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Nitrogen fixation sensitivity related to water use efficiency at reproductive development in soybean

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Abstract: Soybean [*Glycine max* (L.) Merr.] nitrogen fixation is sensitive differentially to drought among different genotypes at different growth and development stages, which directly affects soybean yield. Acetylene reduction activity (ARA) response to a gradual drought and rewatering period at late podding (late R_3) and late seed fill (late R_5) were evaluated in two different water use efficiency (WUE) genotypes. Drought-stressed plants with high WUE (PI 372413) decreased ARA more insensitively than that of low WUE (PI 548534), and drought-stressed plants with low WUE (PI 548534) maintained low ARA level after stress alleviation at late R_5. The recovery ability of N_2 fixation was a genotypic difference with WUE at late reproductive development (late R_5), especially. Analysing relation between fraction of transpirable soil water (FTSW) and relative ARA, it was confirmed that PI 372413 with high WUE was more insensitive to water deficit and had drought tolerance by N_2 fixation and recovery ability with a threshold of 0.139–0.147 FTSW than PI 548534 with a threshold of 0.192–0.209 FTSW. The ability to recover N_2 fixation following drought during the reproductive developmental stage would be of an important value in the actual planting environment.

Keywords: water stress; acetylene; isotope; soil water use; inoculation

Soybean [*Glycine max* (L.) Merr.] is an important source of high-quality protein, but its yield is continuously impacted by abiotic stress. Water deficit is a major limiting factor, particularly during reproductive development (Oya et al. 2004). Improvement of soybean for drought tolerance is an effective approach to stabilise yield. Drought tolerance is a complex physiological process which caused a quantitative change of composition or new substance under water deficit, such as acetylene reduction activity (ARA) and N_2 concentration, ureides, or amides (Sinclair et al. 2007). Drought tolerance is also reflected by morphological change and performance, such as nodule, canopy wilting, water use efficiency (WUE) (King et al. 2009). Among them, the sensitivity of N_2 fixation to water deficit stress was widely reported in soybean. N_2 fixation was more sensitive to water deficit stress than transpiration, gas exchange, nitrogen (N) uptake and assimilation (Ray et al. 2006). So, researching soybean resources for reduced N_2 fixation sensitivity performance to water deficit is an important part to improve soybean yields under drought, and explore drought tolerance mechanism from another angle.

Contribution of N_2 fixation for soybean growth and yield was affected by environment and genotypes. Shoot N changes in response to water deficit reflected genotypes differences in sensitivity of N_2 fixation to drought. Changes of soybean shoot N in response to drought were used to evaluate the sensitivity of genotypes to drought (Sinclair et al. 2007). Genotypic differences for the sensitivity of

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N_2 fixation to soil drying were strongly correlated with the concentrations of shoot N and shoot ureides under well-watered (WW) conditions and with concentrations of shoot ureides under drought conditions (King et al. 2014). The sensitivity of N_2 fixation and well-watered shoot ureide concentrations have a linear relationship among soybean genotypes (Vadez and Sinclair 2001). In addition, soybean N_2 fixation response to drought at different developmental stages has been reported rarely (Denison and Sinclair 1985). Mastrodomenico et al. (2013) evaluated ARA and N redistribution response to drought at different reproductive developmental stages in one genotype. ARA measuring methods affected the results of N_2 fixation activity at different developmental stages. Soybean sustains high N_2 fixation activity under WW at the late seed developmental stage by nondestructive ARA methods (King et al. 2014). Conversely, N_2 fixation peaked soon after flowering and rapidly declined during seed fill by measuring detached root segments ARA (Gomes and Sodek 1987).

Selection for drought tolerance of N_2 fixation has also been researched for developing germplasm with superior yields under drought (Chen et al. 2007). Soybean N_2 fixation ability during late seed development and ability to recover N_2 fixation after drought are different at reproductive developmental stages in the different genotypes, and this phenomenon could be a useful trait in a physiological breeding program. The genotypes with different WUE screened by pot experiment and $\delta^{13}C$ result were studied to N_2 fixation changes at different reproductive developmental stages. It is supposed that high WUE genotypes maintain insensitive to soil water status by N_2 fixation response to soil water, the objective is to explore a relationship between WUE and N_2 fixation to soil water in different soybean genotypes, and provided a new physiological trait to evaluate plants drought stress response.

