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BRIEF COMMUNICATION

Nodule functioning in trifoliate and pentafoliate mungbean genotypes as influenced by salinity

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Abstract

Two genotypes of mungbean differing in the leaf shape, K-851 (trifoliate) and a mutant (pentafoliate), were exposed to salinity (0, 2.5, 5, 10 dS m^{-1}) for 3, 6 and 9 d at flowering stage (40 - 45 d after sowing) to see the effect of salinity on nodule functioning. In both the genotypes, osmotic potential (ψ_s) and relative water content (RWC) of nodules decreased significantly, and proline content increased with increasing the salinity. The ψ_s of nodules was more decreased in the mutant than in genotype K-851, while reverse was true for RWC and proline accumulation. A remarkable increase in ethylene evolution was noticed from nodulated roots with the increasing level and duration of salinity and was much higher in K-851. A sharp decline in leghemoglobin content and acetylene reduction assay (ARA) of the nodules was observed with the salinity and this decrease was more marked in K-851 than the mutant. N content declined while Na$^+$/K$^+$ ratio and Cl$^-$ content increased significantly. The mutant maintained better N status but lower Na$^+$/K$^+$ ratio and Cl$^-$content in nodules than K-851. Nodule dry matter also declined with salinity and the decrease was more expressed in K-851. Thus the functioning of nodules in the mutant was better than in the genotype K-851 under stress conditions.

Additional key words: ethylene, minerals, mutant, N$_2$-fixation, nodule water status, *Vigna radiata*.

Salt stress is a major constraint that limits crop production in a large part of world, especially when nitrogen nutrition in plants depends upon symbiotic fixation of atmospheric nitrogen (Abd-Alla *et al.* 1998). Therefore the possibility of introducing plants capable of survival under these conditions is worth to be investigated. The necessity to use saline water for irrigation also requires a understanding of plant salt tolerance (Cordovilla *et al.* 1995). Ionic and osmotic effects have been identified as possible causes of salt toxicity in various crops (Greenway and Munns 1980). The salt tolerance may vary with plant species and also during plant ontogeny (Ashraf and Waheed 1993). Mungbean is one of the major pulse crop cultivated on a large scale in arid and semi-arid regions. Since more information is available on responses of plant shoots than of the underground parts to salinity, we put an emphasis on testing function of mungbean nodules as affected by single saline irrigation.

Two phenotypically different genotypes, trifoliate (K-851) and a pentafoliate (mutant) were raised in earthen pots (30 cm in diameter) lined with polyethene bags each containing 5 kg of dune sand [93.3 % sand + 3.0 % silt + 3.7 % clay, saturation capacity 25 %, pH 8.2, EC 0.8 dS m^{-1} at 25 °C, 10.3 mg(N) kg^{-1}, 2.5 mg(P) kg^{-1}, 18.0 mg(K) kg^{-1}]. Before sowing the surface sterilized seeds were inoculated with peat-based suitable *Rhizobium* strain (S-24) obtained from the Department of Microbiology, CCS HAU, Hisar. After germination, two

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Abbreviations: ARA - acetylene reduction assay; DAS - days after sowing; DAT - days after treatment; d.m. - dry matter; RWC - relative water content; ψ_s - osmotic potential.

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plants per pot were maintained. Before salinity treatments, whenever needed, the pots were irrigated from the surface only. However, after the salinity treatments, they were irrigated by 50 % from the surface and by 50 % from the subsurface through a slightly inclined embedded plastic feeder tube having a pad of glass wool at the lower end. In this way, uniform required EC was maintained. Each pot was supplied with equal quantity of N free nutrient solution at a regular interval of 15 d.

The chloride dominated salinity solution was prepared by using mixture of NaCl, MgCl, $MgSO_4$ and $CaCl_2$, where Na:Ca+Mg was in the ratio of 1:1, Ca:Mg in the ratio of 1:3 and Cl:SO_4 in the ratio 7:3 on meq basis and applied at flowering stage (40 - 45 d after sowing). Desired salinity (0, 2.5, 5.0, and 10 dS m^{-1}) was established. The sampling was done at 3, 6 and 9 d after treatments (DAT).

The osmotic potential (ψ_s) of nodules was determined with vapour pressure osmometer (*Model 5100-B*, Wescor, Logan, USA). The relative water content (RWC) of nodules was measured according to Weatherley (1950). Nitrogenase activity of nodules was measured as acetylene reduction assay (ARA) described by Hardy *et al.* (1968) and expressed as nmol(C_2H_4 produced) g^{-1}(d.m.) s^{-1}. For ethylene evolution, the excised nodulated roots were incubated in dark at 25 ± 1 °C in air-tight vials of 50 cm^3 with subaseal and containing a wet pad of cotton to maintain high humidity (Fearn and La Rue 1991). One cm^3 of gas sample from each vial was taken and assayed for ethylene production by gas chromatograph (*Nucon 5700*, Delhi, India). For dry matter determination the nodules were oven dried at 85 °C to a constant mass. Leghemoglobin (Lb) content in detached and washed nodules was estimated according to Hartree (1955). Free proline of nodules was estimated spectrophotometrically according to Bates *et al.* (1973). The sodium, potassium, nitrogen, and chloride contents of nodules were determined in the oven dried ground material. The material was digested in 5 cm^3 of H_2SO_4 and $HClO_4$ (9:1) mixture and diluted to the desired volume. Sodium and potassium contents were estimated using Flame Photometer (*Model CL26D*, *Elico*, Delhi, India). The total nitrogen was estimated by micro Kjeldahl technique. Cl content was estimated by an ion analyser (*Model LI-126*, *Elico*, Delhi, India).

