Intraspecific Variability for Nitrogen Fixation in Southernpea (Vigna unguiculata (L.) Walp)\textsuperscript{1}

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Abstract. The extent of intraspecific variability for N\textsubscript{2} fixation among 100 southernpea (cowpea) genotypes was determined, and a screening technique was developed to measure the character. Significant differences in N\textsubscript{2} fixation efficiency were found among host plant genotypes following application of a standard commercial mixed strain Rhizobium inoculant. These differences were obtained whether the criterion used was nodule mass, nodule number of nitrogenase activity, as measured by the acetylene reduction assay. This variability is evidence of genetic control of the trait and suggests the possibility of breeding for increased N\textsubscript{2} fixation efficiency in cowpea.

One of the most distinctive and important features of legumes is their ability to fix atmospheric nitrogen. Considerable effort has been expended toward increasing the efficiency of the symbiotic relationship responsible for N\textsubscript{2} fixation. However, these efforts have concentrated on the bacterial component of the relationship, while the role of the host plant has been essentially ignored. Intraspecific variability for nitrogen fixation among host genotypes has been reported in the forages Medicago sativa L. (3) and Trifolium (2) and recently in several seed legumes including Glycine max (L.) Merrill (5), Vicia faba L. (1), and Phaseolus vulgaris L. (6). The objective of this investigation was to determine the extent of intraspecific variability.

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for nitrogen fixation in southernpea.

Materials and Methods

Initial screening. One hundred southernpea lines representing diverse genetic backgrounds were chosen for the initial screening and planted in the greenhouse on June 4, 1976. Two plants of each line were grown in 1.5 liter pots containing a mixture of 1 builders sand: 1 coarse vermiculite. Three seeds per pot were planted and thinned to a single plant following emergence. All seeds were treated at planting with a commercial mixed strain cowpea *Rhizobium* inoculant. Watering followed a strict regime of 2 applications of nitrogen free Sloger’s solution (4), followed by a thorough flushing of the rhizosphere with tap water. Plants were grown under a temperature regime of about 18°C (night) and 27°C (day) with an average day length of 13 hr. After 6 weeks the plants were severed at the soil line, and the tops dried and weighed. The intact root-nodule complex was then carefully removed from the growth medium and immediately placed into 0.48 liter septum fitted, gas tight Tall canning jars, which were then injected with 25cc of acetylene (5%). Following a 1 hr incubation at 25°C, 3 cc of the gas mixture was removed and injected into gas tight vacutainers for later ethylene determination, using an H-flame ionizing gas chromatograph. The root-nodule complex was then removed from the jars and put into labeled plastic bags and frozen for later determination of nodule number and nodule mass.

Greenhouse verification trial. A replicated greenhouse verification trial was carried out using 5 high N2 fixing genotypes, ‘H-Brown Crowder’, ‘H-Knuckle Purple Hull’, H-TXCS-5, ‘H-Calico Crowder’, H-TXCS-8, and 4 low N2 fixing lines, L-TX 266, L-TX 460, ‘L-Chinese Red’, and ‘L-Bush Purple Hull’, selected from the initial screen. Selection was based primarily on nitrogenase activity as measured by the acetylene reduction assay. Nodule number, nodule mass, and plant dry weight were secondary criteria for selection. The 9 selected lines were planted in the greenhouse on Oct. 3, 1976, and handled as before. A randomized block design with 4 plants per rep and 4 replications was used.

Field verification trial. Four lines previously identified as high fixing and 3 as low fixing were both planted and inoculated by hand in the field in a Lufkin fine sandy loam at College Station, Texas on May 15, 1977. H-TXCS-5 and L-TX 266 were not included in this trial due to insufficient seed supply. The experimental design was a randomized block with 4 replications. Seeds were planted in rows 102 cm apart and 2 m long. The resulting stand was thinned to 15 cm between plants. A banded preplant application of ON-19.8P-OK superphosphate fertilizer was applied at the rate of 45 kg/ha. Irrigation water was applied as needed. Four plants were harvested from the center of each block on June 23 when all genotypes had reached full flower. The plots were irrigated one day prior to harvest to facilitate in the removal of the intact root-nodule complex. Harvest procedure was as before, except that sharpshooter spades were used to dig up roots.

Results

Initial screening. Results from the initial screening indicated wide variability for all indices measured (Table 1). Among the 100 genotypes screened, plant specific activity ranged from 0.6 to 43.3 μmoles C2H4 produced per plant per hr. Similar variability was observed in nodule mass (0.1 – 3.0 g) nodule number (10-146) and plant dry weight (2.5-8.4 g). Among the 4 indexing criteria, specific activity was the most consistent indicator of N2 fixation among entries and their relationship to the check genotypes. Although high nodule mass was generally associated with high plant specific activity, exceptions were observed. Such a relationship was not as obvious when comparing nodule number with activity. However, values obtained for plant dry weight generally showed a positive relationship with activity rate. Differences in plant dry weight were significant and ranged from 2.2 g for ‘L-Chinese Red’ and 8.2 for ‘H-Knuckle Purple Hull’.

