BRIEF COMMUNICATION

Water relations and nitrogen fixation in potassium fed *Vigna radiata* nodules

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Abstract

Drought created by withholding the irrigation at 30 and 45 d after sowing significantly decreased relative water content (RWC) and osmotic potential (ψ_s) of *Vigna radiata* (L.) Wilczek cv. MH-83-30 nodules. Potassium fed plants showed higher RWC, whereas ψ_s was further declined irrespective of soil moisture levels. The nitrogenase activity and leghemoglobin content of nodules markedly decreased under drought and nodules of potassium fed plants showed better recovery after rehydration. The proline content significantly increased under drought but declined upon reirrigation. Also, the C, N and K contents of nodules significantly declined under drought.

Additional key words: carbon, drought, nitrogen, potassium, proline, rehydration.

The osmotic adjustment is important for water uptake and growth in plants under water stress conditions (Girma and Krieg 1992, Bordeleau and Prevost 1994, Volaire and Thomas 1995). Increased solute concentration, particularly, K in leaf of alfalfa contributes to osmotic adjustment in NO_3^--fed plants (Antolín and Sánchez-Diaz 1992, Antolín *et al.* 1995). However, the work on water relations and stress metabolites is mainly confined to the leaves rather than nodules of leguminous crops, which are intolerant to water stress (Antolín and Sánchez-Diaz 1992, Sprent 1992). Mungbean is generally grown under rainfed conditions and subjected to frequent drought. Therefore, the present investigation was aimed at understanding the role of added K on nodule water status and N_2- fixation under water stress.

The plants of mungbean [*Vigna radiata* (L.) Wilczek] cv. MH-83-30 were raised in earthen pots with dune sand in greenhouse. Before sowing the seeds were surface

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sterilized with 80 % ethanol and then inoculated with *Rhizobium* sp. S 24. Each pot (2 plants pot^{-1}) received equal amount of N-free nutrient solution at regular intervals. Potassium was added in the form of KCl at 0 (K_0), 2.56 (K_1) and 3.84 mmol dm^{-3} (K_2) (0.65 mmol dm^{-3} K was in dune sand). The soil saturation capacity of dune sand was 25 %. The control plants were grown at soil moisture content (SMC) 12.0 ± 0.5 %. Drought was created by withholding the irrigation at vegetative [30 d after sowing (DAS)] and at flowering (45 DAS) stages. Half of the stressed plants (SMC 3.5 ± 0.5 %) were re-irrigated to SMC equal to control and plant recovery was seen after 2 d.

The osmotic potential (ψ_s) of expressed tissue sap was measured with *Vapor Pressure Osmometer* (*Model 5100-B*, *Wescor*, Logan, USA). The relative water content (RWC) of nodules was determined by relative turgidity technique. Total nitrogenase (N_2-ase; EC 1.18.2.1) activity of nodules was measured by acetylene reduction assay of Hardy *et al.* (1968). The extraction of the leghemoglobin (Lb) was done in 0.1 M phosphate buffer (Nandwal *et al.* 1991) and was estimated by the method of Hartree (1955). The proline content of nodules was extracted in 3 % sulfosalicylic acid and determined according to Bates *et al.* (1973). 100 mg of powdered dry mass nodules was taken for the estimation of C and N contents using the standard procedures and K content by flame photometry.

To analyze the data statistically complete randomized design (2-factorial) was used and the significance of critical difference (CD) was tested at 5 % level.

Drought significantly decreased RWC and ψ_s of nodules. Upon rehydration the nodules of the untreated and K-fed plants showed recovery of RWC and ψ_s, however, these values were lower than their respective controls (Table 1). These results support the findings of Maribona *et al.* (1992) and Nandwal *et al.* (1993) in pea and pigeonpea, respectively. Under drought decrease in ψ_s of nodules was accompanied by the accumulation of proline. K-fed plants also showed increase in K and C contents (Table 1). Addition of K enhanced RWC of nodules, but decreased their ψ_s. Thus accumulation of K in nodules contributes in maintaining the nodule water status during drought. Antolín and Sánchez-Diaz (1992) and Antolín *et al.* (1995) also reported similar increase in K concentration in NO_3^--fed and N_2-fixing alfalfa plants and suggested that K may contribute to osmotic adjustment in leaf.

Under drought N_2-ase activity of nodules decreased considerably (Table 1). Such inhibitory effect of moisture stress on N_2-ase activity has also been reported earlier by Nandwal *et al.* (1991) and Swaraj *et al.* (1995) in pigeonpea and chickpea, respectively. K-fed plants maintained higher total N_2-ase activity of nodules (Table 1) under control as well as drought conditions.

