Yield and N_2 fixation of backcrossed supernodulating soybean mutants

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Abstract

Growth, nodulation, nitrogen fixation and grain yield of backcrossed materials of supernodulating mutants and wild types of soybean [Glycine max (L.) Merr.] were compared in three field experiments with N levels of 0 and 200 kg N/ha for two years. A backcrossed intermediate supernodulating line, PS47, fixed more N_2 and yielded as much as wild types under both N fertiliser conditions, confirming the previous findings that intermediate supernodulators are promising for commercial use. However, a backcrossed extreme supernodulating line, PS55, showed retarded growth and low yield, attributed to the excessive nodulation. Dry matter production and yield of barley (Hordeum vulgare L.) following PS55 were much greater than that of barley following other soybean genotypes. However, this N benefit was not sufficient to compensate for the low yield of PS55 itself. Therefore, we conclude that extreme supernodulation does not appear to be agronomically beneficial at this stage.

Key words: Soybean, supernodulation, nitrogen fixation.

[Glycine max (L.) Merr.], like other legumes, preferentially utilises nitrate (NO_{3-}) from the soil rather than developing a symbiosis with Rhizobium or Bradyrhizobium to fix atmospheric nitrogen (N_2). Both nodule formation and N2 fixation are inhibited by $NO_{3-.}$ Studies have shown that manipulation of the host plant, rather than the rhizobial strain, would be more likely to alleviate the inhibition (10). Several soybean mutants from cv. Bragg that nodulate in the presence of NO_{3-} have been isolated and characterised (2). These supernodulating soybean mutants, also described as supernodulators or nitrate tolerant symbiotic (nts) mutants can produce up to 10 times the number of nodules of wild types and display increased N_2 fixation. Evaluation of the supernodulating soybean mutants both in glasshouse and field (5, 7) have revealed that the intermediate supernodulators can grow as vigorously as, and yield as much as, their parent cultivars. However, the yield of the extreme supernodulators has been consistently low. It has been suggested that this is due to other mutations unrelated to nodulation (10). Thus, backcrossed lines which remove other possible deleterious mutations of the supernodulating soybean lines have been developed to test this hypothesis and improve their yield (9). Though the low yield of soybean supernodulators makes them unattractive commercially, they are potentially useful as N contributors in cropping systems because their higher dependence on fixed N_2 may spare more soil N for intercropped or subsequent cereal crops. Such a benefit has been found in field experiments but the results are not consistent (9).

The objectives of this study were to evaluate:

growth and yield;

- nodulation and N_2 fixation; and,

- N benefit to a subsequent barley (Hordeum vulgare L.) crop, of backcrossed supernodulating soybean mutants.

Materials and methods

The performance of two supernodulating mutants, PS47 [intermediate supernodulator (sn), designated 2X] and PS55 (extreme sn, designated 6X) (L. Song, pers. comm.) was compared with that of four wild-type (wt) cultivars and two wild-type lines, PS16 and PS31 (L. Song, pers. comm.) in three field experiments. A non-nodulating (non-nod) mutant, nod49 (4), was also included in all experiments for comparative purposes. Experiment 1 (Exp. 1) was established in early December 1994 with eight genotypes. Both experiment 2 (Exp. 2) and 3 (Exp. 3) were established in November 1996 with, respectively, four and five genotypes. In all three experiments, each of the genotypes was either grown with N fertiliser (Nitram at a rate of 200 kg N/ha, 200 N) or left unfertilised (N0). A split-plot design with four replications was used for all the experiments with the N treatment as the main plot. All the seeds (including nod49) were inoculated with commercial inoculant containing Bradyrhizobium japonicum CB1809 at a rate of about 10 times that recommended for commercial use to accentuate the discrepancy in nodulation between the supernodulators and wild types. All experiments were carried out on the Redland Bay Experimental Farm, The University of Queensland (27o37'S, 153o19'E, altitude 5 m).

At R3 (pod-set) stage (6), nodulation and relative ureides (RU%) (total ureide nitrogen as a proportion of total sap soluble nitrogen) in xylem sap, which are highly correlated with the proportion of plant N derived from N2 fixation, were assessed (8). Dry matter production was determined by successive plant harvests from R1 (early flowering) to R5 (pod-fill) stages. Grain yield was determined from three 1 m-rows from each plot. In Exp. 3, four days after the harvest of soybean, barley cv. Grimmett was planted to assess the N benefit from the previous soybean plants. Before the planting of barley, soil samples were taken and the mineral nitrogen, ammonium (NH4+)-N and NO3--N, were analysed by the steam distillation method (1).

