Characteristics of Growth and Yield Formation in the Improved Genotype of Supernodulating Soybean (Glycine max L. Merr.)

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Abstract: Most of the supernodulating soybean lines were agronomically inferior to conventional genotypes. Our previous tests with 'En6500', a supernodulating mutant derived from a variety 'Enrei', revealed that its low growth and yield were not necessarily due to supernodulation. In our attempts to improve the yield of En6500 through crossing with Enrei, we recently succeeded in selecting a supernodulating line showing vigorous growth. Field experiments with this new supernodulating line 'En-b0-1-2' for three years revealed that it yielded much more than En6500. When the overall yield level was low, it even tended to yield more than Enrei. En-b0-1-2 thus showed a remarkably higher productivity than other supernodulating lines reported so far. Its improved yield was largely due to: (a) better seed filling, (b) vigorous vegetative growth during flowering period, and (c) high leaf area index and leaf N content that enabled production of more photosynthates to enhance N fixation and dry matter accumulation during the period of pod and seed development.

Key words: Genotype, Growth, Nitrogen fixation, Root nodule, Soybean (Glycine max L. Merr.), Supernodulation, Yield.

Soybean (Glycine max L. Merr.) needs to accumulate a large amount of nitrogen (N) during reproductive growth because of high protein content of its grains. Improvement of N fixation by symbiotic rhizobia is considered an effective means for enhancing its productivity without high input of N fertilizer and consequent heavy pollution load on the environment. Several methods to improve N fixation have been proposed including the selection of rhizobial strains with high-energy efficiency and high N fixation capacity. Topdressing of N fertilizer at flowering time and use of slow-release N fertilizers have also been tried to improve N utilization, so as not to inhibit nodulation and N fixation through an increased soil inorganic N status.

Another approach to enhance N fixation is the use of supernodulating mutants isolated from various soybean varieties (Carroll et al., 1985; Gremaud and Harper, 1989). Such mutants can form a large number of nodules in soils with a wide range of inorganic N levels. Supernodulating lines were initially expected to be useful not only to gain a better understanding of autoregulation mechanisms of nodulation, but also to enhance soybean productivity through a higher N fixation capacity. However, the so-called 'supernodulating' or 'extremely supernodulating' lines that have more than several times as many nodules as parental lines, are in fact inferior in growth and seed yield, and do not seem to have any agronomic benefit. Indeed only a few genotypes that are 'intermediate' in supernodulation and form about twice as many nodules as parental lines, sometimes yielded similarly to their parents and have been found to be of some agricultural use (Herridge et al., 1990; Wu and Harper, 1991; Hussain et al., 1992a,b; Pracht et al., 1994; Song et al., 1995; Zhao et al., 1998; Herridge and Rose, 2000). The reduced yield in supernodulating lines has been attributed to: (a) high consumption of carbohydrates to form and maintain nodules and to fix N in nodules, and (b) low ability for absorption of nutrients and water by small root systems because of too many nodules (Gremaud and Harper, 1989; Hansen et al., 1989; Ohyama et al., 1993; Takahashi et al., 1995).

Occurrence of other deleterious genetic alterations has also been suggested to account for the low productivity (Pracht et al., 1994).

A supernodulating mutant 'En6500' isolated from a major Japanese soybean cultivar 'Enrei' (Akao and Kouchi, 1992) is also inferior to its parent variety in terms of growth, seed size and yield. However, its defects are not necessarily due to the supernodulation, as they persisted even under non-inoculated, or non-nodulating conditions (Francisco et al., 1992; Takahashi et al., 1995).

In our attempts to improve growth and yield of En6500, we recently succeeded in developing a vigorously growing genotype of supernodulating soybean 'En-b0-1-2'. The objective of this study was: (a) to assess the yield performance of En-b0-1-2 under field conditions, and (b) to clarify the characteristics of growth and yield.

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Abbreviations: LAI, leaf area index; NAR, net assimilation rate; RGR, relative growth rate.
formation of this supernodulating genotype in comparison with the previous genotypes. The latter objective is the first step of our studies to discover the cause of the high productivity of improved supernodulating genotypes.

