Research Article

Genotypic differences in water status, membrane integrity, ionic content, N_2-fixing efficiency and dry matter of mungbean nodules under saline irrigation

Neeraj Kumar[1], Sunder Singh[1], Ajit Singh Nandwal[1], Radhey Shyam Waldia[2] and Manoj Kumar Sharma[3]

[1]*Department of Botany and Plant Physiology*
[2]*Department of Plant Breeding*
[3]*Department of Soil Science, CCS Haryana Agricultural University, Hisar - 125 004, Haryana, India*

ABSTRACT

A pot experiment was conducted under natural conditions of screen house to evaluate the effect of saline irrigation given at flowering stage (30-35 DAS) on nodule functioning and their tolerance in two mungbean genotypes viz. Asha and Muskan based on various physiological traits. The pots containing sandy soil (*Typic Torrispamments*) were saturated with Cl^- dominated saline irrigation to maintain ECe of 2.5, 5.0, 7.5 dS m^{-1} as compared to control. In both the genotypes osmotic potential (Ψs) and relative water content (RWC %) of nodules decreased significantly, while a sharp rise in proline and total soluble sugars contents were observed with the increasing level of saline irrigation after 10 and 20 days of treatment. A marked increase in hydrogen peroxide (H_2O_2), lipid peroxidation (MDA content) and relative stress injury (RSI %) was noticed in nodules which were much higher in Muskan. The decrease in Ψs of nodules was more pronounced in Asha than in Muskan, while reverse was true for RWC and proline accumulation. A sharp decline in acetylene reduction assay (ARA) for N_2-fixation, leghemoglobin content and dry matter of the nodules was observed, but was more in Muskan than in Asha. Nitrogen (N) content declined while Na^+/K^+ ratio and Cl^- content increased significantly. The genotype Asha maintained better N_2 -fixing efficiency but lower Na^+/K^+ ratio and Cl^- content in nodules than Muskan. Though the nodule functioning was further deteriorated at 20 DAT in both the genotypes yet the tolerance capacity of nodules in Asha was better than in Muskan under saline conditions which is correlated with the compensatory mechanism i.e. osmoregulation in nodules. **[Physiol. Mol. Biol. Plants 2008; 14(4) : 363-368]** *E-mail : nandwal@hau.ernet.in*

Key words : *Leghemoglobin, Lipid peroxidation, Minerals, N_2 –ase activity, Vigna radiata, Water relations*

Abbreviations : *ARA - Acetylene reduction assay, DAT - Days after treatment, DM - Drymatter, H_2O_2 - Hydrogen peroxide, LHb - Leghemoglobin, MDA - Malondialdehyde, Ψs - Osmotic potential, RSI - Relative stress injury, RWC - Relative water content, TBA - Thiobarbituric acid*

Soil salinity is a major abiotic stress in agriculture worldwide. Its deleterious effects on plants, in general, are attributed to increase in osmotic pressure of rooting medium, ion toxicity and ionic imbalance. Salinity-caused reduction in growth is the consequence of alterations in several physiological processes (Nandwal *et al*., 2000 a, b; Kukreja *et al.,* 2005; Farooq and Azam, 2006) including nodulation and N_2-fixation in various legumes (Nandwal *et al*., 2007). As a consequence of these primary effects, secondary stresses such as oxidative damage often occurs (Bartels, 2001; Sairam *et al*., 2002; Kukreja *et al.,* 2006; Nandwal *et al*., 2007). Plants have evolved diverse strategies of acclimatization and avoidance to cope with adverse environmental conditions. These include accumulation of compatible solutes like proline, glycinebetaine and total soluble sugars.

In arid and semi-arid regions, insufficient precipitation results in extensive reliance on irrigation and a considerable proportion of underground water in most of these regions is of poor quality. Mungbean is an important pulse crop, but owing to poor quality of water/ soil, the productivity of this crop is not optimal under such conditions. Selection and breeding programmes to

Correspondence and Reprint requests : Ajit Singh Nandwal
Fax : (+91) 1662-234952

increase salt tolerance will be more successful if selection is based directly on the physiological mechanism(s) or character(s) conferring tolerance (Noble and Rogers, 1992, Sairam *et al*., 2002). As tolerance in plants is combined characteristics of both morphological and physiological features, combination of certain characters may be used for improving salt tolerance in mungbean that may vary with plant species and also during plant ontogeny.