Field verification trial. Significant variability for all indexing criteria for the 7 genotypes tested was observed in the field verification trial (Table 2). As expected, nodule activity in the field was substantially higher than that obtained in the greenhouse studies. This activity ranged from 30.9 μmoles C2H4 for L-TX 460 to 118.6 for ‘H-Brown Crowder’. In the field, as in the greenhouse verification trial, significant differences were observed between those genotypes classified as high in activity and those as low in the initial screening. ‘H-Brown Crowder’ exhibited the highest activity in both trials. Significant differences were found among genotypes in nodule mass and nodule number. Nodule mass ranged from 0.78 g for L-TX 460 to 2.38 g for ‘H-Brown Crowder’. In the field, there were significant differences between those genotypes classified as high and low in the initial screen. Although there were significant differences among genotypes for nodule number, there was no distinct separation between the high and low groups. Significant differences among genotypes were observed for plant dry weight, which varied from 34.7 for ‘L-Bush Purple Hull’ to 55.2 g for H-TXCS-8. With the exception of ‘L-Chinese Red’ genotypes classified as low fixing, based on specific activity in the initial screening, were significantly lower in dry weight.

Discussion

The consistent performance of individual genotypes in the initial screening and the subsequent replicated experiments, demonstrated the validity of the proposed N2 fixation screening technique. Differences between tests are to be expected due to environmental fluctuation such as day length and light intensity. However, the critical factor in any given screening is not the magnitude of the differences but the relative position among entries and their relationship to the check genotypes. Among the 4 indexing criteria, specific activity was the most consistent indicator of N2 fixation followed by nodule mass, plant top dry weight, and nodule number. Although definite trends were observed for nodule mass, nodule number and dry weight, relative to activity, inconsistencies, especially for nodule number, cast doubt on their value as N2 fixation indices in an initial screen, especially when cost is considered. These parameters, however, must ultimately be determined for any genotype identified as potentially useful.

Our results show that it is possible to use the acetylene reduction assay to screen large numbers of southernpea genotypes for N2 fixation capacity using as few as 2 plants/genotype. This technique makes possible continuous screening without

dependency upon growing season. We are suggesting the use of
this technique to predict a genotype's N\textsubscript{2} fixing potential, at
least in separating high and low fixing genotypes. However, the
ultimate test of any genotype selected in the screen is its field
performance.

More important than the screening technique, however,
was the genetic variability observed for the N\textsubscript{2} fixation trait
in southernpea. This variability was found among only 100 geno-
types, suggesting that even greater variability may be present,
within the species. Variability is evidence for genetic control
of the trait and suggests the possibility of breeding for in-
creased N\textsubscript{2} fixation in southernpea.

\begin{table}
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\begin{tabular}{|l|c|c|c|c|}
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Genotype\textsuperscript{2} & Specific\textsuperscript{3} activity & Nodule mass (g) & Nodule no. & Dry wt per plant (g) \\
\hline
H-Brown Crowder & 6.8\textsuperscript{a} & 0.33b & 18.7d & 6.8bc \\
H-Knuckle Purple Hull & 6.6b & 0.38ab & 29.4cd & 8.2a \\
H-TXCS-5 & 6.4ab & 0.38ab & 65.3a & 6.6cd \\
H-Calico Crowder & 6.3ab & 0.28c & 48.6b & 6.4cd \\
H-TXCS-8 & 5.9b & 0.38a & 61.4a & 7.2b \\
L-TX 266 & 1.2c & 0.26c & 60.9a & 5.8ef \\
L-TX 460 & 1.1c & 0.34ab & 31.6c & 6.1e \\
L-Chinese Red & 0.9c & 0.29c & 38.4bc & 2.2g \\
L-Bush Purple Hull & 0.5c & 0.16d & 40.0bc & 5.4f \\
\hline
H-Brown Crowder & 118.6a & 2.38a & 110.9bc & 51.6a \\
H-Calico Crowder & 95.8ab & 1.54c & 159.3a & 54.2a \\
H-Knuckle Purple Hull & 86.5ab & 2.17ab & 128.8b & 54.8a \\
H-TXCS-8 & 72.5b & 1.88bc & 165.4a & 55.2a \\
L-Chinese Red & 43.4c & 1.11d & 90.1ed & 49.1a \\
L-Bush Purple Hull & 36.2c & 0.80d & 75.8d & 34.7b \\
L-TX 460 & 30.9c & 0.78d & 103.3bcd & 40.1b \\
\hline
\end{tabular}
\caption{Mean specific activity, nodule mass per plant, nodule number per plant and plant dry weight per plant top for 9 southernpea genotypes from the greenhouse verification trial and 7 from the field verification trial.}
\textsuperscript{2} Genotypes selected from the initial screen.
\textsuperscript{3} Specific activity equals \(\mu\)moles of C\textsubscript{2}H\textsubscript{4} produced per plant per hr.
\textsuperscript{x} Mean separation in columns by Duncan's multiple range test, 5\% level.
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