Short Communication

Dinitrogen Fixation in Male-Sterile Soybeans

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ABSTRACT

Partial male-sterile (ms/ms) soybeans (Glycine max L. Merr.) and their fertile isoline (Ms/Ms) were grown in adjoining field plots. From 62 until 92 days after emergence, the nitrogenase activity, assayed by acetylene reduction, of the average male-sterile plant was approximately twice that of the average fertile plant. At approximately 100 days after emergence, the assayable nitrogenase activity of the fertile plants fell to zero, whereas the nitrogenase of the partial male-sterile plants continued to be active for two additional weeks. Thus, this male-sterile plant seems to fix dinitrogen both at a higher rate and over a longer duration than does its fertile isoline.

Partial male-sterile soybean plants were first described in 1970 (3). The homozygous recessive ms/ms plants are easily recognized, because they produce very few pods, and their leaves usually remain green long after the fertile isoline has reached full maturity (8, 12). In 1978, it was reported that homozygous ms/ms plants fix the same amount of dinitrogen as do heterozygous (Ms/ms) fertile plants (12). Subsequently, it has been assumed that partial male-sterile soybean plants fix dinitrogen at approximately the same rate as do their fertile isolines (2, 11). This assumption, however, is seemingly inconsistent with the reports that senescence is delayed in depodded soybean plants (7) and that the duration of dinitrogen fixation is prolonged in fertile soybean plants that exhibit delayed leaf senescence (1). Thus, one might expect that a partial male-sterile plant displaying delayed leaf senescence would fix more dinitrogen than would a normal fertile plant. Because of these apparent inconsistencies, we investigated both the rate and the duration of dinitrogen fixation in ms/ms (male-sterile) soybeans and in their Ms/Ms (fertile) isoline.

MATERIALS AND METHODS

Plants. 'Rampage' and Rampage ms/ms, varieties of soybean (Glycine max [L.] Merr.), provided by Dr. Reid Palmer, were grown at the Hinds Experimental Agricultural Farm in Ames, IA. The genetic symbol ms represents male sterility.

Plants were dug from the field throughout the growing season. To minimize damage to the root system, a trench about 0.5-m deep and 0.5 to 0.7 m from the row was dug around a group of six to eight plants. With a spading fork, soil was carefully removed from the sides of the root clump until the first nodules were exposed. Then, depending upon soil conditions, one of two strategies was used to remove the rest of the soil from the roots: the soil was crumbled off carefully by hand; or a 1 x 0.5-m board was slipped under the plants and surrounding soil, the entire clump was placed in the shade, and the soil was carefully washed from the roots and nodules with running water from a hose. Individual plants were weighed and assayed for acetylene reduction activity.

Assay for Acetylene Reduction. The roots of an intact plant were placed in a wide-mouth (85-mm) quart jar fitted with a notched rubber stopper with septum. Modeling clay was used to form a seal between the stem of the plant and the notch in the rubber stopper (5). Air (50 ml) was removed from the jar with a syringe, and 50 ml acetylene was injected through the septum. Gas samples were removed from the jar at three or more 10-min intervals with a 10-μl Hamilton syringe, and the gas mixture was analyzed immediately on a gas chromatograph equipped with a hydrogen flame detector. The ratio of acetylene to ethylene was determined from the heights of the respective peaks. A correction for the effect of diffusion on peak height was made.

Analysis of Data. Data were analyzed by two-way analysis of variance. For these calculations, logarithmic transformations of the raw data for total nitrogenase activity, nodule specific activity, nodule dry weight, and partitioning efficiency were used to obtain homogeneity of variance. In addition, t tests comparing the means of the two genotypes at each time point were performed. The efficiency with which each plant fixed dinitrogen was estimated. This partitioning efficiency is defined as μmol ethylene formed per h per g fresh weight.

RESULTS AND DISCUSSION

A partial male-sterile soybean plant produces only a few pods. Thus, the fraction of photosynthate normally consumed by pod filling in the fertile plant is largely unused in the male-sterile plant. This unused photosynthate might be repartitioned so as to increase other plant activities, including dinitrogen fixation. On the other hand, the male-sterile plant requires less nitrogen for seeds than does the fertile plant; therefore, it is conceivable that the male-sterile plant might fix less dinitrogen than might the fertile plant.

