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EFFECT OF SALINITY ON THE FOLIAR FEATURES OF MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK] GENOTYPES

KANTA RANI, S. BABBER AND HANS R. DHINGRA*

Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar-125004

Effect of chloride dominated salinity (0, 4.0, 6.0 and 8.0 dSm^{-1}) on foliar features of four genotypes of mungbean (Km-2, MH85-111, MH98-6 and Asha) was studied. Mungbean stomata are amphistomatous and frequency of epidermal cells and stomata on lower surface was more than the upper surface. Salinity induced a decline in stomatal index and frequency of trichomes. However, the size of stomata remained unaffected by salinity. Salinity did not cause any major change in venation pattern but for the number of areoles/mm^2 which increased significantly. Salinity did not affect the size of primary, secondary veins and number of veinlets.

Key words : Mungbean, salinity, stomata, foliar features, trichomes

Environmental stress conditions are major factors limiting plant productivity and plant distribution (Boyer, 1982). Different biotic and abiotic stresses have great impact on various physiological and biochemical factors of plant and they show a rapid molecular response to changing environmental conditions (Lichtenthaler, 1996). Among abiotic stresses, salinity (both soil and H_2O) is one of the major factors responsible for deterioration of soil health and affects crop yield adversely. Reduction in crop yield under saline condition can be ascribed to its adverse effects on fitness of male and female reproductive structures (Dhingra and Varghese, 1993; Dhingra and Varghese, 1997) and availability of limited amount of photosynthates. The upset in the water balance and other metabolic changes induced by salinity are very often accompanied by structural adaptations. Reported decreased rates of transpiration and other related processes under saline conditions may be partly due to structural and physiological modifications of stomata (Gale. and Poljakoff. Mayber, 1970). Stomata are the orifaces on the surfaces of leaves which regulate not only rate of transpiration but also exchange of gases during photosynthesis. Rate of transpiration is also regulated by the presence of trichomes on the leaf surface (Fahn, 1990). Inspite of great relevance of foliar features in relation to functions and physiology of plants, no meticulous attempt has been made to study stomatal characteristics of plants inhabiting saline habitats. Present study therefore, was taken up to assess the effect of salinity on foliar features of four genotypes (Km-2, MH85-111, MH98-6 and Asha) of mungbean differing in their salinity tolerance status.

MATERIALS AND METHODS

Surface sterilized seeds of four genotypes of mungbean (*Vigna radiata* (L.) Wilczek) viz. Km-2, MH85-111, MH98-6 and Asha were inoculated with broth of *Rhizobium leguminosarum* strain S-24 and sown in pots (30cm. dia) lined with a polythene bag. Each bag had a central drainage hole and was filled with 8 kg of thoroughly washed acid treated river sand. Fifteen days old plants were exposed to chloride dominated salinity (0, 4.0, 6.0 and 8.0 dSm^{-1}) which was created by adding appropriate amounts of different salts viz., NaCl, $CaCl_2$, $MgCl_2$ and $MgSO_4$ to the nitrogen free nutrient solution (Wilson and Reisenaur, 1963). The quantities of salts were adjusted in such a way that ratio proportion of Na : Ca + Mg was 1:1 and Ca : Mg was 1:3 and Cl : SO_4 was 7:3 on milliequivalent basis. For stomatal studies the 3rd or 4th fully expanded leaf from the top of 30d old plants was collected and carefully scraped mechanically in order to remove upper and lower epidermal peel and stained with Delafield's hematoxylin. These peels passed through ethyl alcohol-xylene series and

e-mail : hrdhingra@hau.ernet.in
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mounted in DPX. The stomatal index was calculated by using Salisbury (1928) formula. Average area of individual leaflet of 3rd/4th fully expanded leaf from the top was recorded by leaf area meter. The venation pattern was studied by following the leaf clearing technique of Mohan Ram and Nayyar (1978) using phenol, trichloroacetic acid mixture. Data were subjected to statistical analysis using complete randomized block design (CRD) for two factors (Gomez and Gomez, 1984)

RESULTS

The leaves of mungbean are amphistomatous and the stomata were anomocytic type since these were surrounded by 3-4 subsidiary cells. Epidermal cells were convoluted in outline. Frequency of epidermal cells and stomata on lower surface in general was more as compared to upper surface and values were comparable in the four genotypes. Salinity affected the frequency stomata as well as epidermal cells adversely, reduction in stomatal frequency being more in Km-2 and MH85-111 genotypes over Asha and MH 98-6 on upper leaf surface whereas reduction on the lower side was nearly identical in all genotypes (Table 1). Convoluted feature of epidermal cells was almost lost due to salinity. The stomatal index decreased with the increasing level of salinity, but effect was less