The experiment was completely randomized according to a 4 × 2 × 3 factorial design with three replicates per treatment. The data were statistically analysed and significance was tested at 5 % level of critical difference (CD).

Increasing salinity resulted in a considerable decrease of osmotic potential (ψ_s) and RWC, irrespective of genotypes, and this effect was more pronounced as the duration of salinity exposure increased from 3 to 9 DAT. The mutant showed more negative values of ψ_s than K-851 (Table 1). However, nodules of mutant maintained higher RWC than those of K-851 (Table 1). Similar results were reported by Abd El-Samad and Shaddad (1997) in soybean.

Proline content of nodule increased with increasing salinity. At salinity 10 d Sm^{-1} and 9 DAT, proline content increased from 54.9 to 80.6 % in the mutant and from 44.0 to 74.4 % in K-851 as compared to their respective controls (Table 1). The concentration of proline was widely documented to increase in response to salt stress (*e.g.* Hamada and Khulaef 1995, Zayed and Zeid 1998), however, its role under salt stress has been not satisfactorily elucidated. From 3 to 9 DAT, the mutant accumulated more proline than K-851. This contributed to higher osmoregulation (lower ψ_s at the same RWC) in the mutant in comparison with K-851.

An increased salinity induced ethylene evolution. At 10 dS m^{-1}, rate of ethylene evolution increased about 3 to 7 fold in the mutant and 5 to 11 fold in the K-851 depending upon the duration of exposure (Table 1). Ethylene production showed a positive correlation with the level of salt stress and its duration thus might be used as a parameter for the evaluation of salt resistance in crop plants. In conformity, Feng and Barker (1992) advocated that increasing salt concentrations gradually raised ethylene production rates which appeared to be a factor in promoting early senescence of plant tissues. Genotype (K-851) showed significantly higher ethylene production than the mutant, also suggesting that nodule water status plays key role in ethylene production under saline conditions.

Nitrogenase activity in nodules in terms of acetylene reduction assay (ARA) decreased about 3 to 6 times in the mutant and 3 to 10 times in the K-851 at 10 dS m^{-1} depending upon the duration of exposure to salinity (Table 1) and proved the mutant to be comparatively more tolerant than K-851 in this respect. Earlier studies have described a correlation between the inhibition of N_2-fixation and reduction in leghemoglobin content of nodules (Abd-Alla *et al.* 1998). Serraj *et al.* (1994) concluded that such adverse effects were associated with the change in O_2 permeability of nodule. In addition to change in O_2 permeability and leghemoglobin content, it was suggested that better nodule water status achieved through osmoregulation also improves ARA in nodules under salt stress (Fernandez-Pascual *et al.* 1996). In our experiment, the mutant had higher nodule dry matter as compared to the K-851 (Table 1) indicating tolerance of the nodulation in the former to salt stress. The above finding suggests the possibility of a compensatory mechanism which enables partially mitigate the imbalance in N_2-fixation. On the other hand, the indirect effects of the higher ethylene production in K-851 may be an additional factor decreasing nodulation and N_2-ase activity consistently to the earlier reports (Fearn and La Rue 1991, Rawal *et al.* 1995).

Leghemoglobin content was reduced under salinity by 12.4 to 83.3 % and by 14.9 to 91.3 % in the mutant and K-851, respectively (Table 1). The differences between the genotypes were not statistically significant.

Table 1. Effect of salinity (0, 2.5, 5.0, and 10.0 dS m^{-1}) on osmotic potential (ψ_s) [-MPa], RWC [%], proline content [μg g^{-1}(d.m.)], ethylene production [pmol(C_2H_4) g^{-1}(d.m.) s^{-1}], N_2-ase activity [nmol(C_2H_4) g^{-1}(d.m.) s^{-1}], leghemoglobin content [mg g^{-1}(d.m.)], dry mass [mg plant^{-1}], N content [mg g^{-1}(d.m.)], Na^+/K^+ ratio, and Cl^- content [μmol g^{-1}(d.m.)] in nodules of *Vigna radiata* L.

