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Short Communications

Molecular markers for screening salinity response in *Sorghum*

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Genomic DNAs from two salinity tolerant (IS 23190-Zm 90 & IS 23217-Zm 138), one moderately tolerant (IS 3566) and one sensitive(IS 213253-Zm 321) accessions of *Sorghum* were subjected to RFLP and RAPD analyses. A combination of digestion with *Dra* I and probing with BADH 1 distinguished the sensitive from the tolerant and moderately tolerant accessions in the RFLP patterns. A primer having CGGCTAGGT sequence provided distinct RAPD pattern for the tolerants (presence of specific amplified products a, b & c), moderately tolerant (presence of only c) and sensitive (lacking a, b & c) genotypes.

Keywords: RAPD, RFLP, salinity tolerance, Sorghum

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Molecular markers have found wider application in genetics and crop improvement by providing more exact methods for marker assisted selection (MAS). In the context of breeding for quantitative traits, like salinity tolerance in crop plants, such markers have been invaluable. However, these are available only in few systems, such as rice, soybean, *Triticum* and tomato[1-7].

The expression of betaine aldehyde dehydrogenase (BADH) gene under salt stress was investigated in sorghum using two cDNA clones (BADH 1 & BADH 15) putatively encoding this enzyme and 2- to 3-fold increase was observed in the level of BADH mRNA in response to salinization[8]. The suitability of using these two cDNA clones and some random primer-generated RAPD patterns as markers has been tested for screening salinity tolerance in sorghum by using already characterized tolerant and sensitive varieties and the results are reported here.

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Two tolerant (IS 23190-Zm 90 & IS 23217-Zm 138), one moderately tolerant (IS 3566) and one sensitive (IS 213253-Zm 321) genotypes of sorghum (*Sorghum bicolor* L.) have been used in the present work. Selection and characterization of these lines was based on the prior screening (under 17 dsm^{-1} salinity stress) of 17 accessions, obtained from Genetic Resources Unit (GRU), International Crops Research Institute for Semi Arid Tropics (ICRISAT), Patancheru, India, using plant phenotypic (plant height, leaf index, percentage of dry leaves and flowering ability) and chemical parameters (quantities of proline, Na^+, K^+, Mg^{2+}, SO_4^{2-} and Cl^-)[9].

Genomic DNA from leaves was isolated using CTAB method[10]. For RFLP analysis, DNA was digested with *Eco* RI, *Eco* RV, *Hind* III and *Dra* I (M/s. Bangalore Genei Pvt. Ltd., India). Southern blots were prepared by the alkaline transfer method[10] using Hybrid N^+ membranes (M/s. Boehringer Mannheim, Germany)[11]. cDNA clones of BADH (BADH 1, 800 bp & BADH 15, 1500 bp, provided by Dr Peter B Goldsbrough, Purde University, USA) were used as probes. Hybridization and detection of probes were carried out following chemiluminiscent detection method using DIG high prime DNA labeling and detection kit of Boehringer Mannheim, Germany.

For RAPD analysis, genomic DNA was amplified using *Taq* DNA polymerase and six random 9-mer primers (RC 11–TGACACCTC; RC 14–CTGCT-GAAG; RC 15–CGGCTAGGT; RC 16–GAGCGTTGT; RC 20–ACCCGGACA & RC 41–ACAGCACCC). DNA was subjected to 40 cycles of amplification in a 25 µL reaction volume, containing 25 ng DNA, 10 m*M* Tris-HCl *p*H 8.3, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.001% gelatin, 200 µ*M* each of dATP, dCTP, dGTP and dTTP 15 ng random 9-mer primer and 0.5 units of *Taq* DNA polymerase. The reaction mixture was overlaid with 50 µL of mineral oil. Five cycles of hot start was initially carried with each cycle programmed as–1 min at 94°C, 1 min 30 sec at 30°C and 2 min at 72°C. This was followed by amplification for 35 cycles and each amplification cycle was programmed as–1 min at 94°C, 1 min 30 sec at 36°C and 2 min at 72°C. A final extension was done for

