

PHENOTYPIC AND GENOTYPIC DIVERSITY INVESTIGATIONS IN SUGARCANE FOR DROUGHT TOLERANCE AND SUCROSE CONTENT

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Abstract

Ten elite sugarcane clones were tested for genetic diversity through RAPD, sucrose synthase activity was determined via TRAP and drought tolerance was examined with the help of STS techniques / field trial. RAPD study revealed that genetically most similar genotypes were Thatta-10 and AEC82-223 (80.4%) and most dissimilar genotypes were AEC71-2011 and NIA-2004 (49.8%). On the basis of dendrogram, the varieties could be divided into four clusters (A to D). Variety AEC82-223 produced a specific allele of 311bp with primer B-02. Primer sucrose synthase amplified three alleles which were polymorphic and allelic size were 561, 327 and 222bp. Of 10, seven varieties tagged the specific gene responsible for drought tolerance in the genome. L116 containing a different allele of 912bp amplified with DREB-2 showed the specificity of the variety. Maximum sugar recovery % (14.82) and cane yield (t/ha) (156 t/ha) were recorded in AEC81-0819.

Introduction

Sugarcane (*Saccharum* spp. hybrids) is a highly polyploidy, heterozygous and genetically complex crop of major economic importance in tropical and sub tropical countries (Khan *et al.*, 2002 and 2012; Babar *et al.*, 2011; Khan *et al.*, 2012). Sugarcane cultivars are derived from a few inter-crossings of *S. sinense*, *S. barberi* and *S. spontaneum* (Singh *et al.*, 2004; Khan *et al.*, 2009). Sucrose content is a critical character and plays pivotal role in the sugarcane breeding programs. Cultivars differ in both maximum sucrose accumulation capacity and accumulation dynamics during growth (Menossi *et al.*, 2008). Emphasis was given to identify genotypes able to produce more sucrose early in the crop season to allow for continuous sugar production throughout the year (Terzi *et al.*, 2009). The internodes mature progressively towards the base of the culms with an increasing concentration of sucrose at the base. Sucrose content in the mature internodes can reach around 16-18% of the culms dry weight while lower sucrose levels are observed in younger internodes where glucose and fructose are predominant (Terzi *et al.*, 2009). The improvement of modern cultivars could be achieved by identifying genes associated with important agronomic traits, such as sucrose content. These genes can then be used to generate transgenic plants or can serve as molecular markers for map-assisted breeding (Lingle 1997). Variability in gene pool is characterized by contrasting growth, development, and physiology, all of which affect the carbohydrate metabolism (Harvey & Botha, 1996).

Sucrose synthase is a major enzyme of sucrose metabolism in sugarcane (Lingle & Dyer, 2001). This gene is homologous to the maize gene that produces the Shrunken-1 phenotype. The presence of multiple copies of the sucrose synthase gene in sugarcane is not surprising in this complex polyploidy (Lingle 1997). It is generally known that in sugarcane, increase in sugar content is favoured by low temperature and low water precipitation. Sugars that transduce stress signals or act as osmoprotectants, like fructose, raffinose and trehalose could be central players during this process (Gupta & Kaur, 2005). According to Alexander *et al.*, (1972) and

Zhu *et al.*, (2000), under drought condition, plant start consuming its own sink for its survival thus reducing sucrose concentration in cane stalk. However, Terzi *et al.*, (2009) reported no reduction in sucrose concentration in drought tolerant plants.

Drought tolerance is polygenic and complex trait interplay with the environment makes phenotypic evaluation difficult. Hence, the use of DNA markers can help breeders in improving the speed as well as reliability of the process. Gene tagging and DNA fingerprinting is particularly suitable for pyramiding of desired traits.

This work was focused on evaluation of 10 sugarcane genotypes through RAPD, TRAP, STS markers and their field performance to assess the genetic diversity for drought tolerance and sucrose content endowed with high cane/sugar yield. RAPD primers were used to evaluate highly polymorphic alleles for the estimation of genetic diversity. TRAP markers were use to assess the genetic polymorphism for sucrose synthase gene and drought tolerance was examine through DREB2 sequences via STS method. In this paper we have tried to establish the association of DREB sequences with sucrose synthase gene for harvesting good sugar yield.