Hence the present study was aimed to assess the effect of single saline irrigation with different levels of electrical conductivity (ECe) on water and N status and the functional behaviour of determinate type of nodules in two different genotypes of mungbean because in literature the more emphasis has been given to aerial plants parts than nodules. A comparison of the performance of nodules in two mungbean genotypes *viz*. Asha (Check) and new variety Muskan (MH 96-1) would highlight the component(s) of the mechanism(s) responsible for the adaptation of saline irrigation conditions, and further that would be of value in programme conducted to breed salt tolerant mungbean varieties.

MATERIALS AND METHODS

Two mungbean [*Vigna radiata* (L.) Wilczek] genotypes, Asha and Muskan (MH 96-1) 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, ECe_2 0.8 dS m^{-1} at 25 ^oC, 10.3 mg (N) kg^{-1}, 2.5 mg (P) kg^{-1}, 180 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 Haryana Agricultural University, Hisar, India. After germination, two plants per pot were maintained. Before salinity treatments, whenever needed, the pots were irrigated from the surface only. However, after the salinity treatments, whenever needed, the pots 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 days. The chloride dominated salinity solutions were prepared by using chloride salts of Na^+, Ca^{2+}, Mg^{2+} and sulfate salt of Mg. The ratios of Na (Ca^{2+}+Mg^{2+}) and Ca^{2+}:Mg^{2+} was kept at 1:1 and 1:3, respectively, whereas C1:SO_4^- was maintained at 7:3. The ratios of salts were based on meq basis and irrigation was applied at flowering stage (30-35 days after sowing). Desired salinity (0 (control), 2.5, 5.0 and 7.5 dS m^{-1}) was applied. The sampling of the plants was done at 10 and 20 days after treatments (DAT).

The osmotic potential (Ψs) 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). These measurements were made between 08:00 and 10:00 AM (local time) during a sunny day. Leghemoglobin (LHb) content in detached and washed nodules was estimated according to Hartree (1955). Nitrogenase (N_2-ase) activity of nodules was measured using acetylene reduction assay (ARA) described by Hardy *et al*., (1968) and expressed as μ mol C_2H_4 produced g^{-1} DW h^{-1}. Free proline of nodules was estimated spectrophotometrically according to Bates *et al.* (1973). The lipid peroxidation was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). H_2O_2 content of nodules was determined by method of Patterson *et al.* (1984). The relative stress injury (RSI %) in nodules was evaluated by (Sullivan, 1972). The sodium, potassium, nitrogen and chloride contents of nodules were determined in the oven dried ground material. The material was digested in 5 ml 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) and further expressed in Na^+/K^+ ratio. The total nitrogen (mg g^{-1} dw) was estimated by micro Kjeldhal technique. Cl content was estimated by an ion analyser (*Model L1-126, Elico*, Delhi, India) and expressed as μ moles g^{-1} dw.

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

RESULTS AND DISCUSSION

There was a considerable decrease of osmotic potential (Ψs) and RWC in both the genotypes with increasing levels of saline irrigation and this effect was more pronounced as the duration of salinity exposure increased from 10 to 20 DAT. The genotype Asha showed more negative values of Ψs i.e. from -1.12 to -

1.39 MPa at 10 DAT and from -1.15 to -1.47 MPa at 20 DAT as compared to from -0.94 MPa to -1.28 MPa at 10 DAT and from -0.98 MPa to -1.47 MPa at 20 DAT in Muskan, respectively, with increase in salinity level from 0 to 7.5 dS m^{-1}. However, nodules of Asha maintained significantly better RWC than those of Muskan (Table 1). The proposed reason for decreasing Ψs is that plant adjusts to physiological drought conditions caused by salinity to maintain pressure potential (Wright *et al.,* 1997, Nandwal *et al*., 2000b). Decline in Ψs can be result of either simple passive concentration of solutes due to dehydration or net accumulation of proline and total soluble sugars (TSS). Similar results were reported by Abd El-Samad and Shaddad (1997) in soybean and Nandwal *et al.,* 2000 (a) in trifoliate and pentafoliate mungbean genotypes.

