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Effects of drought stress on legume symbiotic nitrogen fixation: Physiological mechanisms

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Drought stress is one of the major factors affecting nitrogen fixation by legume-rhizobium symbiosis. Several mechanisms have been previously reported to be involved in the physiological response of symbiotic nitrogen fixation to drought stress, i.e. carbon shortage and nodule carbon metabolism, oxygen limitation, and feedback regulation by the accumulation of N fixation products. The carbon shortage hypothesis was previously investigated by studying the combined effects of CO_2 enrichment and water deficits on nodulation and N_2 fixation in soybean. Under drought, in a genotype with drought tolerant N_2 fixation, approximately four times the amount of ^{14}C was allocated to nodules compared to a drought sensitive genotype. It was found that an important effect of CO_2 enrichment of soybean under drought was an enhancement of photo assimilation, an increased partitioning of carbon to nodules, whose main effect was to sustain nodule growth, which helped sustain N_2 rates under soil water deficits. The interaction of nodule permeability to O_2 and drought stress with N_2 fixation was examined in soybean nodules and led to the overall conclusion that O_2 limitation seems to be involved only in the initial stages of water deficit stresses in decreasing nodule activity. The involvement of ureides in the drought response of N_2 fixation was initially suspected by an increased ureide concentration in shoots and nodules under drought leading to a negative feedback response between ureides and nodule activity. Direct evidence for inhibition of nitrogenase activity by its products, ureides and amides, supported this hypothesis. The overall conclusion was that all three physiological mechanisms are important in understanding the regulation of N_2 fixation and its response of to soil drying.

Keywords: Carbon limitation, Drought, Legumes, Nitrogen fixation, Oxygen, *Rhizobium,* Ureides

Symbiotic N_2 fixation by legume-rhizobium associations plays an important role in sustaining crop productivity and maintaining soil fertility, especially on marginal lands and in smallholder farming systems. It is even anticipated that the importance of legumes and N_2 fixation will continue to increase with the growing needs for sustainable agricultural practices and environmental concerns. One of the major approaches for the optimization of symbiotic N_2 fixation is to increase the land area under legumes and enhance their adaptation and resistance to environmental stresses. Symbiotic N_2 fixation by legumes is particularly sensitive to various abiotic stresses such as drought, water-logging, soil salinity, acidity, high/low temperature, low phosphorus and other nutrient limitations.

The establishment and activity of the legume-rhizobium symbiosis are very sensitive to drought stress[26,34,38]. In soybean, N_2 fixation has been found extremely sensitive to drought and starts decreasing before leaf gas exchange, at relatively high soil water contents[4,14,31]. This may constitute a serious constraint on N accumulation and yield potential of soybean subjected to soil drying[31].

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Although drought stress is known to affect all the steps of nodule formation and functioning, most of the work on the mechanisms of drought effects on N_2 fixation has focused on nitrogenase activity. Three major hypotheses are considered in the present work for analyzing drought stress effects on nitrogenase activity: O_2 limitation, regulation by N metabolism and carbon shortage.

The main purpose of this paper is to report some of the recent physiological data about drought-stress effects on N_2 fixation and to discuss the three hypotheses of the possible mechanisms that trigger the inhibition of nitrogenase activity by water deficits.

Nodule metabolism and carbon limitation of nitrogen fixation

It has long been observed by Huang *et al.*[11] that the inhibition of nitrogenase activity by water deficits could be partially reversed by exposing the plants to high CO_2. These authors also concluded that the decline in soybean N_2 fixation under drought was associated with a decline in photosynthesis. However, the decrease in N_2 fixation under drought was found to precede the decrease in photosynthesis[4,31], and the specific nodule activity was never found to increase in response to short-term CO_2 enrichment[5,39].

