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Divergent selection for dinitrogen fixation and yield in soybean

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Summary. Nitrogen fixation is generally considered to be a major parameter of productivity in soybean (*Glycine max*). The aim of the investigations reported here was to analyse the genetic behaviour of this trait in view of its possible use as an indirect criterion of selection for productivity. Divergent selection for nitrogen fixation rate was carried out on F_2 populations obtained from crosses between high-yielding cultivars that are well adapted to French climatic conditions. The genetic component of nitrogen fixation and yield was isolated through the analysis of (1) the nitrogen fixation potentials of the genotypes under controlled conditions and (2) the field yields under favourable conditions. Divergent selection resulted in two groups of genotypes whose nitrogen fixation abilities are significantly different. The F_6 filial progeny obtained by single seed descent from the two groups displayed significantly different abilities for nitrogen fixation and for field productivity. The gain achieved for the nitrogen fixation activity with respect to the mean value of the parents ranged from 20% to 33% for the positive selection, depending on the crosses. The occurrence of positive and significant correlations between the level of nitrogen fixation activity in F_2 plants and N_2 fixation or yield in the F_6 generation corroborates the relatively high heritability of this trait and suggests its possible use as an indirect selection criterion for yield.

Key words: Dinitrogen fixation – Yield – Soybean – Heritability

Introduction

Symbiotic nitrogen fixation and inorganic nitrogen assimilation are the two pathways of nitrogen nutrition in legumes. These two nitrogen sources can be complementary or antagonistic. In most soils where the nitrate content is moderate, the proportion of nitrogen which is derived from symbiotic fixation is about 50% (Hardarson et al. 1984; Vasilas and Ham 1984; Bergersen et al. 1985) and can reach 75% in sandy loamy soils (Matheny and Hunt 1983). The highest rate of nitrogen fixation was observed to occur at the end of flowering (Lawn and Brun 1974) and during pod filling (Imsande 1988). The nitrogen assimilated between R_3 and R_7 seems to be the predominant source of nitrogen for pod development (Zapata et al. 1987). Ronis et al. (1985) observed a highly significant correlation between seed yield and the amount of nitrogen in the seed originating from N_2 fixation. The relationships between N_2 fixation and productivity remain difficult to ascertain as a result of the strong environmental component involved in the expression of these two factors. Divergent selection for the nitrogen fixation rate under controlled conditions allowed the characterization of relationships between the N_2 fixation potential of F_2 plants in phytotronic chambers and the yield of field-grown F_4-derived lines (Burias and Planchon 1990). The results suggest the occurrence of (1) a close relationship between the nitrogen fixation ability of the genotypes and the yield and (2) a good heritability of N_2 fixation. The aim of the investigations presented here was to better assess the heritability of N_2 fixation and the parameters of nodulation through analysis of the progeny obtained by single seed descent after divergent selection in F_2.

Materials and methods

Plant material

Three F_2 populations (210 plants) derived from crosses between 'Weber' (always female, maturity group I) and the cv 'Maple

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Arrow' (maturity group 00), 'Jiling 14' (maturity group I), 'Kingsoy' (maturity group II) and each parent (10 plants) were analysed in 1987 for their N_2 fixation ability. For each cross a selection from ARA estimates (μmoles C_2H_4 h^{-1} kg^{-1} of fresh weight) of the best and the worst plants was achieved with a selection pressure of 11% in each case, that is with 43 plants out of 210. For each F_2-selected plant, the F_6 filial progeny was obtained by single seed descent. The F_6 plants were analysed for their N_2 fixation ability, and F_6 lines issued from one F_5 plant were field grown in 1.5-m rows spaced 50 cm apart with 5 cm intervals within the row. From the positive or the negative selections for the N_2 fixation rates in the F_2 populations, two groups of genotypes were obtained.

Dinitrogen fixation measurements

The plants were grown under soilless culture conditions in continuously aerated (4 l min^{-1} air flow rate) 20-l, 20-cm-deep tanks. The IRAT (Tropical Agronomy Research Institute, Paris, France) nutrient solution enriched in magnesium was used with a variable supply of nitrate, and the stability of the nitrate content was checked daily (Burias and Planchon 1990).