MATERIAL AND METHODS

WUE evaluation of different genotypes. Ten soybean genotypes (MG 0) with possible extreme ^{13}C and N from USDA National Germplasm Collection, and non-nodulating soybean Harsoy NN as control cultivar were planted in the plastic pots (18 cm diameter, 20 cm high), and plus empty pots as a control for four replications in the greenhouse at Fayetteville, USA. The pot mixture was a 1:8 blend of soil and mixing (lb2, Sun Gro Co. Bellvue, USA). Three or four seeds were sown in each pot, inoculated with *Bradyrhizobium japonicum* (USDA 110), soil in each pot was saturated with deionised water, added 1 L of N-free Hoagland's nutrient solution (pH 6.8) (De Silva et al. 1996) and drained overnight. One plant per pot was retained after emergence, passed through a 1 cm hole in a plastic bag wrapped pot, which prevented water evaporation from the soil surface, Tare pot, and each drained pot weights were recorded.

Plants maintained WW through four weeks after sowing by weighing pot and watering to 60–70% of pot-capacity weight every day. Pot-capacity weight represented only the weight of the soil mix and water. After four weeks, each harvested plant was dried and weighed at the R_2 (Fehr and Caviness 1977), then WUE was calculated as dry shoot weight divided by total transpiration, total transpiration was total water weight calculated by weighing pot weight. Dried shoots were grinded by grinder and Geno grinder (SPEX Geno Grinder 2010, Metuchen, USA), and weighed at 3–5 mg per plant for measurement of $\delta^{13}C$ isotope, each sample was measured in PDZ Europa ANCA-GSL elemental analyser and PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) by the UC Davis Stable Isotope Facility and reported in $\delta^{13}C$ and $\delta^{15}N$ notation.

N_2 fixation response to soil water. PI 372413 and PI 548534 screened through the above experiment were used to evaluate N_2 fixation response to water deficit at late R_3 and late R_5 by growth chamber (model PGW36, Conviron, Canada). A completely random experimental design consisted of four replications of a water drought (WD) treatment and a WW treatment for each genotype, water amount under WD treatment decreased the recorded pot weight to 50 g below that of the previous day, formed a slowly gradual water deficit. Only one empty pot without seed as a control for water loss, the growth chamber accommodated 25 pots. Special pots were 10 cm diameter, 40 cm high polyvinyl chloride (PVC) pipes, and sealed at the bottom and top with special lids for the non-destructive ARA measurements (King et al. 2014). Treatments before emergence were the same as above. Plants were thinned to one per pot after emergence and grown in a growth chamber maintained at 25 °C with a 16 h photoperiod (6:00 h to 22:00 h) and photosynthetically active radiation of 600 μmol/m^2/s after sowing, plants were in 12 h photoperiod (6:00 h to 18:00 h) until 25 days after sowing (DAS). Plants were maintained WW until 45 DAS by watering to maintain a pot-capacity weight status (60–70%). An

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additional 100 mL of N-free nutrient solution was added to each pot 37, 38, 59, and 60 DAS.

At 16:00 h 45 DAS (late R_3), all pots had been just watered to 70% of pot capacity; the treatments were initiated. Four plants of each genotype were kept well water at 70% of pot-capacity weight from 45 DAS by daily additions of water at 16:00 h. Four plants for each genotype subjected to the WD treatment were watered at 16:00 h daily from 46 to 52 DAS. Then plants were rewatered at 70% of pot-capacity weight at 16:00 h of 53 DAS. ARA was measured for plants between 9:30 and 11:30 h daily for the consecutive day from 46 DAS to 54 DAS, was measured again at 57 DAS after rewatering. Similarly, treatments were initiated at 16:00 h 63 DAS (late R_5), WD treatments were watered at 16:00 h daily from 64 to 70 DAS, and rewatered at 71 DAS.

