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Physiological Characterization of Mustard (*Brassica* sp.) Genotypes for Their Salt Tolerance

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Abstract: A sand culture experiment was carried out during 2002-2003 to evaluate the effect of different levels of salinity on some metabolic activities and yield of three mustard genotypes viz., Safal, Binasarisha-4 and Binasarisha-5. The salinity treatments were EC0 (control or no salt), EC5, EC10 and EC15 dS m^{-1}. Results reflected that salinity treatment caused decrease in NR activity and reduced the accumulation of chlorophyll, nitrate, amino acid, soluble protein and sugar. Root volume and weight and the accumulation of potassium and zinc were decreased while nitrogen, phosphorus and sodium accumulations increased. Genotypes Safal and Binasarisha-5 had higher accumulation of almost all the biochemical and chemical substances compared to Binasarisha-4 at salt stress condition. In all the genotypes of mustard yield was reduced at salt stress condition. But in genotype Safal dry matter accumulation and seed yield were better than those of the other two. From the above results it was indicated that Safal and Binasarisha-5 performed better with respect to biochemical point of view as well as yield under salinity stress.

Key words: Salinity, mustard, K^+, Na^+, root volume, root weight

INTRODUCTION

Like other environmental stresses salinity also results in agricultural losses[1]. It affects nutrient uptake[2] and metabolic activities in the plant[3]. However, the magnitude of the effect of salinity varied with the plant. species, type and level of salinity[4] There are inter-species, intra-species and intercultivar variations, even individual lines also differ at different ontogenetic stages in salt tolerance which provide scope for selection of genotypes for salt tolerance[5,6]. Mustard is one of the important oilseed crops in Bangladesh. Under salt stress condition the yield of the crop is very poor which results a bad impact for the economy of the country. In Bangladesh, over 30% of cultivable areas are in the coastal zone which faces acute salinity problem. Moreover, the salt affected areas are increasing day by day. So selection of mustard varieties tolerant to salt environment will allow the proper use of vast saline areas of Bangladesh after the end of monsoon season.

Recent strategies of this Institute are to develop salt tolerant genotypes of mustard to cope with the need of the country for better utilization of salt affected areas by fitting salt tolerant genotypes. The objective of this experiment was to evaluate genotypes of mustard for their salt tolerance.

MATERIALS AND METHODS

For this experiment, three newly developed genotypes of mustard viz., Safal (Mother-YS-52, *B. campestris* L. var. Yellow sarson), Binasarisha-4 and Binasarisha-5 (Mother Nap-3, *B. napus* L.) were taken. Binasarisha-5 was considered as a salt tolerant check because no established salt tolerant genotypes of mustard was available. But this genotype performed slightly better in saline soil in field condition.

Ten kilogram acid washed sand was taken in earthen pots which were lined with polyethylene bags to avoid contact or leaching losses of salt. Ten seeds of each genotype were sown in each pot. This was a Complete Randomized Design experiment with three replications. Four seedlings were allowed to grow and the remaining seedlings were removed 7 Day After Germination (DAG). In the experiment Modified Latcombe nutrient solution[7] with the following composition was used. To maintain 60 kg N ha^{-1} KNO_3, Ca $(NO_3)_2$ and $NaNO_3$ @ 5, 2 and 4 mM, respectively (jointly equal to 60 kg N ha^{-1}) and $MgSO_4$, $7H_2O$ @ 0.75 mM to maintain 40 kg S ha^{-1} were applied as N and S sources. For P, B, Fe, Cu, Mo, Mn, Cl and Zn @ 1, 0.092, 0.0967, 0.0025, 0.00016, 0.092, 0.18 and 0.0077 mM kg^{-1} sand from KH_2PO_4, H_3BO_3, Fe-EDTA, $CuSO_4.5H_2O$, $NH_4Mo_7O_2$, $MnSO_4$. $4H_2O$, Kcl and $ZnSO_4$.

