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IMPROVEMENT OF SALT TOLERANCE IN PEARL MILLET: PHYSIOLOGICAL AND BIOCHEMICAL PERSPECTIVE

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Abstract: The present study was undertaken to assess the response of eight pearl millet lines collected from ICRISAT, Hyderabad for salt tolerance in terms of physiological and biochemical traits. The experiment was conducted in randomized complete block design in pots filled with soil having which have $EC_2 - 1.97 \text{ dS m}^{-1}$ in 3 replications at ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal during Kharif 2015. Osmotic stress was imposed by applying saline irrigation water of EC_{iw} 3, 6 and 9 dS m^{-1} along with best available water (BAW) having $EC_{iw} 0.6 \text{ dS m}^{-1}$ (control). Out of 8 pearl millet lines, ICMB 07999 and 03222 lines showed mean RWC more than 75 per cent. Maximum electrolyte leakage was observed in ICMB 03222 (42.25 %) at $EC_{iw} - 9 \text{ dS m}^{-1}$. Chlorophyll concentration was reduced with increasing salinity in all the lines and found maximum in ICMB 01222, ICMB 07999 and ICMB 06888 ($37.5 \mu\text{g ml}^{-1}$) and minimum in ICMB 05888 ($30.7 \mu\text{g ml}^{-1}$). Highest proline content was recorded in ICMB 07999 ($6.38 \text{ mg g}^{-1} \text{ FW}$) at $EC_{iw} - 9 \text{ dS m}^{-1}$. ICMB 9522 and ICMB 0611 were found to be the promising ones maintaining lower Na^+/K^+ with relatively higher yield at $EC_{iw} - 9 \text{ dS m}^{-1}$ in comparison to others.

Keywords: Pearl millet, Salinity, Na^+/K^+ , Saline irrigation, Electrolyte leakage.

Introduction: Inter-sectoral competition for good quality land and water resources has resulted in enhanced interest in the utilization of salt affected soils and poor quality waters for food production to feed the teeming millions. The agriculture sector would be left with no other alternative than to use poor quality water sources to meet the irrigation requirements. Crop tolerance to irrigation water salinity is of high importance due to the extent and the constant increase in salt-affected areas in arid and semi-arid regions where irrigation facilities are limited^[1]. Salt tolerance has been identified as a polygenetically regulated, stage specific phenomenon and is expressed by different genetic, developmental, physiological and biochemical interactions within the plant, and in addition between genotypes and external environments. Accumulation of excess salts results in disturbed metabolic processes which commonly manifested in nutrient imbalance, reduced nutrient uptake including K^+ , specific ion toxicity, distinctly changed concentrations of key biomolecules,

inhibited plant growth to osmotic stress and ultimately poor productivity.

Pearl millet (*Pennisetum glaucum*) is a warm season coarse grain cereal generally considered as fairly tolerant to salinity, could be an alternative crop option for salt affected areas. An understanding of the desirable physiological and biochemical responses under stress conditions, and identification of parent lines which are superior in these attributes, are of great value in breeding programmes related to stress (drought and/or salt) environments. Therefore, the present investigation was carried out to explore the genotypic variability in pearl millet and to identify the key physiological and biochemical traits influencing crop growth and development during the stress periods.

Materials and Methods

To study the response of salinity stress on eight pearl millet lines procured from ICRISAT, Hyderabad, an experiment was conducted at ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal during Kharif

2015 in randomized complete block design with replication using 20 kg capacity clay/porcelain pots filled with 16 kg soil (field capacity 28% v/v) having bulk density of 1.45 g/cc which have porosity approximately 40 per cent. The net house was cautiously covered with a high quality polythene sheet to restrict the entry of rain water and maintains the desired salinity stress in the pots as per treatments by protecting from rains. Nine seeds of each line were sown in three equally spaced hills in each pot and irrigated with deionized water to field capacity previously estimated for the soil. Osmotic stress was imposed by applying saline irrigation water of EC_{iw} 3, 6 and 9 $dS m^{-1}$ along with best available water (BAW) having EC_{iw} 0.6 $dS m^{-1}$ (control).

