

# Soybean Nitrogen Fixation at Different Reproductive Stages and Water Regimes

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## Introduction

In soybean (*Glycine max* [L.] Merr.), N<sub>2</sub> fixation is a primary source of N nutrition during seed development. N<sub>2</sub> fixation is recognized as a drought sensitive mechanism; however, N<sub>2</sub> fixation response to drought at different reproductive stages is not well documented. We tested the hypothesis that drought during late reproductive stages will cause irreparable damage to N<sub>2</sub> fixation due to the breakdown of essential leaf proteins [1] and the inability of N<sub>2</sub> fixation to recover.

## Material and Methods

- Growth chamber experiment repeated in two trials using Hendricks cultivar (MG 0).
- Moderate drought stress at flowering (R2), early seed-fill (R5), late seed-fill (R6) and a well-watered control treatment.
- Well-watered plants watered to 85% of pot capacity.
- Stressed plants watered daily for 5d to maintain transpiration at 40% of controls [2].
- After the drought period, plants were rewatered and kept well-watered until maturity.
- Nitrogenase activity was measured weekly throughout the entire plant cycle using the acetylene reduction assay (Fig. 1).
- Data were analyzed using analysis of variance, and means separated using LSD<sub>α=0.05</sub>.



Figure 1. Soybean nitrogenase activity was measured with a non-destructive, flow-through system (A). Ethylene was quantified by gas chromatography (B).

## Results and Discussion

- Control plants peaked N<sub>2</sub> fixation at R3-R4 (40 d), maintained high activity during R5 and decreased after R6 (60 d) (Figure 2).
- Drought stress reduced N<sub>2</sub> fixation to about 40% of the control plants regardless the developmental stage.
- After rewatering, N<sub>2</sub> fixation recovered from drought at R2 and R5 stage and had higher activity than control plants during mid seed-fill (55 d) (Figure 2).
- Drought stress at R5, prolonged high N<sub>2</sub> fixation activity during late seed-fill (66 d) (Figure 2).
- After drought stress at R6 stage, N<sub>2</sub> fixation did not recover and decreased activity compared with control plants (Figure 2).
- Drought stress at R6 stage decreased yield by reducing individual seed mass (Table 1).
- Drought stress at R5 decreased seed number, but compensated seed yield loss by increasing individual seed mass (Table 1).
- Drought stress at R2 decreased plant biomass, but increased harvest index (Table 1) and nitrogen harvest index (Figure 3).

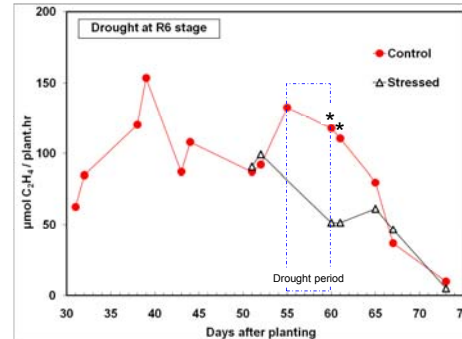
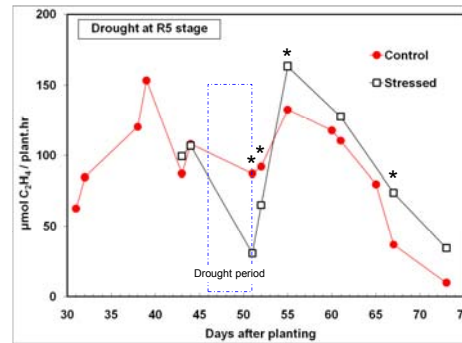
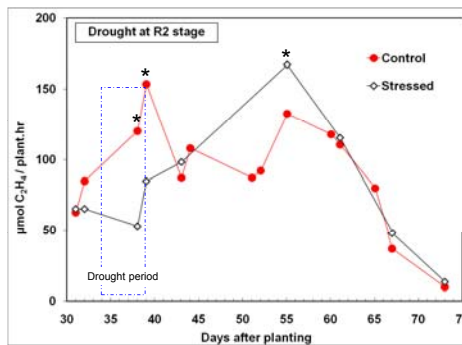


Figure 2. N<sub>2</sub> fixation response to drought at different reproductive stages and N<sub>2</sub> fixation recovering from drought after rewatering. Dates marked with \* indicate significant treatment difference (P ≤ 0.05)

Treatment	Seed yield	HI	Biomass	Seed Number	Individual seed mass
	g plant <sup>-1</sup>		g plant <sup>-1</sup>	Number seed.plant <sup>-1</sup>	mg seed <sup>-1</sup>
Control	7.6 A	0.51 B	14.9 A	57 AB	133 B
R2	6.7 B	0.57 A	11.8 B	49 BC	132 BC
R5	7.2 AB	0.56 A	12.9 A	46 C	163 A
R6	6.8 B	0.52 B	13.1 A	60 A	116 C

Table 1. Seed yield, harvest index, plant biomass, seed number and individual seed mass of plants grown under different water regimes. Numbers followed with different letters are significantly different (P ≤ 0.05).

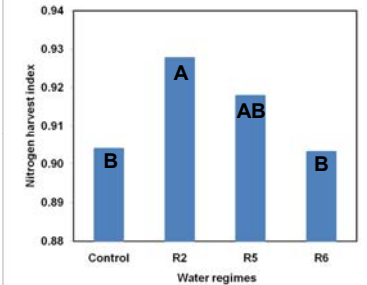


Figure 3. Nitrogen harvest index response to drought at different reproductive stages. Bars containing different letters are significantly different (P ≤ 0.05).

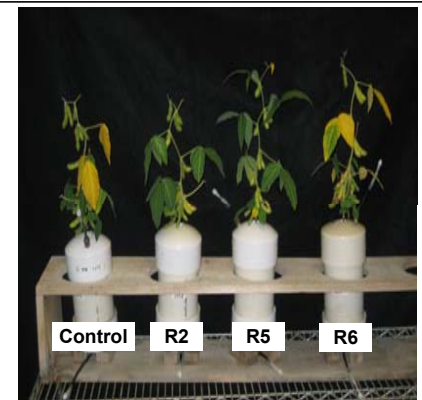


Figure 4. Visual differences in soybean at 66 d after emergence when exposed to drought stress at different developmental stages.

## Conclusions

- Soybean plants drought stressed during R2 and R5 were able to recover N<sub>2</sub> fixation activity after rewatering.
- Although N<sub>2</sub> fixation recovered from drought at R2, plant biomass did not recover and had increased NHI.
- After drought stress at R5, N<sub>2</sub> fixation recovered and had prolonged activity through late seed-fill.
- Plants drought stressed at R6 were incapable of recovering N<sub>2</sub> fixation after rewatering, which resulted in early senescence compared with plants stressed at R2 and R5 (Figure 4), supporting our hypothesis.

## References

- [1] Sinclair, T. R., and C. T., de Wit. 1976. Analysis of the carbon and nitrogen limitations to soybean yield. *Agronomy J.* 68:319-324.
- [2] Ray, J.D. and T.R., Sinclair 1997. Stomatal closure of maize hybrids in response to drying soil. *Crop Sci.* 37:803-807.

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