Proline content of nodule increased under saline irrigation. At salinity 7.5 dS m^{-1} and 10 DAT, proline content increased from 14.71 % to 121.37 % in Asha and from 21.15 % to 90.36% in Muskan as compared to their respective control (Table 1). Thus genotype Asha showed a significant higher accumulation of proline than Muskan. The concentration of proline is widely documented to increase in response to salt stress (Sairam *et al*., 2002, Nandwal *et al.,* 2000 b). Similarly, total soluble sugar (TSS) content increased significantly from 25.00 % to 103.37 % and 34.96 % to 90.84 % in Asha and Muskan, respectively, with increase in the salinity level. It is evidenced that these metabolites are used as an indicator of osmotic stress in the plants. These osmolytes contributed better osmoregulation in the genotype Asha in comparison with Muskan. The H_2O_2, lipid peroxidation (in term of MDA) and RSI increased continuously from 6.73 % to 40.24 %, 52.72 % to 89.47 %, 19.16 % to 65.49 % and from 32.67 % to 71.90 %, 25.21 % to 65.9 %, 19.60 % to 75.88 %, in nodules of genotypes Asha and Muskan, respectively. Similar results were noticed at 20 DAT (Table 2). The increase in relative stress injury (RSI %) of nodule was associated with the increase in lipid peroxidation and can be used for screening of salt tolerance lines (Farooq and Azam, 2006). Increased electrolyte leakage from the tissues is usually an expression of changed physical properties of cell membrane. It is apparent that salinity induces an increase in H_2O_2 content and consequently lipid peroxidation and membrane injury which are seems to be closely associated with the tolerance/ susceptibility of mungbean nodules in addition to water and N status

Table 1. Changes in osmotic potential (Ψs) (-MPa), relative water content (RWC %), proline content (mg g^{-1} dw), total soluble sugars (TSS) (mg g^{-1} dw) in nodules of mungbean genotypes under saline conditions.

Parameters	Genotypes	10 DAT Salinity [dS m^{-1}] 0	2.5	5.0	7.5	20 DAT Salinity [dS m^{-1}] 0	2.5	5.0	7.5
Ψs	Asha	1.12	1.17	1.27	1.39	1.15	1.22	1.32	1.47
	Muskan	0.94	1.04	1.13	1.28	0.98	1.06	1.25	1.47
RWC	Asha	93.9	91.0	84.4	80.2	93.7	90.1	86.2	83.8
	Muskan	92.1	90.4	82.4	78.7	89.9	87.3	81.3	79.3
Proline	Asha	70.7	81.1	141.0	156.5	75.4	86.4	255.4	268.4
	Muskan	62.9	76.3	107.3	119.9	69.7	84.5	147.9	184.9
TSS	Asha	38.9	52.5	67.6	74.2	41.7	61.9	74.7	83.8
	Muskan	28.8	36.0	45.4	58.6	31.0	45.5	65.9	74.9

CD at 5%	Ψs	RWC	Proline	TSS	Ψs	RWC	Proline	TSS
Genotype	0.32	0.72	0.54	0.43	0.27	1.0	17.5	1.2
Salinity	0.46	1.02	0.77	0.77	0.38	1.5	24.8	1.6
Genotype x Salinity	0.58	1.11	1.09	0.93	0.54	1.7	35.1	2.3

Table 2. Changes in H_2O_2 content (moles g^{-1}dw) x 10^{-4}, relative stress injury (RSI %), and lipid peroxidation [μmoles (MDA) g^{-1} dw] in nodules of mungbean genotypes under saline conditions.