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$7H_2O$, respectively were applied initially once in every week and then twice in a week to the pot till maturity of the crop. Salinity levels, viz. EC0(control), EC5 EC10 and EC15 dS m^{-1} were used. Calculated amounts of salt, considering 0.06% salt ≡ EC1dS m^{-1}, as NaCl, were added to the sands, in the respective pots to achieve appropriate salinity. The calculation of salt was done on the basis of weight of sand/pot and salinity was achieved using salts following the methods of Ponnamperuma[8] and Michael[9].

Ten percent equivalent for 1 dS m^{-1} was also determined from a calibration curve in an EC instrument (Salinity Bridge, Cat No. 5500, Soil Moisture Equipment Corp., Santa Barbara, Calif., USA). The required amounts of salts were applied in four equal splits at weekly intervals with a view to developing salinity gradually as in the field condition. The first split of salt solution was applied at 15 day old plants and the remaining salt solutions were applied in the subsequent weeks. Sixty percent field capacity were maintained by adding deionized water during application of nutrient and salt solutions.

Observations were recorded for some physiological and biochemical parameters on specified dates of experiment. Chlorophyll[10], amino acid (R.K.Dutta, personal communication), soluble protein[11], sugars[12-14], nitrate[15], nitrate reductase activity[16] were determined at 45 DAG. After harvest, from the shoot sample amount of sodium, potassium and zinc were measured by atomic absorption spectrophotometer (Model 2348, Perkin Elmer, USA) following ASI method.

Phosphate was determined following vanado-molybdate-nitric acid yellow colour method[17] and nitrogen following micro kjheldahl[18] method. Also dry matter accumulation and seed yield, root volume (using water displacement method in a measuring cylinder), root weights were recorded at that stage. All the data were subjected to statistical analyses following DMRT.

RESULTS AND DISCUSSION

Results showed that salinity caused decrease in chlorophyll content by 16 to 43% than that at the control. Genotypes also showed significant variation with respect to chlorophyll content. Among them Binasarisha-5 had the highest chlorophyll content over that in the other genotypes (Table 1). Such salinity induced reduction in chlorophyll content was in confirmation with the works of Garg *et al.*[19] and Parti *et al.*[20]. Decrease in chlorophyll content due to salt stress might be associated with the decrease in soluble protein and amino acid levels as was reported by Fletcher and Arnold[21].

Results reflected that salinity affected Nitrate Reductase (NR) activity and nitrate content. They were decreased than those of the control by 46 to 72% and 23

Table 1: Effects of salinity levels on biochemical parameters of mustard genotypes

Characters	Total chlorophyll content (mg g^{-1} fw)	Nitrate reductase activity (μ mol NO_2^- g^{-1} fwh^{-1})	Nitrate content (μg^{-1} fw)	Total amino acid content (mg g^{-1} fw)	Soluble protein content (mg g^{-1} fw)	Reducing sugar content (mg g^{-1} fw)	Total sugar content (mg g^{-1} fw)
Treatment							
T_0	1.22a	4.56a	321.43a	20.10a	31.33a	1.99a	21.76a
T_1	1.02b	2.96b	246.68b	16.63b	25.85b	1.24b	18.78b
T_2	1.01b	2.16c	184.81c	15.46b	23.63c	0.95c	14.12c
T_3	0.69c	1.53d	120.24d	10.34c	21.70c	0.63d	10.96d
$LSD_{(0.05)}$	0.102	0.521	30.25	2.41	2.21	0.160	1.763
Variety							
Safal	0.92b	4.12a	183.36b	16.41a	29.23a	1.56a	17.61a
Binasarisha-4	0.97b	2.20b	224.94a	12.24b	23.76b	0.87c	13.04b
Binasarisha-5	1.06a	2.09b	246.57a	18.24a	23.90b	1.17b	18.57a
$LSD_{(0.05)}$	0.88	0.45	26.20	2.09	1.911	0.14	1.527
CV%	10.44	19.11	14.24	15.88	8.85	13.55	11.04

Values with different letter within a column differ significantly at 5% level of probability as per DMRT

