Assessment of Opportunities to Map Pearl Millet Tolerance to Salinity during Germination and Early Seedling Growth

R Mukhopadhyay[1], CT Hash[2*], AG Bhasker Raj[2] and **PB Kavi Kishor[1]** (1. Department of Genetics, Osmania University, Hyderabad 500 007, India; 2. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, AP, India.)
*Corresponding author: c.hash@cgiar.org

Introduction

Pearl millet, *Pennisetum glaucum* (L.) R. Br., tolerates drought, low soil fertility, low soil pH and responds well to water and favorable soil conditions. Soil salinity greatly hampers pearl millet productivity, delaying germination, reducing seed germination percentage, and severely affecting subsequent growth (Ashraf and Idrees 1992).

This study was undertaken to assess opportunities for using existing pearl millet mapping populations (Hash and Witcombe 1994, Hash et al. 2001) and other pearl millet genetic stocks available at ICRISAT-Patancheru to generate molecular markers for genomic regions contributing to salinity stress tolerance during germination and early seedling growth. Salinity tolerance during these early growth stages is critical to crop establishment in saline soil conditions and soil-free *in vitro* screens can be used to assess this on the large numbers of entries required for phenotyping a mapping population progeny set.

Materials and Methods

Twenty-eight inbred pearl millet genotypes (LGD 1-B-10, ICMP 85410-P7, Tift $23D_2B_1$-P1-P5, WSIL-P8,81B-P6, ICMP 451-P8, ICMP 451-P6, H 77/833-2-P5(NT), H 77/833-2, PRLT 2/89-33, W 504-1-P1, P310-17-Bk, PT 732B-P2, P1449-2-P1, ICMB 841(=841B)-P3, 863B-P2; IP 18293-P152, Tift $238D_1$-P158, Tift 186, Tift 383, ICMB 89111, ICMB 90111, ICMB 92666, ICMB 95333, 843B, ICMB 98004, ICMB 99022 and ICML 22) obtained from ICRISAT were tested for salt stress tolerance over a range of salt concentrations (0 mM to 150 mM NaCl). The first 18 lines are nine parental line pairs of ICRISAT pearl millet mapping population progeny sets (Hash and Witcombe 1994, Hash et al. 2001); Tift 186 and Tift 383 are forage hybrid pollinators from Tifton, Georgia, USA, used as control lines; and the following four inbreds are maintainer lines of male-sterile lines used as testers in line × tester trials assessing the opportunities to use the ICRISAT pearl millet mapping populations to map various secondary target traits (Hash et al. 2001). The final four lines are ICML 22, derived from an oasis landrace accession (IP 2696) from Chad that was expected to possess some degree of salt tolerance; 843B, and two lines near-isogenic to 843B (ICMB 98004 and

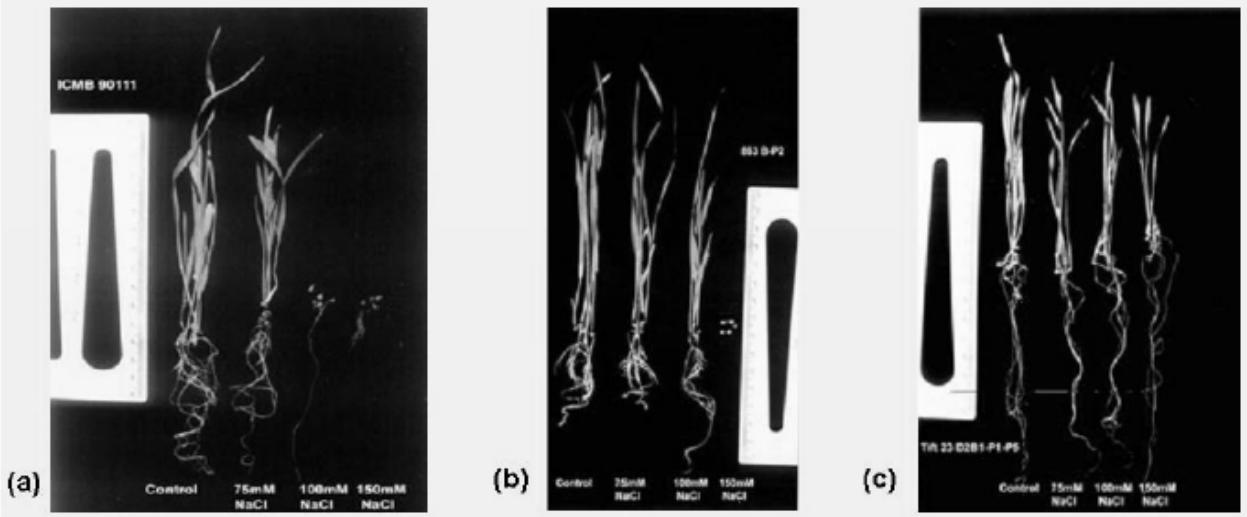

Figure 1. Ten-day-old seedlings of pearl millet inbreds ICMB 90111 (a), 863B-P2 (b) and Tift $23D_2B_1$-P1-P5 (c) grown in 0 mM, 75 mM, 100 mM and 150 mM NaCl media. ICMB 90111 was classified as sensitive to salinity stress during germination, whereas 863B-P2 was classified as moderately tolerant, and Tift $23D_2B_1$-P1-P5 was classified as tolerant.

