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Salt-induced Injury Symptom, Changes in Nutrient and Pigment Composition, and Yield Characteristics of Mungbean

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ABSTRACT

Influence of NaCl salinity was studied on four mungbean [*Vigna radiata* (L.) Wilczek] genotypes to decipher the changes in visual signs of salt damage, growth and seed yield, mineral nutrient and pigment composition at early vegetative (EVS), late vegetative (LVS) and reproductive (RS) stages of growth. Although the genotypes exhibited sensitivity to salinity at all stages, salt tolerance (mM_{50}; NaCl level in mmol L^{-1} at which growth or yield reduces to 50%) ranged from <39 (NM-98) to >51 (NM-54). Most salt tolerant genotype (NM-54) displayed reduced tip burning, low chlorosis and necrosis of young leaflets (two top trifoliate leaves) increased total plant dry mass, greater number and area of green leaves, and seed yield per plant. Tissue analysis revealed that Na^+ and Cl^- content of young leaflets was lowest in NM-54, while highest in NM-98 (Na^+) and NM-92 (Cl^-). However, old leaflets (two bottom green leaves) were indiscriminately affected. Enhanced chlorosis and necrosis of leaves were directly related to increased ionic content of leaves. Although the levels of leaf N, P, K^+ and Ca^{2+} were reduced due to salinity, marked genotypic differences were evident. NM-54 excelled the other genotypes in greater nutrients content of young leaflets at all stages. Chlorophyll (Chl)-a, -b and carotenoid (Car) content decreased due to salinity in young and old leaflets at all stages. NM-54 followed by NM-89 showed lowest reduction in the pigment content of young leaflets. Chl-a:b ratio of young leaflets increased in the sensitive genotypes but remained steady in the tolerants. Enhanced chlorosis and necrosis of leaflets were ascribed to excess-ion induced loss of chlorophyll and mineral nutrient deficiency. Although ion-toxicity is a noxious factor, greater nutrient and pigment contents of young leaves is crucial for better growth and improved salt tolerance of genotypes. Reduced chlorosis and necrosis of leaves can be taken as important visual criteria of salt tolerance at all stages.

Key Words: Carotenoid; Chl-b; Growth; Ion-toxicity; Nutrients; Visual symptoms; Mungbean

INTRODUCTION

Increased soil salinity is one amongst the many devastating environmental adversaries that has multifarious effects on plant growth and development (Shannon, 1977; Flowers, 2004). Manifestation of enhanced salt tolerance by any species is important at any stage, but is more meaningful at critical stages. For example, salinity applied at initial stages impairs the speed of germination, seedling growth and final crop stand in the field (Bayuelo-Jimenez *et al.*, 2002; Murillo-Amamdor *et al.*, 2002a), while at seed/grain filling it determines the economic yield.

Plants challenged with salinity display many visual signs of salt injury. Qualitative effects are symptomatic i.e. stunted growth (Srivastave & Jana, 1984), chlorosis of green parts (James, 1988; Pentalone *et al.*, 1997; Husain *et al.*, 2003), leaf tip burning (Wahid *et al.*, 1999b), scorch (Barroso & Alvarez, 1997) and necrosis of leaves (Volkumar *et al.*, 1998; Chen *et al.*, 2003). Quantitative ones include reductions in dry mass, elongation and expansion growth of leaves (Neumann *et al.*, 1988), tissue ionic and nutrient contents (Misra *et al.*, 2001) etc. Suppression in growth is usually ascribed to a reduced capacity of the green parts to photosynthesize under salinity (Morant-Manceau *et al.*, 2004), which, in addition to other factors, is more related to increased chlorophyll fluorescence (Murillo-Amamdor *et al.*, 2002b) and changes in overall chlorophyll content (Zayed & Zeid 1997; Husain *et al.*, 2003). Appraisal of morphological and physiological criteria of salinity tolerance has proven beneficial in increasing our understanding of salt tolerance in many plant species (Wahid *et al.*, 1999b; Murillo-Amamdor *et al.*, 2002a; Morant-Manceau *et al.*, 2004).

Legumes are important agricultural plants and entail one of the rich sources of proteins. Efforts to understand the salinity tolerance mechanisms of leguminous species have revealed that toxic ions are the main reason for reduced growth. Salt tolerance is achieved by the acquisition of succulence and selective transfer of K^+ to young parts in lupin (Jeschke *et al.*, 1986) and mungbean (Zayed & Zeid, 1997), enhanced Na^+ removal from the xylem stream in soybean (Lacan & Durand, 1995), restricted Na^+ uptake into the shoot, maintenance of high K^+:Na^+ ratio in clover shoot (Shannon & Nobel, 1995) and high photosynthetic rate in cowpea (Plaut *et al.*, 1994).

Mungbean [*Vigna radiata* (L.) Wilzeck] is one among the widely used protein sources. It shows suppressed growth even in marginally saline areas and most cultivars exhibit a

salt tolerance threshold to <2 dS m^{-1} (Minhas *et al.*, 1990). Mungbean shows completely inhibited seed germination in NaCl solution with osmotic potential of -1.5 MPa (Goertz & Coons, 1991; Wahid *et al.*, 1999a; Murillo-Amamdor *et al.*, 2002b). Failure to maintain pressure potential (Neumann *et al.* 1988; Misra *et al.*, 2001) and reductions in chlorophyll and nutrient content of leaves (Zayed & Zeid, 1997; Misra *et al.*, 2001) are important factors that render it salt-sensitive.

In this study four newly evolved mungbean genotypes were investigated with the objective to determine their salt tolerance potential and the mechanism(s) involved. Yardsticks of these determinations were visual symptoms like chlorosis, necrosis and tip burning of trifoliate young and old leaves, changes in vegetative and reproductive growth, quantification of the levels of mineral elements and photosynthetic pigments at three phenological stages, and pod and seed yield at maturity. Such a study was imperative in view of the dearth of such information on most legume species.

