Nitrogen distribution index of *Cajanus cajan* L. during drought and rehydration

A.S. NANDWAL

Department of Botany, CCS, Haryana Agricultural University, Hisar - 125004, India

Abstract

Relative competition among various plant parts for N during water stress, *i.e.* nitrogen distribution index (NDI) was determined in relation to specific nitrogenase activity (SNA) and nodule and soil nitrogen in both indeterminate (H-77-216) and determinate (ICPL-151) types of pigeonpea (*Cajanus cajan* L.) under greenhouse conditions. Two levels of water stress, *i.e.* moderate (soil ψ_w -0.77 MPa) and severe (soil ψ_w -1.34 MPa) were created by witholding the irrigation at vegetative (40 DAS) and flowering (70 DAS) stages. At vegetative stage under moderate stress the highest NDI was in nodules of cv. H-77-216 and in leaf of cv. ICPL-151. Under severe stress both the cultivars showed negative values of NDI, with maximum loss of N from root and nodules. Cultivar ICPL-151 behaved differently at flowering and vegetative stages. Very high loss of N from different plant parts was seen at flowering under severe stress. All the plant parts showed gain in N during rehydration. Loss and gain in N at both the stages under stress and rehydration respectively, correlated with available N in soil. Specific nitrogenase activity (SNA) and nodule N were maximum at moderate stress and related with NDI values of leaf and nodules.

Introduction

Legumes such as pigeonpea are generally grown in tropical conditions, where they experience frequent drought of varying intensity and duration during growth and development. Among the physiological processes in legumes, N_2-fixation is one of the most sensitive to water stress (Sprent 1976, Weiz *et al.* 1985, Durand *et al.* 1987, Venkateswarlu *et al.* 1989, Nandwal *et al.* 1991 b). Nitrogen accumulation and its partitioning and redistribution of different plant parts have been studied by various researchers (Chapman and Muchow 1985, Sinclair *et al.* 1987, De Vries *et al.* 1989).

Received 18 May 1992, *accepted* 20 August 1992.

Abbreviations: C.D.- critical difference; DAS - days after sowing; NDI - nitrogen distribution index; SMC - soil moisture content [%]; SNA - specific nitrogenase activity; ψ_w - water potential [MPa]

Acknowledgement: The author thanks to Council of Scientific and Industrial Research (CSIR), New Delhi, India for awarding the Senior Research Fellowship.

Nitrogen accumulation in grain legumes results from symbiotic nitrogen fixation as well as the uptake of nitrogen from soil. Egli *et al.* (1978) and Selamat and Gardner (1985) showed that pods competed with vegetative parts for nitrogen if N stress period was long, while no competition was evident during short period of stress. Most of the work on nitrogen partitioning and redistribution have been confined to pod formation and seed setting under N and water stresses. It is documented that partitioning and remobilisation of nitrogen was dependent on the growth of the species (Cure *et al.* 1985, De Vries *et al.* 1989). This experiment was performed because a short span of water stress of various intensities does occur during the growth period of this crop, therefore, relative competition among various plant parts for N during stress conditions was determined. Symbiotic nitrogen fixation and N of soil were included for supporting information.

Material and methods

Pigeonpea (*Cajanus cajan* L.) cultivars H-77-216 (indeterminate) and ICPL-151 (determinate) of the same duration were grown in earthenpots (diameter 30 cm) filled with 5.0 kg of sandy soil [with 72 mg(N) kg^{-1}(soil)] under natural conditions of greenhouse. The seeds before sowing were surface sterilized with 1 % of sodium hypochlorite solution and then inoculated with *Rhizobium* culture PH-8666. After thinning two plants per pot were maintained. The plants were supplied with N-free nutrient solution (Wilson and Reisenauer 1963) every two weeks. Water stress was created by withholding the irrigation at vegetative (40 DAS) and flowering (70 DAS) stage as described earlier (Nandwal *et al.* 1991a, b). The stress levels were described as moderate (SMC 5 ± 0.5 % and soil ψ_w -0.77 MPa) and severe (SMC 3 ± 0.5 % and soil ψ_w -1.34 MPa). The control plants were maintained at 50% of soil saturation capacity (20 %), corresponding to SMC 10 ± 0.5 % and soil ψ_w -0.37 MPa. At vegetative stage moderate and severe stress levels were identified at 2 and 3 d after withholding the irrigation whereas at flowering stage, the levels were identified at 3 and 5 d due to different temperatures during the respective periods. At vegetative stage the maximum and minimum temperature recorded were 39.4 and 28.2 °C whereas at flowering stage were 28.7 and 11.5 °C. The experiment was conducted under the average daily irradiance of 1135 ± 25 μmol m^{-2} s^{-1} and 980 ± 30 μmol m^{-2} s^{-1} at respective growth stages.