Results and discussion

Growth and yield

In Exp. 1, crop growth rate (CGR) was calculated for nod49, Manark, PS47 and PS55, between R2 and R4, a period of linear growth. With 0 N, PS55 had a significantly lower CGR (11.2 g/m2/d) than Manark (16.2 g/m2/d) and PS47 (20.8 g/m2/d). However under 200 N, the CGR of PS55 (17.2 g/m2/d) was similar to that of ?Manark (16.6 g/m2/d). The CGR of Manark and PS47 increased only a little with 200 N, while that of PS55 increased greatly.

PS47 outyielded all the other genotypes under 200 N in both Exp. 1 and 2 (Table 1). The yield of PS55 was much lower than that of the wild types under both N conditions in the 3 experiments, except at 200 N in Exp. 1.

The finding that PS55 did not grow as vigorously, and yield as much as, wild types under 0 N was similar to that of the previous studies on the extreme supernodulators conducted both in glasshouse and field (3, 9, 10). Several hypotheses have been proposed for the retarded growth and low yield of soybean extreme supernodulators, including other adverse mutations induced by mutagenesis, or excessive nodulation (5, 9, 10). Since PS55 is a backcrossed extreme supernodulator, its retarded growth and lower yield are more likely to have been caused by its altered nodulation rather than other mutations. The better performance of PS55 under 200 N, where its nodulation was inhibited, supported this point. Furthermore, uninoculated PS55 and other extreme supernodulators grew as vigorously as wild types in glasshouse experiments with adequate N supply (L. Zhao, unpublished). The extreme supernodulators were found to have higher respiration than wild types, particularly in their roots and nodules (5). There are also reports of reduced root growth as a result of supernodulation (5, 10). Our glasshouse studies showed that the extreme supernodulators had lower root/shoot ratio than wild types and that the nodule weight of the extreme supernodulators made up a higher portion of total root weight than that of wild types, especially under low N conditions. The extreme supernodulators also had a very small root diameter and a short tap-root compared with wild types. These results suggest that excessive nodulation retards root growth. No N or other nutrient defici-ency symptoms were ever observed in supernodulators. Therefore, retarded growth does not appear to be due to nutrient limitation.

Growth of all the genotypes was restricted in the 0 N treatment especially during later stages of growth. In particular, nod49, the non-nodulating mutant, produced much less grain, an effect that was obviously due to N-stress, as evidenced by chlorosis which developed about 40 days after planting in all three experiments.

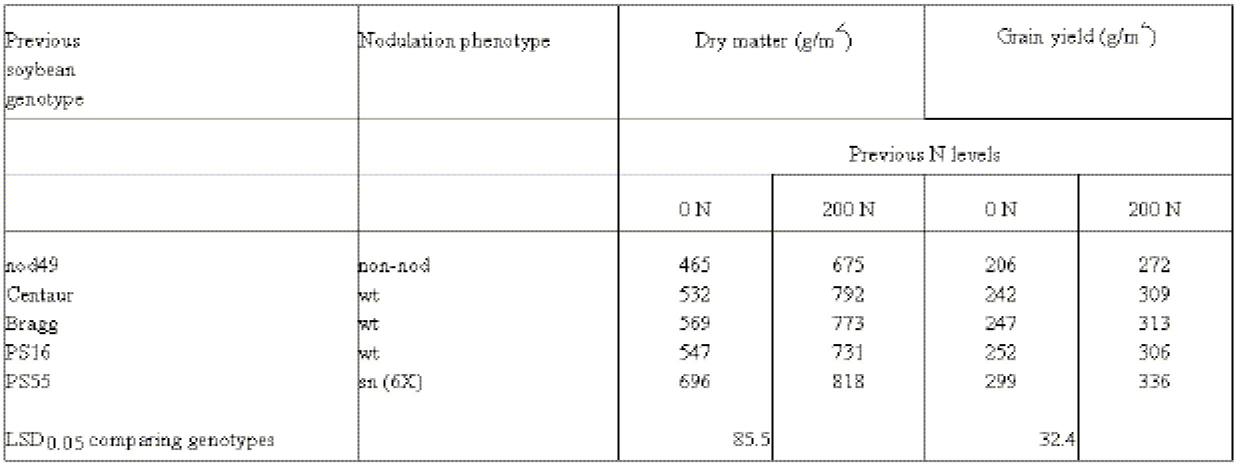
Table 1. Grain yield of nine soybean genotypes as affected by N fertilisation in three experiments