MATERIALS AND METHODS

Plant material and growth conditions. Selected healthy mungbean seeds surface sterilized with 0.1% (w/v) $HgCl_2$ (for 3 minutes followed by four repeated washing with sterilized distilled water) were directly sown in pots (30 cm x 15 cm) lined with double layer of polythene sheets, filled with 10 kg of sandy loam, soil and kept in a greenhouse. The experimental design was completely randomized with three replications. After germination and thinning, five plants of uniform size were maintained in each pot. Salt (NaCl) solution was gradually added to the pots @ 20 mmol NaCl L^{-1} per day to achieve 40, 80, and 120 mmol L^{-1} levels, based on full field capacity of soil, and a control (no salt added) at early vegetative (EVS), late vegetative (LVS) and reproductive (RS) (i.e. at 30, 60 and 80 days after emergence of seedlings respectively) stages of growth. The physico-chemical characteristics of the soil were: sand 40%, silt 30%, clay 29%, textural class loam, organic matter 1.51%, pH 7.6, ECe 1.2 dS m^{-1}, cation exchange capacity 13.2 meq per 100 g soil, sodium adsorption ratio 0.07 Watering was carried out to administer the soil moisture to field capacity. Plants were grown for 15 d under applied salinity at each growth stage and then harvested.

Visual symptoms growth and yield characteristics. Two uppermost trifoliate leaves were marked as young and two lowermost green leaves as old for symptomatic and other determinations. These leaves were visually evaluated for symptoms of salt injury as tip-burning, chlorosis (yellowing) and necrotic spots as reported elsewhere (Wahid *et al.* 1999b). Leaf number per plant was counted and their area was determined using a leaf area meter (Model Li-3000, Licor, Lincoln, USA). For taking dry mass, the harvested plants were transferred to paper bags and kept in an oven at 70°C for seven days. One set of plants receiving salt treatments at RS was saved for the determination of pod and seed yield. The pods were counted and seed number per pod was determined from the harvested plants from each treatment. Salinity tolerance limits i.e. mM_{50} (NaCl level in at which growth and yield parameters reduce to 50%), of genotypes were computed based on the growth and yield characteristics at above defined stages.

Mineral nutrient analysis. For the determination of Na^+, K^+ and Ca^{2+} and P from leaves, the dried powdered material (0.5 g) was digested in HNO_3:$HClO_4$ mixture (3:1 ratio) at 280°C for 2 h, or until a sample has become clear, cooled and made up to 50 mL using deionized water. Na^+ and K^+ were determined on flame-photometer (Sherwood Model 410, Cambridge, UK), and Ca^{2+} on atomic absorption spectrophotometer (Perkin Elmer, Model 303, New York). Total P was estimated colorimetrically using Barten's reagent and total N by microKjeldahl method. For Cl^- determination, dried powdered material (0.5 g) was boiled in distilled water in a screw capped test tubes for 1 h, cooled and filtered, volume made up to 50 mL and determinations made on chloride analyzer (Model-VC-HI Central Kagaku Corporation, Japan).

Chlorophylls and carotenoids determination. Fresh excised trifoliate leaves wrapped in black plastic bags, put in ice bucket were brought to laboratory. Each leaf sample (0.5 g) was extracted in 80% acetone (100 mL), vacuum filtered and filtrate immediately determined for chlorophyll (Chl) and carotenoid (Car) using spectrophotometer (Hitachi Model-U 2001 Japan). Amount of each Chl species was computed following the formulae of Arnon (1949), and that of Car as described by Davies (1976).

Statistical analysis. Analysis of variance was performed using COSTAT software to determine statistically significant differences and interaction of various factors. Duncan's new multiple range test was applied to compare the treatment means. Linear correlation was established between mean values of different variables at the highest salinity level (120 mmol L^{-1}). For this purpose, apparent symptoms (graded to obtain values) and quantitative characters of leaves of each genotype within a growth stage were arranged in ascending order. The correlation coefficients (r) were computed cumulative of all growth stages (n=12). The "r" carrying single or double asterisk given in results section are different at $p<0.05$ and $p<0.01$, respectively.

RESULTS

Visual symptomatic studies. Visual symptoms of ionic injury were evident in the form of tip burning, chlorosis and necrotic spots on leaflets of both the ages. Although ionic injury also occurred at lower salt levels, its extent was greater at higher levels (data shown in Table I pertain to 120 mmol L^{-1}). Genotypes revealed remarkable differences at all the growth stages for these symptoms. Chlorosis was low on

Table I. Symptoms and degree of salt injury on young and old trifoliate leaves of four mungbean genotypes under 120 mmol L^{-1} NaCl level at early vegetative (EVS), late vegetative (LVS) and reproductive (RS) stages of growth

Sign of salt injury	*Degree*	*EVS*		*LVS*		*RS*	
		Young leaflets	**Old leaflets**	**Young leaflets**	**Old leaflets**	**Young leaflets**	**Old leaflets**
Chlorosis	Low	NM-54	NM-92	NM-54	NM-92	NM-54	NM-92
	↓	NM-98	NM-98	NM-89	NM-98	NM-89	NM-98
	↓	NM-89	NM-89	NM-98	NM-89	NM-98	NM-89
	High	NM-92	NM-54	NM-92	NM-54	NM-92	NM-54
Necrosis	Low	NM-54	NM-92	NM-54	NM-92	NM-89	NM-92
	↓	NM-98	NM-89	NM-89	NM-89	NM-54	NM-89
	↓	NM-92	NM-54	NM-92	NM-98	NM-92	NM-98
	High	NM-89	NM-98	NM-98	NM-54	NM-98	NM-54
Tip burnng	Low	NM-98	NM-92	NM-54	NM-92	NM-89	NM-92
	↓	NM-54	NM-89	NM-98	NM-98	NM-54	NM-98
	↓	NM-89	NM-98	NM-89	NM-89	NM-98	NM-54
	High	NM-92	NM-54	NM-92	NM-54	NM-92	NM-89

Table II. Analysis of variance (mean squares) of some growth characteristics of mungbean genotypes under increased salinity

Source of variation	*df*	*Shoot dry weight (g $plant^{-1}$)*			*Number of green leaves per plant*			*Leaf area (cm^2 $plant^{-1}$)*		
		EVS	**LVS**	**RS**	**EVS**	**LVS**	**RS**	**EVS**	**LVS**	**RS**
Genotypes (G)	3	0.07ns	1.69**	1.62ns	33.19**	57.80*	17.47**	31.24**	59.35**	6.64**
Salinity (S)	3	11.10**	25.65**	38.39**	180.47**	433.03**	217.64**	581.08**	1626.86**	29.57**
G x S	9	0.01ns	0.46ns	0.59ns	1.01ns	0.86*	1.40ns	3.79*	2.63ns	0.65**
Error	32	0.04	0.18	0.61	0.81	0.08	1.04	1.64	5.29	0.09

