Genetics of Promiscuous Nodulation in Soybean: Nodule Dry Weight and Leaf Color Score

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Abstract

The symbiotic relationship between the soybean plant and rhizobium results in fixation of atmospheric nitrogen (N₂) in the root nodules, with the result that nitrogenous fertilization of the soybean is unnecessary. The effectiveness of nodule formation and N₂ fixation with rhizobial strains is under genetic control with two general categories identified: (1) promiscuous, which produces functional nodules with cowpea-type rhizobial strains; and (2) nonpromiscuous, which forms no or nonfunctional nodules with these strains. The segregation pattern of this promiscuity trait was studied using nodule dry weight (NDW) and leaf color score (LCS) as indicators of N₂ fixation effectiveness. Individual plants in each of six populations [P₁ = nonpromiscuous, P₂ = promiscuous, F₁ = P₁ × P₂ (and the reciprocal cross), BC₁(P₁) = F₁ (female) × P₁, BC₁(P₂) = F₁ (female) × P₂, F₂] were scored for these characters after inoculation with a rhizobial strain that would distinguish between both types. For NDW, nonpromiscuity was found to be partially dominant (h/d = 0.37), controlled by four loci. For LCS, nonpromiscuity was shown to be almost completely dominant (h/d = 0.74), controlled by two loci. LCS was a more meaningful estimate of N₂ fixation because it represented the total effectiveness of nodulation to provide nitrogen for the plant.

Legumes, including soybean [Glycine max (L.) Merrill], symbiotically associate with rhizobia to form nodules that fix atmospheric nitrogen (N₂) for the plant. Symbiosis often negates the need for application of inorganic nitrogenous fertilizers. Soybean genotypes fall into one of two categories relative to their responses to cowpea-type rhizobia. Promiscuous genotypes form effective (functional) nodules capable of fixing N₂ when supplied with compatible rhizobial strains of the cowpea-type (Pulver et al. 1985). In contrast, nonpromiscuous soybean genotypes similarly inoculated with cowpea-type rhizobial strains either form ineffective (nonfunctional) nodules or no nodules.

Nonpromiscuous genotypes are usually inoculated with compatible commercial inoculants of rhizobia (Bradyrhizobium japonicum type) in order to form functional nodules. However, in some tropical soybean production regions, particularly Africa, both commercial B. japonicum-type rhizobial inoculants and inorganic nitrogenous fertilizers are not readily available. Cowpea-type rhizobia, not B. japonicum, are ubiquitous in tropical soils in Africa (Kueneman et al. 1984), making the use of promiscuous cultivars very desirable since inoculant or nitrogen fertilizers are not necessary for maximum production. Therefore improved soybean cultivars selected for promiscuous nodulation with rhizobia already present in the soil are useful in these production situations. Knowledge of the promiscuity trait is essential for plant improvement programs designed to introgress promiscuity into nonpromiscuous lines with superior agronomic performance (Kueneman et al. 1984).

Information on the inheritance of the promiscuous trait is very limited. Therefore a study of the segregation pattern of promiscuous nodulation using two measures of N₂ fixation effectiveness, nodule dry weight (NDW) and leaf color score (LCS) per plant, was conducted. Individual plants of six populations [P₁ = nonpromiscuous, P₂ = promiscuous, F₁ = P₁ × P₂, BC₁(P₁) = F₁ (female) × P₁, BC₁(P₂) = F₁ (female) × P₂, BC₁(P₁) = F₁ (female) × P₁, BC₁(P₂) = F₁ (female) × P₂, F₂] were scored for these characters after inoculation with a rhizobial strain that would distinguish between both types. For NDW, nonpromiscuity was found to be partially dominant (h/d = 0.37), controlled by four loci. For LCS, nonpromiscuity was shown to be almost completely dominant (h/d = 0.74), controlled by two loci. LCS was a more meaningful estimate of N₂ fixation because it represented the total effectiveness of nodulation to provide nitrogen for the plant.
(female) × P2, and F3] associated with a promiscuous × nonpromiscuous cross were classified for NDW and LCS.