The experiments were carried out under conditions of a 14-h day photoperiod at 650–700 μE m^{-2} s^{-1} irradiance (value measured 50 cm above the tanks) and 25°/20 °C light/dark temperatures. A double inoculation was made on the seeds (10^6 bacteria per seed) and in the hydroponic solution (10^6 per liter) with commercial strain G49 (Lipha, Lyon, France).

Symbiotic N_2 fixation was determined at stage R5 (Fehr and Caviness 1977) by measuring the acetylene reduction activity (ARA) of the root system with the in situ method of Balandreau and Dommergues (1971), which was chosen because it is not destructive. The root system of the whole plant was introduced into 580-ml plasma bottles with 100 ml of N-free nutrient solution. A Terostat 9010 (Teroson, Asnières, France) gasket ensured airtightness at the level of the collar. Forty-eight millilitres of air comprising 10% of the total gas volume was replaced with acetylene using a 50-ml sterile hypodermic syringe. The validity of these comparative measurements has been determined (Burias and Planchon 1990). Two plants for each F_6 line were evaluated.

A 5-ml gas sample was then taken and analysed by solid-gas chromatography with an injection of 1 ml using an Intersmat IGC 120 FB chromatograph (Delsi, Suresnes, France) with a flame ionization detector and a stainless steel column filled with 0.25 to 0.18-mm particle size (60–80 mesh) Porapak T. Two plants of each F_6 line were evaluated. The acetylene reduction assay of nitrogenase is a useful tool for the detection of nitrogenase activity in soybean and is well adapted to genetic analysis because of its simplicity, high sensitivity and nondestructive character. The reduction in nitrogenase activity upon exposure of nodules to saturating concentrations of acetylene is less dramatic in soybean (Weisz and Sinclair 1987) than in white clover (*Tripolium repens* L.). The acetylene technique remains adequate for comparative assessments (Hassan et al 1987) and for the analysis of large numbers of genotypes.

The necessity of obtaining progeny required the use of nondestructive methods and excluded any determination of plant dry weight during the experiments. The data presented (N_2 fixation, nodule volumes, nodule dry weights) take plant size into account and are expressed per unit fresh weight of the whole plant. Nodule sampling at stage R5 afforded volume and dry weight determinations, and subsequent plant growth from R5 stage to maturity was ensured by means of a further calcium nitrate supply ($[NO_3]=6.4$ m*M*).

Yield determinations

The experiments were carried out at the Agricultural Experiment Station of the Ecole Nationale Supérieure Agronomique de Toulouse, France (43°36'N, 1°27'E) on a Typic Udifluvent soil (ph 6.0, organic matter 1.5% and total N 1 g kg^{-1}). The plots were inoculated with Strain G49. The yield of each F_6 line was evaluated from the field experiments (two blocks of one replication of four 1.5-m rows) using the weight of the seeds at 14% relative humidity. The seeds were sown on May 10, 1990. The crop received 200 mm of irrigation water and 220 mm of rainfall. The average monthly temperatures were in the range 17.5 °C (May) to 22.5 °C (July), and the average monthly incident radiation was in the range 340×10^6 J m^{-2} (August) to 560×10^6 J m^{-2} (May).

Data analysis

As the investigations were carried out on F_2 and F_6 generations, the environmental effects were assessed on the pure parent lines ('Weber', 'Maple Arrow', 'Kingsoy' and 'Jiling 14') with five replications of two plants for N_2 fixation. The analysis showed that, at the 0.05 threshold, there was no significant effect of the tank culture on the determination of N_2 fixation. There was no significant block effect on the field yield.

Individual data points were used in the orthogonal polynomial regression analysis to determine the statistical relationship between seed yield and date of maturity for each cross and over all crosses (Steel and Torrie 1980). The regression lines and coefficients were derived from the statistically significant best-fit equations for each cross and for all crosses.

Simple correlation coefficients were determined using the values calculated with respect to the mean of the genotypes with the same data of maturity (Table 1).