Mean square values significant at ** $P < 0.01$, * $P < 0.05$, ns non-significant

Table III. Salinity induced changes in some pod and seed yield characteristics of mungbean genotypes at maturity

Genotypes	*NaCl levels (mmol L^{-1})*	*Number of pods per plant*	*Number of seeds per pod*	*Number of seed per plant*	*1000 seed weight (g)*	*Seed yield (g $plant^{-1}$)*
245/7	Control	8.00±1.00	7.67±0.33	61.67±9.68	63.00±2.66	4.07±0.77
	40	6.00±1.00	4.67±0.58	28.33±7.67	41.73±3.45	1.87±0.87
	80	3.67±0.58	3.67±0.58	13.67±4.04	24.37±2.64	0.90±0.90
	120	2.67±0.58	3.00±1.00	8.33±4.04	18.20±1.20	0.55±0.27
241/11	Control	8.33±0.58	7.67±0.33	63.67±0.58	64.03±3.00	4.20±0.27
	40	5.67±1.15	5.33±0.67	30.67±9.81	44.47±3.26	2.02±0.04
	80	3.33±0.58	3.33±0.58	11.33±4.04	29.90±2.88	0.75±0.05
	120	2.33±0.58	2.67±0.58	8.33±2.52	20.53±1.40	0.42±0.27
NM-89	Control	8.67±0.58	8.33±0.58	72.33±8.5	64.30±2.74	4.77±0.56
	40	5.33±0.67	5.00±1.00	27.09±8.19	45.93±3.85	1.78±0.45
	80	4.67±0.58	4.67±0.67	22.00±2.00	31.57±2.87	1.45±0.75
	120	3.33±0.58	3.33±0.33	11.33±1.33	22.33±1.46	0.75±0.17
NM-54	Control	9.00±1.00	8.33±0.58	75.33±3.32	64.23±2.95	4.97±0.88
	40	7.33±0.67	7.00±1.00	51.67±5.62	51.70±2.39	3.41±0.37
	80	5.33±0.33	5.33±0.33	28.67±3.18	35.10±2.85	1.89±0.21
	120	4.33±0.58	4.33±0.58	18.67±2.31	29.70±1.73	1.23±0.15
		Significance of variance sources				
SOV	df					
Genotypes (G)	3	6.06**	6.02**	654.51**	132.00**	2.82**
Salinity (S)	3	65.39**	49.19**	7631.19**	3825.89**	33.42**
G x S	9	0.56ns	0.58ns	55.56ns	11.94ns	0.24ns
Error	32	0.52	0.46	56.58	7.51	0.24

Mean square values significant at ** $P < 0.01$, * $P < 0.05$, ns non-significant

young leaflets of NM-54 at all stages and high in NM-92, while this trend was just reversing for old leaflets of these genotypes. Necrosis was low on young leaflets of NM-54 at EVS and LVS and in NM-92 at RS; however, old leaflets showed no specific trend. Tip burning of young leaflets was low in NM-98 at EVS, NM-54 at LVS and NM-89 at RS, but high in NM-92 at all stages. For old leaflets, NM-54 at EVS and LVS and NM-89 at RS depicted high leaflet tip burning.

Growth, yield and salt tolerance. Although applied salinity significantly reduced the shoot dry weight of the genotypes at all growth stages (Fig. 1), the difference among genotypes was significant at LVS only, and there was no interaction of salinity and genotypes at any stage (Table II). All the genotypes displayed a similar reduction in the shoot dry weight except at LVS, where NM-89 indicated a lowest reduction in this parameter compared with other genotypes (Fig. 1). Number and area of green leaves were more affected by salinity than the total shoot dry weight, as was evident from a significant effect of salinity in inducing remarkable effect on the genotypes at all stages. However, salinity and genotypes interaction was apparent at LVS for number of leaves, and at EVS and RS for leaf area (Table II). NM-54 excelled the others in maintaining highest number and area of green leaves at all salt levels (120 mmol L^{-1}) or growth stages. Contrarily, NM-98 was greatly affected even at lower salinity levels at all stages (Fig. 1).

Substrate salinity significantly reduced the pod and seed production but significant genotypic differences were evident. All the genotypes indicated a reduction in the number of pods per plant, number of seeds per pod and seeds per plant but with significant differences among genotypes and salt levels. All these parameters were most affected in NM-92 followed by NM-98, whereas NM-54 was the least affected. This led to a marked reduction in the final seed yield per plant of all the genotypes as was further evident from a well-marked reduction in 1000 seed weight as well (Table III).

Genotypes showed variability in NaCl tolerance limits (mM_{50}), which was derived on the basis of above-given growth and seed yield attributes. NM-54 with mM_{50} of 51.6 (average of the growth stages) was ranked highly salt tolerant followed by NM-89 (Table IV). Contrarily, NM-98 with an average mM_{50} of 38.8 (giving ~25% less mM_{50} than NM-54) was ranked salt sensitive.

Nutrient relations. All the genotypes displayed the accumulation of Na^+ and Cl^- in leaflets as the salt levels progressed (Table V). The old leaflets had greater content of both the ionic species than young ones, irrespective of the growth stage. However, the overall level of Na^+ was lesser than Cl^-. NM-54 had the lowest content of Na^+ and Cl^- in the young leaflets compared to other genotypes, while the older leaflets displayed no remarkable genotypic difference. Na^+ and Cl^- contents of NM-54 (highly salt tolerant genotype) were up to 27 and 44 mg g^{-1} dry weight respectively in young leaves, while these values were 44 and 55 for old ones (Table V). On the contrary, young leaflets of sensitive genotypes accumulated as high as 40 and 60 mg g^{-1} dry weight of Na^+ and Cl^-, respectively, while these values were up to 36 and 62, respectively for old leaflets. Data revealed a significant difference among the genotypes, salinity levels and their interaction, except a non-significant interaction at LVS and RS for Na^+ content of old leaflets. The levels of N, P, K^+ and Ca^{2+} of mungbean genotype got reduced at all growth stages, but significant genotypic differences were discernible. Overall content of these nutrients were greater in younger than older leaves (Fig. 2). Although N-content of the genotypes was reduced, young leaflets of NM-54 had the highest and that of NM-98 the lowest value of this parameter in the young leaflets at all growth stages. We noted a significant difference of genotype and salinity levels for N content for young and old leaflets. However, there was no interaction of salinity and genotypes at any stage or leaflets of different ages (Table VI). P content of both leaflets although decreased with a concomitant increase in root zone salinity, the young leaflets had greater P-content than the old ones at all growth stages (Fig. 2). Genotypes, salinity levels and their interaction were significant for young leaflets at all growth stages. The old leaves had no significant difference among genotypes at EVS and LVS, but a significant one at RS. However, no salinity and genotypes interaction was evident at any stage (Table VI).