Fully expanded leaves were sampled for measurement of chlorophyll content as described [2] using DMSO. Relative Water Content (RWC) was measured following procedures [3] and membrane injury was estimated [4]. Freshly harvested leaves were weighed and analyzed for proline [5]. For Na^+ and K^+ content, 100 mg of oven dried and well ground plant material was digested with 10 ml of $HNO_3:HClO_4$ (3:1) di-acid mixture and readings were taken with flame photometer (PFP7, Jenway, Bibby Scientific, UK) using standard NaCl and KCl. The data were analyzed statistically using randomized block design and the significance was tested at 5% level of critical difference (OPSTAT software, CCS HAU, Hisar).

Result and Discussion

The resultant soil salinity (EC_2) at final harvest was found to be 1.59, 1.99 and 2.42 $dS m^{-1}$ with different salinity irrigation water (EC_{iw}) of 3, 6 and 9 $dS m^{-1}$, respectively as against the EC_{iw} of 4.4 $dS m^{-1}$ in BAW treated pots (Fig. 1). The ability of plants to tolerate stress is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast function and maintain ion homeostasis [6]. Plant height decreased with increase in saline irrigation level. Pearl millet line ICMB 95222 showed lesser reduction at $EC_{iw} - 9 dSm^{-1}$ in comparison to other lines (data not shown).

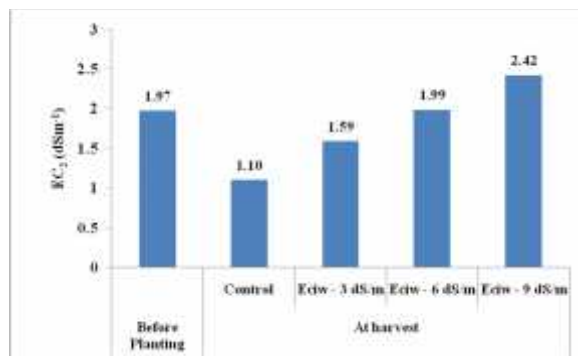


Figure 1: Soil salinity buildup (initial and final) in experimental pots with different salinity irrigation water used for raising pearl millet crop.

The decrease in relative water content (RWC) could be attributed to early symptoms of stress conditions. Pearl millet lines, ICMB 07999 and 03222 showed mean RWC more than 75% at $EC_{iw} - 9 dS m^{-1}$. Maximum reduction in RWC was noticed in ICMB 07444 (30.35 %) and ICMB 01222 (30.42 %) while minimum reduction was observed in ICMB 07999 (11.95 %) and ICMB 06111 (13.73 %) at $EC_{iw} - 9 dS m^{-1}$ (Table 1). Electrolyte leakage (an indicator of membrane damage) increased with increase in saline irrigation and found maximum in ICMB 03222 (42.25 %) at $EC_{iw} - 9 dS m^{-1}$. Total chlorophyll concentration, known as an index for evaluation of source, therefore decrease in concentration can be considered as a stomata non-limiting factor under stress conditions. Reduction in chlorophyll concentration was observed in all the tested lines, being maximum in ICMB 01222, ICMB 07999 and ICMB 06888 ($37.5 \mu g ml^{-1}$) and minimum in ICMB 05888 ($30.7 \mu g ml^{-1}$). Proline accumulation is an important physiological index for plant response to abiotic stresses [7]. The accumulation of compatible solutes such as proline (key osmolytes that contribute to osmotic adjustment) which are non-toxic to cytoplasmic functions even at higher concentrations and allow additional water uptake from the environment and thereby turgor maintenance [8-9]. Highest proline content was recorded in ICMB 07999 ($6.38 mg g^{-1} FW$) and minimum in ICMB 06888 ($4.56 mg g^{-1} FW$) and ICMB 01222 ($4.60 mg g^{-1} FW$) under stress conditions (Table 1).

Table 1: Effect of saline irrigation on the physiological and biochemical parameters in pearl millet lines.