		3 DAT				6 DAT				9 DAT			
		0	2.5	5.0	10.0	0	2.5	5.0	10.0	0	2.5	5.0	10.0
ψ_s	mutant	1.08	1.26	2.17	2.54	1.13	1.97	2.01	2.49	1.08	2.06	2.19	2.37
	K-851	1.04	1.20	2.09	2.19	1.04	1.73	2.23	2.23	1.16	1.86	2.11	2.09
RWC	mutant	91.42	89.23	85.77	85.12	91.02	90.64	86.81	83.39	92.79	90.87	83.44	81.18
	K-851	90.50	88.24	84.67	83.65	90.50	88.16	85.70	77.85	90.17	88.29	80.39	77.32
Proline	mutant	364	446	524	564	364	490	522	680	346	547	571	625
	K-851	334	397	437	481	345	453	491	576	328	505	555	572
Ethylene	mutant	3.46	5.11	5.31	9.55	3.36	5.11	8.02	23.89	3.19	5.07	9.74	24.74
	K-851	4.31	6.24	14.61	22.19	4.06	5.42	15.08	34.81	3.72	5.02	20.21	42.83
N_2-ase	mutant	7.24	5.03	3.39	2.30	7.11	4.31	2.88	1.46	6.14	3.01	1.91	1.09
	K-851	5.05	3.40	2.98	1.70	4.52	2.63	1.76	1.18	3.99	1.81	1.24	0.39
Lb	mutant	13.73	12.04	10.53	6.76	15.22	10.68	8.37	6.12	12.63	5.90	4.25	2.10
	K-851	12.38	10.85	10.30	6.17	12.57	9.72	8.15	4.03	12.65	5.63	3.14	1.08
Dry mass	mutant	93	89	82	72	103	96	87	79	114	101	95	85
	K-851	86	78	73	67	91	82	79	69	105	89	84	72
N	mutant	57.1	56.4	52.7	42.3	57.1	52.4	52.1	39.4	57.3	47.0	45.7	37.1
	K-851	52.7	45.2	40.7	32.8	47.4	40.1	39.5	31.3	41.5	31.1	29.1	23.6
Na^+/K^+	mutant	0.36	0.50	0.66	0.81	0.39	0.67	0.76	0.64	0.41	0.72	0.77	0.74
	K-851	0.34	0.59	0.73	0.80	0.40	0.81	0.81	0.72	0.39	0.76	0.85	1.01
Cl^-	mutant	112	151	250	404	119	172	272	430	117	191	306	492
	K-851	122	158	259	427	127	171	295	447	120	202	331	519

CD at 5 %:	ψ_s	RWC	Proline	Ethylene	N_2-ase	Lb	Dry mass	N	Na^+/K^+	Cl^-
Salinity	0.07	1.75	16.16	1.64	0.58	0.43	4.73	1.6	0.11	15.7
Genotype	0.05	0.91	11.43	1.16	0.41	NS	3.87	1.1	0.07	11.3
Interaction	0.08	1.98	22.86	2.31	0.83	0.61	6.82	2.2	0.13	21.1

A parallel rise in Na^+/K^+ ratio in nodules was observed with the increasing salinity and with the duration of exposure. These results are in agreement with the findings of Velagaleti *et al.* (1990), Manchanda *et al.* (1991), Sharma (1996), and Zayed and Zeid (1998) that Na^+ content in different plant parts increased, whereas that of K^+ content decreased under salinity. It was also observed in lupin nodules under salt stress (Fernandez-Pascual *et al.* 1996). Overall, K-851 showed significantly higher Na^+/K^+ under salinity than the mutant (Table 1). An increase in Cl^- content in nodules with increasing level and duration of salinity was also observed and it was higher in K-851 than in the mutant (Table 1). The ability of the mutant to restrict Na^+ and Cl^- accumulation correlated with the salinity tolerance according to Noble and Rogers (1992).

Nitrogen content of nodules was found to decrease with increasing level of salinity. Decrease in N content of mutant nodules at 10 dS m^{-1} was from 25.94 to 35.21 % whereas in K-851 37.76 to 43.28 % depending upon the duration of exposure to salinity (Table 1). Our results are in agreement with earlier studies conducted on various legumes (Hafeez *et al.* 1988, Zayed and Zeid 1998). The observed decrease in N_2-ase activity of nodules under salinity, could be the reason for low N content. However, the possibility of enhanced leaching of free amino acids due to changes in permeability of nodules cannot be ruled out.

Saline irrigation resulted in considerable decrease in dry matter of nodules per plant and this reduction was 4.3 to 25 % in the mutant and 9.3 to 31 % in K-851 at 10 dS m^{-1} from 3 DAT to 9 DAT (Table 1). However, in salt treated plants the dry matter accumulation in the existing nodules continued to increase but comparatively less than control.

It is concluded that functioning of nodules in the pentafoliate mutant is relatively better under salinity as compared to the trifoliate K-851 owing to better nodule water status, biomass production, N_2-ase activity, N content and lower Na^+/K^+ ratio, Cl^- concentration, and ethylene production.

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