Parameters	Geno-types	10 DAT Salinity [dS m^{-1}]				20 DAT Salinity [dS m^{-1}]			
		0	2.5	5.0	7.5	0	2.5	5.0	7.5
RSI	Asha	34.5	38.5	43.7	52.4	34.5	39.9	45.5	54.1
	Muskan	33.6	40.2	48.6	59.1	34.9	42.4	50.6	62.1
H_2O_2	Asha	117.3	125.2	138.6	164.5	201.8	322.1	407.8	380.8
	Muskan	106.4	162.5	169.5	201.6	325.4	366.9	435.9	468.4
MDA content	Asha	855.6	1019.5	1215.3	1615.2	2221.3	2387.8	2485.7	2559.2
	Muskan	1114.2	1478.0	1721.5	1915.2	2420.6	2532.7	2617.2	2796.8

CD at 5%	RSI	H_2O_2	MDA content	RSI	H_2O_2	MDA content
Genotype	0.956	21.5	126.7	1.393	19.17	145.2
Salinity	1.356	34.20	179.3	1.970	27.88	205.3
Genotype x Salinity	1.918	47.12	253.5	2.786	33.61	223.1

Table 3. N_2 –ase activity (μ moles C_2H_4 g^{-1} dw h^{-1}), leghemoglobin (LHb) (mg g^{-1} dw), dry matter (DM) (mg $plant^{-1}$) and nitrogen (N) (mg g^{-1} dw) of nodules of mungbean genotypes as affected by saline irrigation.

Parameters	Geno-types	10 DAT Salinity [dS m^{-1}]				20 DAT Salinity [dS m^{-1}]			
		0	2.5	5.0	7.5	0	2.5	5.0	7.5
N_2 –ase	Asha	32.7	34.2	20.4	15.3	35.1	35.3	15.4	8.2
	Muskan	24.2	27.6	16.1	9.1	30.2	32.2	11.5	5.1
LHb	Asha	6.53	3.65	2.82	2.16	4.82	2.63	2.13	2.02
	Muskan	5.93	2.58	2.08	2.04	3.70	1.11	0.92	0.85
DM	Asha	79.0	82.0	59.0	41.0	86.0	89.0	47.0	30.0
	Muskan	59.0	66.0	56.0	35.0	70.0	78.0	43.0	21.0
N	Asha	84.20	78.40	61.60	53.20	86.80	81.20	47.20	44.80
	Muskan	80.00	71.20	61.60	44.80	80.80	75.60	43.20	34.80

CD at 5%	N_2–ase	LHb	DM	N	N_2 –ase	LHb	DM	N
Genotype	0.05	0.04	2.63	1.45	0.07	0.06	2.01	1.08
Salinity	0.04	0.05	1.97	4.93	0.06	0.07	2.18	4.62
Genotype x Salinity	0.07	0.07	3.32	5.17	0.11	0.11	2.85	4.93

Table 4. Changes in Na^+/K^+ ratio (10^{-2}) and Cl^- content (m moles g^{-1} dw) in nodules of mungbean genotypes under saline irrigation.

Parameters	Geno-types	10 DAT Salinity [dS m^{-1}]				20 DAT Salinity [dS m^{-1}]			
		0	2.5	5.0	7.5	0	2.5	5.0	7.5
Na^+/K^+	Asha	64.75	72.90	114.20	146.30	75.00	97.20	160.10	187.15
	Muskan	72.75	96.80	118.75	152.65	81.15	120.30	176.80	236.60
Cl^-	Asha	1.10	1.30	1.90	2.40	1.40	1.70	2.10	3.60
	Muskan	1.40	1.70	2.10	2.50	1.40	2.40	3.20	3.90
CD at 5%			Na^+/K^+	Cl^-			Na^+/K^+	Cl^-	
Genotype			4.89	0.24			5.84	0.41	
Salinity			6.90	0.35			8.26	0.58	
Genotype x Salinity			9.76	0.45			11.7	0.71	

and Na^+/K^+ ratio parameters. Due to membrane injury the nodule functioning in both the genotypes was further deteriorated at 20 DAT, however the effect was more pronounced in Muskan. This relationship was confirmed during investigations when the leakage of ions increased in nodules at high salinity levels. The result also suggest that increased lipid peroxidation might be mediated through H_2O_2 accumulation in nodules. Sharp increase in RSI (%), H_2O_2 accumulation and lipid peroxidation under saline irrigation, were noticed as the characteristic of stress induced senescence in nodules.