Leaflets of genotypes, irrespective of their age, showed a sharp reduction in K^+ content under increased salt levels, resulting in marked differences of genotypes and salinity levels at all stages, with an interaction of salinity and genotypes for young leaflets at LVS and RS (Table VI). Genotypes indicated significant difference for old leaves at EVS, but not at later stages. However, applied salinity affected the level of this nutrient at all stages. NM-54 had the highest K^+ content in young leaves at all salinity levels, while the reverse trend was evident in NM-98 at EVS and RS, and in NM-92 at LVS (Fig. 3). Ca^{2+} level in both the leaflets got reduced by salinity. Young leaflets showed greater Ca^{2+} than the old ones (Fig. 2), resulting in significant differences among genotypes and salinity levels at all stages. However, there was no interaction of salinity and genotypes for Ca^{2+}, except at LVS for young and at RS for old leaflets (Table VI). Comparison of genotypes showed that young leaflets of NM-54 exhibited the greatest Ca^{2+} content at stages and salinity levels while NM-98 did so for old leaves.

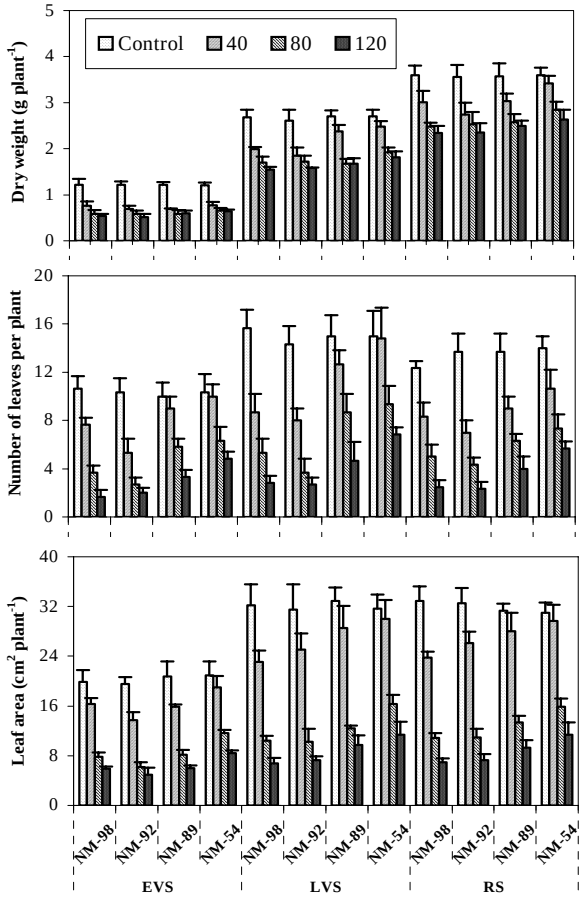
Chlorophylls and carotenoid content. Photosynthetic pigments of the genotypes revealed marked changes under increased salinity in the leaflets of both the ages at all growth stages (Fig. 3). In young leaves, Chl-a was relatively less affected than Chl-b under salinity, as the difference among the genotypes was seen at EVS and LVS but not at RS. NM-54 had the highest Chl-a at all stages and salt levels (Table VII). Contrarily, Chl-b was more affected particularly

Table IV. Salt tolerance limits and ranking of mungbean genotypes under NaCl salinity

Genotype	*Vegetative growth*		*Reproductive growth*		*Mean*	*Rank*
	EVS	**LVS**	**RS**	**Seed yield**		
NM-98	43.2a	37.7a	42.7a	31.5a	38.8a	S
NM-92	42.0a	44.7b	43.3a	29.4a	39.9a	S
NM-89	42.3a	50.6c	52.7b	38.2b	46.0b	Mt
NM-54	47.7b	54.7d	56.7c	47.1c	51.6c	Ht

Means sharing same letter differ non-significantly
S, sensitive; Mt, medium tolerant; Ht, highly tolerant

Fig. 1. Changes in shoot dry weight, number and area of green leaves of mungbean genotypes under salinity at early vegetative (EVS), late vegetative (LVS) and reproductive (RS) stages of growth



in young leaflets of sensitive genotypes (NM-98 & NM-92), thus showing a highly significant difference at EVS and LVS and a significant one at RS. NM-54 maintained the greater content of Chl-b than the other genotypes (Fig. 3). Chl-a:b ratio increased in all the genotypes under salinity with the exception of NM-54, which maintained a steady Chl-a:b ratio at all the stages under salt stress (Fig. 3). However, the magnitude of changes in Chl-a:b ratio indicated no significant difference among genotypes and salt levels at EVS, but there was a significant difference among genotypes at LVS and RS, and a similar difference for salt levels at RS (Fig. 3). Salinity although reduced the Car content of young leaflets of the genotypes at all growth stages, NM-54 was the least affected than other genotypes. However, there was no difference among the genotypes; rather salinity induced a significant Car of the leaflets at various growth stages (Fig. 3).

Chl-a content of old leaflets of genotypes were reduced to >50, >35 and >33% at EVS, LVS and RS respectively at the highest salt level (Fig. 3). For these leaves the genotypes indicated no difference at EVS and LVS but a significant one at RS. However, salinity treatment showed significant differences (Table VII). A high magnitude of changes in Chl-a content of these leaves resulted in an interaction of genotypes and salinity at EVS and RS. Applied salinity reduced the Chl-b content greater than Chl-a (Fig. 3). The effect of salinity on this pigment was more pronounced in NM-98 at EVS, NM-54 at LVS and NM-89 at RS. Chl-a:b ratio of the genotypes varied considerably at all stages with significant difference at LVS and RS but not at EVS (Table VII). Although this ratio increased in all the genotypes with increased salinity levels, a relatively lesser increase was noted in NM-92 at all the stages for these leaflets (Fig. 3). Car content of old leaflets was affected more than young ones. The genotypes had no significant difference for the Car, but the salinity levels indicated a significant difference at all stages (Table VII). However, NM-54, among the genotypes, displayed the highest Car content at all growth stages under increased salinity, though the content was much less than young leaflets of this genotype (Fig. 3).