Table 1. Effect of salinity on stomatal index in different genotypes of mungbean

Genotype	No. of stomata per mm^2									
	Upper epidermis					Lower epidermis				
	Levels of salinity (dSm^{-1})					Levels of salinity (dSm^{-1})				
	Control	4.0	6.0	8.0	Mean	Control	4.0	6.0	8.0	Mean
Km-2	68.58	51.21	42.31	31.92	48.51	125.78	109.97	108.51	100.80	111.27
MH85-111	63.41	51.56	48.04	38.29	50.33	108.34	106.29	100.97	87.75	100.84
MH 98-6	53.24	50.24	47.37	45.12	48.99	104.41	104.14	101.58	101.95	103.02
Asha	55.12	53.41	45.60	36.82	47.74	116.70	106.48	100.60	95.46	104.81
Mean	60.09	51.61	45.83	38.04		113.81	106.72	102.92	96.49	
CD at 5%	Genotype = NS		Salinity = 2.91		G×S = 5.81	Genotype = NS		Salinity = NS		G×S = NS
	No. of epidermal cells per mm^2									
	Control	4.0	6.0	8.0	Mean	Control	4.0	6.0	8.0	Mean
Km-2	191.17	180.41	173.56	155.85	175.25	214.00	198.21	197.00	195.12	201.08
MH85-111	190.48	187.63	186.48	180.78	186.34	214.92	209.85	203.41	203.41	207.90
MH 98-6	188.39	185.65	176.02	170.03	180.02	207.60	195.97	193.00	170.32	191.72
Asha	185.95	184.66	178.56	171.19	179.34	202.29	193.92	188.41	175.95	190.14
Mean	188.99	183.84	178.66	169.46		209.70	199.49	195.46	186.20	
CD at 5%	Genotype = NS		Salinity= 9.0		G×S = NS	Genotype = NS		Salinity = 13.9		G×S =NS
	Stomatal index									
	Upper epidermis					Lower epidermis				
Km-2	26.43	22.10	19.64	16.99	21.26	36.94	35.60	35.45	33.99	35.49
MH85-111	24.98	21.57	20.51	17.51	21.14	33.52	33.57	33.12	30.08	32.57
MH 98-6	22.05	21.33	21.22	20.97	21.39	33.43	34.72	34.51	37.43	35.02
Asha	22.89	22.72	20.36	17.73	20.93	36.54	35.46	34.84	35.21	35.51
Mean	24.08	21.90	20.43	18.30		35.11	34.84	34.48	34.18	
CD at 5%	Genotype = NS		Salinity = 1.58		G×S=NS	Genotype = NS		Salinity = NS		G×S=NS

pronounced on lower leaf surface than upper surface. The frequency of epidermal cells was less affected compared to the stomatal frequency. Like stomatal frequency, the epidermal frequency decreased with increasing level of salinity and results varied in both upper and lower epidermis (Table 1).

There was no significant difference in the length and width of stomata of upper and lower surface (Table 2). Number of trichomes was more on lower leaf surfaces than the upper ones. On the upper leaf surface number of trichomes in Km-2 and Asha was comparable and higher than those of MH85-111 and MH98-6. Like the frequency of stomatal and epidermal cells, trichome frequency also decreased on both surfaces with increasing level of salinity and reduction was maximum in case of Asha and MH98-6 genotypes followed by MH85-111 and Km-2. Length of trichomes was highest in MH98-6 on both leaf surfaces while the values were comparable in other three genotypes. Salinity, however, did not

Table 2. Effect of salinity on stomatal size and trichome characteristics in different genotypes of mungbean