The N_2-ase activity of nodule markedly decreased from 53 % to 77 % and 62 % to 83 % in the genotypes Asha and Muskan respectively, under saline irrigation from 10 to 20 DAT (Table 3). Similarly, application of saline irrigation significantly reduced the leghemoglobin content of nodules in the genotypes Asha and Muskan, ranging from 45.43 % to 58.09 % and by 70.1 % to 77.02 %, respectively. Salinity stress induced decrease in water status and LHb content and an increase in lipid peroxidation of nodules therefore can at least partially explain the inhibition of N_2-ase activity. A synergistic effect of natural and induced nodule senescence was observed at 20 DAT, because the crop was near maturity.

At 10 DAT nitrogen content of nodules was found to decrease with increasing level of salinity. The decrease in N content of nodule ranged from 3.45 % to 34.48 % and 3.33 % to 46.67 % in the genotype Asha and Muskan, respectively. Asha had more N content in nodule, which may be due to better plant water status, N_2-ase activity and LHb content of nodules (Table 3). Our results are in agreement with earlier studies conducted on various legumes (Zayed and Zeid, 1998; Nandwal *et al.,* 2000 a&b; Nandwal *et al.,* 2007). The decrease in N_2-ase activity and leghemoglobin content 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 treatments significantly reduced the dry matter of nodules plant^{-1}. Irrespective of treatments genotype Asha maintained more than 25 % higher nodule drymatter than Muskan (Table 3). The results indicate that overall genotype Asha maintained higher N_2-fixing efficiency i.e. in term of N_2-ase activity, LHb and N contents and dry matter of nodules as compared to Muskan and were directly correlated with H_2O_2 accumulation and lipid peroxidation. Further the above findings in nodules of genotypes Asha suggest the possibility of existence of a compensatory mechanism that enables this salt-affected genotype to partially mitigate the imbalance in N_2-fixation, perhaps by osmoregulation / water status as discussed earlier. This compensatory mechanism seems to be less effective in genotypes Muskan where nodulation and N_2-fixation were affected more adversely than in genotype Asha.

The Na^+/K^+ ratio of nodules markedly increased with saline irrigation and with the duration of exposure in both the genotypes. Na^+ content in nodules increased, whereas that of K^+ content decreased under salinity. Earlier Fernandez-Pascal *et al.* (1996) and Sharma (1996) also reported that Na^+ concentration in plant parts increased under salinity. Overall, Muskan showed significantly higher Na^+/K^+ ratio under salinity than Asha (Table 4). The changes Na^+/K^+ in ratio were also reflected in the changes in Cl^- concentration. The increase Na^+/K^+ ratio was correlated with the drastic reduction in Ψs and RWC, occurred under the influence of various salinity levels. Thus the cumulative effect of salt and reduction of Ψs and RWC caused maximum membrane injury in nodules, confirming collective injurious effects of salinity and water deficiency in this study. An increased in Cl^- content in nodules with increasing level and duration of salinity was also observed and it was higher in Muskan than in the Asha (Table 4). The ability of genotype Asha to restrict Na^+ and Cl^- accumulation in nodules correlated with the salinity tolerance and nodule dry matter. Based upon the better water and N status, N_2-ase activity, LHb content, dry matter of nodules and lower Na^+/K^+ ratio and membrane injury, the nodule functioning in genotype Asha was identified relatively more tolerant than Muskan under saline conditions.

REFERENCES

Abd EL-Samad HM and Shaddad MAK (1997). Salt tolerance of soybean cultivars. *Biol. Plant.* 39: 263-269.

Bartels D (2001). Targeting detoxification pathways: an efficient approach to obtain plants with multiple stress tolerance. *Trends Plants Sci*. 6(7): 284-286.