Correlations. Results were validated by establishing correlations between different variables at the highest salinity levels for the young leaflets in view of their importance to plant survival under salinity. There was a positive correlation of increased chlorosis with Na^+ (r=0.769**) and Cl^- (r=0.742**). A positive relationship of mM_{50} was evident with increase in Chl-b (r=0.731**) and Car (r=0.739**), but not with Chl-a (r=0.489ns). However, increased mM_{50} had an inverse relationship with higher Chl-a:b ratio (r=-0.688*). Increased necrosis indicated a week but positive correlation with excess of Na^+ (r=0.621*) and Cl^- (r=0.592*), but a strong inverse correlation with reduced contents of N (r=-0.824**), P (r=-0.885**), K^+ (r=0.911**) and Ca^{2+} (r=-0.854**). Likewise higher mM_{50} exhibited a negative relationship with chlorosis (r=-0.728**) and necrosis (r=-0.744**) of young leaflets.

DISCUSSION

Nature, extent and timing of salinity application are important to determine the salt tolerance ability of any

Table V. Ionic relations (mg g^{-1} dry weight) of young and old leaves of four mungbean genotypes under NaCl salinity at various growth stages

Genotype	*NaCl (mmol L^{-1})*	*Early vegetative stage*				*Late vegetative stage*				*Reproductive stage*			
		Na^+		Cl^-		Na^+		Cl^-		Na^+		Cl^-	
		Young leaves	Old leaves	Young leaves	Old leaves	Young leaves	Old leaves	Young leaves	Old leaves	Young leaves	Old leaves	Young leaves	Old leaves
NM-98	Cont	4.3±0.5	5.6±0.6	9.66±1.4	11.7±1.2	5.3±0.5	6.1±0.7	10.0±1.0	12.0±1.2	6.0±0.6	6.2±0.3	10.3±1.0	11.9±0.8
	40	13.4±2.2	16.4±1.0	23.4±3.2	28.2±1.2	15.4±2.2	18.5±2.8	25.1±4.2	31.7±3.7	17.2±2.0	17.8±1.8	26.1±2.4	32.2±2.5
	80	20.7±1.9	24.8±0.3	35.3±2.2	43.3±0.5	22.9±1.8	26.4±0.9	37.3±2.5	44.3±2.3	24.2±2.9	25.1±2.3	38.6±2.2	44.9±2.5
	120	30.8±1.9	34.7±2.7	51.2±2.6	60.8±4.8	34.0±2.7	35.9±2.7	53.6±2.1	54.2±3.4	34.1±3.0	35.8±2.8	54.3±2.8	61.6±4.3
NM-92	Cont	4.2±1.0	5.7±0.8	9.9±0.8	11.6±1.7	5.1±0.4	6.3±0.7	10.5±0.8	11.0±1.3	5.8±1.2	6.4±1.0	10.6±1.5	11.1±1.7
	40	11.7±1.3	14.7±1.2	20.4±2.3	25.7±2.1	12.4±1.9	16.8±2.0	21.2±1.8	29.5±3.6	12.4±1.7	17.4±1.2	22.5±2.0	30.4±2.0
	80	23.2±2.3	26.6±1.9	39.9±3.0	46.6±3.5	23.6±2.3	29.5±2.6	40.3±4.3	49.6±2.3	25.2±1.4	29.7±3.0	41.5±2.9	52.3±4.6
	120	31.5±2.4	33.6±2.1	53.3±1.7	58.9±3.6	33.5±2.1	33.9±2.5	54.2±3.5	56.0±3.7	34.0±1.8	34.6±1.3	55.1±4.4	60.8±2.1
NM-89	Cont	4.3±0.4	5.5±0.8	10.4±0.8	11.1±0.9	5.4±0.8	6.6±1.1	10.6±1.5	11.6±1.8	5.5±0.6	6.8±0.6	10.6±0.6	11.9±1.7
	40	9.3±1.2	16.2±1.7	19.8±2.2	28.4±2.9	10.0±0.5	18.1±1.9	20.2±2.3	31.7±3.3	12.2±1.7	18.7±2.5	20.4±1.6	33.1±4.4
	80	19.1±1.5	23.0±2.3	34.7±2.9	41.5±1.9	21.0±1.5	26.0±2.4	34.7±1.6	46.6±2.5	22.4±2.1	26.9±1.5	35.4±1.9	47.4±3.0
	120	28.6±2.3	31.2±1.6	50.1±4.1	55.4±3.6	29.7±2.6	34.0±3.3	51.2±3.2	59.5±5.8	30.3±3.6	34.0±1.8	51.3±3.2	61.2±3.6
NM-54	Cont	4.5±0.4	5.8±0.7	9.8±0.9	11.8±0.6	5.6±1.1	6.5±0.8	10.4±0.8	12.5±2.3	5.9±0.7	6.7±0.8	11.2±0.9	11.7±1.3
	40	8.7±1.1	16.3±1.3	16.2±2.1	29.1±2.6	9.8±0.8	20.1±2.7	16.7±1.9	36.1±3.8	10.5±1.9	22.6±1.3	16.3±0.9	39.6±2.3
	80	16.8±1.4	29.3±2.1	29.4±2.5	48.5±1.2	17.0±0.6	30.1±2.6	29.6±2.9	53.0±4.0	17.3±2.6	31.0±2.4	30.1±2.4	54.3±4.2
	120	25.2±3.2	35.2±2.1	42.3±3.0	60.7±2.3	26.4±3.2	38.6±2.0	42.9±3.7	65.1±5.2	26.6±1.8	39.8±2.8	43.8±3.8	65.8±3.8
		Significance of variance sources											
SOV	df												
Gen (G)	3	38**	14**	98**	65*	54**	16**	116**	79**	68**	36**	118**	56**
Salinity (S)	3	1397**	1781**	3555**	538**	1487**	1911**	3674**	5130**	1478**	1932**	3604**	5894**
G x S	9	7**	6*	18**	56**	10**	5ns	20*	20*	11**	7ns	22**	22*
Error	32	3	2	5	17	3	4	7	7	3	3	4	8