The plants were harvested when the soil of different sets of pots subjected to water stress for the moderate and severe stress at defined values of soil ψ_w and SMC. Harvested plants were separated into leaf, stem root, nodules and reproductive parts, and oven-dried at 85 °C.

The soil ψ_w was measured with *HR-33T Dew Point Hygrometer* (*Wescor*, USA) using sensor *PT-51-05*, whereas soil moisture content (SMC %) of mixed soil of the pot was determined gravimetrically. Half of the severe stressed plants were reirrigated and sampled after 2 d at soil ψ_w of -0.37 MPa to see the rehydrations. Nitrogen content was determined by Micro Kjeldhal's technique. Nitrogen distribution index (NDI) was calculated by modified methods Egli *et al.* (1978) and Selamat and Gardner (1985):

$$NDI = \frac{\text{N in part of treated plant} - \text{N in part of control plant}}{\text{N in the whole treated plant} - \text{N in whole control plant}} \times 100$$

Nodule SNA was determined by acetylene reduction assay (Nandwal *et al.* 1991 b). Available soil nitrogen was estimated by alkaline permanganate method (Subbiah and Asija 1956). The data from five replicates were analysed statistically to calculate the critical difference (C.D.) at P = 0.05.

Results and discussion

NDI at vegetative stage was different under different levels of water stress in two cultivars (Table 1). When control plants were shifted to moderate stress (soil ψ_w -0.37 to -0.77 MPa), the maximum gain of N was in nodules in cv. H-77-216, while in cv. ICPL-151, it was in leaf. Root lost the nitrogen in both the cultivars, but more in cv. ICPL-151. During severe stress (soil ψ_w -0.37 to -1.34 MPa) cv. H-77-216, lost N in leaf, root and nodules and in turn there was a slight accumulation in stem. Cultivar ICPL-151, again showed the highest value of NDI in leaf and loss of N in root and nodules.

Table 1. Effect of water stress on nitrogen distribution index (NDI) in pigeonpea cultivars.

Change in soil ψ_w [MPa] from	to	Cultivar	Leaf	Stem	Root	Nodules	Reproductive parts
Vegetative stage							
-0.37	-0.77	H-77-216	3.80	2.63	-2.92	89.47	-
		ICPL-151	119.34	0.70	-22.33	20.18	-
-0.37	-1.34	H-77-216	-12.69	3.40	-44.44	-46.33	-
		ICPL-151	110.77	0.83	-55.32	-4.73	-
-0.77	-1.34	H-77-216	65.65	-6.26	-146.00	-149.49	-
		ICPL-151	181.80	2.25	-100.00	-21.66	-
-1.34	-0.37	H-77-216	28.59	0.12	34.60	18.14	-
(rehydration)		ICPL-151	39.60	0.14	16.18	9.55	-
Flowering stage							
-0.37	-0.77	H-77-216	63.73	1.23	-20.51	34.43	7.44
		ICPL-151	-70.83	-1.33	-15.60	32.87	16.83
-0.37	-1.34	H-77-216	120.90	25.78	-547.15	-175.76	- 69.84
		ICPL-151	-8.78	-1.42	-77.51	-3.29	-3.87
-0.77	-1.34	H-77-216	-36.03	-0.91	-27.20	-14.97	15.53
		ICPL-151	19.72	-0.22	-53.93	-8.39	8.84
-1.34	-0.37	H-77-216	42.31	0.14	31.09	23.40	0.45
(rehydration)		ICPL-151	10.99	1.05	33.63	15.08	0.28

When the plants of moderate stress were shifted to severe stress (soil ψ_w -0.77 to -1.34 MPa), both the cultivars showed high NDI in leaf (Table 1). Cultivar H-77-216 showed very high N loss in root and nodules followed by stem, whereas in cultivar ICPL-151, NDI values of root and nodules were -100.00 and -21.66, respectively.