Bates LS, Waldren RP and Teare ID (1973). Rapid determination of free proline for water stress studies. *Plant Soil*. 39: 205-207.

Farooq S and Azam F (2006). The use of cell membrane stability (CMS) technique to screen for salt tolerant wheat varieties. *J. Plant Physiol*. 163: 629-637.

Fernandez-Pascual M, De Lorenzo C, De Felipe MR, Rajalakshmi S, Gordan AJ, Thomas BJ and Minchin FR (1996). The possible reasons for relative salt stress tolerance in nodules of white lupin cv. Multolupa. *J. Exp. Bot.* 47: 1709-1716.

Heath RL and Packer L (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophy*. 125: 189-198.

Hardy RWF, Holsten RD, Jackson EK and Burns RC (1968). The acetylene ethylene essay for N_2-fixation: Laboratory and field evaluation. *Plant Physiol*. 44: 1185-1207.

Kukreja S, Nandwal AS, Kumar N, Sharma SK, Sharma SK, Kundu BS, Unvi V and Sharma PK (2006). Response of chickpea roots to short-term salinization and desalinization: Plant water status, ethylene evolution, antioxidant activity and membrane integrity. *Physiol. Mol. Biol. Plants*. 12: 67-73.

Kukreja S, Nandwal AS, Kumar N, Sharma SK, Sharma SK, Unvi V and Sharma PK (2005). Plant water status, H_2O_2 scavenging enzymes, ethylene evolution and membrane integrity of *Cicer arietinum* roots as affected by salinity. *Biol. Plant*. 49(2): 305-308.

Nandwal AS, Godara M, Kamboj DV, Kundu BS, Mann A, Kumar B and Sharma SK (2000a) Nodule functioning in trifoliate and pentafoliate mungbean genotypes as influenced by salinity. *Biol. Plant.* 43(3): 459-462.

Nandwal AS, Godara M, Sheokand S, Kamboj DV, Kundu BS, Kuhad MS, Kumar B and Sharma SK (2000b). Salinity induced changes in plant water status, nodule functioning and ionic distribution in phenotypically differing genotype of *Vigna radiata* L. *J. Plant Physiol*. 156: 352-359.

Nandwal AS, Kukreja S, Kumar N, Sharma PK, Jain M, Mann A and Singh S (2007). Plant water status, ethylene evolution, N_2-fixing efficiency, antioxidant activity and lipid peroxidation in *Cicer arietinum* L. nodules as affected by short-term salinization and desalinization. *J. Plant Physiol.* 164: 1161-1169.

Noble CL and Rogers MJE (1992). Arguments for the use of physiological criteria for improving the salt tolerance in crops. *Plant Soil*. 146: 99-107.

Patterson BD, Machae EA and Ferguson IG (1984) Estimation of hydrogen peroxide in plant extracts using titanium (IV). *Anal. Biochem*. 139: 487-492.

Sairam RK, Veerabhadra Rao K and Srivastava GC (2002). Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci*. 163: 1037-1046.

Sharma SK (1996) Effect of salinity on uptake and distribution of Na^+, Cl^- and K^+ in two wheat cultivars. *Biol. Plant*. 38: 261-267.

Sullivan CY (1972). Mechanism of heat and drought resistance in grain sorghum and methods of measurement. In: *Sorghum in Seventies* (Eds. NGP Rao and LR House). Oxford and IBH Publishing CO., New Delhi, PP. 247-264.

Weatherley PE (1950). Studies on the water relations of the cotton plant. I. The field measurement of water deficit in leaves. *New Phytol*. 40: 81-97.

Wright PR, Moran J and Jessop RS (1997). Turgor maintenance by osmoregulation in *Brassica napus* and *Brassica juncea* under field conditions. *Anal Bot.* 80: 313-319.

Zayed MA and Zeid IM (1997-98). Effect of water and salt stress on growth, chlorophyll, mineral ions and organic solutes contents and enzymes activity in mungbean seedlings. *Biol. Plant*. 40: 351-356.