Table 2: Effects of salinity on ion accumulation, root, volume, root weight and yield in mustard genotypes

Character	K (%)	Na (%)	P (%)	Zn ppm	N (%)	Root volume (cc)	Root weight (g)	Total dry matter/plant (g)	Seed yield matter/plan
Treatment									
T_0	3.08a	0.73b	0.19c	114.44a	2.66b	4.36a	2.39a	9.94a	2.38a
T_1	3.0a	1.14a	0.24b	61.11c	2.91b	4.02a	1.64b	8.33b	1.89b
T_2	2.75b	1.27a	0.25b	75.56b	3.35a	2.59b	1.14c	3.92c	1.08c
T_3	2.0c	1.30a	0.28a	50.33d	3.70a	2.19c	0.93c	2.22d	0.97c
$LSD_{(0.05)}$	0.66	0.19	0.03	14.87	0.42	0.40	0.14	0.61	0.15
Variety									
Safal	3.13a	1.06a	0.29a	80.83b	3.44a	4.36a	2.36a	7.48a	1.69a
Binasarisha-4	2.86a	1.12a	0.20c	110.25a	3.01b	3.0b	1.140b	5.13c	1.51b
Binasarisha-5	2.89a	1.14a	0.24b	42.75c	3.01b	3.19b	1.10b	5.70b	1.55b
$LSD_{(0.05)}$	0.15	0.17	0.03	7.4	0.36	0.3	0.35	0.53	8.9
CV%	6.1	6.44	9.96	10.2	11.71	10.1	13.7	7.3	0.13

Values with different letter within a column differ significantly at 5% level of probability as per DMRT to 62%, respectively at 5 to 15 dS m^{+} of salinity. Similar salinity induced decrease in NR activity had reported in *Brassica juncea* by Garg *et al.*[22] and Sureena *et al.*[23] and nitrate content in sunflower by Jaykumar *et al.*[24].

Among the genotypes Safal had the highest NR activity along with the lowest of nitrate content.

While the lowest NR activity with the highest nitrate content were recorded in the genotype Binasarisha-5. As NR is a nitrate inducible enzyme[25] the decrease in NR activity and the subsequent increase in nitrate is interrelated. Data showed that genotype Safal had the lowest nitrate content along with higher NR activity which indicated its higher efficiency of reducing nitrate to nitrite at salt stress condition.

It was observed that salinity caused reduction in the accumulation of amino acid and soluble protein by 17 to 48% and 17 to 31%, respectively at 5 to 15 dS m^{-1} of salinity than those of the control treatment (Table 1). Similarly, genotypes also showed variability in respect of soluble protein and amino acid content. Soluble protein content was recorded the highest in the genotype Safal and that was the lowest in Binasarisha-4. On the other hand, the lowest accumulation of amino acid was recorded in the genotype Binasarisha-4 and the highest of that was recorded in the genotype Binasarisha-5 which was statistically similar to that in Safal (Table 1). Similar, decrease in soluble protein and amino acid content in salinity stress condition had reported by Saha and Gupta[26] This decrease in soluble protein content might be associated with salinity induced decrease in leaf area and chlorophyll content[27] which resulted poor synthesis of sugar that might limits energy supply.

Result showed that reducing and total sugar contents decreased by 37 to 68% and 13 to 49%, respectively at 5 to 15 dS m^{-1} of salinity over those of the control. There was also significant variation among the genotypes in respect of sugar accumulation. The highest accumulation of reducing sugar was in Safal and the lowest of that was recorded in Binasarisha-4. While total sugar accumulation was recorded the highest in the genotype Binasarisha-5 which was statistically similar to that in Safal (Table 1). Similar decrease in reducing sugar accumulation due to salt treatment had reported by Garg *et al.*[19]. On the other hand Paek *et al.*[28] had reported that salinity had no effect on reducing sugar accumulation. While Surrena *et al.*[23] reported salinity induced increase in total sugar content in *Brassica juncea*. The decrease in sugar accumulation due to salt treatment might be associated with salinity induced decrease in pigment content which impaired metabolic activities in plants[29].

