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Review article

Feedback regulation of symbiotic N_2 fixation under drought stress

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Abstract – Although drought inhibition of N_2 fixation is general among rhizobium-legume symbioses, a large genetic variation has been found among legume species and cultivars in the N_2 fixation sensitivity to water deficits. Legume species that transport ureides from the nodules were found to be much more drought-sensitive than those that transport amides. It was concluded that a feedback mechanism involving ureide level may control N_2 fixation under drought. Consistent with this observation, the drought tolerance of N_2 fixation in soybean was associated with low concentrations of ureides in plant tissues. Experimental evidence for a direct inhibition of nitrogenase activity by ureides application supported the feedback hypothesis, although the exact nature of the chemical compound involved in the signal is still unknown. The basis for ureide accumulation is hypothesized to result from decreased ureide catabolism in the leaf. We showed recently that Asparagine (Asn) chelates Mn ions, which are the co-factor of the enzyme allantoate amidohydrolase, and as a result may cause an inhibition of ureide breakdown in the shoot. These data are consistent with the hypothesis that Asn would be a signal for the feedback inhibition of N_2 fixation.

N_2 fixation / drought stress / feedback / ureides / soybean

Résumé – Régulation « feedback » de la fixation symbiotique de N_2 sous déficit hydrique. Une large variabilité génétique a été mise en évidence parmi les espèces et cultivars de légumineuses pour la sensibilité de la fixation de N_2 au déficit hydrique. Les espèces de légumineuses qui transportent l'azote sous forme d'uréides se sont avérées plus sensibles que les espèces à amides. Il en a été déduit qu'un mécanisme de régulation de type « feedback » impliquant les uréides serait responsable de l'inhibition de la fixation de N_2 par déficit hydrique. Des résultats expérimentaux montrant une inhibition de l'activité nitrogénase par l'application d'uréides exogènes constituent une preuve supplémentaire en faveur de l'hypothèse de régulation « feedback ». Le mécanisme physiologique responsable de l'accumulation des uréides est supposé résulter d'une diminution du catabolisme foliaire des uréides, mais la nature exacte de la molécule chimique impliquée dans le signal reste encore inconnue. Nous avons montré récemment que l'asparagine (Asn) pourrait induire une inhibition de la dégradation des uréides dans les feuilles, et par conséquent, leur accumulation dans les différents tissus de la plante. Ces résultats soutiennent l'hypothèse de l'intervention de Asn comme signal de régulation du métabolisme des uréides et de la fixation symbiotique de N_2 sous déficit hydrique.

fixation de N_2 / déficit hydrique / feedback / uréides / soja

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1. INTRODUCTION

The drought inhibition of symbiotic N_2 fixation is widespread among rhizobium-legume symbioses, which seriously limits legume yields in many arid and semi-arid regions of the world. Drought stress affects all physiological processes in plants as stress develops. A critical question regarding N_2 fixation is whether the effect of the stress is first perceived in other physiological processes and the changes in N_2 fixation are a consequence of these other changes, or rather, the stress is directly and initially perceived by N_2 fixation mechanisms. In fact, several studies have shown that N_2 fixation is more sensitive to soil dehydration than leaf gas exchange [7, 25], nitrate assimilation [14] and dry matter accumulation [25, 30].

The fact that N_2 fixation is more sensitive to decreasing soil water content relative to leaf gas exchange constitutes an important constraint on N accumulation and yield potential of legumes subjected to soil drying [25, 30]. For cool-season food legumes, Beck et al. [2] concluded that even if the drought stress effects on N_2 fixation do not always directly affect grain yield, drought may result in a significant decrease in the total N balance.

Although water deficit is known to affect all the steps of nodule formation and functioning, most of the previous work on the mechanisms of drought effects on N_2 fixation has focused on nitrogenase activity, rather than nodulation. Three major hypotheses have been proposed to explain drought stress effects on nitrogenase activity: carbon shortage, oxygen limitation and regulation by N metabolism. All these physiological mechanisms have been recently discussed in detail (see review by Serraj et al. [23]).

The purpose of the present paper is to review some of the most recent data on the N feedback regulation of N_2 fixation under drought. An early report by Pate, Gunning, and Briarty [12] suggested that lower rates of water movement out of the nodule during drought stress may restrict the export of products of N_2 fixation, thus inhibiting nitrogenase activity via a feedback mechanism. Several authors have also proposed that N_2 fixation in legumes might be regulated under other stress factors by a feedback mechanism involving N metabolism and the pool of soluble N in the plant [4, 10, 11, 24]. Here we review new evidence on the interaction between nodule function under drought and ureide metabolism at the whole plant level.