Before the calculation of the correlation coefficients, a control of the normality of the distributions was carried out (with or without logarithmic transformations).

The means were compared using the tests of Student-Fisher or Newman and Keuls.

Results

Dinitrogen fixation and nodulation parameters in F_2 populations

The nitrogen fixation rate of cv 'Maple Arrow' was significantly higher than that of the other three cultivars, whereas the lowest value was relative to 'Kingsoy'. Likewise, significant differences were observed between the cultivars for the nodulation parameters (number of nodules, nodule volume and nodule dry weight; Table 2). However, there were no clear-cut relationships between the nitrogen fixation rate and the nodulation parameters at the level of the cultivars as the superiority of some cultivars appeared to be more closely associated with a higher nitrogenase efficiency, which was expressed by the specific fixation activity (ARA per unit nodule volume, Table 2). The mean value of nitrogen fixation in F_2 populations did not differ significantly from that of the two

Table 1. Relationship between N_2 fixation, nodulation parameters, yield and maturity (days after 1st september) of F_2 and F_6 genotypes

Crosses and generation		Traits[b] N_2 fixation (μmol $C_2H_4h^{-1}$ kg^{-1})	Nodule dry weight (g kg^{-1})	Nodule volume (10^3 mm^3 kg^{-1})	Yield (g row^{-1})	
W×MA	F_2	NS[a]	NS	NS		
	F_6	NS	NS	NS	y=227.3+5.6×	$R^2=0.12$*
W×K	F_2	NS	NS	NS		
	F_6	NS	NS	NS	y=352.6+3.4×	$R^2=0.21$*
W×J14	F_2	NS	NS	NS		
	F_6	NS	NS	NS	y=253.8+5.3×	$R^2=0.21$*
All crosses	F_2	NS	NS	NS		
	F_6	NS	NS	NS	y=241.5+6.5×	$R^2=0.30$**

*,** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively
W, Weber; MA, Maple Arrow; K, Kingsoy; J14, Jiling 14
[a] Regression straight lines were not significant at $P \leq 0.05$
[b] Expressed per whole plant fresh weight unit

Table 2. Variation in N_2 fixation activity and nodulation parameters among four soybean genotypes and F_2 populations

Genotypes and and F_2 populations[a]	Plants (*n*)	Traits[b] N_2 fixation (μmol C_2H_4 h^{-1} kg^{-1}		Nodule number (10^3 kg^{-1})		Nodule dry weight (g kg^{-1})		Nodule volume (10^3 mm^3 kg^{-1})		Specific N_2 fixation[c] (nmol C_2H_4 h^{-1} mm^{-3})
		X	SE	X	SE	X	SE	X	SE	
Weber	20	55b	1.6	2.4c	0.15	1.1a	0.07	6.8a	0.22	8.1
Maple Arrow	20	70c	2.4	1.3a	0.04	0.9a	0.09	7.9b	0.06	8.8
Kingsoy	20	47a	1.8	2.8c	0.16	2.0c	0.08	12.7c	0.48	3.7
Jiling 14	20	54b	2.1	1.9b	0.09	1.6b	0.07	8.4b	0.20	6.5
W×MA	70	66b	4.2	1.2a	0.10	0.8ab	0.06	5.4a	0.52	11.8
W×K	70	46a	1.8	2.4b	0.24	0.6a	0.06	5.1a	0.56	9.0
W×J14	70	61b	3.9	3.3b	0.41	1.1b	0.12	8.2b	0.84	7.4

Values followed by the same letter are not significantly different at the 0.05 level according to Newman and Keuls test
[a] Mean of the total F_2 populations
[b] Expressed per whole plant fresh weight unit
[c] Specific nitrogen fixation (N_2 fixation/NV)

parents, which suggests additive control of the genes involved in the expression of the fixation activity.