Pearl millet lines/Treatments	Relative water content (%)		Electrolyte leakage(%)		Chlorophyll content ($\mu g ml^{-1}$)		Proline content ($mg g^{-1} FW$)	
	Control	$EC_{iw} 9 dSm^{-1}$	Control	$EC_{iw} 9 dSm^{-1}$	Control	$EC_{iw} 9 dSm^{-1}$	Control	$EC_{iw} 9 dSm^{-1}$
ICMB 01222	91.03	63.34	9.42	28.64	50.70	37.42	1.05	4.60
ICMB 05888	90.55	69.85	5.35	38.48	49.85	30.70	1.16	4.68
ICMB 95222	81.85	68.16	6.31	32.33	50.74	34.11	1.07	4.72
ICMB 06111	84.71	73.08	6.73	34.30	53.40	33.72	1.99	5.15
ICMB 07999	89.81	79.08	9.59	30.99	46.43	37.87	1.45	6.38

ICMB 03222	91.21	75.45	5.84	42.25	50.41	33.92	0.84	4.66
ICMB 07444	92.30	64.29	4.37	35.33	52.01	35.14	1.60	5.20
ICMB 06888	89.35	73.75	6.48	38.42	52.36	37.37	1.12	4.56
CD @ 5%	V – 0.128; T – 0.064		V – 0.0685; T – 0.0342		V – 0.115; T – 0.057		V – 0.006; T – 0.003	
	V × T – 0.181		V × T – 0.097		V × T – 0.162		V × T – 0.008	

Mean yield reduction of 20.82, 50.88 and 72.14 per cent was recorded when saline irrigation of 3, 6 and 9 dS m⁻¹ was applied in comparison to BAW (6.82 g/plant). The pearl millet lines i.e. ICMB 95222, ICMB 06888 and ICMB 07999 performed relatively well and recorded highest grain yield under control conditions (9.96, 9.441 and 8.53 g/plant) respectively.

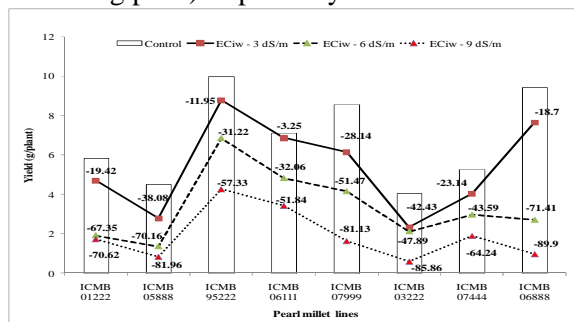


Figure 2: Effect of saline irrigation on yield (g/plant) of pearl millet lines. Line graph showing the per cent yield reduction with different levels of saline water in comparison to control (BAW).

Concomitant reduction in pearl millet yield was noticed with the increase in irrigation water salinity Pearl millet lines ICMB 05888, ICMB 03222 and ICMB 06888 showed more than 80 % reduction in their yields whereas the minimum per cent reduction in yield was obtained in ICMB 06111 (51.84 %) and ICMB 95222 (57.33%) at EC_{iw} – 9 dS m⁻¹ (Figure 2). A scatter diagram plotted on the basis of Na⁺/K⁺ and yield potential at EC_{iw} – 9 dS m⁻¹ clearly differentiates the promising lines as shown in Figure 3. Pearl millet Entries ICMB 95222, ICMB 06111 were found promising as these maintain lower Na⁺/K⁺ with higher yield potential at EC_{iw} – 9 dS m⁻¹ than other pearl millet lines. Reduction in yield due to abiotic stresses has already been reported by various workers and found that stresses reduced yield by reducing the number of filled grains per panicle^[10]. Reduction in seed weight may possibly be due to decreased pollen viability or decreased receptivity of the stigmatic surface or both^[11].

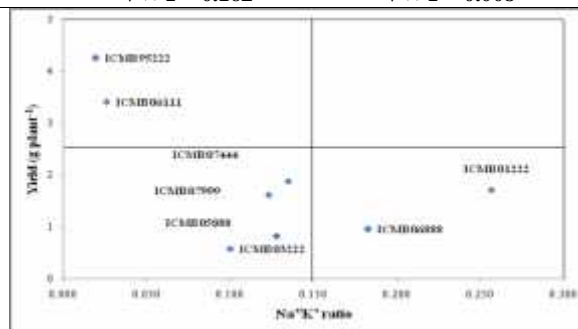


Figure 3: Scatter diagram showing the comparative performance of pearl millet lines and Na⁺/K⁺ ratio under saline irrigation of EC_{iw} – 9 dS m⁻¹.

Conclusion: Plant responses to salt stress are a complex phenomenon being governed by polygenic traits. The genetic basis of the physiological and biochemical expressions of tolerance may not be the same in different lines of the same species or across a range of species. These can be used as useful selection criteria in pearl millet developmental programmes for improving the adaptation strategies under stress environments grown under arid and semi-arid climatic conditions.

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