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Effect of water stress on functioning and structure of *Cicer arietinum* L. nodules

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Abstract

Chickpea (*Cicer arietinum* L. cv. 235) plants were grown in sand culture at moisture equal to 45 - 50 % of sand saturation capacity under greenhouse conditions. 60 d after sowing, pots were divided into four lots, leaving one as control and sand moisture content of others was brought to 25 - 30 % (S_1), 12 - 15 % (S_2) and 5 - 6 % (S_3) of sand saturation capacity, by withholding the water supply and then maintaining the required levels gravimetrically till the harvest. Relative water content of leaves and nodule water content were measured as indices of water stress. With increase in the severity and duration of water stress nitrogenase activity and nitrogen and leghemoglobin content of the nodules decreased and the ratio of leghemoglobin components I and II were changed. Nodules developed under limited water availability showed decreased branching, breakdown of the endodermis, greater compactness and decreased vacuolation of cells in the central symbiotic tissue as compared to the control.

Key words: chickpea, leghemoglobin components, nitrogen, nitrogenase, nodule anatomy.

Introduction

Chickpea plants face frequent and prolonged periods of drought. In addition they depend largely on the symbiotic N_2-fixation, a process very sensitive to water stress (Albrecht *et al.* 1984, Sprent 1976, 1992, Sheoran *et al.* 1988, Nandwal *et al.* 1991, 1993). It has been postulated that part of the effect of drought on N_2-fixation is mediated through the development of a barrier to O_2 diffusion into the nodules (Pankhurst and Sprent 1975, Ralston and Esmande 1982, Swaraj *et al.* 1984, Sprent 1992). Permeability of nodules to O_2 is determined by the area of intercellular spaces in the uninfected cortical region which will ultimately be determined by the density

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Abbreviations: ARA - acetylene reducing activity; Lb - leghemoglobin; RWC - relative water content

** To whom correspondence should be sent of cells in this region. Present investigations were, therefore, undertaken to understand the influence of prolonged periods of moisture stress on structure and functioning of chickpea nodules related to change in leghemoglobin components.

Materials and methods

The plants of chickpea (*Cicer arietinum* L.) cultivar C-235 were raised in sand culture using earthen pots, filled with 5 kg of dune sand. While watering, half of the quantity of water was applied through the plastic tube reaching a 3/4 depth of the pot and the remaining half through sand surface. The seeds were surface sterilized with 80 % ethanol and at the time of sowing each seed was inoculated with 0.5 cm^3 of a *Bradyrhizobium* spp. (*Cicer*), strain Ca-545 (Department of Microbiology, CCS HAU Hisar - 125 004, India). Plants were supplied with nitrogen free nutrient solution (Wilson and Reisenauer 1963) at appropriate intervals and grown at moisture content 45 - 50 % of sand saturation capacity. 60 d after sowing, the pots were divided into four lots, one lot serving as the control. Water supply in other three lots was withheld till the required sand moisture level was attained: 25 - 30 % (S_1), 12 - 15 % (S_2) and 5 - 6 % (S_3), correspondingly sand moisture content was 12, 8, 4 and 2 % in control, S_1, S_2 and S_3, respectively. The sand moisture level in each treatment was maintained gravimetrically. The plants were sampled 95, 110, 120 and 135 d after sowing (DAS).

At 95, 110 and 135 DAS the first fully expanded leaf, *i.e.* fourth leaf from the top was used for determination of relative water content (RWC) as described by Weatherley (1950). The nodules from plant roots were excised, sand attached to their surface was removed gently with a filter paper and immediately their fresh mass recorded. The nodules were dried at 90 °C, to a constant mass and nodules water content (NWC) was calculated.

Nitrogenase (EC 1.18.2.1) activity of the nodules was estimated as acetylene reducing activity (ARA) (Hardy *et al.* 1973).

At 95 and 120 DAS the leghemoglobin (Lb) was extracted by homogenizing 1.0 g of nodules in chilled phosphate buffer (0.1 M, pH 7.0) and isolated by $(NH_4)_2SO_4$ fractionation as described previously (Swaraj and Garg 1977).