More recently, comparisons of photosynthate allocation patterns during drought among genotypes that differ greatly in their drought tolerance of N_2 fixation were investigated in ^{14}C labeling studies[20]. Under drought, drought-tolerant (Jackson) allocated approximately four times the amount of ^{14}C per gram of nodules as the drought-sensitive genotype (KS4895) and Jackson had approximately twice the acetylene reduction activity (ARA) as KS4895. Consistent with the ^{14}C labeling results under drought individual nodule mass increased for Jackson but decreased for the drought sensitive genotype, indicating a greater allocation of photosynthate supply to nodules.

Based on the observation of a rapid decline of nodule sucrose synthase activity in soybean plants subjected to water deficits[6], a recent model has suggested that sucrose synthase down-regulation produces a parallel increase in nodule sucrose content[29] and a depletion of substrates for bacteroids respiration, which would induce a transient accumulation of oxygen in the infected region and a closure of the oxygen diffusion barrier[1,7]. According to this model, the observed drought-induced decline of nitrogen fixation would result from the depletion of respiratory substrates and the concomitant closure of the oxygen diffusion barrier.

Exposing soybean plants to elevated atmospheric CO_2 has been shown to result in N_2 fixation changing from being drought sensitive to being very drought tolerant[24]. Since the elevated CO_2 also resulted in substantial increases in total non-structural carbohydrates (TNC) in the nodules, it is conceivable that increased availability of carbon in the nodules overcame the normal response to water deficits. This conclusion is contradicted, however, by the fact that nodules on plants exposed to ambient CO_2 when subjected to drought also accumulated high levels TNC. Therefore, accumulation of carbon, per se, in the nodules does not necessarily result in drought tolerant N_2 fixation.

An especially intriguing result from the experiment in which soybean plants were subjected to elevated CO_2 was the substantial influence on the levels of ureides in both the nodules and shoot. In these experiments, increased CO_2 resulted in much lower concentrations of ureides in the plant (Fig. 1). If the main effect of elevated CO_2 under drought is to increase N-sink strength[12], our speculation is that under elevated CO_2 leaves have an increased capability to catabolize ureides which results in lower levels of ureides for feedback inhibition of nodule activity. The concept of regulation of nitrogen fixation by the N-sink has been proposed by several authors[10,18,30,33]. Although no mechanism for feedback inhibition of nitrogen fixation has been yet demonstrated in legume nodules, indirect physiological evidence is consistent with this hypothesis[10,16,32,36].

Role of nodule oxygen permeability

Substantial research during the eighties and early nineties has concluded that drought-induced decreases in nitrogenase activity are associated with a nodule oxygen limitation[4,17,38]. According to this hypothesis, nodule permeability to oxygen (Po) would be first affected by water deficits, which would restrict nodule respiration and trigger the decline in N_2

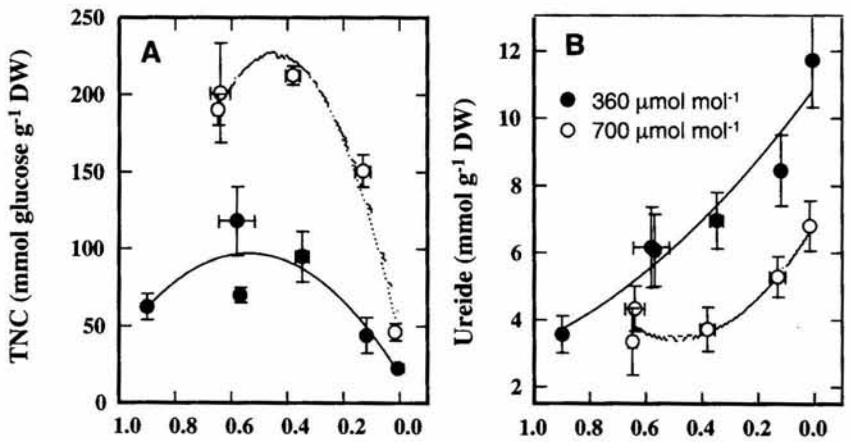

Fig. 1 — Plot of (A) total non-structural carbohydrates (TNC) and (B) ureide in soybean leaves as a function of transpirable soil water (FTSW) for plants exposed to two atmospheric CO_2 concentrations during a dehydration cycle. Each point represents the mean (± SE) of 5 replicates. From data of Serraj *et al.* (1998).

fixation. However, the mechanism involving Po alteration in the drought stress inhibition of nodule activity has been disputed in recent reports in which it was concluded that nodule Po inhibition by drought stress was a consequence rather than a cause of the nitrogenase activity inhibition[3,19].