2. ACCUMULATION OF N COMPOUNDS UNDER DROUGHT

Although ureides (allantoin and allantoic acid) are the main product of N transport from the nodules in some grain legumes like soybean [6], the level of ureides in the plant has surprisingly been found to increase as the soil dried and N_2 fixation rates declined [3, 18]. Moreover, a strong association was discovered among grain legume species between the type of nitrogenous compounds exported from nodules and the sensitivity of N_2 fixation to drought [26]. Ureide transporting species were found to be drought-sensitive, whereas amide transporting species were relatively drought-tolerant. We concluded that decreased phloem transport in the ureide-transporting species with soil drying may result in a negative feedback on N_2 fixation [26].

Soybean usually exports more than 80% of the N compounds out of the nodules in the form of ureides. They are transported in the xylem to the shoots, where they are catabolized [31]. During the inhibition of N_2 fixation by water deficits, accumulations of high levels of ureides have been measured under both controlled environments [3, 18] and field [15, 20]. Furthermore, the drought tolerance of N_2 fixation in the soybean cultivar Jackson was associated with low concentrations of ureides in xylem exudates and petiole under well-watered conditions and a low ureide accumulation during soil drying [19, 20].

Ureide accumulation was recently compared in nodule, root and shoot tissues in a drought-sensitive soybean cultivar exposed to soil dehydration [22]. Under well-watered conditions, ureide concentration was very low in nodule, root and leaf tissues [22]. During soil dehydration, ureides accumulated mainly in leaves and nodules. Linear regressions between ureide concentration and leaf water potential showed that the drought-induced increase in ureide of nodules was higher relative to changes in leaves [22].

Our recent data indicated that not only ureides but also Asn accumulated in nodules upon water deficit or treatment with Alac or Asn [29]. Therefore, both compounds might be involved in a feedback mechanism to the nodules. Since part of the nitrogen is exported from nodules as amides, the possibility of an increase in amide production under water deficit may be worth exploring. Part of this accumulation might also be explained by a feedback from the shoots, where Asn or Asp could be products of ureide degradation. The inhibition of ureide degradation in shoots by Asn [29] was also a sufficient factor in a feedback inhibition of N_2 fixation, and Asn appeared to be the compound triggering this feedback. Because Asn also increases under water deficit, it may be an important factor explaining the accumulation of ureides in shoots under water deficit.

3. EFFECTS OF UREIDES AND VARIOUS N COMPOUNDS ON NODULE ACTIVITY

In order to examine the effects of ureide accumulation on nitrogenase activity, we compared the effects of 10 mM of various N compounds: allantoic acid (Alc), allantoin (Aln), asparagine (Asn), Urea and other compounds (KCl, malate) on acetylene reducting activity (ARA) after 4-d of treatment [22]. All N compounds showed an inhibition effect on ARA, while non-nitrogenous compounds showed no significant effect. Among N-compounds, urea prompted the smallest decrease on ARA, i.e. only 25%. By contrast, there was a 90% inhibition of ARA by Asn. Ureides induced a sharp 70% inhibition of ARA compared to control, with no difference detected between Aln and Alac.

The effects of Aln and Alac on nodule ARA were further compared at different concentrations. Both ureide compounds resulted in large ARA declines compared to control plants [22]. The ARA decline increased with ureide concentration, with no significant difference between the effects of Aln and Alac at any concentration. To investigate the reversibility of ureide effect, ARA recovery was studied after treatment with 2.5 mM and 5 mM Alac [22]. The recovery of ARA from ureide inhibition was dependent on the concentration applied. Removing ureides prompted only a small ARA recovery in the 5.0 mM treatment, while ARA increased up to 80% of control in plants formerly treated with 2.5 mM. Ureides were measured in shoots and nodules at the end of the recovery experiment. Both Alac concentrations treatments showed an important ureide accumulation in shoots, i.e. five-fold increase with 5 mM Alac and four-fold increase with 2.5 mM Alac. Plants recovered from the 5.0 mM Alac treatment showed a three-fold increase in shoot ureide compared to control, while the 2.5 mM treatment showed a decreased shoot ureide concentration compared to control. By contrast, nodules did not show any treatment difference for ureide concentration regardless of Alac concentration [22].