	Size of stomata (µm) upper epidermis									
	Length					Width				
	Control	4.0	6.0	8.0	Mean	Control	4.0	6.0	8.0	Mean
Km-2	23.56	21.05	19.80	19.00	20.85	11.20	11.40	8.90	8.50	10.00
MH85-111	20.46	19.86	19.80	16.84	19.24	11.25	10.70	10.20	10.02	10.54
MH 98-6	19.75	19.16	18.76	17.69	18.82	11.00	10.23	9.96	9.72	10.23
Asha	19.65	19.23	18.96	18.93	19.19	10.65	9.35	9.24	9.10	9.58
Mean	20.85	19.83	19.33	18.09		11.03	10.42	9.58	9.34	
CD at 5%	Genotype = 1.09		Salinity=1.09		G×S=NS	Genotype = NS		Salinity = 0.92		G×S= NS
	Size of stomata (µm) lower epidermis									
	Length					Width				
	Control	4.0	6.0	8.0	Mean	Control	4.0	6.0	8.0	Mean
Km-2	19.11	18.80	17.55	15.37	17.71	9.55	9.12	9.60	9.50	9.44
MH85-111	20.54	19.95	19.73	19.30	19.88	11.63	10.47	9.42	9.40	10.23
MH 98-6	20.00	19.10	19.25	18.75	19.28	11.40	11.36	10.40	10.15	10.83
Asha	20.07	19.71	18.96	18.76	19.38	10.91	10.71	9.23	9.12	9.99
Mean	19.93	19.39	18.87	18.05		10.87	10.42	9.66	9.19	
CD at 5%	Genotype =NS		Salinity = NS		G×S= NS	Genotype = NS		Salinity = 1.04		G×S=NS
	No. of trichomes per mm^2									
	Upper epidermis					Lower epidermis				
Km-2	7.31	7.92	6.09	4.26	6.40	9.75	9.14	8.53	5.48	8.23
MH85-111	6.09	5.48	4.87	3.04	4.87	8.53	7.92	6.70	4.87	7.01
MH 98-6	6.09	5.48	4.26	3.04	4.72	9.14	7.92	6.78	4.26	7.01
Asha	7.42	6.15	4.26	3.04	5.22	10.36	8.53	6.70	4.26	7.46
Mean	6.73	6.26	4.87	3.34		9.45	8.38	7.16	4.72	
CD at 5%	Genotype = 0.94		Salinity = 0.94		G×S= NS	Genotype = NS		Salinity = 1.00		G×S = NS
	Size of trichomes (µm)									
	Upper epidermis					Lower epidermis				
Km-2	285.00	279.00	261.00	250.00	268.75	270.00	269.00	253.00	239.00	257.75
MH85-111	265.00	257.00	234.50	210.00	241.63	250.00	246.00	220.00	201.00	229.25
MH 98-6	338.05	328.00	282.50	264.00	301.14	316.50	281.70	261.50	252.00	277.93
Asha	240.00	237.00	225.00	207.00	227.25	241.00	229.00	221.00	206.00	224.25
Mean	282.01	273.25	250.75	232.75		269.38	256.43	238.88	224.50	
CD at 5%	Genotype =45.31		Salinity= NS		G×S= NS	Genotype = 35.95		Salinity = NS		G×S=NS

Table 3. Effect of salinity on leaflet area, number of areoles, veinlets and their width in different genotypes of mungbean

Genotype	Average area of leaflet (cm^2)					No. of areoles per mm^2				
	Levels of salinity (dSm^{-1})					Levels of salinity (dSm^{-1})				
	Control	4.0	6.0	8.0	Mean	Control	4.0	6.0	8.0	Mean
Km-2	11.08	12.87	9.27	7.75	10.24	31.70	55.60	60.10	68.29	53.92
MH85-111	10.29	11.37	9.30	6.78	9.43	41.25	56.09	61.23	72.56	57.78
MH 98-6	11.33	12.83	8.97	7.60	10.18	43.53	59.51	68.35	72.48	60.97
Asha	18.73	16.30	10.81	9.66	13.88	40.24	50.00	59.17	63.41	53.20
Mean	12.86	13.34	9.59	7.95		39.18	55.30	62.21	69.18	
CD at 5%	Genotype = 1.82		Salinity =1.82		G×S = NS	Genotype = 4.15		Salinity = 4.15		G×S = NS
	No. of veinlets entering/areole					No. of vein endings/areole				
	Control	4.0	6.0	8.0	Mean	Control	4.0	6.0	8.0	Mean
Km-2	1.67	1.72	1.47	1.60	1.61	1.62	1.50	1.57	1.50	1.55
MH85-111	1.57	1.70	1.65	1.50	1.60	1.71	1.44	1.25	1.50	1.39
MH 98-6	1.72	1.57	1.68	1.50	1.62	1.50	1.33	1.44	1.28	1.39
Asha	1.80	1.50	1.67	1.53	1.62	1.71	1.63	1.55	1.37	1.56
Mean	1.69	1.62	1.62	1.53		1.63	1.47	1.45	1.41	
CD at 5%	Genotype = NS		Salinity=0.06		G×S=0.11	Genotype =0.10		Salinity=0.10		G×S=NS
	Width of primary vein (µm)					Width of secondary vein (µm)				
	Control	4.0	6.0	8.0	Mean	Control	4.0	6.0	8.0	Mean
Km-2	82.20	72.50	68.75	61.75	71.31	23.66	21.70	18.75	13.91	19.51
MH85-111	78.33	73.60	69.22	58.75	69.98	25.62	23.25	17.68	14.50	20.26
MH 98-6	78.12	70.23	65.16	53.71	66.81	39.00	34.75	28.20	18.62	30.14
Asha	81.25	70.00	62.75	50.11	66.03	23.50	19.75	14.11	10.86	17.05
Mean	79.98	71.58	66.47	56.08		27.95	24.86	19.69	14.47	
CD at 5%	Genotype =NS		Salinity=4.6		G×S= NS	Genotype = 3.13		Salinity=3.13		G×S=NS

