 kg N/ha fertilizer level, while apparently more N_2 fixed was recorded for Dunadja at the high fertilizer N level. Also, significant difference was observed between the N yields of the soybean cultivars (Table 4). However, on the average, inorganic N application did not have much influence on the total N yield. No significant N x cultivar interaction was observed.

In Experiment I, there were significant differences in nodule dry weight among the soybean cultivars at the lower levels of inorganic fertilizer application (Table 3). In addition, nodule weights were on the average reduced when the inorganic fertilizer N level was increased from 20 to 100 kg N/ha. The mean nodule dry weights for all cultivars were 189 and 97 mg/plant for the 20 and 100 kg N/ha applications, respectively. However, no significant change in the dry weights of nodules were found in Dunadja and Chippewa at both of these N levels. Specific nodule activity as measured by the ^{15}N method was on the average reduced by 50% by increased N fertilizer application (Table 3). This did however not occur in Dunadja and Evans, which had similar specific nodule activities at both N fertilizer levels.

Confirming results of Patterson and LaRue[8], who found great variation in N_2 fixation between various maturity groups of soybean, the results obtained in Experiment I by several direct as well as indirect methods show that cultivars are different in the extent to which they support N_2 fixation. Although N_2 fixation is reserved exclusively for procaryotes which possess the nitrogenase enzyme, the present investigation shows that the amount of fixed nitrogen is influenced by the host plant. In addition, results of Experiment I have clearly shown that certain cultivars can fix N_2 to quite similar extents at both low and high soil inorganic N levels, while others can fix high amounts of N_2 only when the soil N levels are low. Dunadja in this experiment, although low in N_2 fixed, maintained almost the same ability to fix atmospheric nitrogen at low and high inorganic N levels. This cultivar was at the same time responsive to N fertilizer and produced higher yield at 100 kg N/ha fertilizer application than at 20 kg N/ha (Table 5).

The results obtained in Experiment II, using both the N difference (Table 6) and isotope methods (Table 7) confirm the observations of Experiment I, showing that there are large differences in the amounts of N_2 fixed by different genotypes of a legume species. Although in this experiment Dunadja did not fix as much N_2 at the lower inorganic N level as Chippewa and Evans, it essentially fixed the same amount of N_2 at both 20 kg N/ha and 100 kg N/ha as estimated by the N difference method, while N_2 fixed in Evans was reduced from 125 to 94 kg N/ha, with a more drastic reduction of 74 to 30 kg N/ha for Chippewa when inorganic N level was raised from 20 to 100 kg N/ha (Table 6). The isotopic parameter also revealed differences in N_2

Table 5. Total dry matter and pods dry matter yields of eight nodulating and one non-nodulating soybean cultivars at 20 and 100 kg N/ha fertilizer application (Exp. I)

		Total dry matter yield (ton/ha)		Pods dry matter yield (ton/ha)	
Cultivar	kg N/ha	N_{20}	N_{100}	N_{20}	N_{100}
Ada		8.5	8.1	2.6	2.4
Altona		9.0	8.3	2.5	2.3
Chippewa		11.6	11.6	1.9	1.9
Dunadja		10.3	11.9	2.3	2.8
Evans		12.0	10.1	3.2	3.2
ISZ-10		8.7	9.9	2.5	2.2
Kalitur		9.6	9.4	0	0
Pannonia		9.6	10.7	2.1	2.2
Non-nod.		11.8	10.6	2.0	1.7
Means		10.1	10.1	2.1	2.1
LSD 0.05 between:					
N means		1.43		0.1	
Cultivar at same N		2.09		0.6	

Table 6. Nitrogen yield and fixed nitrogen (estimated by the difference method) of three nodulating and one non-nodulating soybean cultivars at 20 and 100 kg N/ha fertilizer application (Exp. II)

		Nitrogen yield (kg/ha)		Fixed N (difference method) (kg/ha)	
Cultivar	kg N/ha	N_{20}	N_{100}	N_{20}	N_{100}
Chippewa		210	190	74	30
Dunadja		203	229	67	69
Evans		261	254	125	94
Non-nod.		136	160	–	–
Mean		202	208	89	64
LSD 0.05 between:					
N means		28			
Cultivar at same N		57			

Table 7. Percentage of nitrogen derived from fertilizer*, percentage of nitrogen derived from atmosphere and amount of fixed nitrogen (estimated by the ^{15}N methodology) of three nodulating and one non-nodulating soybean cultivars at 20 and 100 kg N/ha fertilizer application (Exp. II)

		Ndff (^{15}N method) (%)		Ndfa (^{15}N method) (%)		Fixed N (^{15}N method) (kg/ha)	
Cultivar	kg N/ha	N_{20}	N_{100}	N_{20}	N_{100}	N_{20}	N_{100}
Chippewa		1.21	7.23	53	38	111	72
Dunadja		1.22	6.91	52	40	106	92
Evans		1.07	8.47	58	27	151	79
Non-nod.			11.61				
Means				54	35	123	81
LSD 0.05 between:							
cultivars		0.37	2.10				

* weighted averages of leaves, stems and pods.

Table 8. Total dry matter and pods dry matter yields of three nodulating and one non-nodulating soybean cultivars at 20 and 100 kg N/ha fertilizer application (Exp. II)

		Total dry matter yield (ton/ha)		Pods dry matter yield (ton/ha)	
Cultivar	kg N/ha	N_{20}	N_{100}	N_{20}	N_{100}
Chippewa		10.0	9.8	1.6	1.6
Dunadja		8.7	10.1	2.6	2.8
Evans		10.2	10.8	3.2	3.3
Non-nod.		10.7	10.4	1.5	1.7
Mean		9.9	10.3	2.2	2.3
LSD 0.05 between:					
N means		0.9		0.3	
Cultivar at same N		1.8		0.6	

fixed by these three cultivars in Experiment II although the differences were smaller than those found by the N difference method (Table 7). Here again, the reduction in N_2 fixed in Dunadja due to a higher level of inorganic N was not as large as in the other two varieties, and in fact, it fixed a higher amount of N_2 as well as derived a higher proportion of its total N from N_2 fixation at 100 kg N/ha fertilizer application than both Chippewa and Evans (Table 7). Dunadja was again responsive to N fertilizer and produced higher yield at 100 kg N/ha fertilizer application than at 20 kg N/ha (Table 8). Differences between the amount of N_2 fixed in the two experiments were probably due to late nodulation in Experiment I caused by low temperature.

Both Experiment I and II reveal that a cultivar like Dunadja is able to fix efficiently in situations where the soil inorganic N levels are high, or where there is the need to apply high amounts of N fertilizers. The results obtained show that there exists a big genetic variability in legumes as regards their ability to support N_2 fixation. The genes for N_2 fixation associative traits may be selected for by appropriate testing of legumes. The isotopic methods in our opinion offer the greatest potential for final evaluation of lines, since it alone is able to distinguish between N_2 fixed and N derived from other sources, such as soil and fertilizer.

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