ICMB 99022) derived by backcrossing ICML 22 (as the donor of oligogenic downy mildew resistance) to recurrent parent 843B (CT Hash unpublished).

Seeds of these 28 inbred pearl millet lines were surface-sterilized and germinated on filter-paper boats in balanced nutrient solutions (Hoagland and Arnon 1938) of pH 6.7 at 20°C, containing four different concentrations of NaCl (0 mM, 75 mM, 100 mM and 150 mM) in triplicates for each RCBD experiment. Fifteen seeds were germinated in each culture tube and the seedlings were allowed to grow for 10 days at 25°C under continuous fluorescent light (30 μE m^{-2} S^{-1}) in the same nutrient solutions. The experiments were repeated 4 times for each line and the genotype × salinity treatment means of each experiment were taken for the statistical analysis. The seed germination percentage of each pearl millet inbred was determined for each treatment 10-days after sowing. For genotype × salinity level treatment combinations, shoot and root length (cm), fresh weight (mg), and dry weight (mg) were recorded for 10-day old seedlings. Experimental data were analyzed statistically using the GenStat software package (GenStat 1995) to ascertain the levels of significance for each source of variation (replications, genotypes, salinity levels,

Table 1. Best linear unbiased predictions (BLUPs) for germination percentage of 28 pearl millet inbreds screened *in vitro* in Hoagland's solution in three treatments varying in NaCl concentration and in a non-saline control.

Entry name	0 mM NaCl	75 mM NaCl	100 mM NaCl	150 mM NaCl
LGD 1-B-10	96.8	88.3	75.3	0.0
ICMP 85410-P7	100.0	99.3	0.0	0.0
Tift $23D_2B_1$-P1-P5	91.8	89.3	90.3	78.3
WSIL-P8	95.3	92.5	0.0	0.0
81B-P6	100.0	92.0	1.3	0.0
ICMP 451-P8	92.5	89.0	87.5	0.0
ICMP 451-P6	99.8	81.3	75.3	0.0
H 77/833-2-P5(OT)	99.0	94.8	87.5	1.3
H 77/833-2	100.0	92.0	68.3	0.8
PRLT 2/89-33	73.8	36.8	4.3	0.5
W 504-1-P1	86.3	75.3	68.0	0.0
P310-17-Bk	96.0	96.0	90.0	72.8
PT 732B-P2	87.0	72.3	75.8	12.3
P1449-2-P1	95.8	75.3	0.0	0.0
841B-P3	100.0	97.0	91.0	76.5
863B-P2	93.8	93.0	82.0	0.0
IP 18293-P152	23.3	0.0	0.0	0.0
Tift $238D_1$-P158	90.3	87.5	0.0	0.0
Tift 186	97.5	70.0	73.0	0.0
Tift 383	99.5	94.8	91.8	10.0
ICMB 89111	52.5	43.3	19.0	0.0
ICMB 90111	100.0	33.0	0.0	0.0
ICMB 92666	97.3	79.5	88.3	0.0
ICMB 95333	99.3	98.8	98.0	53.0
ICML 22	85.0	79.8	74.5	63.5
843B	93.8	92.3	89.0	0.0
ICMB 98004	80.0	73.5	56.0	0.0
ICMB 99022	89.5	81.8	47.0	0.0
Salinity level grand mean	89.83	78.49	54.74	13.17
SE	±1.69	±4.71	±1.30	±1.19
CV (%)	3.77	11.99	4.74	18.11
F-ratio	95.84	24.71	867.08	508.19
$h^{2,1}$	0.96	0.86	1.00	0.99
$h^{2,2}$	0.99	0.96	1.00	1.00

$h^{2,1}$ = operational heritabilities calculated on entry mean basis
$h^{2,2}$ = operational heritabilities calculated on plot basis

genotype × salinity level interactions, and error) in the experiment.

Results and Discussions

Genotype × salinity level interactions were significant for all observed traits, indicating that the genotypes differed in their tolerance to the salinity treatments (Tables 1 and 2). The pearl millet inbreds were categorized as sensitive, moderately tolerant and highly tolerant to salinity based on their relative abilities to maintain high germination levels and good early seedling growth (Fig. 1) across NaCl levels of 75 mM, 100 mM and 150 mM, respectively. Seven of the pearl millet inbred lines were categorized as sensitive (ICMB 90111, PRLT 2/89-33, P1449-2-P1, Tift $238D_1$-P152, 81B-P6, WSIL-P8 and ICMP 85410-P7), fifteen as moderately tolerant, and five as highly tolerant (Tift $23D_2B_1$-P1-P5, ICMB 841-P3, P310-17-Bk, ICML 22 and ICMB 95333).

