

The effect of water stress on nitrogen metabolism of horsegram *Dolichos biflorus* L.

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Abstract

Horsegram plants were raised in fertile soil in earthen pots and subjected to various durations of water stress (7, 14 and 21 days). Total nitrogen contents were reduced in water-stressed plants but differently in different plant parts. Nitrate content and nitrate reductase activity decreased with stress. This species possesses a good capacity for accumulation of free proline under water stress. Analysis of amino acid composition also revealed marked changes in the levels of various amino acids in water-stressed plants. Accumulation of γ -aminobutyric acid in water-stressed plants was observed.

Key words: horsegram, *Dolichos biflorus*, water stress, nitrogen metabolism, proline, amino acids, nitrate reductase

INTRODUCTION

Some of the more striking metabolic changes in plant leaves subjected to moisture stress are those involving proteins (Todd and Basler 1965). There are several reports indicating drought-induced alterations in nitrogen metabolism in crops like wheat, soybean and barley. However, very little attention in this respect has been paid to hardy pulse crops like horsegram (*Dolichos biflorus* L.). This crop is cultivated in low rainfall tracts in India and hence it was thought worthwhile to investigate the influence of moisture stress on a few aspects of nitrogen metabolism in this legume.

MATERIAL AND METHODS

Plants of *Dolichos biflorus* L., were raised from healthy seeds (collected locally), in fertile soil in earthen pots under greenhouse conditions. The plants were equally watered (two litres per pot, thrice a week) for 8 weeks so as to stabilize them. After 8 weeks water was withheld from various pots so that at the time of harvest there were pots receiving no water for 7, 14 and 21 days. The plants which received a regular water supply (control) and the plants exposed to water stress were used for the estimation of total nitrogen, nitrate and free proline contents. Total nitrogen was estimated by the method of Hawk et al. (1948). The zinc dust method of Wooley et al. (1960) was used for the estimation of nitrates. The activity of nitrate reductase in leaf sample was assayed according to the *in vitro* method described by Evans (1982). The free proline contents in leaves, stems and roots was determined according to the method of Bates et al. (1973). Free amino acids were analysed in leaf samples from water stressed plants using an amino acid analyzer.

RESULTS AND DISCUSSION

The influence of water stress on the total nitrogen content in different parts of horsegram plants is recorded in Table 1. It is evident from the Table that the values of nitrogen in the leaves and root tissue of the water-stressed plants were less than those of the control, although the effect was not proportionate to the duration of water stress. Stem tissues accumulated nitrogen under stress conditions. The negative influence of water stress on total nitrogen content is reported in a few papers (Pade and Singh 1969, Popov 1969, Hsiao 1973, Trung et al. 1984). Although the N content in leaves and roots was lowered due to water stress in horsegram leaves and roots, the decrease was relatively very small (in the range 8.6 to 31.8% in leaves and 39.5 to 73.8% in roots, respectively), which suggests that the nitrogen uptake in this plant is only very slightly reduced, even under severe water stress. However, the same cannot be said about N translocation within the plants since there was marked accumulation of nitrogen in the stem tissue. This may be due to either retranslocation of nitrogen from the leaf tissue or inhibition of nitrogen transport from stem to the leaf tissue due to water stress.

It can be seen from Table 1 that accumulation of free proline was considerably stimulated in horsegram stem and roots due to water stress. According to Paleq and Aspinall (1981), accumulated proline acts as a compatible solute regulating and reducing water loss from the cell during water deficit. They suggest that the proline concentration may adjust rapidly to changes in the aqueous environment of the cell. Schobert and Tschesche (1978) showed that proline affects the solubility of various substances and protects bovine albumin from denaturation by $(\text{NH}_4)_2\text{SO}_4$ or ethanol. This

information supports earlier empirical observations of increases in the cytoplasmic "bound-water" fraction in the presence of proline (Palfi et al. 1974b, Savitskaya 1976) which may be related to significant adaptive phenomena. Proline can also serve as a source of carbon and nitrogen for biosynthesis of various compounds during a post-stress recovery. Proline accumulation under conditions of water stress has been reported for many legume species. Palfi et al. (1974a) studied proline accumulation in *Pisum sativum*, *Lens culinaris*, *Medicago sativa*, *Trifolium repens* and *Phaseolus vulgaris*. Jager and Meyer (1977) reported a 983 per cent increase of proline ($\mu\text{m shoot}^{-1}$) in *Phaseolus vulgaris* and Chu et al. (1978) reported a 350 per cent increase of proline in *Vicia faba*. In the present investigation, in the leaves of horsegram plants stressed 7 and 14 days, proline increase was 42.9% and 103.9%, respectively, which is rather low. At the same time it is interesting to note that the roots accumulated higher amounts of proline than the leaves and stem. Although leaves are generally regarded as the site of proline accumulation under stress conditions, in some plants like cotton, proline accumulates in the stem tissue also (Janagoudar et al. 1983). The higher proline contents in the horsegram roots might be due to its translocation from the leaves and this can be certainly regarded as an adaptive feature of the species since proline accumulated in the roots can help in recovery processes of the leaves following resumption of water supply.