**Materials and Methods**

1. Development of supernodulating soybean lines 'En-bO-1' and 'En-bO-1-2'

Soybean cultivar 'Enrei' (determinate, maturity group IV) and its supernodulating mutant 'En6500' were crossed in 1992, and 25 F1 seeds were obtained. Of 244 F2 plants, 57 plants indicated supernodulating phenotype. Growth and yield of the supernodulating lines were then compared in the F3 generation, and the highest yielding line was selected and named 'En-bO-1'. A supernodulating plant showing vigorous growth was selected in the F5 generation, fixed genetically, and named 'En-bO-1-2'. The generation of En-bO-1-2 was Fg, FlO, and Fll in 1996, 1997, and 1998, respectively.

2. Selection of high yielding supernodulating soybean lines (1996)

The main purpose of the experiment in 1996 was to select supernodulating lines with vigorous growth and high yield. Enrei, En6500, five supernodulating progenies of Enrei×En6500 including En-bO-1 and En-bO-1-2, and 65 supernodulating progenies of [(Enrei×En6500)×Enrei]×Enrei (F3) were grown in an upland field of the National Agriculture Research Center in Tsukuba. Details of cultivation are given in Table 1. Nitrogen, phosphorus and potassium were applied as a compound fertilizer and incorporated into the soil up to a depth of 18 cm. Plots were over-seeded and seedlings were thinned. Plants were sampled at the full maturity stage. Whole plant weight, grain yield and single seed weight were measured.

3. Experiments on the selected supernodulating line 'En-bO-1-2' (1997 and 1998)

The primary purpose of the experiments in 1997 and 1998 were to clarify the characteristics of the improved supernodulating line En-bO-1-2 under two different conditions of soil. Field experiments were conducted under upland fields in Tsukuba and in fields under paddy-upland crop rotation in Shintone. The experimental fields were rotated and therefore they were not the same every year even at the same location. Cultivar Enrei, supernodulating line En-bO-1-2 and a non-nodulating line 'EnI282' derived from Enrei (Francisco and Akao, 1993) were tested in all experiments. Other supernodulating lines, En6500 and En-bO-1, were included in only a few trials. Details of cultivation and sampling at both sites are given in Table 1. Compound fertilizer was incorporated into the soil up to a depth of 18 cm. Plots were over-seeded and seedlings were thinned. Plants were sampled at R2 (full bloom stage, Fehr et al., 1971), R3.5 (stage when leaf area index (LAI) reached its maximum), and R8 (maturity) stages. After measuring plant height and the number of nodes, plants were separated into various parts and leaf area was measured. Samples were then dried at 75°C for 48 h and weighed. The number and dry weight of nodules sampled from the top 15 cm soil in Tsukuba field were recorded in 1997. Leaf N content in Enrei and En-bO-1-2 was measured using an N-G analyzer (Sumigraph NC-800, oxygen gas combustion – gas chromatography system, Sumika Chemical Analysis Service, Ltd.).

4. Data analysis

All the experiments were arranged in a randomized block design. The data were analyzed by analysis of variance and following Tukey's honestly significant difference (HSD) test. The analysis was conducted for each year and each location separately.
1. Yield and yield components

Evaluation of 72 soybean lines in 1996 showed that all supernodulating progenies yielded more than En6500 (Fig. 1). While most of the supernodulating lines yielded less, some progenies yielded more than Enrei. Among the supernodulating progenies, En-b0-1 recorded the highest grain yield.

Further field evaluation of En-b0-1-2 and other selected lines in 1997 and 1998 showed that grain yields of En-b0-1-2 were similar to Enrei in both locations (Table 2). On the other hand, En6500, the original supernodulating line, yielded 62% less than Enrei. The supernodulating line En-b0-1 also yielded 15-19% less than Enrei. It is noteworthy that En-b0-1-2 showed similar grain yield to that of Enrei even when the grain yield of Enrei was about 5t ha⁻¹ in 1997. Insofar as the yield components are concerned, En-b0-1-2 seemed to be superior to Enrei in pod number or 100-seed weight. In contrast, En6500 was always inferior to Enrei especially in 100-seed weight due to partial seed filling (with several depressions on the seed surface).

2. Nodulation

Nodule number per plant in supernodulating lines was 11-14 times more than in Enrei. Nodule dry weight per plant was also more, but the single nodule weight was much less in supernodulating lines than in Enrei (Table 3).

