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Fertilizer Induced Amelioration of Nitrogen Fixation under Moisture Stress in Chickpea

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Abstract: The investigations were carried out on chickpea (*Cicer arietinum* L.) cultivar HC-1 under pot culture with the objective to ameliorate the deleterious effects of water stress using phosphorus and potassium alone and in combinations at vegetative and reproductive stages. At both the stages, water stress-induced decline in relative water content (50-60%) decreased the nitrogen fixation in terms of ARA (40-47%) and nodule leghaemoglobin content (30-40%). Fertilizer applications considerably improved the RWC of leaf (upto 7%), N_2-ase activity (3-14%) and leghaemoglobin content of nodule (10-25%) under moisture deficit conditions. Phosphatic fertilizers proved better than potassium in ameliorating deleterious effects of water stress on nitrogen fixation. Moreover, their interaction effects were still better.

Key words: Chickpea, nodule leghaemoglobin, moisture deficit, N_2-ase activity, phosphorus, potassium.

Chickpea depend largely on the symbiotic N_2-fixation, a process very sensitive to water stress (Nandwal *et al.*, 1993; Swaraj *et al.*, 1995; Serraj *et al.*, 1997). Response of chickpea to phosphate application is very conspicuous and the growth, nodulation and nitrogen fixation significantly enhanced in plants (Singh, 1971). However, not much emphasis has been given on use of fertilizers in ameliorating deleterious effect of water stress on N_2-fixation. Therefore, present investigation was carried out to study stress-induced changes in nodulation and N_2-fixation, along with fertilizer (phosphorus/potassium) mediated drought amelioration in chickpea.

Materials and Methods

The present investigations were carried out on chickpea (*Cicer arietinum* L.) cv. HC-1. The crop was raised in earthen pots (30 cm dia. and 30 cm depth) lined with polythene bags and filled with 5 kg dune sand. The dune sand was Typic Torrispamments containing 93.3% sand, 3.0% silt, 3.7% clay, 0.06% OC, 10.3 ppm available N, 2.5 ppm available P and 18 ppm available K. The seeds after inoculation with *Rhizobium* culture, strain Ca-181 @ 1 ml/5 seeds, were sown on 3rd November, 1998. *Rhizobium* were procured from the Department of Microbiology, CCS Haryana Agricultural University, Hisar. Each pot had three plants.

After germination potassium and phosphorus were supplied through the rooting medium in the form of muriate of potash and single super phosphate (SSP). Control = without fertilizer, Potassium (K_2O) = 50 kg ha^{-1}, Phosphorus (P_2O_5) = i) 40 kg ha^{-1}, ii) 50 kg ha^{-1}, interactions

(K+P) = i) 50 kg K ha^{-1} + 40 kg P ha^{-1}, ii) 50 kg K ha^{-1} + 50 kg P ha^{-1}. A basal dose of 20 kg N ha^{-1} was given to all pots. The plants were irrigated with canal water at regular interval. Stress was created by withholding irrigation at vegetative (50 DAS) and reproductive stages (95 DAS). The plants were sampled at PWP (obtained at 7 days and 5 days after withholding irrigation at vegetative and reproductive stage, respectively) for various observations. Stressed plants were revived by irrigating plants and sampled after two days. Relative water content (RWC) was calculated following Weatherley (1950). N_2-ase activity was measured by acetylene reduction assay (Hardy *et al.*, 1968) and nodule leghaemoglobin was estimated by the method suggested by Hartree (1955). The data collected were analyzed using factorial completely randomized design (CRD). To compare the treatments, critical difference (CD) at 5% level of significance was used.

Results and Discussion

The leaf RWC decreased progressively with the ageing of plants (Table 1). The RWC declined under water stress by 60% at reproductive and 54% at vegetative stage. There was marginal revival upon re-watering of the plants. The decrease in RWC under water stress has been reported earlier in leaves of *Brassica* (Asharaf and Mahmood, 1990) and pigeonpea (Jaidayal *et al.*, 1993). However, fertilizer application improved the RWC under water stress as well as normal conditions. This improvement with fertilizer might be due to reduction in water loss coupled with increased efficiency of roots to extract more water from deeper soil layers. Increase in RWC, water potential and water retention capacity with application of potassium in combination with phosphorus and nitrogen was observed by Kuhad *et al.* (1997).