The fraction of transpirable soil water (FTSW) was defined as the amount of plant-available soil water relative to the total amount of transpirable soil water. When daily transpiration for WD plants was < 10% of that for the WW plants, FTSW was calculated according to King and Purcell's methods (2017), the FTSW threshold value where ARA began to decline was determined using segmented linear regression. After the final ARA measurement, plants were kept at 70% of pot-capacity weight until physiological maturity. Plants were harvested at maturity and measured seed number and mass. Data were analysed by Excel (Microsoft, Redmond, USA) and SAS software (SAS Institute, Raleigh, USA); significant differences were calculated by ANOVA followed by Fisher's protected *LSD* (least significant difference) at 0.05 level.

RESULTS AND DISCUSSION

WUE analysis of different genotypes. Carbon isotope composition was used widely as a direct or indirect method for the selection of genotypes with improved WUE and productivity in some conditions (Cattivelli et al. 2008). WUE (3.74 ± 0.10 g/kg) and $\delta^{13}C$ (–28.78 ± 0.33) of PI 372413 were highest among eleven accessions, and WUE of PI 548534 and PI 548568 were lowest; however, $\delta^{13}C$ (–30.01 ± 0.30[e]) of PI 548534 was lower (Table 1). PI 372413 and PI 548534 identified as high WUE and low WUE genotypes, respectively, were evaluated for N_2 fixation response to soil dehydration and to rewatering under different reproductive development.

N_2 fixation response to water deficit and rewatering. ARA increased during early reproductive development, and high ARA levels were maintained through late seed development (Nelson et al. 1984). ARA of PI 372413 control plants attained high level (140–145 µmol C_2H_4/plant/h), and ARA of PI 548534 control plants was 114–126 µmol C_2H_4/plant/h at late R_3 and R_5 (Figure 1). Field measurements of N_2 fixation under WW conditions had found high N_2 fixation until the end of seed fill (Leffel et al. 1992). However, ARA in both genotypes declined in two points from late R_3 to early R_5 under WW treatment, coincided with ARA change of Hendricks control plants at early reproductive development (Mastrodomenico et al. 2013). It was unclear what caused the difference ARA decline for WW treatment during reproductive development.

ARA of PI 372413 and PI 548534 at late R_3 decreased to 7 µmol C_2H_4/plant/h and 11 µmol C_2H_4/plant/h on the 7th day (53 DAS) after WD. ARA of PI 372413 and PI 548534 between WW and WD-R_3 was significantly different on the 6th and 5th day separately. PI 372413 restored N_2 fixation faster at the early stage of rewatering than PI 548534 under late R_3 treatment, N_2 fixation response of two genotypes restored similar level to control at the end of 4 days after rewatering. From the 6th day of drought stress at late R_5, ARA of PI 372413 and PI 548534 decreased to 8 µmol C_2H_4/plant/h and 1 µmol C_2H_4/plant/h separately and significantly different with control plant until the 8th day (Figure 1). Stressed plants of PI 372413 recovered ARA to 37% of the control plants 1 day after rewatering, to 62% of the control plants 4 days after rewatering. However, stressed plants

Table 1. Water use efficiency (WUE) and $\delta^{13}C$ difference of eleven accessions

Accession	WUE (g/kg)	$\delta^{13}C$
Harsoy NN	3.60 ± 0.45[ab]	–29.26 ± 0.43[bc]
PI 290118	3.42 ± 0.08[b]	–29.31 ± 0.29[bc]
PI 319537A	3.37 ± 0.10[b]	–29.43 ± 0.37[bcd]
PI 372413	3.74 ± 0.10[a]	–28.78 ± 0.33[a]
PI 437766	3.46 ± 0.19[ab]	–29.92 ± 0.34[e]
PI 438013	3.45 ± 0.11[ab]	–29.66 ± 0.20[cde]
PI 445827B	3.42 ± 0.17[b]	–29.77 ± 0.44[de]
PI 548534	3.35 ± 0.08[b]	–30.01 ± 0.30[e]
PI 548568	3.35 ± 0.06[b]	–29.10 ± 0.39[ab]
PI 592931	3.56 ± 0.16[ab]	–29.87 ± 0.35[de]
PI 612617B	3.44 ± 0.13[b]	–29.18 ± 0.47[ab]

