

Assessment of traits related to nitrogen fixation in soybean (*Glycine max* [L.] Merr.)

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Introduction

A 2-fold increase in food production will be needed to meet the expected increase in global population by 2050 (Ray et al., 2013). Soybean cultivars have a relatively high yield potential, but some factors prevent them from reaching their full capacity. The biological nitrogen fixation (BNF) capacity of modern cultivars has been appointed as one of the major factors that limits nitrogen (N) supply to soybean crop (Salvagiotti et al., 2008). Previous studies showed that various traits directly and indirectly related to BNF need to be evaluated for enhancing di-nitrogen fixation in soybean (Munoz et al., 2016; Hwang et al., 2014; Santos et al., 2013; Herridge et al., 2000). Thus, the objective of this study was to: (1) evaluate traits directly or indirectly related to BNF in 25 soybean genotypes with different genetic background; (2) test their association with BNF; and (3) assess their value for relatively high BNF capacity.

Table 1. Correlation coefficients between leaf and pod atom% ¹⁵N excess and traits related to di-nitrogen fixation.

	Pod AT% ¹⁵ N excess
Days to Maturity	-0.36**
Shoot Dry Mass	-0.51**
Seed Weight	-0.38**
Seed Number	-0.48**
Mean Seed Weight	0.09
Pod %N	-0.28**
Nodule Number	-0.39**
Nodule Dry Weight	-0.30**
	Leaf AT% ¹⁵ N excess
Leaf %N	-0.49**
SPAD readings	-0.45**

** Significant at $p < 0.01$.



Figure 1. Soybean plants 5 wks after germination.

Material and Methods

A total of 25 soybean genotypes with different genetic backgrounds were evaluated under greenhouse conditions in 2014 and 2015. Inoculation with the *Bradyrhizobium japonicum* strain USDA 110 was performed at the seed stage (2×10^5 cells per seed). To assess the N efficiency of each genotype, the ¹⁵N isotope dilution technique was used with the application of a small quantity (7.82 mg kg⁻¹ of potting medium) of ¹⁵N-urea (10 atom%) prior to sowing on the potting medium. Leaf and pod samples were collected at 5 wks after germination and at the R7 developmental stage, respectively. The results from atom% ¹⁵N excess were obtained from each sample using an isotope ratio mass spectrometer and considered as time integrated measurements of di-nitrogen fixation. Ten different traits that interact with N₂ fixation were assessed (i.e., SPAD readings, leaf %N, shoot dry mass, total seed weight, mean weight per seed, total seed number, days to maturity, pod %N, nodule number, and nodule dry weight), and Pearson's correlation coefficient was estimated for each pair-wise trait combination and atom% ¹⁵N excess measurement. Multiple regression analysis was performed to select the traits that better predict the response in N₂ fixation. Genotypes with similar characteristics linked to BNF were grouped using the Ward's method based on the leaf and pod di-nitrogen fixation measurements.

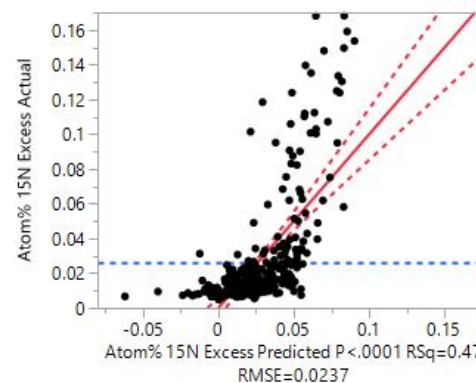


Figure 2. Measurements of time integrated di-nitrogen fixation (pod atom% ¹⁵N excess) vs. the estimated di-nitrogen fixation predicted by the multiple regression analysis.

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Results

As presented in Table 1, leaf atom% ¹⁵N excess was moderately negatively correlated with SPAD readings ($r = -0.45$; $p < 0.001$) and leaf %N ($r = -0.49$; $p < 0.001$), whereas pod atom% ¹⁵N excess was moderately negatively correlated with shoot dry mass ($rc = -0.51$; $p < 0.001$) and seed number per plant ($r = -0.48$; $p < 0.001$). The multiple regression model revealed that the number of days to maturity, shoot dry mass, seed weight, pod %N, and nodule dry weight significantly contributed to pod atom% ¹⁵N excess ($R^2 = 0.47$; $p < 0.001$; Fig. 2). Cluster analysis using the Ward's method grouped the 25 genotypes into three different clusters (Fig. 3), revealing a high level of genetic diversity among the genotypes.

Conclusions

- Diverse traits that are directly and indirectly related to BNF significantly correlate with N₂ fixation.
- Multiple regression analysis might help to select traits that better predict the N₂ fixation capacity.
- Cluster analysis based on multiple measurements of N₂ fixation might help to more efficiently select lines with high BNF capacity.

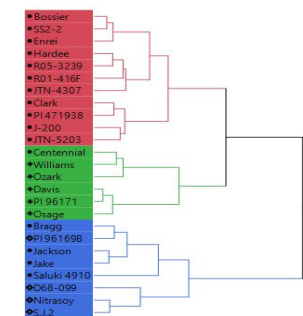


Figure 3. Dendrogram of leaf and pod atom% ¹⁵N excess for 25 soybean genotypes using the Ward's method.

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