Table 1. Effect of water stress on RWC (%) in leaves in chickpea (HC-1) grown at different K and P levels

	Control	K_{50}	P_{40}	P_{50}	$K_{50}+P_{40}$	$K_{50}+P_{50}$	Mean
Vegetative stage							
Control	70.5	71.0	71.5	71.3	73.0	74.3	71.9
Stress	32.2	33.5	35.0	35.6	36.5	38.0	35.1
Revival	65.8	68.0	69.0	69.8	71.0	71.5	69.2
Mean	56.8	57.5	58.5	58.9	60.1	61.2	
Reproductive stage							
Control	65.5	64.2	65.2	65.2	65.8	66.8	65.2
Stress	25.0	26.4	26.4	26.8	27.2	28.3	26.7
Revival	59.4	59.9	61.4	63.1	63.2	64.2	61.8
Mean	49.3	50.2	51.0	51.8	52.1	53.0	

CD at 5%; S = 1.262, T = 1.785, S x T = N.S.
S = 0.921, T = 1.303, S x T = N.S.; S = Stress level, T = Treatment.

Decreased RWC of leaves and soil moisture availability, decreased the nodule development. Drastic reduction (47%) in

Table 2. Effect of water stress on nodule dry weight (mg per plant) in chickpea (HC-1) grown at different K and P levels

	Control	K_{50}	P_{40}	P_{50}	$K_{50}+P_{40}$	$K_{50}+P_{50}$	Mean
Vegetative stage							
Control	85	84	87	92	98	105	92
Stress	45	56	59	62	68	72	60
Revival	53	68	75	77	79	89	74
Mean	61	69	74	77	82	89	
Reproductive stage							
Control	258	265	264	269	272	281	268
Stress	156	165	168	172	175	196	172
Revival	168	179	182	189	192	216	188
Mean	194	203	205	210	213	231	

CD at 5%; S = 1.262, T = 1.785, S x T = N.S.
S = 0.921, T = 1.303, S x T = N.S.; S = Stress level, T = Treatment.

nodule dry weight was recorded under water deficit conditions. On revival, only partial recovery was observed (Table 2). Reduction in nodule number and weight in soybean under drought was observed earlier by Sinclair *et al.* (1988). Fertilizer application improved the dry weight of nodules under water stress. Moreover, this increase in dry weight was considerably higher with phosphorus treatment in comparison to potassium. Phosphorus increased nodulation and subsequently dry matter accumulation under water stress. Kulkarni *et al.* (1986) reported that phosphorus application maintained nodulation, plant dry weight and nitrogen content in groundnut. In the present study, the increase was markedly greater at vegetative stage than at reproductive stage irrespective of the fertilizer used, because at reproductive stage, assimilates are transported more towards the reproductive organs in comparison to nodules. Besides this, senescence of nodules also starts at reproductive stage. Application of P and K together enhanced the dry weight of nodules. Earlier, Singh *et al.* (1993) also reported significant increase in dry weight of nodules and total dry weight of roots with phosphatic fertilizers.

Acetylene reduction assay (ARA) of nodules (mm^3 C_2H_4 evolved kg^{-1} of nodule dry weight s^{-1}) showed a marked decrease (upto 47%) under water stress. This decrease was more at reproductive stage. On revival, there was partial recovery (Fig. 1). Drought has been shown to decrease N_2-fixation rate when measured as ARA in chickpea (Swaraj *et al.*, 1995). More inhibition in N_2-ase activity at reproductive stage pointed to the inadequate supply of photosynthates to nodules in addition to reduced photosynthesis (Kuhad and Sheoran, 1982), nodule water potential (Durand *et al.*, 1987) or accumulation/utilization of soluble nitrogen content of nodules (Nandwal *et al.*, 1991). Fertilizer application enhanced the nitrogenase activity (upto 15%). Phosphorus application, particularly at 50 kg ha^{-1}, was more beneficial than potassium