During plant rehydration (soil ψ_w -1.34 to -0.37 MPa) N was found to accumulate in roots and nodules in both the cultivars (Table 1). It is clear from the data that there was a loss of N from the root and nodules in the medium under stress conditions and this was more in cv. H-77-216 than cv. ICPL-151. The data of available soil nitrogen (Table 2) confirm the findings of N loss of root and nodules in the medium. After rehydration NDI shows gain in N in different plant parts, indicating that there was an uptake of N from the medium which resulted in decrease the available N content of soil (Table 2). The drying/decaying/shedding of nodules and root under stress conditions were the reasons for an increase the N of soil. Redistribution of N and increase in SNA during the moderate stress resulted in increase the NDI in nodules and leaf.

Table 2. Effect of water stress on specific nitrogenase activity (SNA) and nodule and soil nitrogen of pigeonpea cultivars.

Soil ψ_w [MPa]	Cultivar	SNA [μmol C_2H_4 g^{-1}(d.m.)s^{-1}] × 10^{-3}		Nodule nitrogen [mg $plant^{-1}$]		Soil nitrogen [mg N kg^{-1}(soil)]	
		vegetative	flowering	vegetative	flowering	vegetative	flowering
-0.37	H-77-216	6.6	8.5	5.93	9.49	67.0	40.0
	ICPL-151	9.3	7.1	6.46	8.69	64.0	39.0
-0.77	H-77-216	9.8	13.5	8.99	13.65	80.0	56.0
	ICPL-151	11.8	9.6	9.37	11.79	71.0	50.0
-1.34	H-77-216	0.8	1.0	7.51	12.18	97.0	92.0
	ICPL-151	0.8	0.8	7.55	9.23	87.0	91.0
-0.37	H-77-216	3.0	3.8	3.52	6.09	62.0	75.0
rehydration	ICPL-151	2.8	5.1	3.06	4.88	61.0	65.0
C.D. at 5 % level							
Stress		0.09	0.07	1.22	1.55	8.42	6.14
Cultivar		0.05	0.03	NS	NS	NS	NS
Interaction		0.14	0.11	NS	NS	NS	NS

At flowering stage, cv. ICPL-151 showed quite different response of NDI, then at vegetative stage. In addition to root, leaf and stem also showed loss of N, which was not at vegetative stage (Table 1) during the depletion of soil ψ_w from -0.37 to -0.77 MPa. Very high NDI for N loss was seen when plants were shifted from soil ψ_w -0.37 to -1.34 MPa in cv. H-77-216 for root and nodules. Cultivar ICPL-151 also showed all the negative values of NDI at this conditions (Table 1).

When the plants were subjected to severe stress (soil ψ_w -0.77 to -1.34 MPa) cv. H-77-216 showed the negative NDI except in reproductive parts, clearly indicating the loss of N from different plant parts. Similar results were noticed in cv. ICPL-151, except where leaf and reproductive parts showed some gain in N. The results obtained at flowering stage again showed the loss of N from the plant parts in medium which is confirmed from the Table 2. NDI values again showed positive response during rehydration with highest in leaf in cultivar H-77-216 and in root in cv. ICPL-151. This trend was almost reverse at vegetative stage.

SNA and nodule N (Table 2) were significantly enhanced under moderate stress at both the stages, but decreased sharply at severe stress. After rehydration the values of SNA were increased 3 - 6 times as compared to severe stress, but could not reach the value of control. During vegetative stage, cv. ICPL-151 showed significantly more SNA than H-77-216 but reverse was obtained at flowering. The interaction of stress × cultivar was also significant. The increase in SNA at moderate stress was due to an increase in nodule respiration (Nandwal *et al.* 1991b) and higher diffusion of O_2 in the medium (Pankhurst and Sprent 1975). A decrease in SNA under severe stress was accompanied by a concomitant decrease in nodule respiration (Nandwal *et al.* 1991b) and was not due to less supply of photosynthates (Durand *et al.* 1987, Nandwal 1991b). Nodules showed gain in N at moderate stress which was due to high SNA. As expected, where the control plants were grown, the available nitrogen of soil at vegetative stage was higher than at flowering (Table 2). The effect of water stress on the release of N to the soil was significant and at the severe stress values of available nitrogen during both the stages were almost equal. An increase of 44.7 % and 130 % in cv. H-77-216 and 35.9 % and 133 % in cv. ICPL-151 soil nitrogen as compared to control was estimated under severe stress at the respective stages. Slightly higher value of available nitrogen was observed for soil having cv. H-77-216 than cv. ICPL-216. High loss of N in severely stressed plant was due to drying/decaying/shedding of nodules and root as mentioned earlier. Negative NDI for nodules and root at both the stages (Table 1) confirm the findings of changes in soil nitrogen. Another possible reason could be a change in the permeability of root and nodules as reported earlier (Aggarwal and Sinha 1983). After revival decrease in soil nitrogen was due to its utilization for regrowth of the plant.