3. Growth characteristics

A positive significant correlation (r=0.50**) between the main stem length at early growth stage and the grain yield was found in all genotypes in 1996 (Fig. 2). However, En-b0-1-2 and En6500 digressed widely from the line of fit, as their grain yields were very high and low respectively. This result indicates that these two genotypes performed much differently from other lines including Enrei. Dry weight of stem and pod wall at R8 also positively correlated with grain yield (r=0.81**) in 1996 (Fig. 3). Intermediate stem length, heavy weight of stem and pod wall, and high grain yield in En-b0-1-2 suggested that En-b0-1-2 grew moderately until...
Table 3. Nodulation of various soybean genotypes under field conditions in Tsukuba (1997).

| Genotypes | nodule no. (no. plant⁻¹) | whole nodules dry wt. (g plant⁻¹) | single nodule dry wt. (mg nodule⁻¹) |
|-----------|--------------------------|----------------------------------|-----------------------------------|
| En-b0-1-2 | 1748 a                   | 1.14 a                           | 0.67 b                            |
| En-b0-1   | 1484 b                   | 1.35 a                           | 0.91 b                            |
| En6500    | 1443 b                   | 1.11 a                           | 0.79 b                            |
| Enrei     | 128 c                    | 0.19 b                           | 1.46 a                            |

Values denote means of 9 plants measured on 19 August. Means not followed by the same letter are significantly different at P ≤ 0.05 based on Tukey’s HSD test.

Fig. 2. Relationship between seed yield and main stem length in soybean 34 days after sowing (1996).

Fig. 3. Relationship between grain yield, and dry weight of stem and pod wall at maturity (R8) (1996).

flowering and vigorously after flowering as compared with other lines. This growth pattern is clearly shown in Fig. 4. Shoot dry weight in En-b0-1-2 was 68% of that in Enrei at R2, but it increased to 85% at R3.5 and 104% at R8 in 1997 at Tsukuba (Fig. 4). In contrast, En6500 and En-b0-1 weighed only 52% and 81%, respectively, of Enrei even at R8 (Fig. 4).

LAI showed an increase pattern similar to that of shoot dry weight in all the lines (Fig. 5). LAI in En-b0-1-2 was 69% of that in Enrei at stage R2 but it increased to 85% at R3.5. By contrast, the LAI values in En6500 and En-b0-1 were only 41% and 65%, respectively, of that in Enrei even at R3.5. Regardless of the year and location, LAI in En-b0-1-2 was about 81–96% of that in Enrei at R3.5 (data not shown). Among all genotypes tested, En-b0-1-2 seemed to show the highest growth rate in leaf area after the R2 stage.

Relative growth rate (RGR), and net assimilation rate (NAR) tended to be higher in supernodulating lines...
Table 4. Dry matter production characteristics of soybean genotypes under field conditions.

| Site       | Year | Genotype | Shoot dry weight R2 | Shoot dry weight R3.5 | Shoot dry weight R8 | Pod + seed growth rate R3.5 | Leaf area R2-R3.5 | Leaf area R2-R3.5 | Leaf area R2-R3.5 | Leaf area R2-R3.5 |
|------------|------|----------|---------------------|-----------------------|---------------------|-----------------------------|-------------------|-------------------|-------------------|-------------------|
|            |      |          | (g m⁻²)             | (g m⁻²)               | (g m⁻²)            | (m² m⁻¹ d⁻¹)                | (g m⁻² d⁻¹)       | (g m⁻² d⁻¹)       | (g m⁻² d⁻¹)       | (m² g⁻¹)          |
| Tsukuba    | 1997 | En-b0-1-2| 149 b               | 410 ab                | 828 a              | 0 c                         | 0.210 a           | 0.0679 a          | 4.17 ab           | 0.0163 a          |
|            |      | En-b0-1  | 149 b               | 340 bc                | 646 ab             | 24 a                        | 0.119 a           | 0.0553 a          | 3.56 ab           | 0.0155 a          |
|            |      | En6500   | 79 c                | 227 c                 | 411 b              | 19 a                        | 0.068 a           | 0.0701 a          | 4.66 a            | 0.0151 a          |
|            |      | Enrei    | 217 a               | 482 a                 | 794 a              | 9 b                         | 0.194 a           | 0.0332 a          | 3.27 b            | 0.0163 a          |
|            |      | En1282   | 185 ab              | 451 ab                | 746 a              | 7 bc                        | 0.196 a           | 0.0599 a          | 3.66 ab           | 0.0163 a          |
|            | 1998 | En-b0-1-2| 163 b               | 437 b                 | 725 a              | 18 c                        | 0.126 a           | 0.0492 a          | 3.16 a            | 0.0156 a          |
|            |      | En-b0-1  | 178 b               | 466 b                 | 592 a              | 124 a                       | 0.065 ab          | 0.0486 a          | 3.47 a            | 0.0140 a          |
|            |      | Enrei    | 297 a               | 661 a                 | 767 a              | 99 a                        | 0.035 b           | 0.0398 a          | 2.99 a            | 0.0135 a          |
|            |      | En1282   | 268 a               | 572 ab                | 692 a              | 69 b                        | 0.048 ab          | 0.0378 a          | 2.51 a            | 0.0152 a          |
| Shintone   | 1997 | En-b0-1-2| 141 b               | 357 b                 | 832 a              | 0 b                         | 0.196 a           | 0.0709 a          | 4.26 a            | 0.0167 a          |
|            |      | Enrei    | 268 a               | 546 a                 | 880 a              | 7 a                         | 0.100 a           | 0.0545 a          | 3.64 a            | 0.0151 b          |
|            |      | En1282   | 247 a               | 511 a                 | 574 b              | 3 b                         | 0.147 a           | 0.0560 a          | 3.50 a            | 0.0160 ab         |
|            | 1998 | En-b0-1-2| --- ²°              | 416 a                 | 543 a              | 74 a                        |                 |                   |                   |                   |
|            |      | Enrei    | ---                 | 603 a                 | 566 a              | 133 a                       |                 |                   |                   |                   |
|            |      | En1282   | ---                 | 423 a                 | 252 b              | 63 a                        |                 |                   |                   |                   |