For resolution of Lb components (only at 95 DAS) by HPLC, the precipitates were dissolved in 50 mM phosphate buffer containing sodium azide (0.01 %), desalted using *Sephadex G-25* column (1.5 × 10.0 cm) saturated with the same buffer. The protein fractions so obtained were further resolved by HPLC (*Shimadzu HSG* 30 W aqueous gel permeation column and UV detector 280 nm) using 50 mM phosphate buffer (pH 7.0) containing 0.1 % sodium azide as the elution buffer at a flow rate of 1 $cm^3\ min^{-1}$.

Heme proteins were estimated according to Hartree (1955). Total nitrogen content of plant tissue was estimated by Micro Kjeldahl technique.

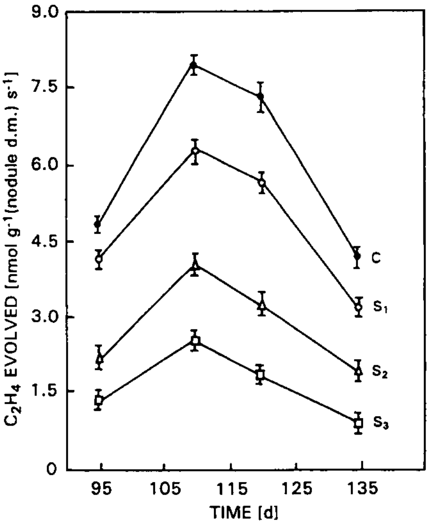
To study anatomical changes longitudinal nodule segments were fixed in FAA (formaldehyde 5 %, acetic acid 10 %, alcohol 35 % and distilled water 50 %) 120 d after sowing. Nodule sections were prepared for light microscopy and staining was done with safranin light green combination.

Results and discussion

Leaf RWC decreased with decreasing moisture level of the sand. Nodule water content decreased with the decrease moisture availability in sand and RWC of leaf (Table 1).

Table 1. Effect of water stress on leaf relative water content, RWC [%], nodule water content, NWC [%] and nodule nitrogen content, N [mg g^{-1} (d.m.)] in 95, 120 and 135 d old plants of chickpea. CD - critical difference at 5 % level.

	95 d			120 d			135 d		
	RWC	NWC	N	RWC	NWC	N	RWC	NWC	N
Control	93.07	87.94	35.8	86.29	84.69	31.9	86.85	88.20	28.0
S_1	85.16	87.55	30.0	83.26	82.50	29.5	82.07	87.20	25.5
S_2	67.05	70.99	28.4	68.48	70.83	24.5	57.26	70.80	22.0
S_3	64.23	67.34	18.0	66.66	67.50	15.4	52.39	67.40	14.0
CD	7.85	9.63	3.81	6.23	5.12	4.02	6.17	4.87	3.21



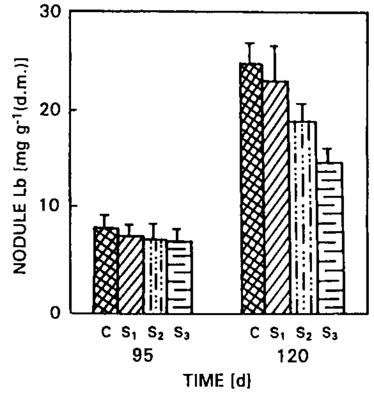


Fig. 1. Effect of water stress on acetylene reducing activity (ARA) of chickpea nodules. Bars indicate standard error.

Fig. 2. Effect of water stress on leghemoglobin content of chickpea nodules. Bars indicate S.E.

ARA of nodules decreased consistently as the water availability decreased and the adverse effects became accentuated as duration of water stress became longer (Fig. 1). Decrease in ARA reflected itself in decreased nodule nitrogen (Table 1).

Drought induced decrease in N_2-fixation rates has been well documented in literature (Sprent 1992). Sinclair *et al.* (1988) reported that great sensitivity of N_2-fixation rates was not due to the drought effect on nodule number. Nandwal *et al.* (1991) found that the decrease in ARA was due to the decrease in water potential of nodulated root.