4. MN APPLICATION DELAYS THE DROUGHT INHIBITION OF N_2 FIXATION

Although the exact pathway for allantoic acid degradation is still controversial, Winkler, Blevins, Polacco and Randall [31] gave evidence that allantoic acid degrades through allantoate amidohydrolase, which is manganese (Mn) dependent [8]. Winkler, Blevins, Polacco and Randall [31] showed that in vitro allantoate amidohydrolase activity in the presence of 1 mM Mn was inhibited by 10 mM Asn or 10 mM boric acid. This inhibition was attributed to the chelation of free Mn, which could be partially overcome by an increase in Mn concentration [8].

Because of Mn's role in the ureide degradation in soybean leaves, Purcell, King and Ball [16] hypothesized that Mn might regulate nitrogen fixation under water deficit. Interestingly, our work showed that there was a major interaction between Mn nutrition and the decline of nitrogen fixation under water deficit [28], in agreement with the data of Purcell, King and Ball [16]. Indeed, a Mn application allowing a leaf Mn concentration of about 21 to 32 $mg \cdot kg^{-1}$ delayed the decline of nitrogenase activity induced by water deficit, which appeared to be linked to an increased leaf Mn availability, leading to an enhanced rate of in situ ureide degradation. The observed increased rate of in situ ureide degradation in leaves of plants grown with supplemental Mn nutrition is consistent with reports from Winkler, Blevins, Polacco and Randall [31] and Lukaszewski, Blevins and Randall [8] showing that the enzymatic degradation of allantoic acid is Mn-dependent.

It appears from these results that Mn has two major effects associated with N_2 fixation: it enhances the overall rate of nodule activity and increases the rate of ureide degradation in leaves, which helps to sustain N_2 fixation under water deficit conditions.

5. UREIDES DECREASE NODULE PERMEABILITY TO O_2

The hypothesis of O_2-limitation of N_2 fixation under drought was previously analyzed by comparing the kinetics of ARA and nodule permeability to O_2 (Po) responses to an osmotic treatment by PEG [17]. The PEG treatment resulted in a decrease in calculated Po that paralleled ARA decrease. Exposure to the PEG treatment for 24 h or more resulted in very low nodule respiration and nitrogenase activity. However, increasing pO_2 failed to recover nodule activity [17], indicating that serious disruptions in nodule functioning had taken place.

Ureide treatment of hydroponically-grown soybean plants also resulted in a continual decrease in Po, simultaneous to the inhibition of nitrogenase activity over a 5-d treatment period [22], suggesting that a pO_2 limitation within the nodules could possibly be involved in the ureide inhibition of nitrogenase activity. It was thus hypothesized that the feedback response involving ureides might be based on the regulation of nodule oxygen permeability [11, 13, 27].

Our results showing a decrease in nodule Po that paralleled ARA inhibition associated with the Asn and ureide treatments [22] indicated that the inhibition of nitrogenase activity could be driven by a pO_2 limitation within the nodules. However, if such a mechanism really occurred, increased pO_2 would help to overcome the inhibitory effects of ureides on nodule activity. Increasing pO_2 5 d after imposition of the ureide treatment failed to induce a recovery of nodule activity. This indicates that by this time serious disruptions in nodule functioning had taken place, and that mechanisms other than O_2 diffusion may be involved in the long-term response to the ureide inhibition. However, we do not know whether O_2 enrichment would have helped to fully recover ARA in the initial stages of inhibition by Asn or ureide. The lack of response to pO_2 after exposing the roots to ureides for 5 d is similar to the results obtained with plants that had low activity after being subjected to prolonged osmotic stress [17]. Consistent with the conclusion of this work, oxygen limitations seem to be less important in limiting nodule activity in the case of severe stages of water deficit stress or exposure to ureide.

6. CO_2 ENRICHMENT DECREASES THE DROUGHT-INDUCED UREIDE ACCUMULATION

The interaction of CO_2 enrichment with the response of N_2 fixation and ureide accumulation to water deficits was recently studied on soybean [21]. Consistent with previous works, N_2 fixation under ambient CO_2 was found very sensitive to soil drying and decreased in response to soil drying before the other measured processes. In sharp contrast, N_2 fixation became highly tolerant to soil drying under CO_2 enrichment treatment (700 Φmol $CO_2 \cdot mol^{-1}$). Only in the final stage of soil drying when the drought stress was quite severe did N_2 fixation under the 700 Φmol $CO_2 \cdot mol^{-1}$ finally decrease [21].