Table 1

Effect of water stress on total nitrogen (in g (100 g)⁻¹ dry tissue) and free proline content (in mg (100 g)⁻¹ dry tissue) in different parts of horsegram (*D. biflorus* L.)

Water stress (days)	Nitrogen			Free proline		
	leaf	stem	root	leaf	stem	root
0	4.40	0.61	3.82	77	70	110
7	3.00	0.74	1.00	110	205	252
14	4.02	1.00	1.84	157	202	280
21	3.01	1.84	2.31	78	187	217

Each value is a mean of three determinations.

The nitrate content and nitrate reductase activity in the leaves of water-stressed horsegram are shown in Table 2. It is evident from the data that the nitrate content and the nitrate reductase activity decreased with stress. Nitrate is the major form in which soil nitrogen is available to most plant species. Nitrate reductase occupies a key role in plant nitrogen metabolism and many workers consider it to be a rate limiting step in nitrogen nutrition. Since legumes have a nitrogen fixation capacity through a symbiotic association with *Rhizobium* it is quite obvious that the enzyme plays rather a secondary role.

Our findings recall the work of Shaner and Boyer (1976a, b) who suggested that nitrate reductase activity in plants is regulated by the nitrate flux which becomes reduced in water stressed plants, leading to a lowering of enzyme activity. Although both nitrate content and nitrate reductase activity showed a reduction in horsegram leaves under water stress, it is seen that the nitrate content was reduced by only 50% to that of the control while there was almost an 89% decrease in enzyme activity after 14 days of stress. This indicates that poor substrate availability is not the sole reason for the decline in enzyme activity and other factors like enzyme synthesis and availability of NADH may come into the picture (Sinha and Nicolas 1981). Although some workers have postulated a possible link between proline accumulation and decrease in nitrate reductase activity (Sinha and Rajagopal 1975), our observations indicate that the rise in the proline level in horsegram leaf tissue is not sufficient to prevent the decline of nitrate reductase activity under the conditions of water stress.

Table 2

Effect of water stress on nitrate content and nitrate reductase activity in the leaves of horsegram (*Dolichos biflorus* L.)

Water stress (days)	Nitrate content %	Nitrate reductase $\mu\text{M NO}_2 \text{ g}^{-1} \text{ fresh wt. min.}^{-1}$
0	0.72	5.84
7	0.66	1.34
14	0.31	0.66
21	NR	NR

NR — not recorded. Each value is a mean of three determinations.

The effect of water stress on the free amino acid content in leaves of *D. biflorus* is shown in Table 3. It is clear from the table that 14 days of water stress caused a considerable increase in the levels of aspartic acid, threonine, asparagine, glutamine, lysine, histidine and gamma amino butyric acid. We also noticed a moderate increase in the levels of other amino acids such as cystine and isoleucine. Severe water stress (21 days) caused a considerable increase in the levels of serine, asparagine, glutamine, glycine, alanine, valine, isoleucine, leucine, phenylalanine and arginine. This stress also caused a marginal increase in the levels of other amino acids like aspartic acid and threonine. Severe water stress caused a decrease in the level of amino acids like cystine, lysine and histidine. The level of these amino acids increased under mild water stress (14 days). In view of the key role of amino acids in nitrogen metabolism, there have been a number of attempts to study the fate of these compounds under stress conditions, while studying accumulation of low molecular weight solutes in water-stressed tropical legumes, Ford (1984) observed that free amino acid levels were low. Of the seven species analysed by