Different letters within columns and same genotype indicate significant differences (P < 0.05; Fisher's test)

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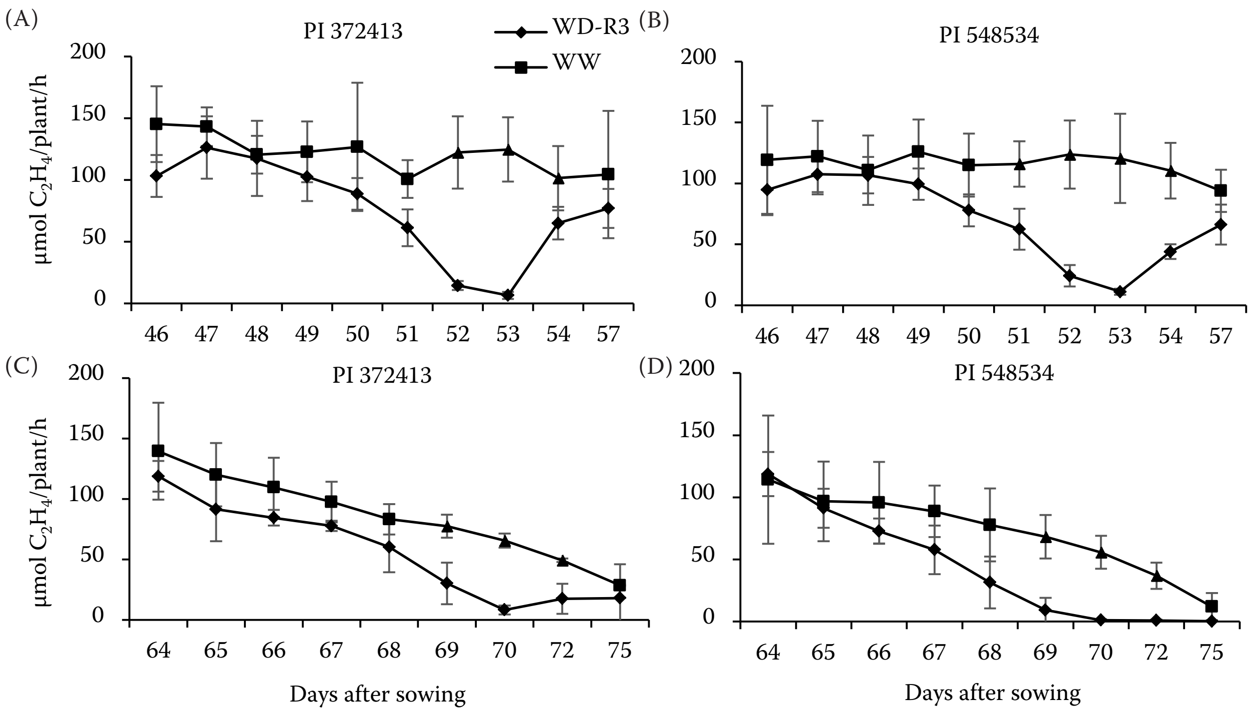

Figure 1. Acetylene reduction response to drought *versus* days after planting at late R_3, and late R_5. (A, B) denote acetylene response of PI 372413 and PI 548534 at late R_3; (C, D) denote acetylene response of PI 372413 and PI 548534 at late R_5. The day drought period for each developmental stage was decided by fraction of transpirable soil water (FTSW) = 0. Data marked with a solid triangle on the WW line indicated a significant difference (P < 0.05; Fisher's test). WD – water drought; WW – well-watered conditions