References

Aggarwal, P.K., Sinha, S.K.: Dynamics of roots and shoot growth during water stress and recovering in maize and cucumber seedlings. - Plant Physiol. Biochem. **10**: 95-106, 1983.

Chapman, A.L., Muchow, R.C.: Nitrogen accumulated and partitioned at maturity by grain legumes grown under different water regimes in a semi-arid tropical environment. - Field Crop Res. **11**: 69-79, 1985.

Cure, J.D., Raper, C.D., Patterson, R.P., Robarge, W.P.: Dinitrogen fixation in soybean in response to leaf water stress and seed growth rate. - Crop Sci. **25**: 52-58, 1985.

De Vries, J.D., Bennett, J.M., Boote, K.J., Albrecht, S.L., Maliro, C.E.: Nitrogen accumulation and partitioning by three grain legumes in response to soil water deficits. - Field Crop Res. **22**: 33-44, 1989.

Durand, J.L., Sheehy, J.E., Minchin, F.R.: Nitrogenase activity, photosynthesis and nodule water potential in soybean plants experiencing water deprivation. - J. exp. Bot. **38**: 311-321, 1987.

Egli, D.B., Leggett, J.E., Duncan, W.G.: Influence of N stress on leaf senescence and N redistribution in soybeans. - Agron. J. **70**: 43-47, 1978.

Nandwal, A.S., Bharti, S., Kuhad, M.S., Sheoran, I.S.: Water relations and gaseous exchange studies in pigeonpea under depleting soil water potential. - Plant Physiol. Biochem. **29**: 75-78, 1991a.

Nandwal, A.S., Bharti, S., Sheoran, I.S., Kuhad, M.S.: Drought effects on carbon exchange and nitrogen fixation in pigeonpea (*Cajanus cajan* L.). - J. Plant Physiol. **138**: 125-127, 1991b.

Pankhurst, C.E., Sprent, J.I.: Effect of water stress and the respiratory and nitrogen fixing activity of soybean root nodules. - J. exp. Bot. **26**: 287-304, 1975.

Selamat, A., Gardner, F.P.: Nitrogen partitioning uptake and redistribution in non-nodulating peanut related to nitrogen stress. - Agron. J. **77**: 859-862, 1985.

Sinclair, T.R., Muchow, R.C., Bennett, J.M., Hammond, L.C.: Relative sensitivity of nitrogen and biomass accumulation to drought in field grown soybean. - Agron. J. **79**: 986-991, 1987.

Sprent, J.I.: Water deficits and nitrogen fixing root nodules. - In: Kozlowski, T. (ed.): Water Deficits and Plant Growth. Vol. IV. Pp. 291-315. Academic Press, New York - London - Toronto 1976.

Subbiah, B.V., Asija, C.L.: A rapid procedure for the estimation of available nitrogen in soils. - Curr. Sci. **25**: 259-262, 1956.

Venkateswarlu, B., Maheswari, M., Saharan, N.: Effect of water deficit on $N_2(C_2H_2)$ fixation in cowpea and groundnut. - Plant Soil **114**: 69-74, 1989.

Weiz, P.R., Denison, R.F., Sinclair, T.R.: Response to drought stress of nitrogen fixation (acetylene reduction) rates by field grown soybean. - Plant Physiol. **78**: 525-530, 1985.

Wilson, D.O., Reisenauer, H.M.: Cobalt requirement of symbiotically grown alfalfa. - Plant Soil **19**: 364-373, 1963.

Communicated by J. POSPÍŠILOVÁ