1) Within the same year and site or column, means not followed by the same letter are significantly different at P≤0.05 based on Tukey's HSD test.
2) Data were not taken.

Fig. 6. Nitrogen content of soybean leaves at growth stage of R3.5. Vertical bars represent standard errors of the mean.

than in Enrei in both years and locations, though most of them were not statistically significant (Table 4). Thus, in comparison with Enrei, En-b0-1-2 grew slowly until flowering and vigorously after flowering as evidenced by an increased RGR and NAR (Table 4).

4. Nitrogen content in leaf
Leaf nitrogen content at R3.5 tended to be higher in En-b0-1-2 (5.05-5.86%) than in Enrei (4.63-5.26%) (Fig. 6).

5. Morphological characters and course of growth stage
Main stem length in En-b0-1-2 was similar to that in Enrei but it was less in En-b0-1 (73-84% of Enrei) and En6500 (61%) (Table 5). Likewise, total node number in En-b0-1-2 was 3-9% more than in Enrei. En-b0-1 (78-81% of Enrei) and En6500 (79%), however, had fewer nodes than Enrei due to fewer branches (Table 5). En-b0-1-2 flowered 2-5 days later than Enrei while En6500 and En-b0-1 flowered around the same time as Enrei (Table 6). En-b0-1-2 matured 9-20 days later while En-b0-1 matured 3-8 days earlier than Enrei. En6500, however, matured around the same time as Enrei.

Discussion
Field experiments for three years at two locations consistently showed that our new supernodulating line ‘En-b0-1-2’ was highly productive as compared with other supernodulating lines reported so far. Several researchers (Herridge et al., 1990; Wu and Harper, 1991; Pracht et al., 1994; Song et al., 1995; Zhao et al., 1998; Oya and Ishii, 1999) reported that super-
hyper-nodulating lines were largely inferior to conventional nodulating soybean cultivars in grain yield or shoot dry weight. The higher shoot weight, pod number and grain yield of En-bO-l-2 in some trials indicate that this line is worthy of further attention by agronomists and plant breeders. En-bO-l-2 tended to yield better than Enrei, especially when the yield level was low, e.g. Tsukuba field in 1996 and Shintone field in 1998. The yield of the non-nodulating line Enl282 grown in Shintone field in 1998 was only 36% of Enrei's yield, hence available soil N fertility in this field was considered low. Under such conditions, En-bO-l-2 seemed to show a definite yield advantage. On the other hand, no significant yield differences could be observed between En-bO-1-2 and Enrei under fertile soil N conditions, where the yield of Enl282 was 90% or more than that of Enrei.