On rehydration, the K-fed plants showed better improvement in N_2-ase activity. Marked decrease in leghemoglobin content (Lb) was observed under drought (Table 1), as was also reported by Bordeleau and Prevost (1994). It has been suggested that water stress triggers the synthesis of component II and degradation of component I of leghemoglobin protein (Swaraj *et al.* 1995). Change in ratio of two components of Lb effects O_2 binding capacity of Lb and thus, adversely affects N_2-fixation. During recovery Lb content further declined. The Lb content of K-fed plants was higher.

Table 1. Effect of K on dry mass, RWC, ψ_s and proline, C, N, K and leghemoglobin contents and N_2-ase activity of mungbean nodules under water stress and recovery.

		30 DAS control	stress	recovery	45 DAS control	stress	recovery
Dry mass	K_0	36	12	8	49	29	17
[mg plant^{-1}]	K_1	50	16	11	62	35	25
	K_2	49	32	12	59	39	23
C.D. at 5 %		K = 7.3	S = 7.3	K×S = 12.5	K = 8.1	S = 8.1	K×S = 13.7
RWC	K_0	92.20	69.12	81.77	89.54	65.77	80.64
[%]	K_1	95.41	69.88	86.93	92.52	67.46	82.40
	K_2	93.59	71.64	86.41	92.40	68.21	84.86
C.D.		K = 1.55	S = 1.55	K×S = 2.69	K = 2.02	S = 2.02	K×S = 3.51
ψ_s	K_0	1.02	2.03	1.50	1.21	2.34	1.72
[-MPa]	K_1	1.14	2.18	1.35	1.24	2.66	1.73
	K_2	1.14	2.24	1.46	1.25	2.79	1.71
C.D.		K = 0.09	S = 0.09	K×S = 0.16	K = 0.06	S = 0.06	K×S = 0.09
Proline	K_0	891.51	1667.79	1163.54	488.22	6204.09	1132.90
[µg g^{-1}(d.m.)]	K_1	705.19	1574.89	1014.51	455.94	6013.12	1022.70
	K_2	583.24	1595.45	912.96	432.40	5368.25	1041.41
C.D.		K = 78.38	S = 78.38	K×S = 135.8	K = 295.37	S = 295.37	K×S = 487.5
Carbon	K_0	0.324	0.187	0.222	0.286	0.198	0.217
[g g^{-1}(d.m.)]	K_1	0.356	0.209	0.256	0.326	0.214	0.241
	K_2	0.356	0.197	0.265	0.319	0.214	0.239
C.D.		K = 0.027	S = 0.027	K×S = 0.048	K = 0.023	S = 0.023	K×S = 0.034
Nitrogen	K_0	0.067	0.036	0.027	0.042	0.025	0.033
[g g^{-1}(d.m.)]	K_1	0.072	0.058	0.036	0.056	0.036	0.044
	K_2	0.075	0.050	0.043	0.063	0.037	0.048
C.D.		K = 0.011	S = 0.011	K×S = 0.019	K = 0.01	S = 0.01	K×S = 0.017
Potassium	K_0	0.103	0.053	0.027	0.081	0.061	0.046
[g g^{-1}(d.m.)]	K_1	0.140	0.078	0.036	0.099	0.070	0.067
	K_2	0.137	0.078	0.043	0.113	0.091	0.070
C.D.		K = 0.021	S = 0.021	K×S = 0.037	K = 0.011	S = 0.011	K×S = 0.020
Leghemoglobin	K_0	14.2	6.4	5.2	12.8	7.2	7.0
[mg g^{-1}(d.m.)]	K_1	18.4	7.2	5.4	14.0	8.4	7.8
	K_2	18.8	7.9	7.0	15.6	9.8	8.7
C.D.		K = 1.4	S = 1.4	K×S = 2.3	K = 1.2	S = 1.2	K×S = 2.1
N_2-ase	K_0	174.1	7.6	10.7	142.1	9.2	33.6
[pmol(C_2H_4)	K_1	242.1	10.7	21.3	155.8	16.8	48.9
plant-1(d.m.) s-1]	K_2	233.7	15.3	19.7	160.4	30.11	47.4
C.D.		K = 12.2	S = 12.2	K×S = 23.9	K = 10.7	S = 10.7	K×S = 19.7

K - effect of potassium, S - effect of stress, K×S - interaction

Nodule proline content significantly increased under drought (Table 1). However, this gain was lost upon rewatering. The lower level of proline in K-fed plants may be ascribed to their better water status.

The contents of C, N and K of nodules significantly declined under drought, but the nodules of K-fed plants maintained higher level of these elements irrespective of soil moisture contents, than the untreated plants (Table 1). The N and K contents in nodules further declined after re-irrigation, however, the C content of nodules increased as found earlier by Nandwal *et al.* (1992, 1996) in pigeonpea. Application of K increased the N content of nodules due to the increase in N_2-ase activity of nodules.

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