Due to salt treatment accumulation of potassium and zinc were decreased while it increased the accumulation of sodium, phosphorus and nitrogen (Table 2). Genotypes also differed significantly with respect to ion accumulation. Among them Safal had the highest accumulation of potassium, phosphorus and nitrogen while Binasarisha-4 had accumulated the lowest of those (Table 2). Quayyum *et al.*[30] observed that Na^{+} accumulation was higher in salt tolerant and susceptible genotypes in NaCl treated rice plants but susceptible one had more amount of this element. This might be because the excess Na^{+} restricted uptake of K^{+} in the plant. Similarly, Yang *et al.*[31] observed increased Na^{+} accumulation with increased salinity in leaves of less tolerant S*orghum bicolor* but not in the tolerant genotypes. On the other hand, increase in the accumulation of nitrogen and phosphorus with increase in salt levels might be the concentration effect of the poor morphological growth where these elements were not being utilized properly by the plants.

It is apparent that at salt stress condition root volume and root weight were decreased by 8 to 50% and 31 to 61%, respectively at 5 to 15 dS m^{-1} of salinity than those of the control. It was further noticed that the root volume and weight were the highest in the genotype Safal compared to those of the other two genotypes. Similar decrease in root volume and weight due to salinity stress had reported earlier[32] in rice and that the genotypes which had the higher root volume and weight i.e. in which development of root system was better, the ability of those genotypes to sustain in salt stress condition was also better.

Interaction effects between genotypes and salinity levels also showed significant variations among the genotypes with respect to the parameters studied. It was observed that among the genotypes Safal showed better performances with respect to almost all the parameters studied (Fig. 1A-I).

Dry matter accumulation and seed yield were recorded the highest in the control treatment followed by those at 5 dS m^{-1} of salinity. Among the genotypes, Safal had the highest seed yield and dry matter content followed by those of the genotype Binasarisha-5. The lowest seed yield and dry matter content were recorded in the genotype Binasarisha-4. Similar decrease in dry matter accumulation and seed yield due to salt treatment were reported by Ashraf *et al.*[33, 34] in *Brassica* species. Further Sharma and Manchanda[35] reported that at EC 9.5 and EC11.5 dS m^{-1} the seed yield of yellow sarsan decreased by 19 and 20%, respectively compared with a reduction of 29 and 40% in toria indicating that the former was more tolerant to salinity than that of the latter. The decrease in dry matter accumulation and seed yield might be associated with the salinity induced decreased synthesis