Materials and Methods

Seeds of a nonpromiscuous parental cultivar, Soma (P1), used in this study were obtained from the Crop Breeding Institute, Harare, Zimbabwe. Soma was released for commercial production in Zimbabwe in 1995 (Gwata and Nziramasanga 2001). This cultivar was developed using a modified single seed descent (SSD) breeding method (Poehlman and Sleper 1995) in which a single plant was selected from the F_8 generation of a superior breeding line. The harvested seeds from the selected plant were multiplied for subsequent yield trials conducted for three seasons and then distributed as a new cultivar. In the SSD and other plant breeding methods for pedigree (self-pollinating) systems, cultivars are generally released for commercial production after 11 or 12 generations of selfing (Poehlman and Sleper 1995). The flowers of this cultivar are white, while the hilum color is black. Seeds of a promiscuous cultivar Sam-Soy (P2) were obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria. Similarly this promiscuous cultivar was derived from a single plant selected from a superior breeding line developed using pedigree system plant breeding procedures (Kueneman et al. 1984). Sam-Soy has purple flowers and a brown hilum color. Purple is dominant over white flower (Hartwig and Hinson 1962) and black is dominant over brown hilum color (Specht and Williams 1978). Both traits were used to validate cross-pollinations.

In 1999, the F_1 generation of the nonpromiscuous (P1) × promiscuous (P2) cross, as well as the reciprocal cross, was made in the greenhouse. In 2000, the P_1, P_2, backcross [BC_1(P_1) = F_1 (female) × nonpromiscuous (P_2), BC_1(P_2) = F_1 (female) × promiscuous (P_2)] and F_2 generations were developed. The F_2 generation was obtained by mixing the seed from at least 45 F_1 plants (derived from both reciprocal crosses P_1 × P_2 and P_2 × P_1). In 2001, seeds of all generations [P_1, P_2, F_1, F_2, BC_1(P_1), and BC_1(P_2)] of the cross were planted in Conetainers (Stuewe and Son, Inc., Corvalis, Oregon) (4.0 cm diameter × 20.5 cm depth) filled with sterile washed sand in the greenhouse with day/night temperatures of approximately 28°C/20°C and a 14 h photoperiod of natural light in Gainesville, FL. One week after emergence, 0.1 g of micronutrient fertilizer (including 3.72% iron, 9.28% magnesium, 0.002% molybdenum, and 2.32% manganese) was applied to each plant. Starting at 2 weeks after emergence, 0.1 g of nitrogen-free (0-10-20) fertilizer was applied at weekly intervals to each plant. Previous work showed that these were appropriate levels of micro- and macronutrient fertilizer. At planting, seeds of all generations were inoculated with a cowpea-type rhizobial strain, AB-16D (obtained from NiFTAL, Paia, Hawaii), which was known to form functional nodules with Sam-Soy (promiscuous) but not with Soma (nonpromiscuous).

Six weeks after emergence, the leaves of plants from each generation (Table 1) were scored for LCS using a chlorophyll meter (Minolta Chlorophyll Meter Spad-502, Minolta Co., Ltd., Tokyo, Japan). This apparatus quantitatively records numerical leaf color units ranging from high (green) to low (yellow). The apparatus determines the transmittance of light through the sample leaf at two wavelengths (650 nm and 920 nm). By comparing the transmittance at these two wavelengths, the instrument automatically calculates a numerical value, which is linearly related to the leaf chlorophyll content (Markwell et al. 1995). Each time the meter is used it is calibrated by pressing on the finger rest to close the head. After the meter beeps, a display is shown indicating the meter is ready for the first sample. In a previous study designed to examine the relationship between LCS and leaf chlorophyll content per unit leaf weight in soybean, we found a high positive correlation (r = 0.88; df = 19; P < .01) between the two variables. After scoring for LCS, the roots of each plant were washed and carefully rinsed with water. The nodules were harvested, oven dried at 65°C for 4 days, and weighed to determine the total NDW per plant. A positive correlation between NDW and N_2 fixation in soybean (Shiraiwa et al. 1994) and in other leguminous species such as common bean (Rosas and Bliss 1986), cowpea (Ankomah 1995), and peanut (Hafner et al. 1992) was reported. In addition, shoot (plant) dry weight was found to be correlated positively with NDW (Sinclair et al. 1991) and with both leaf chlorophyll content and shoot total nitrogen in soybean (Mirza et al. 1990). Therefore NDW was assumed to be a reliable measure of N_2 fixation in the present study.