Materials and Methods

Plant material: Double budded vegetative seed of 10 sugarcane varieties viz., GT11, AEC71-2011, AEC1026, AEC81-0819, NIA-2004, BL4, Thatta-10, L116, AEC92-105 and AEC82-223 were sown at the experimental Farm of NIA Tando Jam. Experimental layout was RCB design with 4 replications. Each plot size was 8 x 10m with a row to row distance of one metre. Sowing was done in September 2008 and 2009. Three irrigation treatments (well-watered, moderate and drought-stressed) were initiated at 180 days after planting. The fully irrigated treatment received 30 irrigations, 24 and 18 irrigation treatment designated as moderate and drought treatment respectively. Normal agronomic practices were followed through out the growth period. Observations for seven important agronomic characters viz., plant girth (cm), tiller number, weight per stool (kg), sugar recovery %, fiber %, cane yield (t/ha) and sugar yield (t/ha) were recorded at plant maturity.

DNA extraction: DNA was extracted from fresh leaves of sugarcane using MATAB method (Bibi *et al.*, 2009). The concentration of the extracted DNA was measured with spectrophotometer (BIOMATE 3) at absorbance 260/280 nm. The quality of DNA was further checked on 0.8% agarose gel (Khan *et al.*, 2009).

DNA amplification with random (RAPD) primers: For RAPD analysis, the PCR reaction was carried in 25 μ l reaction mixture containing 1 μ M of primer (Gene link) in 1x reaction buffer, 0.33mM of each dNTPs, 2.5mM MgCl₂, 0.1u/ μ l of Taq polymerase and 2.6ng/ μ l of template (Genomic DNA). The amplification reaction was performed in the Eppendorf Master Cycler with an initial denaturation for 5 minute at 94°C, then 33 cycles: 1 minute denaturation at 94°C; 1 minute annealing at 40°C; 2 minute extension at 72°C. A final extension was carried out at 72°C for 10 minute.

DNA amplification with specific (STS) primers: National Center for Biotechnology Information (NCBI) website was used to obtain the EST nucleotide sequences of model plant *Arabidopsis thaliana* for drought tolerance (DREB) expression and a 18-nucleotide base pair primer was designed using a primer design software 'Primer3' for the selection of drought tolerant genotypes through STS (Olson *et al.*, 1989). PCR reaction was carried out in 25 μ l reaction mixture containing 2.6ng of template (Genomic DNA), 2.5mM MgCl, 0.33mM of each dNTPs, 2.5U of Taq polymerase and 0.25 μ M of each primer in a 1X PCR reaction buffer. The amplification reaction was performed in the Eppendorf Master Cycler with an initial denaturation for 4 minutes at 94°C, then 30 cycles: 1 minute denaturation at 94°C; 2 minute annealing at 55°C; 3 minutes extension at 72°C and a Final extension was carried out at 72°C for 7 minutes.

DNA amplification with specific (TRAP) primers: For this study, primers were designed and reported by Khan *et al.* 2011. PCR was optimized for TRAP study in sugarcane with one fixed primer and two arbitrary primers. PCR reaction was carried out in 10 μ l reaction mixture containing 0.33mM dNTPs, 2.5mM Mg, and 0.15 μ M of each primer, 0.5U of Taq polymerase, 0.5% gelatin and 50ng of template DNA. The best amplifications were obtained by using following PCR programmed in Eppendorf Master Cycler: initial denaturation for 4 minute at 94°C, then 35 cycles: denaturation at 94°C for 45 seconds; 45 seconds annealing at 45°C; for 1 minute extension at 72°C with a Final extension at 72°C for 7 minutes.

Gel electrophoresis: Amplified products were analyzed through electrophoresis on 1.5% agarose gels containing 0.5X TBE (Tris Borate EDTA) and 0.5 μ g/ml ethidium bromide to stain the DNA. Gel electrophoresis was done at 72 volts for 2 hours and a photograph was taken under UV light using gel documentation system.

Data analysis: Three stools were randomly taken from each plot to determine sugar contents according to sugarcane laboratory manual for Queensland Sugar Mills (Anon., 1970) while three rows from each plot were harvested to record yield data. The mean and variance were computed from each treatment. Data were analysed following Steel & Torrie

(1980). Molecular data were recorded for the presence of bands as (1) and absence as (0) from RAPD, TRAP and STS of amplification profile. Coefficient of similarity among cultivars was calculated according to Nei & Li (1979). Similarity coefficient was utilized to generate a dendrogram by means of Un-weighted Pair Group Method of Arithmetic means (UPGMA).