Consequently, we measured both the rate and duration of nitrogenase activity in the two genotypes (Fig. 1). The rate of dinitrogen fixation in both genotypes was still very low at 50 DAE; however, by 62 DAE, the total nitrogenase activity of the average male-sterile plant was approximately twice that of the average fertile plant (Fig. 1). This 2-fold difference in nitrogenase activity continued until the activity of the fertile plants approached zero at beginning maturity (96 DAE). The male-sterile plants, on the other hand, retained both nitrogenase activity and green leaves at 105 DAE. The analysis of variance F test for differences in total nitrogenase activity between the two genotypes from 62 to 92 DAE was significant at the 0.0001 level. This difference in nitro-

1 Supported in part by the Iowa Soybean Promotion Board. Journal Paper No. J-10410 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 2298.

2 Abbreviation: DAE, days after emergence.
Table 1. Temporal Profile of Plant Fresh Weight, Nodule Mass, Specific Activity, and Partitioning Efficiency in Dinitrogen Fixation

Data provided in Figure 1 and Table 1 were obtained from the same plants and are expressed on a per-plant basis.

<table>
<thead>
<tr>
<th>DAE</th>
<th>Stage of Fertile Plants</th>
<th>Plant Fresh Weight</th>
<th>Nodule Dry Weight</th>
<th>Nodule Specific Activity</th>
<th>Partitioning Efficiency</th>
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<tr>
<td></td>
<td>Sterile</td>
<td>Fertile</td>
<td>P value</td>
<td>Sterile</td>
<td>Fertile</td>
</tr>
<tr>
<td>62</td>
<td>R4-R5</td>
<td>166</td>
<td>197</td>
<td>NS</td>
<td>289</td>
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<tr>
<td>68</td>
<td>R5</td>
<td>230</td>
<td>248</td>
<td>NS</td>
<td>581</td>
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<tr>
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<td>R5</td>
<td>224</td>
<td>274</td>
<td>NS</td>
<td>889</td>
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<tr>
<td>77</td>
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<td>259</td>
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<tr>
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<tr>
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<td>112</td>
<td>R8</td>
<td>350</td>
<td>ND</td>
<td>ND</td>
<td>758</td>
</tr>
</tbody>
</table>

Composite P value (62 through 92 DAE): 0.001 0.0001 NS 0.0001

* See Ref. 4.

b Not statistically different.

c No quantitative determination was made.

![Graph](http://placehold.it/120x120)

**Fig. 1. Temporal profile of nitrogenase activity in msu/msu partial male-sterile and msu/Msu fertile soybeans.** Six to eight plants of each genotype were assayed at each time point. The average nitrogenase activity and its SE for each genotype at a given time point are shown: male-sterile (●); fertile (○). Data shown in this plot were obtained during the 1981 season and are very similar to data obtained during the 1980 season (not presented).

Nitrogenase activity cannot be explained by differences in plant size, because the average msu/msu plant weighed slightly less than did the average fertile plant but reduced acetylene at a higher rate. Thus, the nitrogenase activity of the male-sterile genotype is both greater and more prolonged than is that of the fertile genotype.

Mean values and results of the t tests for the differences between genotypes at each time point are presented in Table 1. The analysis of variance F tests performed on the data from 62 to 92 DAE for differences between genotypes were significant at the 0.0001 level for nodule dry weight and partitioning efficiency and at the 0.001 level for plant fresh weight. The test for overall differences in nitrogenase specific activity between the two genotypes was not significant.

The larger mass of nodules, the higher rate of total nitrogenase activity, the longer duration of nitrogenase activity, and the higher partitioning index found in the male-sterile plant, collectively, strongly suggest that the average msu/msu male-sterile plant directs a greater portion of its photosynthetic into dinitrogen fixation than does a fertile plant. Likewise, these data and those reporting an accumulation of nitrogen in the leaves and stem of the male-sterile plant (12) suggest that dinitrogen fixation is not simply turned 'off' when the plant has acquired a certain level of plant nitrogen. Instead, these findings provide further evidence that, under certain conditions, dinitrogen fixation may be turned off by a substance(s) produced by, or during, seed formation in the normal fertile plant (6, 9, 10).

**LITERATURE CITED**