The association between the induced N_2 fixation sensitivity to drought, and shoot or leaf ureide was also observed in this study. Increased CO_2 resulted in dramatically decreased levels of ureide [21]. Particularly important was the fact that at the end of drought stress, ureide levels under the increased CO_2 had risen to only slightly greater than the levels for the ambient CO_2 under well-watered conditions. Not surprisingly, TNC increased in response to increased CO_2 [21]. These data indicate the possibility that increased TNC resulting from elevated CO_2 might result in decreased ureide levels in the shoots. This is consistent with the hypothesis that N_2 fixation drought tolerance under elevated CO_2 is associated with decreased ureide levels. Under drought stress, ureide concentration increased in nodule, root and shoot tissues, and this accumulation was especially dramatic in nodules, where tremendous amounts of ureides were found. However, exposure to 700 $\Phi mol \cdot mol^{-1}$ [CO_2] resulted in substantially less drought-induced ureide accumulation in leaf and nodule tissues, compared to the ambient [CO_2] treatment.

The great effect of [CO_2] on ureide accumulation in the leaves taken together with the relationship observed between ureide accumulation and a decrease of TNC levels under drought are consistent with the importance of ureide breakdown in the response of N_2 fixation to drought and the hypothesis of feedback inhibition by ureides.

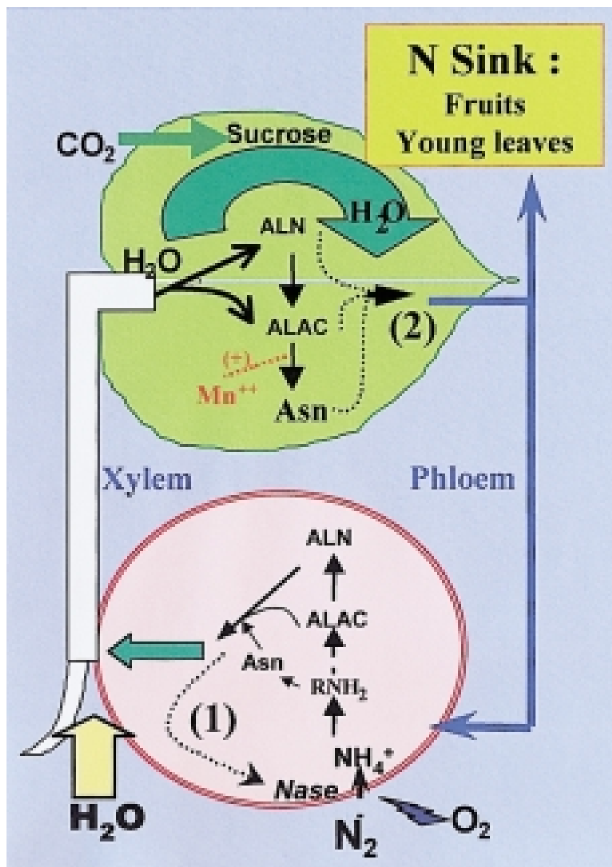

Figure 1. Synthetic scheme of the two possible origins for feedback regulation of nodule activity by N compounds under drought stress.

7. CONCLUSION

The segregation of legume species based on N_2 fixation sensitivity to drought showed an interesting association with the type of nitrogenous compounds exported from nodules. Those species that transported high concentrations of ureides were found to be more drought-sensitive than amide transporters. The association of high ureide concentrations in species with drought sensitivity may result from the low solubility of ureides. Decreased xylem transport in the ureide-transporting species with soil drying might result in a negative feedback on N_2 fixation and, consequently, result in the expressed drought sensitivity of several important grain legume crops.

We suggest that there may be two possible origins for feedback inhibition of nodule activity (Fig. 1): (i) a direct feedback within the nodule from accumulation of nitrogenous compounds, presumably ureides, that fail to be exported in the case of limited water availability; (ii) an indirect feedback coming from the shoot, with several likely compounds as candidates, among which are Asn and ureides, depending on the nature and amount of compound applied and interaction with ureide degradation metabolism.

The first possibility is in agreement with the previous hypothesis that N_2 fixation is regulated by currently fixed nitrogen [5], while the second agrees with results from Neo and Layzell [9] or Bacanamwo and Harper [1] where feedback is driven by compounds originating from the shoot. In fact, under drought it is very likely that both currently fixed nitrogen and nitrogen re-cycled from the shoot may contribute to the feedback regulation of nitrogenase activity.

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