Several factors seem to account for the improved yield of En-bO-l-2. First, seed filling in En-bO-l-2 was much better than in the parental line En6500, which produced several small and empty seeds (Table 2). Second, the vegetative growth of En-bO-l-2 was much better than that of En6500 leading to marked increases in stem and leaf dry weight, LAI, and total node number (Fig.4, 5, Table 4, 5). The improved vegetative growth, in turn, perhaps led to an improvement in pod number and seed weight in En-bO-l-2. Vegetative growth was improved in En-bO-l also, but the magnitude of improvement was much less than in En-bO-l-2. Third, the vigorous growth of En-bO-l-2 after flowering also helped accumulation of more photosynthates. Despite its relatively low vegetative growth during early growth stages as reflected by moderate main stem length (Fig.2), dry matter production and LAI in 1997 and 1998 also confirmed the vigorous growth of En-bO-l-2 after full bloom stage or R2 (Figs.4 and 5, Tables 4 and 5).

The higher yield in En-bO-l-2 may be traced back to its unique growth characteristics in two phases: R2 to R3.5 and R3.5 to R7 (leaf yellowing stage). During the first phase, both vegetative and reproductive stages progressed simultaneously. However, En-bO-1-2

Table 5. Morphological characteristics of soybean genotypes at maturity (R8).

| Site    | Year | Genotype          | Main stem length (cm) | Node no. of main stem | Node no. of branch (plant) | Total node no. |
|---------|------|------------------|-----------------------|-----------------------|---------------------------|----------------|
| Tsukuba | 1997 | En-bO-1-2        | 59.7 a                | 16.6 a                | 23.1 a                    | 39.6 a         |
|         |      | En-bO-1          | 46.3 ab               | 13.3 c                | 18.1 ab                   | 31.4 bc        |
|         |      | En6500           | 33.3 b                | 12.5 d                | 14.7 b                    | 27.2 c         |
|         |      | Enrei            | 54.9 a                | 13.9 bc               | 24.7 a                    | 38.6 ab        |
|         |      | En1282           | 49.0 a                | 14.1 b                | 24.1 a                    | 38.1 ab        |
|         | 1998 | En-bO-1-2        | 66.9 ab               | 15.8 a                | 22.7 a                    | 38.6 a         |
|         |      | En-bO-1          | 51.0 c                | 13.2 c                | 15.6 b                    | 28.8 b         |
|         |      | Enrei            | 69.4 a                | 13.9 b                | 23.0 a                    | 36.9 a         |
|         |      | En1282           | 59.8 b                | 13.9 bc               | 22.3 a                    | 35.8 a         |
| Shintone| 1997 | En-bO-1-2        | 60.4 b                | 16.0 a                | 19.5 a                    | 35.5 a         |
|         |      | Enrei            | 69.8 a                | 14.6 b                | 17.9 ab                   | 32.5 ab        |
|         |      | En1282           | 63.0 b                | 14.8 b                | 14.1 b                    | 28.9 b         |
|         | 1998 | En-bO-1-2        | 43.2 b                | --- 2)               | ---                       | ---            |
|         |      | Enrei            | 59.4 a                | ---                    | ---                       | ---            |
|         |      | En1282           | 43.6 b                | ---                    | ---                       | ---            |

1) Within the same year and site or column, means not followed by the same letter are significantly different at P ≤0.05 based on Tukey's HSD test.
2) Data were not taken.
seemed to allocate more carbon assimilates to vegetative than reproductive organs. The leaf area growth rate tended to be high during this period, while dry weight of pods was low (Table 4). Such vigorous vegetative growth from R2 to R3.5 apparently compensated for low vegetative growth in the earlier stages. Other changes occurred during the second phase from R3.5 to R7. Despite recording the lowest dry weight of reproductive parts at R3.5 among all genotypes tested, final seed yield of En-b0-1-2 was high because of a high increasing rate of pod wall and seed weight, and prolonged seed filling period (Fig. 4, Table 6). En-b0-1-2 also maintained a higher NAR between R2 and R3.5 (Table 4) and a higher leaf N content than Enrei at stage R3.5 (Fig. 6). Leaves of En-b0-1-2 thus remained green for a longer time than Enrei (Table 6). These results suggest, therefore, that photosynthetic capacity of En-b0-1-2 remained high throughout the period of pod and seed development.

The above results clearly showed that field performance of En-b0-1-2 was significantly improved over the parental supernodulating line En6500, and that its yield was similar to or more than that of the parental wild-type line Enrei, especially in fields with low available N. The improved performance of En-b0-1-2 is especially appealing and raises the possibility of producing high soybean yields even in fields with low soil fertility. To practically realize this, however, further studies on a genetic basis of various physiological mechanisms, e.g. N fixation, photosynthesis at each growth stage, are necessary.

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