The relationship between N_2 fixation inhibition by drought and Po was investigated by analyzing the recovery of nitrogenase activity to pO_2 increase. ARA was more responsive to increasing pO_2 in drought stress than in well-watered soybean plants[22] (Fig. 2). The nodules moved from being non-limited by oxygen under well-watered conditions to oxygen limited under drought-stress conditions. However, under the severe drought stress, increasing pO_2 did not overcome the effects of the stress completely, although ARA was doubled at 40 kPa O_2 compared to 20 kPa O_2 (Fig. 2). Therefore, under this more severe stress, O_2 limitation had increased and/or additional factors also inhibited ARA.

The hypothesis of O_2-limitation of N_2 fixation under drought was further analyzed by comparing the kinetics of ARA and Po responses to an osmotic treatment by PEG. The PEG treatment resulted in a rapid and continual decrease in respiration and nitrogenase activity over 30 hr[21]. This was consistent with the results of Purcell and Sinclair[19], showing that decreases in respiration and nitrogenase activity occurred within 4 hr of PEG treatment. The PEG treatment also resulted in a decrease in calculated Po that paralleled ARA decrease. Exposure to the PEG treatment for 24 hr or more resulted in very low nodule respiration and nitrogenase activity. Increasing pO_2 failed to recover nodule activity[21], indicating that serious disruptions in nodule functioning had taken place.

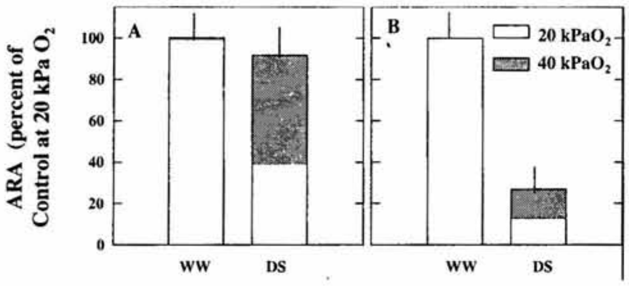

Fig. 2 — Acetylene reduction activity (ARA) of intact nodulated roots in response to pO_2 at two levels of soil moisture. Roots of well-watered (WW) and drought-stressed (DS) plants were exposed to 20 and 40 kPa O_2 as described in Serraj and Sinclair (1996b). Drought stress levels were (A) fraction of transpirable soil water (FTSW) = 0.1 and (B) FTSW = 0.05. Means (± SE) of 3 (WW) or 6 (DS) replicates. From data of Serraj and Sinclair (1996b)

Therefore, it appears that water deficit induced either by soil dehydration or by PEG results in a two-stage inhibition of nodule activity. The first stage clearly involves a rapid and simultaneous decrease in Po and nitrogenase activity. The second stage of inhibition occurs under severe drought stress, when nodule activity is less than half of the initial rates, and when nitrogenase activity may be constrained by factors other than Po, such as leghaemoglobin content or proteolysis[3,9,13].

Ureides metabolism and feedback inhibition of N_2 fixation

It has been suggested by several authors that N_2 fixation in legumes under stress conditions might be regulated by a feedback involving N metabolism[10,18,30]. Although no mechanism for feedback inhibition of nitrogen fixation has been yet demonstrated in legume nodules, there is a convergence of several indirect arguments in its favour[26,36].