him, namely *Lablab purpureus* cv Highworth, *Vigna radiata* cv Berken, *Vigna mungo* cv Regur, *Glycine max* CPI 26671, *Psoralea eriantha*, *Macroptilium atropurpureum* cv Siratro and *Centrosema* sp. aff. *pubescens* cv Belalto, consistently increased levels in dry tissue were found for glycine in five species: *Lablab purpureus* cv Highworth, *Vigna radiata* cv Berken, *Vigna mungo* cv Regur, *Glycine max* CPI 26671 and *Centrosema* sp. aff. *pubescens* cv Belalto. Consistently increased levels in dry tissue were also found for valine in six species: *Vigna radiata* cv Berken, *Vigna mungo* cv Regur, *Glycine max* CPI 26671, *Psoralea eriantha*, *Macroptilium atropurpureum* cv Siratro and *Centrosema* sp. aff. *pubescens* cv Belalto. Ford (1984) also observed decreased levels of serine and alanine in *Lablab purpureus* cv Highworth, *Vigna radiata* cv Berken, *Vigna mungo* cv Regur, *Glycine max* CPI 26671, *Psoralea eriantha* and *Macroptilium* cv Siratro, *Lablab purpureus* cv Highworth, *Vigna radiata* cv Berken, *Vigna mungo* cv Regur, *Glycine max* CPI 26671, *Psoralea eriantha*, *Macroptilium atropurpureum* cv Siratro and *Centrosema* sp. aff. *pubescens* cv Belalto, respectively. A further point of interest observed by him was that the accumulation of valine in the dry tissue of some of the legumes mentioned earlier, coincided with accumulation of γ -lactone in these species. The other species accumulated neither valine nor γ -lactone in their dry tissue.

Table 3
Effect of water stress on free amino acid contents of leaves of *D. biflorus*

Amino acid nmoles g ⁻¹ fresh tissue	Day of water stress		
	0	14	21
Aspartic acid	trace	34	38
Threonine	trace	6	9
Serine	28	24	38
Asparagine	trace	8	19
Glutamic acid	36	18	22
Glutamine	trace	7	16
Glycine	13	5	23
Alanine	65	35	86
Valine	—	trace	13
Cystine	80	100	34
Methionine	—	—	trace
Isoleucine	trace	3	12
Leucine	—	trace	9
Tyrosine	—	trace	trace
Phenylalanine	trace	trace	35
Lysine	12	105	6
Histidine	trace	72	13
Arginine	—	—	60
Gamma amino-butyric acid	18	158	56

— Not detectable.

It is clear from the foregoing account that there is no uniformity among various plant species with respect to changes induced by water stress in free amino acid pool. The trend in amino acid alteration is not similar even among legumes (Ford 1984). Stress-induced, pronounced changes in the composition of amino acid pools are attributed to either a pronounced protein breakdown or to the stress-induced changes in amino acid metabolism (Moris et al. 1969, Steward and Bogges 1977). It is possible that both these processes are affected differently in different plant species. Some of these changes may be of an adaptive nature while other changes may simply represent 'metabolic disturbances'. In the case of horsegram we can notice that there is a general tendency for accumulation of free amino acids under conditions of severe water stress. The increase in asparagine and glutamine levels is certainly an adaptive feature in view of the fact that this may be useful for trapping toxic ammonia liberated during deamination reactions. However, increases in glycine and serine contents in water-stressed horsegram leaves may reflect an increase in the photorespiratory rate.

The increase in the level of γ -aminobutyric acid in water-stressed horsegram plants is quite interesting. γ -Aminobutyric acid is a major constituent of higher plants. The amino group functions in amino acid transamination and thus it supplies nitrogen for other plant needs. Additionally, the resulting semialdehyde can be oxidized to succinic acid which provides another interchange between the amino acid metabolism and the reactions of the Krebs cycle (Rosenthal 1982). In the present investigation it can be seen that there is nearly a 9-fold increase in the level of γ -aminobutyric acid in the leaves of 14-day water-stressed plants while the level of the same compound is increased to about 3-fold over control due to severe water stress (21 days). These observations indicate the possible role of this non-protein amino acid both during drought tolerance and post-stress recovery as indicated by Rosenthal (1982).

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Wpływ stresu wodnego na metabolizm azotu u Dolichos biflorus L.

Streszczenie

Rośliny *Dolichos biflorus* rosły w żywej ziemi we wkopanych w ziemię wazonach. Poddano je stresowi wodnemu trwającemu 7, 14 lub 21 dni. Stres wodny spowodował zmniejszenie się całkowitej zawartości azotu w roślinach, ale objawiło się to różnie w różnych częściach roślin.

Wraz z przedłużaniem się stresu wodnego zmniejszała się zawartość azotu i aktywność reduktazy azotanowej. Badany gatunek ma dużą zdolność gromadzenia wolnej proliny pod wpływem stresu wodnego. U roślin poddanych stresowi wodnemu stwierdzono również wyraźne zmiany w zawartości różnych aminokwasów oraz obserwowano gromadzenie kwasu γ -aminomasłowego.