affect the length of trichome significantly (Table 2).

Area of leaflet was maximum in genotype Asha. Salinity decreased leaflet area and the reduction was the highest in Asha. Typical of dicots, the venation in mungbean is also reticulate type. Venation pattern of mungbean did not reveal major change under various levels of salt treatment. The basic architecture of venation remained the same except the number and size of areole, width of primary and secondary veins (Table 3) with increasing level of salinity. Number of areole/mm^2 was higher and comparable in MH85-111, MH98-6 and Asha than Km-2. It increased significantly with salinity. Among tested genotypes, Km-2 showed maximum increase followed by MH85-111, MH98-6 and Asha. Width of primary vein was comparable in all the tested genotypes but width of secondary vein was higher in MH98-6 than the other three genotypes. The width of primary and secondary veins was reduced by salinity, width of secondary vein being affected more adversely. Among the genotypes the reduction in width of primary and secondary veins was maximum in genotypes MH98-6 and Asha. Salinity had a little effect on the number of veinlets entering/areole and this effect was variable (Table 3).

DISCUSSION

Salinity is known to induce physiological dryness. Water loss from the plant is regulated through stomatal control. Opening of stomata leads to relative transpirational cooling while converse hold true when stomata are closed or showed low stomatal conductance. In addition, opening of stomata raises the possibility of resumption of apoplastic water flow with concomitant entry of large quantities of salts into the plant. Present study has revealed that salinity induced a decrease in frequency of epidermal and stomatal cells, the former being affected more adversely than the latter. Wignarajah *et al.* (1975) also reported that salinity reduced the number of dermal cells drastically. It might be one of the mechanisms of adaptation in order to reduce the transpiration in the physiological dry condition. Devi and Rao (1980) also observed a decrease in stomatal frequency and index under NaCl salinity in groundnut. Similar observations were also reported in chickpea (Purohit *et al.*, 1997) and in mungbean (Kudawala, 2002). Conversely, an increase in the number of stomata per unit area has been reported in cotton (Rozhanovskii, 1948), barley (Strognov, 1964) and wheat (Akram *et al.*, 2002). Salas *et al.* (2001) observed an increase in the stomatal index in tomato grown under saline conditions. Such a variability in results seems to be species specific and depends upon the adaptive potential of the crop. Stomatal index is a relative feature which remains almost constant under a defined environmental condition. Salinity decreased stomatal index which further decreased with increasing level of salinity. Stomatal index of lower surface was affected less severely as compared to upper surface. Decrease in stomatal index of upper surface was maximum in Km-2 and minimum in MH98-6. For the lower surface the reduction of stomatal index was non-significant for all genotypes.

Observed reduction in leaflet area adduces support to the findings of Bray and Reid (2002) in *Phaseolus vulgaris*. Salinity did not induce any significant change in the shape of trichomes but decreased their frequency of distribution. These findings corroborate the reports of Purohit *et al.* (1997) in chickpea and Kudawala (2002) in mungbean who found decrease in frequency of trichomes with the rise in rhizospheric salinity. Like frequency, the length of trichomes on both upper and lower leaf surfaces was also reduced by salinity. Similar results were observed by Kudawala (2002). However, an increase in the length of trichomes of upper and lower surface under chloride dominated salinity was reported in chickpea (Purohit *et al.*, 1997). Such a variation in result might be due to genotypic difference in crops. An increase in the number of areoles per unit area is an indicative of a reduction in the size of areoles. This reduction in size is perhaps due to the retardation in cell elongation under saline condition as reported by Strogonov (1964).

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