Results

Phenotypic data: Data regarding the average performance of all clones for seven traits in three different irrigations are presented in Table 2. Non significant differences were observed in tillers (No), cane girth and fiber % in 30 irrigations. Highest tiller number was observed in L116 and AEC81-0819 (7.67), followed by AEC70-2011 (7.33) and NIA-2004 (7.00), whereas, lowest tiller was observed in Thatta-10 (5.67) in 30 irrigations. These traits showed significant difference at 24 and 18 irrigations confirming the capability of the genotype to combat water stress conditions. Inman-Bamber & Smith, (2005), Da Silva & Da Costa (2004), reported that cane elongation and stalk height were negatively and strongly affected under drought conditions. Silva *et al.*, (2008) reported that in water stress condition cane girth increases as compared to well watered crop. Our result was contrary to their results and we found decrease in the cane girth under water stress condition. Significant difference was observed for weight per stool (kg) and maximum weight was observed in AEC82-1026 (11.33 kg) in 30 irrigations and the same genotype gave only 4.25(kg) weight under 18 irrigation. The genotypes unable to perform better under water stress conditions were due to the heavy leaf necrosis. Clone AEC82-0819 showed good tolerance under water stress condition with only 3.92% reduction and showed heavy leaf rolling which is peculiar phenotypic marker for drought tolerant genotype. Maximum sugar recovery % was recorded in AEC81-0819 in all irrigation levels and under low irrigation the sugar recovery was increased by 0.82 units. This high sugar recovery is because of NCo-310 which is a female parent of this genotype. Similarly the clone NIA-2004 showed high sugar recovery because of this parent. Significantly higher cane yield (t/ha) was recorded in AEC81-0819 (156 t/ha) under all the irrigation levels and lowest cane yield was recorded in L116 (60 t/ha) in 30 irrigation. Highest reduction was observed in clone AEC82-1026 (64%) in 18 irrigations followed by AEC92-105 (61%). Minimum reduction was observed in NIA-2004 and AEC81-0819. Highest sugar yield (t/ha) was observed in AEC81-0819 in all irrigations and lowest (8.04 t/ha) in L116 in 30 irrigations.

Clones AEC82-1026 and AEC92-105 were the product of C147-83 x CP57-614 and exhibited susceptibility to water stress condition whereas, clones AEC81-0819, NIA-2004 and NIA86-328 were the outcome of NCo-310 x CP57-614 and showed tolerance under water stress condition (Tables 1 & 2 and Fig. 2). This also revealed that the genetic makeup of CP57-614 in the background of NCo-310 exhibited drought tolerance and when the same genotype was used in the background of C147-83 susceptible genotypes were harvested. Similar susceptibility was reported in variety N35 (68W1049 x CP57-614) developed in 1999 in South Africa.

Table 1. Putative pedigree of ten sugarcane clones and their salient features.

Clone	Female parent	Male parent	Salient features
AEC82-1026	CI47-83	CP57-614	High yielding and good ratooner
GT-11	CP49-50	Co-419	High yielding and mid maturing
AEC92-105	CI47-83	CP57-614	High yielding, mid maturing and good ratooner
AEC81-0819	NCo-310	CP57-614	Early maturing, high yielding and drought tolerant
Thatta-10	L-113	Unknown (polycross)	Early maturing, high yielding and drought tolerant
AEC82-223	F31-436	F31-412	High yielding, mid maturing and good ratooner
AEC70-2011	Co-547 (mutant)	---	High yielding, late maturing and good ratooner
NIA-2004	NCo-310	CP57-614	Early maturing, high yielding and drought tolerant
L116	CoL-29	Unknown (polycross)	Early maturing and good ratooner
NIA86-328	NCo-310	CP57-614	Early maturing and high yielding

Table 2. Quantitative and qualitative data of sugarcane varieties evaluated at NIA, Tando Jam under different irrigation levels.