Table 2. Best linear unbiased predictions (BLUPs) for shoot and root lengths of 28 pearl millet inbreds screened *in vitro* in Hoagland's solution in three treatments varying in NaCl concentration and in a non-saline control.

	Shoot Length (cm)				Root Length (cm)			
Entry name	0 mM NaCl	75 mM NaCl	100 mM NaCl	150 mM NaCl	0 mM NaCl	75 mM NaCl	100 mM NaCl	150 mM NaCl
LGD 1-B-10	9.1	8.5	5.5	-	6.4	6.3	2.5	-
ICMP 85410-P7	8.8	11.9	-	-	6.7	7.3	-	-
Tift $23D_2B_1$-P1-P5	10.1	7.9	8.9	9.5	10.7	11.9	10.7	8.4
WSIL-P8	10.7	11.1	-	-	6.9	7.6	-	-
81B-P6	9.7	9.9	0.6	-	4.7	8.2	0.6	-
ICMP 451-P8	14.5	10.7	10.6	-	7.7	11.6	16.9	-
ICMP 451-P6	10.0	4.4	8.4	-	12.8	10.2	11.1	-
H 77/833-2-P5(OT)	11.7	8.6	9.2	2.8	12.3	10.5	9.4	3.2
H 77/833-2	5.8	4.3	4.4	0.5	6.3	5.7	9.4	1.1
PRLT 2/89-33	8.6	13.9	2.3	0.4	4.3	7.3	1.5	0.2
W 504-1-P1	8.8	8.8	8.2	-	9.3	9.6	9.4	-
P310-17-Bk	9.4	6.9	8.6	7.1	10.2	7.2	9.2	8.5
PT 732B-P2	10.2	9.0	8.1	4.2	10.5	8.3	8.5	7.1
P1449-2-P1	13.6	11.4	-	-	11.4	12.4	-	-
841B-P3	12.1	9.1	8.4	7.6	7.6	9.6	7.3	5.9
863B-P2	10.4	9.9	7.6	-	8.7	6.1	8.7	-
IP 18293-P152	4.3	-	-	-	1.0	-	-	-
Tift $238D_1$-P158	8.4	7.8	-	-	4.9	4.7	-	-
Tift 186	8.3	8.8	9.4	-	8.6	10.0	9.5	-
Tift 383	10.3	8.5	9.2	2.5	8.7	9.9	12.6	1.9
ICMB 89111	8.5	8.3	7.9	-	9.2	9.9	8.3	-
ICMB 90111	9.8	7.8	-	-	8.9	6.7	-	-
ICMB 92666	10.3	9.6	6.6	-	8.9	9.5	10.1	-
ICMB 95333	10.9	9.8	7.7	8.5	5.1	9.4	10.9	8.5
ICML 22	7.6	7.1	6.4	5.0	6.2	5.7	7.1	4.6
843B	10.3	7.3	8.2	-	5.4	8.7	9.4	-
ICMB 98004	12.2	13.0	8.7	-	10.4	10.0	13.6	-
ICMB 99022	11.5	9.7	6.6	-	8.8	7.5	9.5	-
Salinity treatment grand mean	9.9	9.0	7.3	4.8	7.9	8.6	8.9	4.9
SE	±0.2	±0.3	±0.2	±0.6	±0.2	±0.3	±0.3	±0.4
CV (%)	3.01	5.73	5.90	24.44	3.94	5.92	6.21	17.32
F-ratio	200.38	71.08	119.70	31.36	293.77	63.03	177.45	59.83
$h^{2,1}$	0.98	0.95	0.97	0.88	0.99	0.94	0.98	0.94
$h^{2,2}$	0.99	0.96	1.00	1.00	1.00	0.99	0.99	0.97

$h^{2,1}$ = operational heritablities calculated on entry mean basis
$h^{2,2}$ = operational heritablities calculated on plot basis

Large differences in germination salinity tolerance (Table 1) were detected between members of several pearl millet mapping population parental line pairs (including Tift $23D_2B_1$-P1-P5 and WSIL-P8, ICMB 841-P3 and 863B-P2, and P310-17-Bk and W 504-1-P1 at 150 mM NaCl; and ICMP 451-P8 and 81B-P6, LGD 1-B-10 and ICMP 85417-P7, and PT 732B-P2 and P1449-2-P1 at 100 mM NaCl), indicating that their previously skeleton-mapped pearl millet mapping population progeny sets can be phenotyped to map genomic regions contributing to these differences. Differences between mapping population parental line pairs were also detected for salinity tolerance of early seedling growth (Table 2).

This preliminary study indicates the potential for mapping genomic regions contributing to genetic variation in tolerance of pearl millet to salinity during germination and early seedling growth by combining existing marker data sets with phenotypic data sets produced by screening progeny sets from currently available pearl millet mapping populations. Such QTL mapping could be the next step towards identification of genes contributing to these components of salinity tolerance.

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