Mean square values significant at ** $P < 0.01$, * $P < 0.05$, ns non-significant

Table VI. Analysis of variance (mean squares) of some mineral nutrient contents of young and old trifoliate leaves of mungbean genotypes at various growth stages

Source of variation	*df*	*Young trifoliate leaves*				*Old trifoliate leaves*			
		N	P	K^+	Ca^{2+}	N	P	K^+	Ca^{2+}
		Early vegetative stage (EVS)							
Genotypes (G)	3	1.40**	0.89**	7.86**	9.27**	0.78**	0.06ns	0.94*	0.92*
Salinity (S)	3	15.23**	1.63**	500.79**	230.60**	8.54**	1.22**	294.42**	138.78**
G x S	9	0.11ns	0.19**	1.38ns	0.94ns	0.06ns	0.05ns	0.32ns	0.48ns
Error	32	0.12	0.05	0.64	0.69	0.07	0.03	0.31	0.31
		Late vegetative stage (LVS)							
Genotypes (G)	3	0.96**	0.97**	7.17**	7.39**	0.22*	0.04ns	0.05ns	1.29**
Salinity (S)	3	14.18**	1.52**	498.65**	262.70**	8.79**	0.96**	292.32**	129.74**
G x S	9	0.10ns	1.23**	1.39**	1.38**	0.11ns	0.03ns	1.01**	0.41ns
Error	32	0.12	0.04	0.33	0.45	0.06	0.02	0.21	0.29
		Reproductive stage (RS)							
Genotypes (G)	3	1.58**	0.88**	7.76**	8.23**	0.76**	0.06*	0.59ns	1.32**
Salinity (S)	3	12.02**	1.38**	499.45**	263.55**	8.64**	1.22**	299.28**	125.55**
G x S	9	0.16ns	0.18**	1.38*	1.44ns	0.06ns	0.02ns	0.38ns	0.72**
Error	32	0.09	0.03	0.62	0.66	0.06	0.02	0.22	0.23

Mean square values significant at ** $P < 0.01$, * $P < 0.05$, ns non-significant

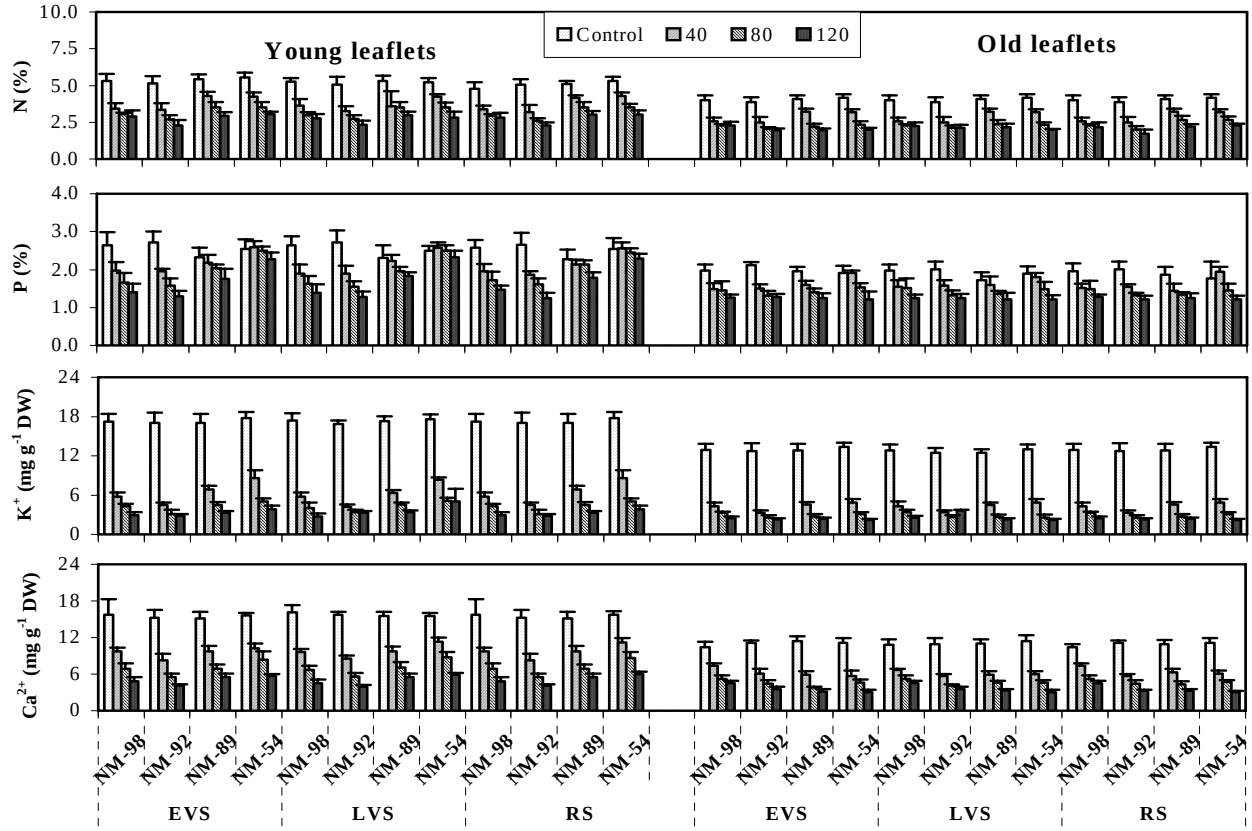
species (Zeng *et al.,* 2001; Bayuelo-Jimenez *et al.,* 2002; Sreenivasulu *et al.,* 1998; Katerji *et al.,* 2003). This study on mungbean genotypes performed at various growth stages showed substantial differences in the signs of salt damage (Table I), reductions in overall growth (Fig. 1) and the pod and seed yield attributes (Table II). The effect was most pronounced on the leaf area per plant (>75 and 55% reduction in sensitive and tolerant genotypes respectively), which severely curtailed the available area for stomatal gas exchange (Sreenivasulu *et al.,* 1998). NaCl tolerance ability (mM_{50}) from ~39 (NM-98) to ~52 (NM-54) substantiated that mungbean (Table IV). Despite a salt sensitive species (Minhas *et al.,* 1990), has scope for improvement and fetching acceptable yield in low salinity profile soils. Consistency in salt tolerance at all stages, as noted in NM-54, is an important finding because the incidence of salinity spell at any growth stage may severely curtail the stand in the field.