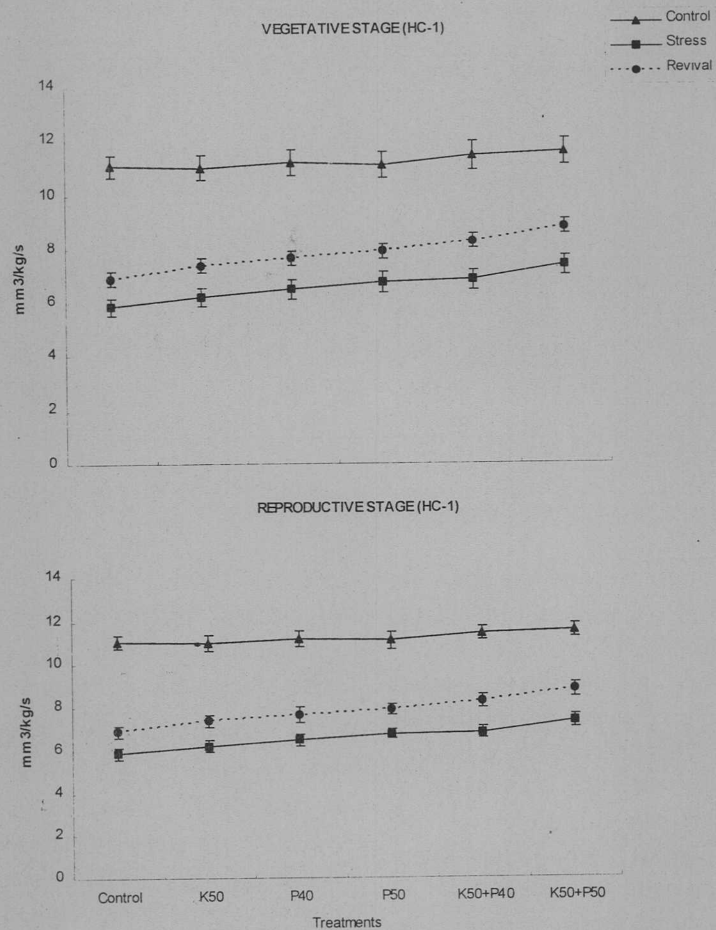

Fig. 1. Effect of water stress and response to fertilizer application on Acetylene Reduction Assay in chickpea cultivar (HC-1).

under water stress condition. However, improvement in ARA was more when P and K were applied together. There was marked reduction (upto 45%) in nodule leghaemoglobin content under water stress condition (Fig. 2), but upon revival, it marginally increased. The leghaemoglobin content was more at vegetative stage than at reproductive stage. Water stress induced onset of nodule senescence may probably be responsible for decline in leghaemoglobin content and consequently inhibition of N_2-fixation. Significant increase in leghaemoglobin content of nodules was observed with fertilizer treatment. Here again phosphorus had an edge over potassium in improving nodule leghaemoglobin under water stress condition at both the stages. However, the interactions were still better than individual fertilizer treatments. Potassium and phosphorus facilitate in improving functional efficiency

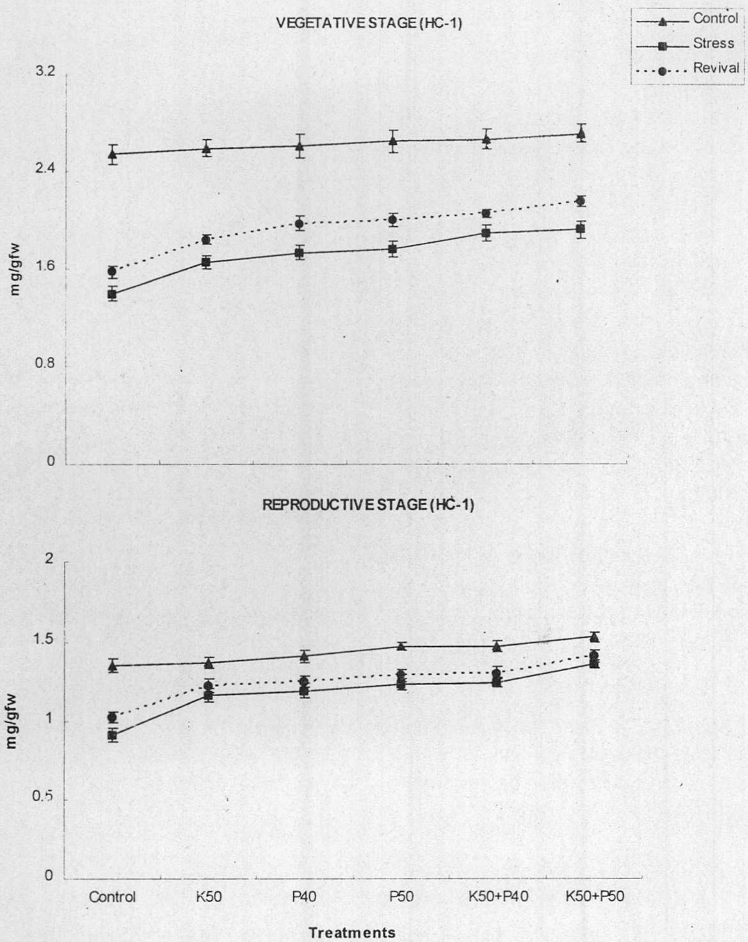

Fig. 2. Effect of water stress and response to fertilizer application on nodule leghaemoglobin content in chickpea cultivar (HC-1).

of nodulated roots and osmotic adjustment under water deficit conditions.

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