At 95 DAS, Lb content showed only a variation of 5 - 10 % among various treatments (Fig. 2). At 120 DAS, Lb content of the nodules showed a considerable decline with decreasing sand moisture (Fig. 2) probably due to onset of premature nodule senescence under water stress.

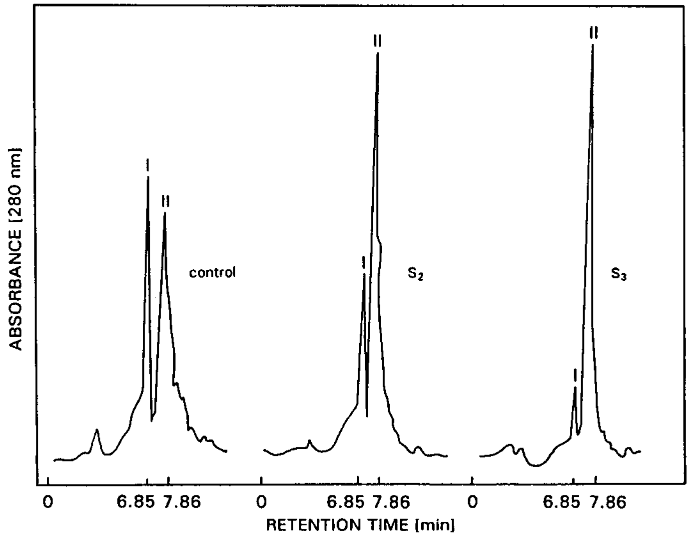


Fig. 3. Effect of water stress on composition of leghemoglobin in chickpea nodules.

Not only the content, but the protein composition of Lb was also affected by limited moisture availability. When proteins precipitating between 50 - 80 % of $(NH_4)_2SO_4$ saturation were resolved by HPLC at 95 DAS, two main protein components were observed (Fig. 3), first one (component I) showing a retention time of 6.85 min and second one 7.86 min (component II). Under control conditions component I constituted 34 % and component II constituted 45 % of Lb proteins. When nodules were subjected to stress (S_2), component I dropped to 17 % of the total Lb protein while component II increased to 65 %. The relative content of component II increased further with increased in stress level (S_3) constituting 72.5 % of Lb proteins while component I declined to 10 %. Thus, it appears from these studies that limitation of moisture availability to plants probably results in faster degradation of component I and preferential synthesis of component II. However, it cannot be ruled out that component I gets converted to component II under stress conditions. This change in the ratio of component I and component II affects the O_2

binding capacity of Lb. From several other legume species (*Glycine max, Lupinus luteus, Phaseolus vulgaris, Pisum sativum, Trifolium repens, Vigna radiata, V. unguiculata*) also multiple components of Lb have been separated by Becana and Sprent (1989). According to them nitrate stress has a major effect on proportions of Lb I and Lb II. Differences in O_2 affinities of different soybean Lb components and between Lb I and Lb IV of *Pisum* (Uheda and Syono 1982 a, b) and between Lb I and Lb II in *Vigna radiata* (Becana and Sprent 1989) have been reported. Holl *et al.* (1983) suggested that this variation in Lb components may have a compensatory value to maintain the overall efficiency of N_2-fixation.

Decrease in Lb content under drought have also observed (Sprent 1976, Swaraj *et al.* 1984, 1986, Venkateswarlu *et al.* 1989, Nandwal *et al.* 1991). Since Lb plays an important role in facilitating diffusion of O_2 to the bacteroids, a decrease in its