The experimental design was a completely randomized design with each genotype representing an experimental unit. Data were subjected to analysis of variance (ANOVA) procedures. Tukey’s method (Ott 1988) was applied to separate the means of the six populations. The dominance relationships (b/d values) were determined using b = d − observed F_1 mean and d = (P_1 mean + P_2 mean)/2 and the locus number (k) was obtained using k = [(P_1 mean − P_2 mean)^2]/8(F_2 variance − F_1 variance) as used by Pfahler and Barnett (1989).

Results

NDW

The mean NDW per plant for the promiscuous parent Sam-Soy (62.0 mg) was about seven times larger than that of the nonpromiscuous parent Soma, indicating that promiscuous genotypes have considerably heavier nodules than non-promiscuous genotypes (Table 1). The mean NDW of the F_1 population (25.0 mg) was significantly different from either parent with partial dominance (b/d = 0.27) for nonpromiscuous trait. The distributions (Figure 1) of BC_1(P_1), BC_1(P_2), and F_2 supported this observation. However, the BC_1(P_1) and F_2 means were not significantly different from the mean of the nonpromiscuous parent (P_1), suggesting that a higher degree of dominance was involved. Calculations estimated that four loci were probably involved in the segregation.
When plants from each generation were classified with the leaf chlorophyll meter, similar frequency distribution patterns for each of the six generations were found. All the plants in the nonpromiscuous parental population (P1) were distinctly yellow, but those of the promiscuous parental population (P2) were green. The mean LCS value of the promiscuous parental population (24.54 units) was three times higher than that of the nonpromiscuous type (Table 1). The mean LCS value of the F1 population (10.20 units) was 37% less than the midparent value (16.32 units), indicating that dominance of nonpromiscuity for this trait was present. Nonpromiscuity was shown to be almost completely dominant ($h/d = 0.74$) and calculations estimated that two loci were involved in the segregation of this trait.

Calculation of phenotypic correlation coefficient ($r$) using data from all genotypes in all generations resulted in a positive correlation ($r = 0.640; \text{df} = 276; P < .01$) between NDW and LCS, indicating that a relationship existed.

**Discussion**

The results of this study indicate that the conclusions of the genetic analysis of the promiscuity trait depend, in part, on the method used to classify the trait. Partial dominance for promiscuity was found in both the NDW and LCS analyses, but the $h/d$ values differed substantially. In addition, considerable differences in the estimated loci number were present, depending on the method used to classify the character. Apparently the genetic expression of the promiscuous trait is quite variable and the method used to determine the expression of this trait in plants considerably alters the estimates of the mode of gene action and loci number.

Nodulation and N$_2$ fixation is a very complex process, making it very sensitive to environmental and genetic factors affecting the plant, bacteria, and interactions (Hungria and Stacey 1997). Nodule formation is a multistep process involving movement of rhizobia by chemotaxis to the root hair (Oke and Long 1999). The attachment of rhizobia to the root hair induces deformation of growth in the root hair and stimulates the division of the host cortical cells, culminating in infection and nodule development (Long 1990). N$_2$ fixation occurs as the rhizobia and plant differentiate and establish a metabolic cooperation (Long 1990).

Various traits associated with root structure, development, and physiology, which are influenced by genetic and environmental factors, influence the fixation process. Genotypic variation and environmental influence for nodule number and weight within nonpromiscuous soybeans have been reported, with highly significant correlation coefficients found between nodule number and weight (Sinclair et al. 1991). In this study, the variances for NDW for 17 soybean genotypes grown in six environments (3 years × 2 locations) showed significant ($P < 0.01$) environmental differences and significant ($P < 0.01$) genotype × environmental interactions. The mean NDW for 5 of the 17 genotypes was

