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## Introduction

The rate of di-nitrogen (N<sub>2</sub>) fixation varies throughout the growth cycle of soybean (Lawn and Brun, 1974; Thibodeau and Jaworski, 1975; Latimore et al., 1977; Imsande, 1989; Keyser and Li, 1992). Restrictions in nitrogen (N) supply in periods of high requirement suggest that N accumulation follows a different pattern from that of symbiosis in soybean (Phillips and DeJong, 1984; Keyser and Li, 1992). To date, a limited number of studies investigated the genetic control of N<sub>2</sub> fixation efficiency in soybean due to assessment difficulties (Nicolás et al., 2002; Santos et al., 2013). Therefore, the objective of this study was to assess the variation in N<sub>2</sub> fixation capacity at the early and late growth stages of soybean.

**Table 1.** Correlation coefficients between leaf (LAT) and pod (LAT) atom% <sup>15</sup>N excess and traits related to di-nitrogen fixation.

	SPD	SDW	-LAT%	-PAT%
SPD	1.00	-0.28**	0.43**	-0.10**
SDW		1.00	0.03 <sup>ns</sup>	0.47**
-LAT%			1.00	0.15**
-PAT%				1.00

+SPD, SPAD meter measurement; SDW, shoot dry weight.\*\* Significance at p<0.01

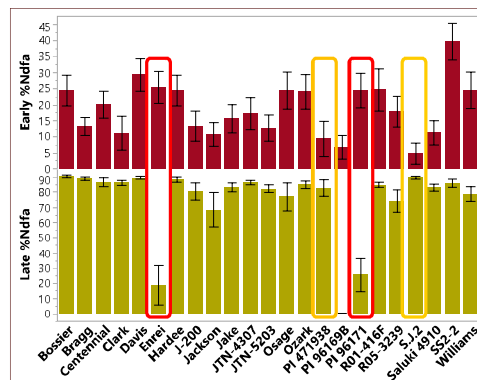
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## Material and Methods

A two-year experiment (2015 and 2016) was carried out under greenhouse conditions to evaluate 22 soybean lines against a supernodulating check ('SS2-2'). The lines were assigned to a randomized complete block with four blocks and four replications per block and inoculated with the *Bradyrhizobium japonicum* strain USDA 110 at the seed stage (2 × 10<sup>5</sup> rhizobia cells per seed). Leaf and pod wall samples were collected at 35 d after emergence and at the R7 growth stage, respectively, to measure the atom% <sup>15</sup>N excess. The percentage of N derived from the atmosphere (%Ndfa) by each genotype was calculated as follows (Unkovich et al., 2008):

$$\%Ndfa = \left( 1 - \frac{\text{atom}\% \text{ } ^{15}\text{N excess N}_2\text{fixing soybean genotype}}{\text{atom}\% \text{ } ^{15}\text{N excess non-nodulating soybean line}} \right)$$



**Figure 1.** Mean %Ndfa measured at the early (35 d after emergence) and late (R7) growth stage of 22 soybean lines and the supernodulating line 'SS2-2' grown under greenhouse conditions.

## Results

Atom% <sup>15</sup>N excess in the leaf (LAT) was significantly correlated with that in the pod wall (PAT). The strongest correlation was found between -LAT and SPAD meter readings as well as between -PAT and shoot dry weight (Table 1). The 23 soybean lines demonstrated different patterns of N<sub>2</sub> fixation activity in the two growth stages (Figure 1). For instance, Enrei and PI 96171 showed high %Ndfa at the early growth stage, but low at the late growth state, whereas S.J.2, PI 471938, and Saluki 4910 showed the opposite trend.

## Conclusions

- Soybean genotypes showed different N<sub>2</sub> fixation capacity according to the time of assessment in plant development.
- Measuring %Ndfa both at the early and late growth stage was necessary for identifying genotypes with different patterns of N<sub>2</sub> fixation.
- Auxiliary traits, such as SPAD meter readings at the early growth stage and shoot dry weight at the late growth stage, might be useful to select genotypes with improved N<sub>2</sub> fixation.

## References

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