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EFFECT OF WATER STRESS ON SOME ENZYMES OF NITROGEN METABOLISM IN PIGEONPEA

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Key Word Index—*Cajanus cajan*; Leguminosae; pigeonpea; water stress; nitrogenase; glutamine synthetase; glutamate dehydrogenase; uricase; allantoinase; allantoic acid.

Abstract—Water stress created by withholding irrigation at flowering stage (70 days) in pigeonpea resulted in decreased water potential of roots, nodules and leaves. The decreased water potential in nodules resulted in decreased activities of nitrogenase, glutamine synthetase, glutamate dehydrogenase and uricase. However, the activity of allantoinase increased under mild stress with a slight decrease under severe stress. This corresponded with a simultaneous increase in allantoic acid content. Uricase and allantoinase could not be detected in roots and leaves of both control and stressed plants. In roots, the activities of GS and GDH decreased under stress, whereas in leaves, their activities were not affected. Although the water potential recovered in different organs of the stressed plant on re-irrigation, the recovery in the case of some enzymes was not complete.

INTRODUCTION

Water stress is now widely known to affect nitrogen-fixing efficiency of legumes root nodules [1–4]. However, as far as the authors are aware, similar work on enzymes metabolizing the fixed nitrogen further has not been reported. Since pigeonpea (*Cajanus cajan*) is now known to produce ureides from fixed nitrogen and further transporting the same to aerial parts [5], studies regarding the effect of water stress on enzymes of ureide metabolism, particularly uricase and allantoinase, would be of great interest. Keeping in view the above, it was thought worthwhile to investigate the effect of water stress on these enzymes, together with nitrogenase, glutamine synthetase (GS) and glutamate dehydrogenase (GDH).

RESULTS AND DISCUSSION

Exposing the plants to water stress by withholding irrigation resulted in considerable decrease in water potential of leaves, roots and nodules (Table 1). At permanent wilting point, the values were −33.2, −25.3 and −21.0 bars for leaves, roots and nodules, respectively. On the fourth day, the plants were re-irrigated and 2 days after, the water potential of different organs became equal to that of control. Such reduction in water potential with decreasing soil moisture and its recovery is well known [3,6].

Nitrogenase activity (μmol C_2H_2 reduced/g dry wt per hr) in nodules decreased considerably under water stress. No activity was observed when the water potential of nodules reached −21.0 bars. At −6.5 and −12.5 bars, the activity was 21.4 and 12.6, respectively. The activity recovered very little even after re-irrigation, although water potential of nodules had become equal to that of control. This is in agreement with earlier reports [1–4, 7, 8]. In general, loss in water content of nodules up to *ca* 25% of fr.wt (equivalent to −10 bars) results in reversible effects on nitrogenase. However, beyond this, the effects become progressively more deleterious and irreversible [3, 7–10]. Engin and Sprent [10], based on the studies conducted in white clover, concluded that the recovery following wilting is a two-step process. The first step involves rehydration of existing nitrogen-fixing tissues and the second involves production of new nitrogen-fixing tissues as a result of renewed growth of nodules meristem.

Table 1. Effect of water stress on the water potential of leaves, roots and nodules

Days after withholding irrigation	Stage of stress	Water potential (−bars)		
		Leaf	Root	Nodule
0	I (Control)	10.7	8.5	6.5
2	II (Mild stress)	22.0	16.4	12.5
4	III (Severe stress)	33.2	25.3	21.0
2 days after revival	IV (Recovery)	10.5	8.7	6.0

In the present study, the recovery seems to be due to rehydration only, as no new nodule was formed in the 2 day period following re-irrigation.

Results presented in Tables 2 and 3 show that water stress resulted in a drastic decrease in the sp. act. of GS and GDH both in roots and nodules. However, in leaves, the activity was unaffected by stress. The recovery in the sp. act. of both enzymes was complete in nodules but not in roots. Glutamate synthase (GOGAT) could not be detected in any of the organs of pigeonpea. Brown and Dilworth [11] were also unable to detect GOGAT activity in cytosol of nodules of various legumes. However, Robertson *et al.* [12] reported considerable GOGAT activity in lupin nodule cytosol. Boland *et al.* [13] also have proposed the universal presence of the GS–GOGAT system of ammonia assimilation in nitrogen-fixing legume nodules. GOGAT may be present but might have been inhibited by glutamate and glycine as suggested earlier [12].

Greater activity of GS in leaves could be of significance for reassimilation of nitrogen, as in pigeonpea, nitrogen is transported to the shoot mainly in the form of allantoic acid [5]. Glutamine synthetase in leaves under water stress could also reassimilate ammonia, which is reported to be produced under stress due to oxidative deamination of amino acids [6].