Genotype	nodulation phenotype	Grain yield (g/m²)					
		Exp. 1		Exp. 2		Exp. 3	
		0 N	200 N	0 N	200 N	0 N	200 N
nod49	non-nod	172	382	199	374	186	311
Manark	wt	365	389	375	414	--	--
Oxley	wt	289	273	--	--	--	--
Centaur	wt	343	402	--	--	232	367
Bragg	wt	--	--	--	--	310	348
PS16	wt	344	349	--	--	315	344
PS31	wt	339	368	--	--	--	--
PS47	sn (2X)	339	441	384	429	--	--
PS55	sn (6X)	214	363	207	359	176	235
$LSD_{0.05}$ comparing genotypes		62.8		23.1		44.9	
comparing genotype X N		64.5		23.5		44.9	

Table 2. Relative ureides present in xylem sap of seven soybean genotypes grown with 0 or 200 kg N/ha in three experiments

Genotype	nodulation phenotype	relative ureides (%)					
		Exp. 1		Exp. 2		Exp. 3	
		0 N	200 N	0 N	200 N	0 N	200 N
nod49	non-nod	16.4	14.7	10.3	14.7	16.8	14.2
Manark	wt	49.6	26.5	33.0	25.4	--	--
Centaur	wt	--	--	--	--	45.2	33.6
Bragg	wt	--	--	--	--	43.7	40.5
PS16	wt	--	--	--	--	49.8	40.0
PS47	sn (2X)	43.9	42.2	47.0	38.3	--	--
PS55	sn (6X)	61.6	56.1	70.9	57.9	78.2	66.6
$LSD_{0.05}$ comparing genotypes		11.4		7.1		14.6	
comparing genotype X N		10.6		9.1		16.6	

Table 3. Dry matter and grain yield of barley as affected by the previous soybean line



Previous soybean genotype	Nodulation phenotype	Dry matter (g/m^2)		Grain yield (g/m^2)	
		Previous N levels			
		0 N	200 N	0 N	200 N
nod49	non-nod	465	675	206	272
Centaur	wt	532	792	242	309
Bragg	wt	569	773	247	313
PS16	wt	547	731	252	306
PS55	sn (6X)	696	818	299	336
$LSD_{0.05}$ comparing genotypes		85.5		32.4	

Nodulation and N_2 fixation (relative ureides, RU%)

In both N treatments, PS47 produced about twice the nodule number of the wild types, while PS55 had 10 times the nodule number of wild types. High N consistently reduced the nodule number of all the nodulating genotypes. Under both N conditions in the 3 experi-ments, RU% was consistently higher for PS55 than for other genotypes (Table 2). PS47 had higher RU% than Manark under both N conditions in Exp. 2 and in the 200 N treatment in Exp. 1. The 200 N treatment reduced RU% of all the nodulating genotypes compared with 0 N treatment, especially in the wild types. These results were in agreement with those reported from previous studies (4, 9, 10).

Nitrogen benefit to subsequent barley crop

Mineral N (NH_{4+}-N and NO_{3-}-N) in the soil after the harvest of soybean was significantly higher after PS55, in both N treatments, than after other genotypes. This is especially evident in the 0 N treatment where mineral N was 28.7 ?g N/g soil after PS55, as compared to other genotypes where mineral N ranged from 13.6 to 16.8 ?g N/g soil.

Both the yield and dry matter of barley following PS55, were higher than those of barley following other soybean genotypes in plots previously unfertilised with N (Table 3). The lowest dry matter and grain yield were recorded for the barley following nod49, the non-nodulating mutant. The results indicate a high N benefit after PS55. Similar results were also obtained by Song et al. (9). The N benefit might be due to the combined effects of spared N and residue N, since the N content of nodules is much higher than that of root and PS55 had much higher nodule mass than wild types. However, the yield difference between PS55 and wild types, which averaged 150 g/m_2 in 0 N treatment, was not offset by the increased barley yield following PS55 of 50 g/m_2.

Conclusion

PS47, the intermediate supernodulator, fixed more N2 and yielded as much as wild types, indicating that the intermediate supernodulators are promising for practical use. The retarded growth and low yield of PS55, the backcrossed extreme supernodulator, under low N con-ditions suggests that excessive nodulation is the reason for its low yield. PS55 benefited the subsequent barley crop by sparing more N or providing more residue N. However, this N benefit does not appear to compensate for the low yield of PS55 itself. Therefore, extreme supernodulation is of questionable value to crop rotat-ions at this stage.

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