Table VII. Analysis of variance (mean squares) of Chl and Car content of young and old trifoliate leaves of mungbean genotypes at various growth stages

Source of variation	*df*	*Young trifoliate leaves*				*Old trifoliate leaves*			
		Chl-a	**Chl-b**	**Chl-a:b**	**Carotenoid**	**Chl-a**	**Chl-b**	**Chl-a:b**	**Carotenoid**
					Early vegetative stage (EVS)				
Genotypes (G)	3	0.23*	0.38**	0.24ns	0.004ns	0.03ns	0.11**	0.11ns	0.002ns
Salinity (S)	3	5.25**	3.51**	0.29ns	0.110**	5.42**	3.52**	0.34**	0.110**
G x S	9	0.13ns	0.12ns	0.04ns	0.002ns	0.07**	0.04ns	0.32*	0.005ns
Error	32	0.06	0.06	0.11	0.002	0.03	0.02	0.08	0.003
					Late vegetative stage (LVS)				
Genotypes (G)	3	0.36*	0.62**	0.93**	0.002ns	0.01ns	0.08**	0.24**	0.004ns
Salinity (S)	3	4.53**	2.43**	0.06ns	0.092**	3.09**	2.66**	0.32**	0.049**
G x S	9	0.09ns	0.04ns	0.04ns	0.001ns	0.07ns	0.04ns	0.05ns	0.005ns
Error	32	0.12	0.05	0.07	0.001	0.03	0.02	0.03	0.003
					Reproductive stage (RS)				
Genotypes (G)	3	0.05ns	0.08*	0.16*	0.002ns	0.11*	0.18**	0.16**	0.002ns
Salinity (S)	3	5.11**	3.63**	0.17**	0.058**	2.60**	2.91**	0.53**	0.060**
G x S	9	0.03ns	0.05ns	0.05ns	0.002ns	0.10**	0.04ns	0.04ns	0.004ns
Error	32	0.04	0.03	0.04	0.002	0.03	0.02	0.03	0.006

Mean square values significant at ** $P < 0.01$, * $P < 0.05$, ns non-significant

Fig. 2. Mineral nutrient content of young and old leaves of mungbean genotypes under salinity at early vegetative (EVS), late vegetative (LVS) and reproductive (RS) stages of growth



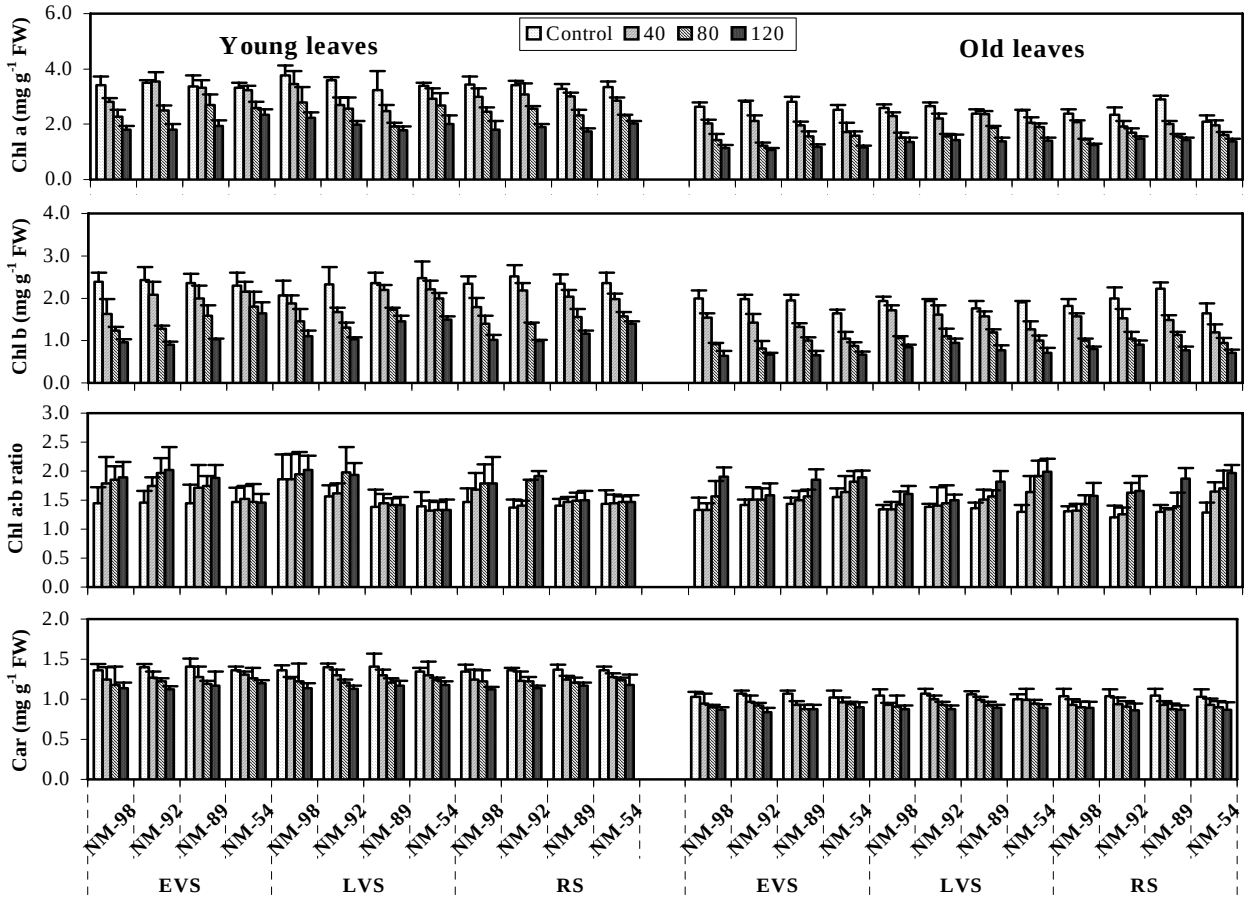
Mechanisms of salt tolerance have been widely studied in majority of crops, including legumes that are quite diverse in nature. Many plant species partition the excess of ions at organ (Wolf *et al.,* 1991; Wahid, 2004), and/or sequester at cell levels (Jacoby, 1999; Chen *et al.,* 2003). Taking the account of visual symptoms, the effect of excess ions is commonly reported in the form of tip burning, chlorosis (Wahid *et al.,* 1997), appearance of scorch (Barroso & Alvarez, 1997) and necrotic spots on whole leaf (Wahid *et al.,* 1999b; Chen *et al.,* 2003). Whether the frequency of