of PI 548534 retained low ARA after rewatering at late R_6 (70–75 DAS). Drought-stressed PI 548534 at late R_5 irreparably decreased nodule activity to accelerate leaf yellow, all of the plants were in the physiological maturity approximately 7 days after rewatering (82 DAS). Mastrodomenico et al. (2013) reported that Hendricks's drought-stressed plants at R_2 and R_5 were able to recover ARA after rewatering. In contrast, plants stressed at R_6 were unable to increase ARA after rewatering and decreased similar to control plants. King and Purcell (2006) concluded that genotypic differences of N_2 fixation sensitivity to drought were likely associated with differences in WW shoot N concentration. Additionally, the sensitivity of N_2 fixation in response to drought was correlated with genotypic differences in the WUE trait. The ability to recover N_2 fixation after drought stress is associated with the amount of photosynthate and N available in the vegetative tissue (Cure et al. 1985). To some extent, as a comprehensive physiological trait, WUE reflected photosynthesis matter and N accumulation level directly.

FTSW thresholds reflected plant N_2 fixation in response to the soil water condition. FTSW thresholds of PI 372413 ranged from 0.139 to 0.147 at late R_3, and R_5 treatments, FTSW thresholds of PI 548534 ranged from 0.192 to 0.209 (Figure 2). FTSW breakpoints of different genotypes increased at late reproductive development, and FTSW breakpoints of high WUE genotype was lower than that of low WUE genotype. The results indicated that a low threshold value would allow plants to continuously accumulate N under WD. N_2 fixation of drought-stressed plants with low WUE was more sensitive to water deficit, especially at late reproductive development. FTSW thresholds ranged from 0.139 for high WUE PI 372413 to 0.209 for low WUE PI 548534 among four treatments. These FTSW threshold values were a medium-range between values of 0.11 for the most drought-tolerant genotypes to 0.19 for the drought-sensitive cultivar (King et al. 2014) and values of 0.11 to 0.33 FTSW (Sinclair et al. 2000). The reason for a narrow values range may be relatively low shoot N concentration of WW plants (King et al. 2014). However, two WUE difference genotypes were unable to be the most drought-tolerant and drought-sensitive genotypes compared with previously published values.

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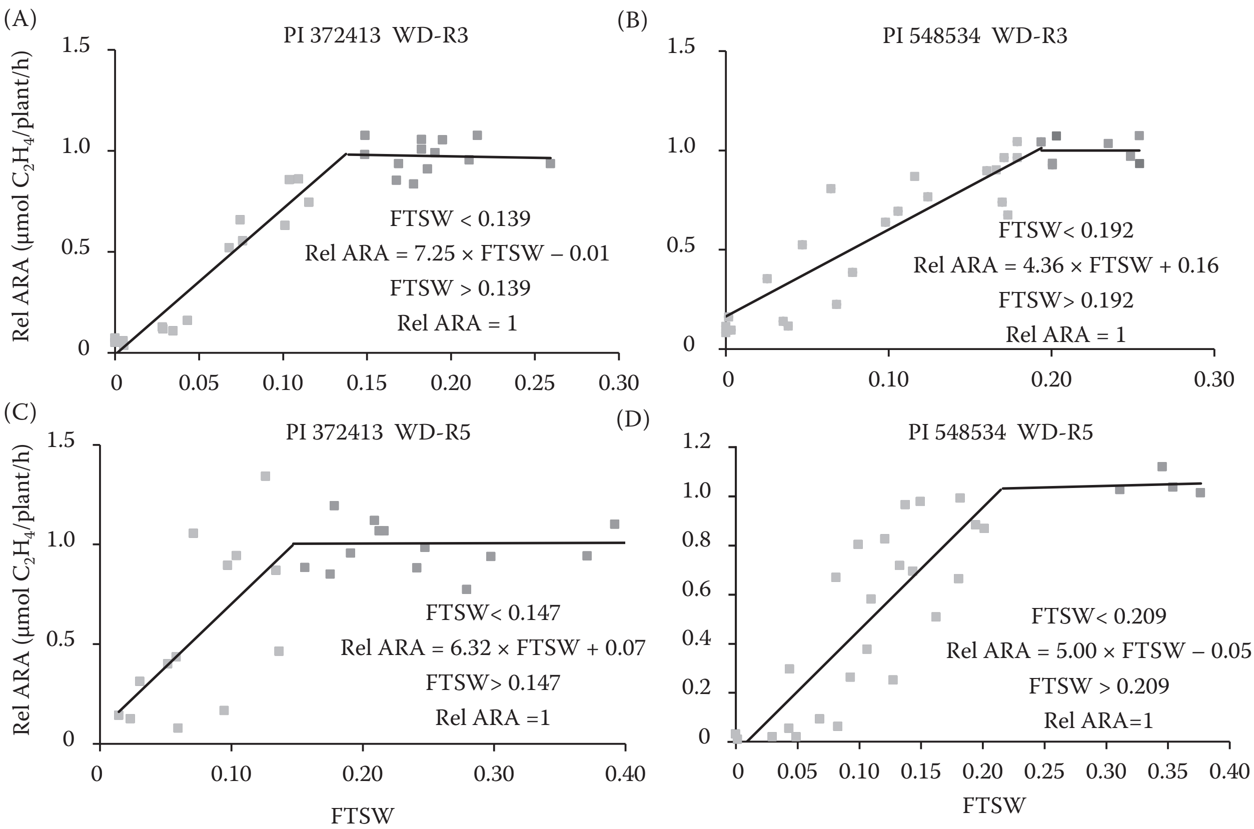