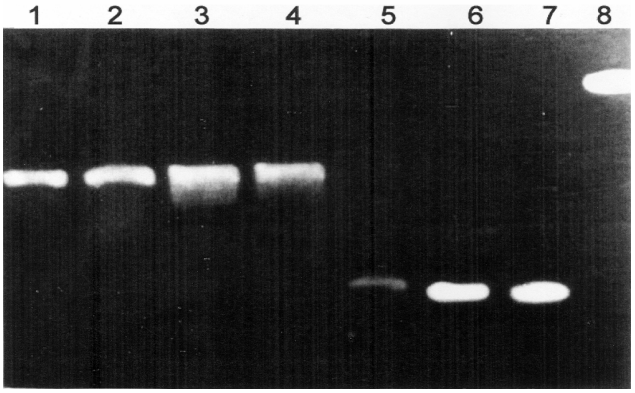

Fig. 1—RELP patterns of four accessions of Sorghum: Lanes 1-4, digests with *Hind* III ; lanes 5-8 digests with *Dra* I. lanes 1 & 5-Moderately tolerant; lanes 2 & 6-Tolerant 1; lanes 3 & 7-Tolerant 2; & lanes 4 & 8- Sensitive.

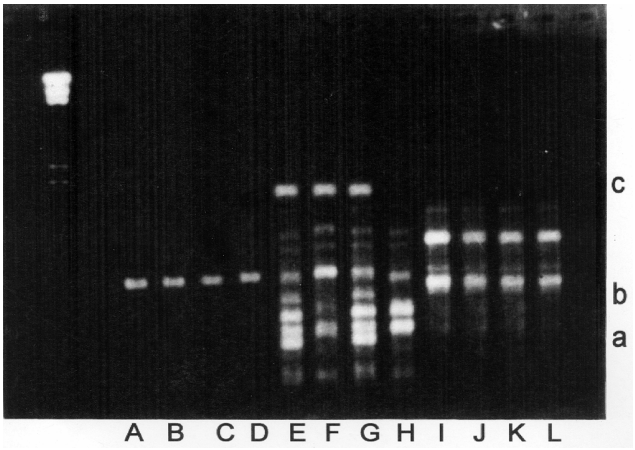

Fig. 2—RAPD patterns in four accessions of Sorghum: DNA size marker-*Hind* III digested λ DNA; A-D, Amplified with primer RC – 16; E-H, Amplified with Primer RC 15; I-L, Amplified with primer RC 20. A, E & I-Tolerant; B, F & J-Moderately tolerant; C, G & K-Tolerant; & D, H & L-Sensitive.

5 min at 72°C. The reaction mixture was kept at 4°C until further use. To each of this reaction mix, 4 μL of 6 × gel loading buffer was added. This sample was loaded in the wells of 1.4% agarose gel and electrophoresed for 2.5 h at 12 V/cm in 1 × TBE buffer. After the run, the gel was stained with ethidium bromide (5 μg mL^{-1}) and was photographed using a UV-transilluminator.

When BADH 1 was used as the probe, a single band was observed in each of the enzyme digests in the four accessions. In the *Dra* I digest, a variation in the band position was noticed, viz. in IS 23253 Zm-321 (sensitive), the band was nearer to the origin as compared to its relatively more distal or anodal position in the other three accessions (Fig. 1; lanes 5-8); DNA digested with other enzymes did not show any variation (Fig. 1; lanes 1-2). With the probe BADH 15, one band in each of the digests with *Eco* RI, and RV, two bands in the *Hind* III digest and three bands in the *Dra* I digest were observed uniformly in all the four accessions.

Of the six primers used, only one (RC 15) gave the clear difference in polymorphism among the four accessions. The RAPD patterns revealed by this primer in the two tolerant accessions, IS 23190 ZM-90 (Tolerant 1) and IS 23217 Zm-138 (Tolerant 2), included bands a, b and c (~ mol wts ranging between 600-2000 bp), whereas only c band was observed in the moderately tolerant accession (IS 3566). The sensitive accession (IS 213253 Zm-321) did not show any of these three bands (Fig. 2; E-H).

It is, therefore, suggested, that BADH 1 and primer RC 15 might be useful as molecular markers (RFLP and RAPD, respectively) to distinguish salinity tolerants and sensitives in sorghum.

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