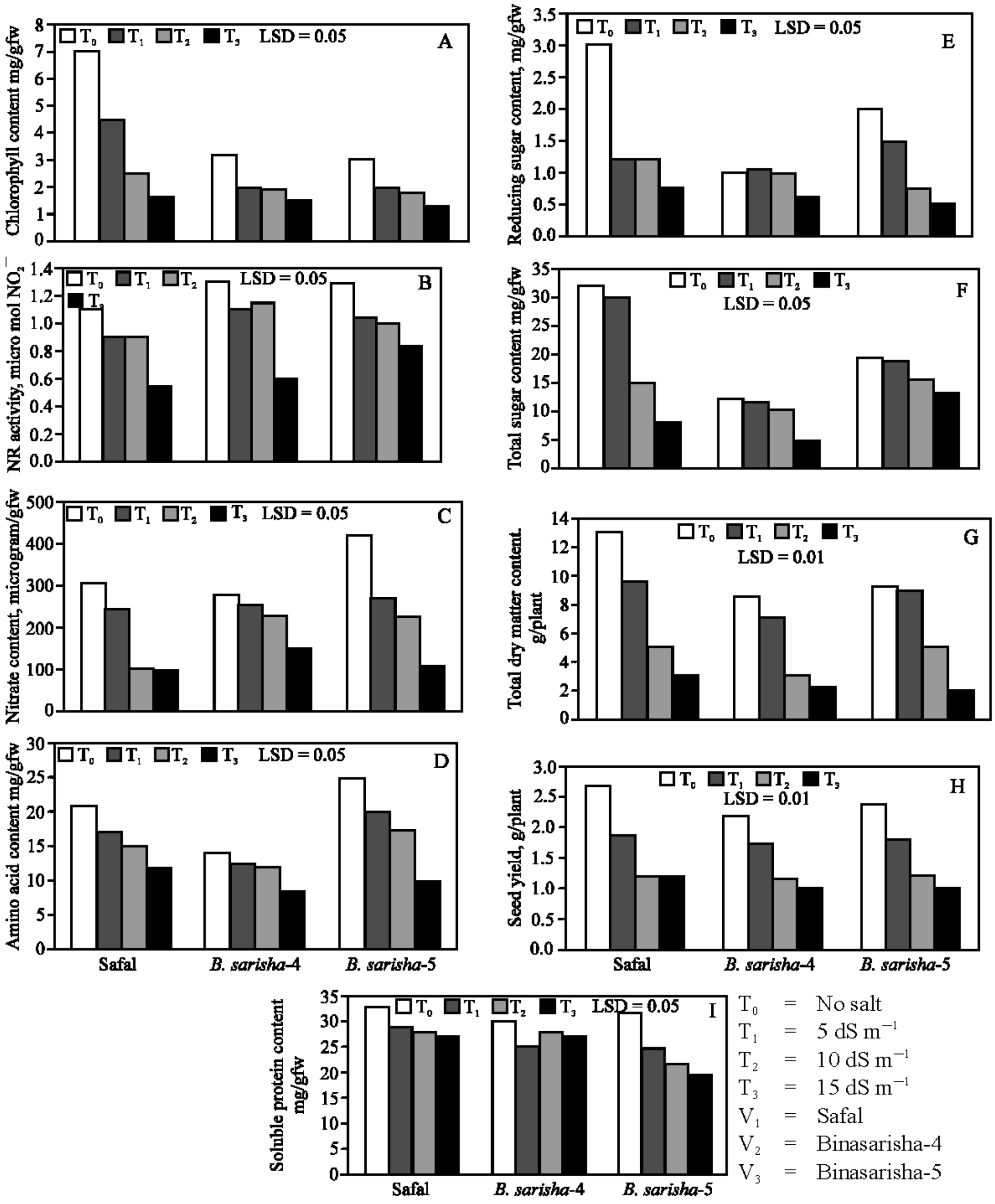

Fig. 1: A: Interaction effects between genotypes and salinity levels on chlorophyll content in mustard
B: Interaction effects between genotypes and salinity levels on NR activity in mustard
C: Interaction effects between genotypes and salinity levels on nitrate content in mustard
D: Interaction effects between genotypes and salinity levels on amino acit content in mustard
E: Interaction effects between genotypes and salinity levels on soluble protein content in mustard
F: Interaction effects between genotypes and salinity levels on reducing sugar content in mustard
G: Interaction effects between genotypes and salinity levels on total sugar content in mustard
H: Interaction effects between genotypes and salinity levels on dry matter accumulation in mustard
I: Interaction effects between genotypes and salinity levels on seed yield in mustard

of biochemical substances, uptake and transport of the cations and anions and the development of root, so development of shoot was also affected and ultimately reduced seed yield[36]. In the experiment salinity did not show any detrimental effect to produce dry matter and seed yield in Safal and Binasarisha-5 which indicated their tolerance to salinity.

From the above findings it is evident that salinity inhibited plant growth and seed yield, it affected chlorophyll synthesis, sugar, amino acid and soluble protein accumulation and NR activity. Further, there were variabilities among the genotypes in responding to salinity and salinity affected overall metabolic processes. Over and above, genotypes Safal and Binasarisha-5 withstood in salt stress condition and could produce maximal yield. Thus, the genotypes Safal and Binasarisha-5 may be recommended for field trial under actual saline condition to test their performance to release as salt tolerant varieties.

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