Dinitrogen fixation and nodulation parameters in F_2- and F_6-selected populations

The positive or negative selection in F_2 resulted in populations that displayed significantly different nitrogen fixation rates (Table 3). The gain obtained in F_6 with respect to the mean of the parents ranged from 20% to 33% for the positive selection, whereas the loss ranged from 16% to 47% for the negative selection. The significant differences observed in nodule volume between these two groups reflect the occurrence of a relationship between this trait and nitrogen fixation. The maintenance of significantly different N_2 fixation abilities and nodule volumes in the F_6 progeny obtained through single seed descent reflects the heritability of these parameters. This result was corroborated by the occurrence of positive and highly significant correlations between the performances of F_6 and F_2 plants ($r=+0.46$, $P \leq 0.01$ and $r+0.39$, $P \leq 0.01$ for N_2 fixation and nodule volume, respectively). These values estimate the heritability percentage in standard units (Frey and Horner 1957) and are likely to be close to those obtained through the conventional (parent-progeny regression) method, since, under controlled conditions, environmental effects are low. The heritability of the nodule number and specific nitrogen fixation activity traits was shown by the +0.42 ($P \leq 0.01$) and +0.37 ($P \leq 0.05$) values of the F_6/F_2 correlation coefficients, respectively.

Table 3. Variation in N_2 fixation and nodulation parameters among F_2-selected and F_6-derived populations

Populations		Traits[c] N_2 fixation (μmol C_2H_4 h^{-1} kg^{-1})		Gain[b]	Nodule number (10^3 kg^{-1})		Nodule dry weight (g kg^{-1})		Nodule volume (10^3 mm^3 kg^{-1})		Specific N_2 fixation[d] (nmol C_2H_4 h^{-1} mm^{-3})
		X	SE		X	SE	X	SE	X	SE	
F_2W × MA	(+)	107b	6.6	–	1.6b	0.23	1.4b	0.17	9.3b	1.1	11.6
	(−)	49a	3.1	–	0.6a	0.17	0.8a	0.17	4.9a	1.4	10.1
W × K	(+)	58b	4.2	–	3.2b	0.81	0.8b	0.13	7.4b	1.4	7.8
	(−)	26a	2.9	–	1.7a	1.18	0.5a	0.09	2.4a	0.6	10.8
W × J14	(+)	86b	4.1	–	5.3b	1.81	2.7b	0.60	16.4b	2.3	5.2
	(−)	47a	4.8	–	4.6c	1.85	1.3a	0.25	11.4a	2.1	4.1
F_6W × MA	(+)	83b	16.8	33*	1.5a	0.19	1.2a	0.17	6.4b	1.6	13.0
	(−)	33a	4.8	−46**	1.4a	0.35	1.0a	0.37	2.7a	1.0	12.2
W × K	(+)	63b	7.4	24*	2.9a	0.32	1.4a	0.26	8.8b	1.6	7.2
	(−)	42a	9.5	−16	2.0a	0.35	1.3a	0.33	5.5a	1.7	7.7
W × J14	(+)	65b	8.1	20*	2.2a	0.30	1.3a	0.20	5.8b	1.3	11.2
	(−)	29a	7.3	−47**	2.9a	0.32	1.2a	0.24	3.8a	1.0	7.6
Correlation F_6/F_2[a]		0.46**			0.42**		0.24		0.39**		0.37*

*,** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively
+, Positive selection; −, negative selection
In a column, between the positive selection and the negative selection values followed by the same letter are not significantly different at the 0.05 level according to Newman and Keuls test
[a] Correlation for all crosses
[b] Gain achieved in F_6 population with respect to the mean fo the two parents
[c] Expressed per whole plant fresh weight unit
[d] N_2 fixation per nodule volume unit

Table 4. Phenotypic correlations between N_2 fixation and nodulation parameters of F_2 populations and yield of F_6 genotypes

Yield of F_6 populations	F_2 populations traits N_2 fixation	Nodule number	Nodule dry weight	Nodule volume	Specific N_2 fixation[b]
W × MA	0.68**	[a]	0.57*	0.40	−0.27
W × K	0.39	[a]	0.20	0.23	−0.23
W × J14	0.63*	[a]	0.29	0.18	0.15
All populations	0.30*	0.20	0.22	0.21	0.03

*,** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively
[a] No normal distributions of values
[b] Nitrogen fixation per nodule volume unit