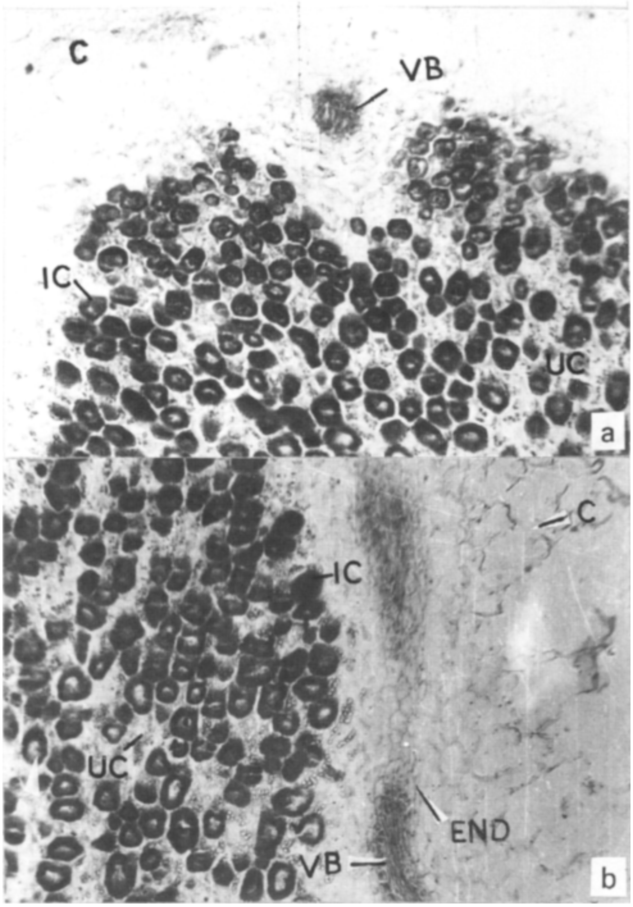


Fig. 4. Longitudinal section of control nodule under control and stressed conditions. *a* - longitudinal section of control nodule showing the distal end with enlarged cortex (× 200), *b* - longitudinal section of stressed nodule showing the shrinked cortex (× 200). *C* - cortex; *END* - endodermis; *UC* - uninfected and *IC* - infected cells of the central symbiotic tissue; *VB* - vascular bundle.

content might contribute to increased resistance to diffusion of O_2 inside the nodules and thus adversely affect N_2-fixation (Aquirreolea and Sanchez-Diaz 1989).

Nodules developed under stress (S_3) showed a number of structural differences from control nodules at 120 DAS. Nodules of the control were predominantly larger in size and profusely branched showing the dichotomous pattern whereas the nodules developed under stress were relatively smaller and branched occasionally. The longitudinal sections clearly reveal their indeterminate nature like nodules of alfalfa and pea (Hirsch 1992). The cortical region, *i.e.* outer to the endodermis, varied considerably being wider in control nodules as compared to stressed nodules (Fig. 4 a, b). Similarly, the nodule meristem and the invasion zone were considerably reduced under stress conditions. Central tissues of the control nodules were highly vacuolated, but the intensity of vacuolation decreased from middle portion towards the distal end because the infected cells differentiate from the distal (apical) to the proximal (basal) part of a nodule, the vacuolation increases in the course of nodule development. On the other hand, stressed nodules experienced a shrinkage in size and were less vacuolated. A comparative study regarding the extent of vacuolization indicate that under controlled condition 60 - 70 % area of the cell was occupied by the vacuole, whereas under stressed condition (S_3) the percentage of vacuolization declined to 50 - 55 % of the cell area. As was apparent from the number of cells per unit area, cells in the central zone of stressed nodules were more compactly arranged as compared to the control. The dimension of central zone cells along the long axis was larger in control nodules as compared to the stressed nodules. Bacteroid containing infected cells in the nodule under stress, except for their smaller size, were not very different from the control. At the proximal end, the area of parenchymatous cells appearing empty and devoid of bacteroids was considerably larger under stressed conditions than that in control. Vascular elements in nodules under stress conditions were poorly developed and showed a decrease in their number. Nodule endodermis in control conditions is continuous but it is distorted and poorly developed under stress.

Greater compactness of cells in nodules under stress conditions may be one of the causes of increased resistance to O_2 diffusion inside the nodules (Pankhurst and Sprent 1975, Weisz *et al.* 1985, Sprent 1992). Greater area of the region of empty cells devoid of any bacteroids under stress conditions might point out to accelerated nodule senescence under conditions of limited moisture availability.

It is concluded from these studies that nodule functioning was highly sensitive to water availability in the rooting medium. Decrease in N_2-fixation under limited water availability can be attributed to changes in Lb composition and greater compaction of cells inside the nodules which might limit O_2 availability to the bacteroids inside the nodules.

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