Clone	Tiller (no.)	Weight (kg.)	cane girth (cm)	Cane yield (t/ha)	Fiber (%)	Sugar Recovery (%)	Sugar yield (t/ha)
30 Irrigations							
AEC82-1026	6.67	11.33a	2.80b	113c	11.74	13.72b	16.54b
GT-11	6.33	8.67b	2.77b	86d	11.95	9.87e	8.48d
AEC92-105	7.00	8.00b	2.79b	135b	11.15	11.58d	16.62b
AEC81-0819	7.67	8.17b	2.74b	156a	10.25	14.82a	22.33a
Thatta-10	5.67	8.50b	2.90b	85d	11.52	13.46b	11.44c
AEC82-223	6.00	8.83b	3.10a	88d	12.93	11.35d	10.68c
AEC70-2011	7.33	6.67c	2.89b	66ef	11.95	12.14c	8.60d
NIA-2004	7.00	7.33bc	2.83b	73e	11.15	14.47a	11.27c
L116	7.67	6.00c	2.65b	60f	10.25	12.59c	8.04d
NIA86-328	6.67	8.00b	3.65a	80d	11.52	11.42d	9.73d
24 Irrigations							
AEC82-1026	5.68b	8.24a	2.18b	80bc	11.80b	13.01b	10.40b
GT-11	6.32b	8.62a	2.76b	85b	11.84b	9.88e	8.40c
AEC92-105	5.24b	6.34	2.10b	95b	12.10ab	11.01d	10.54b
AEC81-0819	7.54a	8.15a	2.75b	154a	10.10c	14.85a	22.86a
Thatta-10	5.62b	7.12ab	2.42b	80bc	11.50b	13.50b	10.80b
AEC82-223	4.26c	6.14b	2.76b	68cd	13.10a	11.18d	7.60c
AEC70-2011	6.53b	6.24b	2.61b	67cd	11.95b	12.15c	8.14c
NIA-2004	6.87b	7.14ab	2.80b	72cd	11.11bc	14.37a	10.36b
L116	7.51a	6.00b	2.61b	60d	12.34ab	12.50bc	7.50c
NIA86-328	6.66b	8.00a	3.10a	73cd	11.41b	11.34d	8.27c
18 Irrigations							
AEC82-1026	3.24cd	4.25d	2.10b	40d	11.81c	13.46c	5.38c
GT-11	6.04b	6.68b	2.35ab	65b	11.83c	10.21f	6.63c
AEC92-105	3.26cd	3.84e	1.19c	52c	12.06c	11.53e	6.00c
AEC81-0819	7.00a	7.85a	2.65ab	135a	10.13	15.64a	21.11a
Thatta-10	4.21c	5.24c	2.21b	70b	11.90c	13.96c	9.77b
AEC82-223	2.84d	3.14e	2.10b	43d	13.09a	11.78e	5.06c
AEC70-2011	3.56c	4.21d	2.24b	60bc	11.94c	12.20de	7.32c
NIA-2004	6.80ab	6.79b	2.68ab	64b	11.03c	14.78b	9.46b
L116	7.00a	4.21d	2.56ab	52c	12.54b	12.68d	6.59c
NIA86-328	6.65b	6.12b	2.88a	60bc	11.48c	11.98e	7.19c

DMR Test: Similar letter showed statistically non significant difference at 5% level

Genotypic data: Primer sucrose synthase amplified three alleles which were polymorphic. The allelic size of SucSy gene were 561, 327 and 222 bp (Fig 1). This showed that sucrose synthesis in sugarcane is controlled by three different alleles. Presence or absence of allelic form manifests the sucrose accumulation in cane.

Allele 'A' was observed in AEC-1026, AEC92-105, AEC71-2011, AEC82-223, AEC81-0819, NIA-2004 and BL4 whereas, allele 'B' was produced by GT11, AEC92-105, Thatta-10, NIA-2004, BL4 and L116. All clones were contained allele 'C' except NIA-2004.

Specific primers corresponding to DREB2 sequences were used to screen the 10 sugarcane varieties for drought tolerance. Of 10, seven varieties viz. GT11, AEC71-2011, Thatta-10, AEC81-0819, NIA-2004, BL4 and L116 tagged the specific gene responsible for drought tolerance in the genome. DNA amplification with DREB2 sequence yields 1.18 to 0.939kbp bands (Fig. 2). L116 contained a different allele of 912bp which is amplified with the same primer (DREB) showed the specificity of the variety and can be used for varietal identifications through STS.

Clones AEC82-1026, AEC92-105 and AEC82-223 were sensitive to drought condition and possessing different alleles for sucrose content. All three clones carry same male parent i.e., CP57-614 whereas the clone AEC81-0819 exhibited drought tolerance also having the same male parent but the female parent is different.