these signs is related to ultimate biomass and seed yield needs thorough investigations. This study indicated the effect of excess ions was apparent on leaves of both the ages; the old leaflets being much more affected than the young ones (Table I). It is noteworthy however that highly tolerant genotype (NM-54) had the lowest content of Na^+ and Cl^- as compared to the others (Table V). This indicated the ability of tolerant genotypes to exclude toxic ions into old senescing parts (Schachtman & Munns, 1992; Wahid, 2004), perhaps by withdrawing ions from the xylem stream (Jeschke *et al.*, 1986; Wolf *et al.,* 1991), so minimizing their accumulation in the young leaflets.

Induced nutrient deficiency is another important effect of increased salinity (Zidan *et al.,* 1992). Salt stress alters the membrane properties leading to reduced uptake of various essential nutrients by the roots and transport to the shoots (Promila & Kumar, 2000; Lauchli & Luttge, 2002). This can be measured readily by tissue analysis or noted from the visual nutrient deficiency symptoms. Deficiency of N and Ca^{2+} leads to the chlorosis (yellowing) and that of P to the necrosis of leaves. Reduction in K^+ levels is due to specific effect of Na^+. Role of K^+ has been implicated in the enzyme activities of respiration and photosynthesis and osmotic adjustment. Its deficiency initially leads to chlorosis and then necrosis (James, 1988; Taiz & Zeiger, 2002). Although the content of all nutrients were severely reduced under salinity, a better tendency of the tolerant genotypes to maintain their greater content in young leaflets emerged as an important strategy to depict low nutrient deficiency symptoms as chlorosis and necrosis (Table I). Both isotope-labeling and tissue-analysis studies have shown that different species have an important tendency of remobilization and utilization of various essential nutrients from aged and senescing leaves to the young growing regions under normal (Durand & Lacan, 1994; Mavrogianopouls *et al.,* 2002; Wang *et al.,* 2003) or stress (Hocking, 1982) conditions. The tolerant genotypes appeared to adopt this strategy in displaying higher content of essential nutrients (Fig. 2) in displaying better growth and seed yield at maturity. This aspect, however, needs to be verified in mungbean and other species.

Stress conditions adversely affect the pigment composition in different plant species, as is quite often noted from the changed coloration (Wahid *et al.,* 1997) or necrosis

Fig. 3. Clorophyll (Chl) and carotenoid (Car) content of young and old leaves of mungbean genotypes under salinity at early vegetative (EVS), late vegetative (LVS) and reproductive (RS) stages of growth



of leaves (Chen *et al.,* 2003). Salinity-induced chlorophyll fluorescence has been used as a tool to ascertain the salt tolerance in various plant species (Misra *et al.,* 2001; Morant-Manceau *et al.,* 2004). In this study, the Chl and Car contents were substantially diminished under salinity in leaflets of both the ages (Fig. 3). Positive correlations of increased mM_{50} and high levels of photosynthetic pigments in young leaflets (cf. results section) revealed that greater contents of Chl and Car are important in salt tolerance. Furthermore, Chl-a:b ratio of young leaves of the sensitive genotypes (NM-92 and NM-98) increased significantly, while the tolerant ones (NM-89 and NM-54) tended to maintain a fairly steady value (Fig. 3). This corroborates the reports for durum wheat (Husain *et al.,* 2003). Increased Chl-a:b ratio was positively related to Na^+ and Cl^- while negatively to increased mM_{50} of young leaflets. This revealed an important role of photosynthetic pigments (mainly Chl-b & Car) in the enhanced salt tolerance of mungbean genotypes (Table IV). This role may be a higher photochemical capacity of the Chl and Car-mediated regulation of nonphotochemical energy dissipation under stress (Rmiki *et al.,* 1999).

Increased necrosis, an important visual symptom (Volkmar *et al.,* 1998), indicated a strong inverse correlated with reduced contents of N, P, K^+ and Ca^{2+} contents. Excess of Na^+ and Cl^- on the other hand was only weakly correlated with necrosis of the genotypes (cf. results section). This showed that appearance of necrotic signs (Table I) was predominantly due to ion-induced nutrient deficiency (Fig. 2). Inverse relationships of higher mM_{50} with increased chlorosis and necrosis are ascribed to reduced dry matter partitioning during whole growth period in sensitive genotypes (Fig. 1). Leaf tip burning a naturally occurring phenomenon, although increased under salinity, sparingly exhibited any relationship with various attributes of young leaflets and thus revealed a little role in the salt sensitivity response.

Ionic-induced deficiency of essential nutrient and loss of Chl and Car hamper the photosynthetic efficiency of the plants in the production and partitioning of photoassimilates (Sreenivasulu *et al.,* 1998; Morant-Manceau *et al.,* 2004) a higher rate of which is imperative in greater dry mass and seed yield. Therefore, sustained levels of all these attributes in young leaves are a requisite at all stages. A severe reduction in pod and seed yield of the genotypes was presumably related to the hampered photosynthetic activity of leaves due to chlorosis and necrosis of the leaves (Table I, II). Moreover, it is documented that photosynthesis in fruit wall contributes significantly (up to 20%) towards seed filling (Wahid & Rasul, 2004); the browning (chlorosis) of pods due to salinity has also partly affected the final seed yield in mungbean genotypes.

In crux, although ion-toxicity mainly crippled the dry mass and seed yield, nevertheless flexibility for salt tolerance indicates a scope for breeding and tailoring of mungbean in moderately saline tracts. Pattern of ion-distribution did not change with the growth stages. Low chlorosis and necrosis of young leaves were taken as important criteria of salt tolerance. Ion-exclusion into senescing leaves together with enhanced tissue nutrients and photosynthetic pigments (mainly Chl-b) of young leaves were important strategies of salt tolerance.

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