A link between ureide metabolism and N_2 fixation inhibition by drought has been previously established[2,27,32]. Inhibition of soybean N_2 fixation by water deficits was associated with increased ureide levels in plant tissues[2,22]. Additionally, large variation has been found among soybean germplasm in terms of N_2 fixation response to drought and ureide contents[20,23].

Differences of N_2 fixation sensitivity to drought among legume species showed an interesting link with the type of nitrogenous compounds exported from nodules[32]. Species that transport high concentrations of ureides were found to be drought sensitive, as in soybean and cowpea where the highest xylem sap ureide concentrations were found. By contrast, species with low or no ureide were discovered to be relatively drought tolerant.

Furthermore a direct effect of ureides on nodule activity was found[25]. ARA and nodule Po were simultaneously decreased within one day upon ureide application in the nutrient solution of hydroponically grown soybeans. The severity of ARA inhibition varied with the concentration of ureides and was partially reversible by removing ureides. Further work is needed to investigate the sequence of events following ureide application and to find out whether or not the response of an exogenous ureide application is comparable to the accumulation and effects of ureides that occur during drought.

The mechanism by which ureide accumulation could trigger the inhibition of nitrogenase activity is

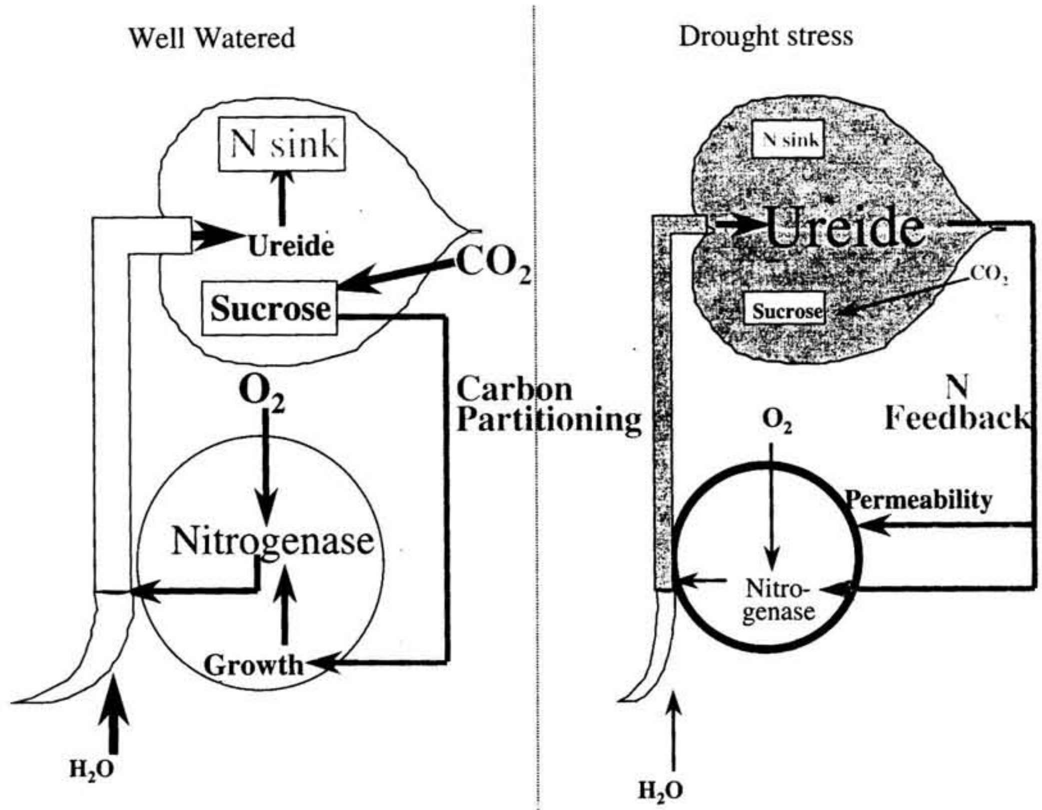

Fig. 3 — Synthetic view of the main physiological effects that affect directly or indirectly the legume symbiotic nitrogen fixation under drought. Fig. 1