All ureide-producing legumes examined so far have shown a high proportion of nitrogen as ureide and glutamine in their xylem sap relative to asparagine, typical of amide-exporting species [14]. Most recent evidence has suggested that the amide group of glutamine is the initial product of ammonia assimilation after nitrogen fixation and ureide formation based on purine biosynthesis requires two atoms of amide nitrogen mainly from glutamine [14]. This indicates that GS plays a vital role even in the species producing and exporting ureides. High activity of GS has been reported in various other legumes also [15, 16].

Water stress again resulted in a drastic decrease in the sp. act. of uricase (allantoin-synthesizing enzyme) in nodules, whereas the activity of allantoinase (allantoin-degrading enzyme) increased under mild stress (Table 4). Even under severe stress, allantoinase exhibited only slight decrease in its activity. The above two enzymes could not be detected in roots and leaves. However, Tajima and Yamamoto [17] have shown the presence of uricase and allantoinase in nodules and leaves of soybean seedlings. Herridge *et al.* [16] also reported high concentrations of uricase and allantoinase in nodules of cowpea, but little uricase was found in extracts of leaflets, pods and seeds. These differences could be either due to the age of the plant or species differences.

It has been shown that severe water stress generally lowers enzyme levels, although moderate to severe stress raises those involved in hydrolysis and degradation [18]. Allantoinase, being a hydrolysing enzyme, was found to increase in activity under mild stress (Table 4). This increased activity under stress corresponded with a simultaneous increase in allantoic acid content in the nodules (Table 5). At the same time, since the solubility of ureides is very low [19], the increased allantoic acid content in nodules could be either due to the increased activity of allantoinase and/or to the reduced translocation of allantoic acid to the roots and leaves. To transport an equivalent amount of nitrogen as allantoin or allantoic acid, rather than as asparagine requires *ca* 2.6 times as much water, assuming both are at saturation and the same temperature [19]. Since the water stress decreases the moisture content of plant organs [6], the decreased water content in nodules might have further reduced the solubility and subsequent translocation of allantoic acid. This would help the allantoic acid to serve

Table 2. Effect of water stress on the sp. act. of glutamine synthetase in leaves, roots and nodules

Stage of stress	Glutamine synthetase (μg γ-glutamyl hydroxamate formed/mg protein per min)		
	Leaf	Root	Nodule
I	12.6	8.75	6.64
II	11.9	2.82	4.62
III	12.0	1.78	1.22
IV	16.8	1.59	6.37

Table 3. Effect of water stress on the sp. act. of glutamate dehydrogenase in leaves, roots and nodules.

Stage of stress	Glutamate dehydrogenase (units/mg protein)		
	Leaf	Root	Nodule
I	10.0	406	58.6
II	6.35	217	36.1
III	7.05	23	9.1
IV	7.65	106	61.5

Table 4. Effect of water stress on the sp. act. of uricase and allantoinase in nodules

Stage of stress	Allantoinase (μg allantoic acid formed/mg protein per min)	Uricase (μg uric acid degraded/mg protein per min)
I	2.58	43.2
II	4.49	11.2
III	2.35	2.7
IV	2.81	11.1

Table 5. Effect of water stress on allantoic acid content in leaves, roots and nodules

Stage of stress	Allantoic acid (μg/g fr. wt)		
	Leaf	Root	Nodule
I	107	96	515
II	130	137	2140
III	475	285	1840
IV	160	150	1110

as a temporary storage product under water stress conditions.

The higher content of allantoic acid in leaves could be due to its lesser utilization as stress resulted in complete cessation of growth [6]. The amount of allantoic acid decreased during revival but it was still more than the control. It seems that under water stress allantoic acid serves as a nitrogen reserve which could support the initial growth after revival until new nitrogen is available from underground parts. In legumes, Mothes [20] has suggested that ureides accumulate when the ratio of nitrogen fixed to nitrogen utilized is increased.

In conclusion, nitrogenase and uricase seem to be the most sensitive enzymes to decreasing water potential followed by GDH and GS. Allantoinase, on the other hand, is least affected by stress.

EXPERIMENTAL

Pigeonpea plants (cv UPAS-120) were raised in earthen pots (30 cm diam) on N-free sand culture in a naturally lighted net-house. The seeds before sowing were inoculated with an effective *Rhizobium* culture (strain IPH-159). At weekly intervals, each pot having one plant was supplied with 500 ml of N-free nutrient soln [21]. Pots are irrigated daily with 500 ml of H_2O. H_2O stress was created by withholding the irrigation at 70 days after sowing (flowering stage). Leaf, root and nodule H_2O potentials were recorded after every 2 days in control as well as stressed plants by using a HR-33T Dew Point Microvoltmeter with C-52 sample chamber. Four days after creating stress, the plants were re-irrigated and H_2O potential recorded as usual 2 days after revival.