Dinitrogen fixation of F_2 plants and yield of F_6 genotypes

The productivity of the F_6 lines was expressed taking their maturity into account: seed yield is highly dependent on the length of development, particularly on the duration of the pod filling phase (Paul et al. 1979; Smith and Nelson 1986). Each genotype was thus characterized by its deviation from the regression line (Table 1). for all of the genotypes and for the genotypes issued from each cross there were positive and highly significant correlations between the yield of F_6 lines grown in soils with low to adequate nitrogen contents and the N_2 fixation performances of the F_2 parent plants grown hydroponically. The correlation between the yield of F_6 genotypes and the other nodulation parameters of F_2 plants was also positive, but was generally not significant at $P = 0.05$ (Table 4). The F_6 yield of the two groups issued from the divergent selection in F_2 was signficantly different for each of the crosses and for all crosses (Table 5).

Discussion

These results reflect the efficiency of selecting for the N_2 fixation rate in F_2 and suggest a possible use of this trait

Tabel 5. Yield of F_6 lines derived from divergent selection for N_2 fixation in F_2

F_2-derived lines	Yield (g row^{-1})[a]		Difference between the two groups[a]
	Positive selection	Negative selection	
W×MA	59	−68	127**
W×K	19	−22	41**
W×J14	30	−27	57**
All crosses	34	−36	70**

** $P \leq 0.01$

[a] Values derived from the regression lines obtained for seed yield versus maturity (deviation value, Table 1) in grams per row

as an indirect criterion of selection for productivity as the yield performances of F_2 plants are not representative of the yield of the derived fixed lines grown at a normal density.

Nodulation and symbiotic N_2 fixation, in soybean as well as in other legume crops, are major factors in productivity. Productivity improvement based on genotypic differences of the host plant for symbiotic N_2 fixation has already been considered (Betts and Herridge 1987; Hardarson 1984; Neuhausen et al. 1988). The selection of the host plant then implies the occurrence of a relationship between nitrogen fixation and the final seed yield, which is difficult to ascertain because of the strong environmental component involved in the expression of these two factors. A good heritability of symbiotic N_2 fixation is then also implied. The investigations reported here, through a limitation of the role of the environmental factors in the expression of the traits, showed the good heritability of nodulation parameters and especially of the nitrogen fixation ability. The nitrogen fixation rate, as a result of both its relationship with productivity and its heritability, constitutes a good early criterion of selection for productivity. The symbiotic fixation rate depends to a large extent on the specific activity of nitrogenase, and thus a selection based on nodule number or mass would be less efficient. The cross value of some genotypes, such as 'Maple Arrow', for nitrogen fixation ability does appear to be related to nitrogenase efficiency. However, the relationships observed between nitrogen fixation and nodule parameters (nodule weight, nodule volume and number of nodules) are in agreement with the results obtained in peanut (Arrendell et al. 1989) and in soybean (Duteau et al. 1986). The occurrence of a genetic variability for nitrogen fixation has been demonstrated in seed as well as in forage legumes (La Rue and Patterson 1981; Tan 1981; Attewell and Bliss 1985). The results presented above suggest how this variability might be used for improving soybean yields. Selection for yield should also effectively select for increased N_2 fixation. These two parameters were closely associated with each other (Ronis et al. 1985). Past emphasis on breeding soybean for increased seed yields has also increased the N_2 fixation of selected genotypes (Coale et al. 1985; Leffel 1989). The good heritability of the nitrogen fixation rate further supports the consideration of such a selection pathway. The ability of the host plant to fix nitrogen appears to be an overall response involving possible compensations between the parameters that contribute to its expression. The elementary factors of N_2 fixation (number of nodules, nodule volume, specific fixation activity) are characterized by a poorer relationship with yield and thus constitute less efficient selection criteria. These parameters, however, display a wide variability (Sinclair et al. 1991) and a relatively high heritability (Greder et al. 1986). The necessary consideration of N_2 fixation activity limits the possibility of screening large numbers of plants, but its efficiency should counterbalance this shortcoming.

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