still unclear. The low solubility of ureides[35] may be important in the association of high ureide concentrations with drought sensitivity. The response of N_2 fixation to provide soybean plants with various nitrogenous compounds further demonstrated the importance of a ureide-related regulation[25,37]. Plants exposed to 10 m*M* allantoic acid had decreased ARA and Po within 3 d of exposure, although plants exposed to 10 m*M* asparagine had a greater decrease in ARA and Po that was initiated after only 2 d of exposure. The severity of the ureide-induced decrease in ARA was dependent on the ureide concentration in the nutrient solution and was partially reversible upon the removal of the ureides from the solution. These results indicated an important role for ureides in influencing nodule activity and Po and showed that asparagine might have a more direct role in this regulation[37].

The hypothesis that the inhibition of N_2 fixation by ureides is alleviated by elevated [CO_2] was recently tested by comparing the short-term responses of acetylene reduction assay (ARA), ureide accumulation and TNC levels to the application of ureide to the nutrient solution of hydroponically-grown soybean under ambient and enriched [CO_2] environments[28]. The data showed that the application of 5 and 10 m*M* ureide to the nutrient solution inhibited nitrogenase activity under both ambient and elevated [CO_2] conditions. However, the ARA inhibition was delayed and the percent of inhibition was significantly decreased under elevated [CO_2]. Under ambient [CO_2], the application of 5 and 10 m*M* Alac resulted in a significant accumulation of ureide in all plant tissues with higher accumulation levels in the leaves than in the other tissues. However, plant exposure to elevated [CO_2] resulted in no accumulation of ureides in response to Alac application in either of plant tissues[28]. In addition, TNC concentrations were consistently higher under elevated [CO_2], compared to the ambient, and for both [CO_2] treatments, the application of Alac induced a significant decrease of TNC concentrations in the leaves and nodules. For both plant parts, a negative correlation was observed between TNC and ureide levels. Overall, the results of this study revealed that elevated [CO_2] prevented ureide accumulation in leaf and nodule, allowing soybean plants to maintain higher N_2 fixation rates after exposure to external ureide application.

Conclusion

The physiological mechanism controlling nitrogen fixation under drought stress is still an open question. None of the three hypotheses concerning the regulation of nodule activity in response to drought stress have been excluded based on recent research. Indeed, O_2 permeability changes, ureide feedback, and carbon shortage have all been shown to be involved in the regulation of N_2 fixation. The more appropriate view now seems to be that all three mechanisms are intimately involved in the regulation of nodule activity under water deficit. Experimental evidence of interactions among mechanisms include between C metabolism and nodule Po[8], between nodule Po and N transport[15], between N transport and C metabolism[24], and between photosynthate allocation to nodules and N_2 fixation[20].

It is interesting to speculate that the common factor involved in all these mechanisms is the water budget of the plant and nodules. Certainly the transport of photosynthate and N compounds in the xylem and phloem is intimately regulated by water flux. Nodule permeability is likely to be directly regulated by water flux between symplast and apoplast and/or by water flux between cells in the nodule. One hypothesis to describe the sequence of responses through the three controlling mechanism in soybean[22] would be as follows: (1) decreased phloem flux from the leaves results in a decreased rate of ureide catabolism, (2) ureide accumulation in the shoot feedbacks into the nodules, (3) accumulation of N_2 fixation products in the nodules results in inhibition of nitrogenase activity and a decrease in nodule Po.

Finally, it is not yet possible to definitely resolve the sequence of mechanisms that regulate the water deficit response of N_2 fixation in soybean. It is clear, however, that there is substantial interaction among a number of key mechanisms and that each of these processes is closely linked to water flux in the plant and nodule. Resolving the interaction of mechanisms resulting in the sensitivity of N_2 fixation to water deficits will likely be important in increasing soybean yields under drought conditions.

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