Estimation of early and late nitrogen fixation in soybean (*Glycine max*[L.] Merril)

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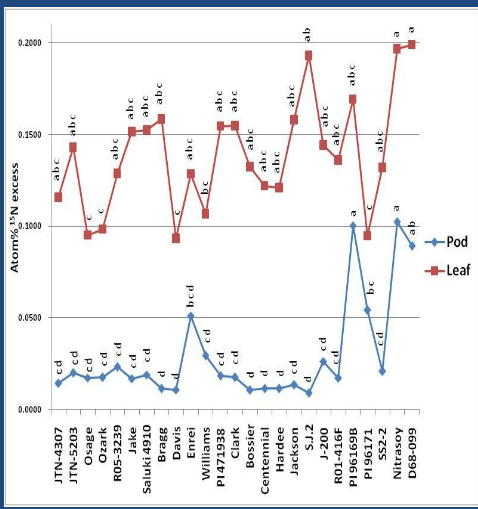
Introduction

Soybean is the most widely cultivated legume worldwide. Although the symbiotic nitrogen (N)-fixing bacteria provide the needed N to the plant, previous studies showed that the high-yielding cultivars rely on N fertilizers in order to achieve their full yield potential (Salvagiotti et al., 2008). Therefore, the improvement of di-nitrogen fixation capacity is considered essential (Herridge et al., 2000) and might be achieved through the evaluation of biological nitrogen fixation (BNF) by analyzing traits related to nodulation (Devine et al., 1996; Santos et al., 2013). The objective of this study was to evaluate the BNF capacity of 25 soybean lines with diverse genetic backgrounds in the early and late growth stages.



+Levels not connected by same letter are significantly different ($p<0.05$)according to Tukey's HSD mean separation test.

Figure 1. Average leaf and pod atom% ^{15}N excess of 25 genotypes evaluated under greenhouse conditions in 2014 and 2015.

Figure 2. Soybean plants five weeks after germination at the early sampling time (left), and soybean root with extensive nodulation at the R7 developmental stage (right).

Material and Methods

A total of 25 soybean genotypes, including seven high-yielding cultivars, 13 high-nodulating cultivars, two plant introductions, one super-nodulating genotype, and two non-nodulating lines, 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 rhizobia cells per seed). A small quantity (7.82 mg kg^{-1} of potting medium) of ^{15}N-urea (10 atom%) was applied and mixed with the potting medium prior to sowing. The most fully developed upper leaf from each plant collected at 5 weeks after germination and pods collected at the R7 developmental stage were analyzed for determining atom% ^{15}N excess using an isotope ratio mass spectrometer. This estimate was used to calculate the percentage of N derived from atmosphere (%Ndfa) as follows:

$$\%\text{Ndfa} = \left(1 - \frac{\text{atom\% }^{15}\text{N excess } N_2\text{fixing soybean genotype}}{\text{atom\% }^{15}\text{N excess non} - \text{nodulating soybean line}}\right)$$

Results

Highly significant differences in atom% ^{15}N excess ($p < 0.001$) as well as different patterns of di-nitrogen fixation capacity were identified among the evaluated genotypes (Fig. 1). 'Davis' showed the lowest leaf atom% ^{15}N excess, whereas S.J.2 the lowest pod atom% ^{15}N excess, indicating their overall high di-nitrogen fixation capacity. Overall, %Ndfa varied from 0 to 53.1 (min, D68-099; max, Davis) at the early developmental stage and from 0 to 91.2 (min, Nitrasoy; max, SJ2) at the late developmental stage.

Conclusions

- Leaf and pod atom% ^{15}N excess were significantly different among the 25 genotypes, suggesting their different capacity of di-nitrogen fixation in the early and late growth stages
- Two estimates of %Ndfa during the plant development might allow a more accurate selection of genotypes for high di-nitrogen fixation capacity.

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