**Table 1.** Nodule dry weight (NDW) and leaf color score (LCS) means and variances

<table>
<thead>
<tr>
<th>Trait</th>
<th>Generation</th>
<th>No. of plants</th>
<th>Mean</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDW (mg)</td>
<td>P$_1$</td>
<td>20</td>
<td>8.0</td>
<td>d 30</td>
</tr>
<tr>
<td></td>
<td>P$_2$</td>
<td>24</td>
<td>6.20</td>
<td>a 530</td>
</tr>
<tr>
<td></td>
<td>F$_1$</td>
<td>45</td>
<td>25.0</td>
<td>c 140</td>
</tr>
<tr>
<td></td>
<td>BC1(P$_1$)</td>
<td>56</td>
<td>14.0</td>
<td>cd 190</td>
</tr>
<tr>
<td></td>
<td>BC1(P$_2$)</td>
<td>48</td>
<td>48.0</td>
<td>b 810</td>
</tr>
<tr>
<td></td>
<td>F$_2$</td>
<td>95</td>
<td>15.0</td>
<td>cd 240</td>
</tr>
</tbody>
</table>

| LCS        | P$_1$      | 20            | 8.11 | b 7.81   |
|            | P$_2$      | 24            | 24.54| a 9.32   |
|            | F$_1$      | 45            | 10.25| b 12.41  |
|            | BC1(P$_1$) | 56            | 9.21 | b 17.15  |
|            | BC1(P$_2$) | 48            | 21.23| a 39.32  |
|            | F$_2$      | 95            | 10.73| b 32.33  |

Means in the same column followed by the same letter were not significantly different (Tukey’s test, $P < 0.05$).

**Figure 1.** Frequency distribution of each generation in the combined crosses. (Vertical dashed line is the midparent value $m = 35$ mg.)
unstable across the six different environments. In addition, the greatest differences (in NDW per plant) between genotypes were expressed in the two most favorable environments. The relationship between the nodule number and root hair number per unit area was not studied, but a relationship between N\textsubscript{2} fixation capacity and root size and weight was not found (Sinclair et al. 1991). Genetic variation among various promiscuous soybean lines and the effectiveness of diverse rhizobial strains in nodulation was reported (Sanginga et al. 2000). The symbiotic effectiveness of 20 cowpea-type rhizobial strains on promiscuous breeding lines evaluated at three field locations in different agroecological regions depended on both the plant genotype and location (Sanginga et al. 2000). However, in our study, environmental effects were minimized. The expression of the nodulation trait in the populations was standardized by using washed sand in Conetainers in the greenhouse with controlled environmental conditions.

The apparent lack of transgressive segregation in the F\textsubscript{2} generation was unexpected. In addition, the greater variance for NDW in the promiscuous parent (P\textsubscript{2}) versus the variance in the F\textsubscript{2} generation was unexpected in presumably homozygous parental populations. The parents were assumed to be homozygous at the major loci conditioning the character, and this assumption in all probability was fulfilled. However, there may have been a number of minor modifying loci present that could alter the expression to a limited extent, which could contribute to increased variance and absence of transgressive segregation. Another important aspect to be considered in interpreting the unexpected variances is that the expression of the character was controlled by the segregation of the host, the effect of environment, and the bacteria and interactions. The environment was highly controlled in the experiment. Probably the rhizobial (bacterial) population may have contained some genetic variation, but the isolation and propagation of the population should have reduced this possibility to an absolute minimum. Moreover, the experiment was conducted under very controlled conditions, with all the biological aspects and environment controlled as much as possible.

The moderate correlation coefficient between NDW and LCS suggests that the two measures of N\textsubscript{2} fixation were different from each other. Since NDW includes nonfunctional nodules, then NDW is not as valid an indicator of N\textsubscript{2} fixation. However, both measures might be required in evaluating promiscuous nodulation under these experimental conditions.

In conclusion, the results suggest that this genetic character is dominant for nonpromiscuity and is controlled by a relatively small number of genes at a small number of loci in the cross reported in this study. The environmental variation observed is probably the combined result of the complexity of the character and the method of classification. Evaluation of the nonpromiscuity trait will be most valuable in breeding lines adapted for production in geographical areas lacking the technology to objectively measure levels of N\textsubscript{2} fixation. Therefore visual scoring of leaf color intensity would be adequate to distinguish N\textsubscript{2} fixing genotypes in segregating populations grown under similar nitrogen-depleted conditions. This would be useful in applied plant breeding programs that require rapid screening methods to select for promiscuous genotypes.

References


Received May 30, 2003

Accepted November 11, 2003

Corresponding Editor: Reid Palmer