Enzyme assays. Plants were sampled on the day on which the H_2O potentials were recorded and separated into leaves, roots and nodules. Nitrogenase activity in freshly harvested nodulated roots was estimated by the acetylene reduction assay [22]. Other enzymes were extracted from freshly harvested nodules, roots and leaves by thorough homogenization in a mortar and pestle at 4° with 0.1 M Pi buffer (pH 7.4) containing 10^{-4} M EDTA and 10 mM mercaptoethanol. The homogenate was passed through eight layers of cheese-cloth and centrifuged at 12 000 *g* for 30 min. The supernatant was used as such for different estimations except for allantoinase where the extract was dialysed for 24 hr before use. Protein was estimated following precipitation with 20% (w/v) TCA using BSA as standard [23]. Enzyme assays were carried out at 30°. NADH–glutamate dehydrogenase (EC 1.4.1.4) was assayed as described previously [24] and expressed in terms of change in $0.01\,A_{340}$ per min. Glutamine synthetase (EC 6.3.1.2) was assayed in the biosynthetic direction by the formation of glutamyl hydroxamate [25], uricase (EC 1.7.3.3) by the conversion of uric acid to allantoin [17] and allantoinase (EC 3.5.2.5) by measuring the glyoxylate freed from enzymically derived allantoic acid [26]. Allantoic acid from different plant organs was extrd and estimated according to ref. [27]. Each value is the mean of three determinations.

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REFERENCES

1. Sprent, J. I. (1976) in *Water Deficit and Plant Growth* (Kozlowski, T. T., ed.) Vol. 4, p. 291. Academic Press, New York.
2. Minchin, F. R. and Pate, J. S. (1975) *J. Exp. Botany* **26,** 60.
3. Huang, C. Y., Boyer, J. S. and Vanderhoef, L. N. (1975) *Plant Physiol.* **56,** 228.
4. Hume, D. J., Criswell, J. G. and Stevenson, K. R. (1976) *Can. J. Plant Sci.* **56,** 811.
5. Luthra, Y. P., Sheoran, I. S. and Singh, R. (1980) *Curr. Sci.* **50,** 270.
6. Hasio, T. C. (1973) *Annu. Rev. Plant Physiol.* **24,** 519.
7. Pankhurst, C. E. and Sprent, J. I. (1975) *J. Exp. Botany* **26,** 287.
8. Sprent, J. I. and Gallacher, A. (1976) *Soil Biol. Biochem.* **8,** 317.
9. Sprent, J. I. (1971) *New Phytol.* **71,** 443.
10. Engin, M. and Sprent, J. I. (1973) *New Phytol.* **72,** 117.
11. Brown, C. M. and Dilworth, M. J. (1975) *J. Gen. Microbiol.* **86,** 39.
12. Robertson, J. G., Warburton, M. P. and Farnden, K. J. F. (1975) *FEBS Letters* **55,** 33.
13. Boland, M. J., Fordyce, A. M. and Greenwood, R. M. (1978) *Aust. J. Plant Physiol.* **5,** 553.
14. Pate, J. S., Atkins, C. A., White, S. T., Rainbird, R. M. and Wood, K. C. (1980) *Plant Physiol.* **65,** 961.
15. Robertson, J. G., Farnden, K. J. F., Warburton, M. P. and Banks, J. M. (1975) *Aust. J. Plant Physiol.* **2,** 265.
16. Herridge, D. F., Atkins, C. A., Pate, J. S. and Rainbird, R. M. (1978) *Plant Physiol.* **62,** 495.
17. Tajima, S. and Yamamoto, Y. (1975) *Plant Cell Physiol.* **16,** 271.
18. Todd, G. W. (1972) in *Water Deficit and Plant Growth* (Kozlowski, T. T., ed.) Vol. 3, p. 177. Academic Press, New York.
19. Sprent, J. I. and Embrapa (1980) *Plant, Cell Environ.* **3,** 35.
20. Mothes, K. (1961) *Can. J. Botany* **39,** 1785.
21. Wilson, D. O. and Reisenauer, H. M. (1963) *Plant Soil* **18,** 364.
22. Hardy, R. W. F., Hoslsten, R. D., Jackson, E. K. and Burns, R. C. (1968) *Plant Physiol.* **43,** 1185.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.
24. Gupta, P. and Sheoran, I. S. (1979) *Phytochemistry* **18,** 1881.
25. O'Neal, D. and Joy, K. W. (1973) *Arch. Biochem. Biophys.* **159,** 113.
26. Ory, R. L., Gordon, C. V. and Singh, R. (1969) *Phytochemistry* **8,** 401.
27. Nirmala, J. and Sivarama Sastry, K. (1972) *Analyt. Biochem.* **47,** 218.