Figure 2. Relative acetylene reduction activity (rel ARA) response to drought for genotypes under different developmental stages. (A, B) denote rel ARA response of PI 372413 and PI 548534 at late R_3; (C, D) denote rel ARA response of PI 372413 and PI 548534 at late R_5. The point at which ARA declines was estimated using the nonlinear model of two segmented regressions where provided a fit estimate of the threshold value for fraction of transpirable soil water (FTSW) (*P* < 0.05; Fisher's test)

WD at R_3 and R_5 affected separately seed number per plant and weight per seed, low seed number per plant directly resulted in a decline of single plant seed yield (Table 2). Seed weight per plant of two genotypes under WD and WW had no significant difference after harvest, but drought stresses more influenced seed yield of PI 548534. WD-R_5 treatment shortened the seed-fill period, and low WUE plants were unable to recover N_2 fixation and C assimilation after rewatering (Brevedan and Egli 2003), these induced weight per seed reduced for plants drought stress. Decreased seed mass for plants stressed at late reproductive development was consistent with accelerated senescence and a shortened seed fill period (De Souza et al. 1997). Cerezini et al.

Table 2. Biomass comparing of two genotypes under different treatments

Accession number	Treatment	Seed weight (g/plant)	Seed number (No./plant)	Weight per seed (mg)
	WD-R_3	9.8[a]	67[a]	147.6[a]
PI 372413	WD-R_5	10.1[a]	85[a]	121.8[b]
	WW	10.1[a]	75[a]	136.3[ab]
	WD-R_3	9.8[a]	70[b]	142.3[a]
PI 548534	WD-R_5	10.5[a]	107[a]	99.0[b]
	WW	11.1[a]	91[ab]	122.3[ab]

Different letters within columns and same genotype indicate significant differences (*P* < 0.05; Fisher's test). WD – water drought; WW – well-watered conditions

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(2016) studied that the N_2 fixation recovery was from 1.3 to 5.5 days, with little or no permanent damage in soybean plants, when plants were in WD after V_3–V_4 until transpiration rate reached 80%. However, plant growth and development stage to drought should be considered (King and Purcell 2005), it was important for soybean genotypic difference of N_2 fixation and single plant seed weight formation.

Two WUE difference genotypes were identified that differ in N_2 fixation sensitivity to drought and rewatering at late R_3 and later R_5. High WUE genotype PI 372413 exhibited drought tolerance in N_2 fixation with a threshold of 0.139 FTSW at late R_3 treatment, and low WUE genotype PI 548534 exhibited drought sensitivity in N_2 fixation with a threshold of 0.209 FTSW at late R_5 treatment. WUE should be a promising characteristic for the identification of soybean genotypes, revealing enhanced drought tolerance with respect to N_2 fixation.

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