On the basis of dendrogram, the varieties could be divided into four clusters, designated A through D (Fig. 3). Cluster 'A' comprised of AEC82-1026 and AEC92-105 because they share the same genetic background. Cluster B contained predominantly GT11 and AEC71-2011 showing more genetic similarity, this might be due to the common origin in the parent i.e. Coimbatore, clone GT-11 contains Co-419 as male parent whereas AEC70-2011 is the mutant of Co-547. Cluster C contained Thatta-10 and AEC82-223. Clone Thatta-10 is developed from the polycross L-113 and AEC82-223 was developed after crossing F31-436 with F31-412 the similarity between these clones could be the parents of Thatta-10 and AEC82-223 may bear similarity in their parentage. Cluster D consisted of AEC82-328, L116, AEC81-0819 and NIA-2004. Despite of having similar parent clone AEC81-0819 and NIA-2004 showed 30% dissimilarity this exhibited the degree of heterozygosity in sugarcane cultivar (Khan *et al.*, 2011) (Table 3). Cluster A and B formed one group which were designated as group 'One'; whereas, Cluster C and D formed the other group 'Two' (Fig. 3).

A total of 70 bands were generated by 12 primers that were used on all 10 genotypes. Forty-eight (68.57%) of these bands were polymorphic while 22 (31.42%) were monomorphic. Thus, the average number of bands produced by each primer was 5.83. The sizes of the amplification products ranged from 220 bp to 4.50 kb. The maximum number of bands (13) was produced by the primer A-10, while the minimum number (1) was produced by the primers B-10. Among the genotypes, L116 gave maximum number of bands and polymorphic loci with all 12 primers. The degree of polymorphism was linked to genetic diversity in sugarcane genotypes.

Mean genetic similarity among the genotypes was 76.02% showing that a large part of the genome is similar. This may be due to the lack of parental diversity. RAPD data revealed that genetically the most similar genotypes were

Thatta-10 and AEC82-223 (80.4%) followed by AEC92-105 and AEC82-223 (79.3%) while most dissimilar genotypes were AEC71-2011 and NIA-2004 (49.8%) (Table 3).

RAPD Primer C-02 amplified the ten sugarcane genotypes and produced five alleles (Fig. 4). Size of the alleles ranged from 307bp-1.495kbp in which three were polymorphic and two were monomorphic. All sugar clones produced the 374bp allele except L116. Allele of 307bp was observed in AEC 1026, AEC81-0819, NIA-2004, BL4, Thatta-10, AEC92-105 and AEC82-223.

RAPD Primer B-02 amplified eleven alleles out of which Ten alleles were polymorphic and one was monomorphic, size of allele ranged between 177-1.64kbp. Variety AEC82-223 produced a specific allele of 311bp (Fig. 5).

Discussion

Significant differences were observed in all phenotypic traits under study. Plant height and plant girth are main traits in determining cane yield (Rehman *et al.*, 1992; Khan *et al.*, 1997). Khan *et al.*, 2004 suggested that plant height and plant girth can only contribute for higher cane yield when number of stalks per stool is taken into consideration. In present study all genotypes showed non-significant difference in stalks per stool. Singh *et al.*, (1985) reported that number of canes were the most important character contributing directly to higher yield. According to Raman *et al.*, (1985) and Javed *et al.*, (2000), number of stalks was the major contributing factor for cane yield. Quebedeaux & Martin (1986), proposed that both the stalk number and weight should be assessed to get an accurate yield potential of any variety. Khan *et al.*, (1997; 2002) reported that excessive stalks in stool showed adverse effect on cane yield due to high intra plant competition. Sugar recovery increased under water stress conditions is due to the association of sucrose gene with drought responses (Silva *et al.*, 2008). Terzi *et al.*, (2009) described that 15 alleles were involved in sucrose accumulation in cane stalk whereas we were able to screen only three important alleles in our study involved in the sucrose synthase activity. Gene expression analysis of sugarcane population for sucrose content indicated a possible overlap of sugar and drought metabolism. This finding may prove to be useful as molecular marker in breeding programme.

In natural environment plants are exposed to various environmental stresses. Drought stress is a major factor limiting the growth of plants (Begg, 1980). Many species can reduce the quality of radiation that they intercept when suffering from drought stress either by paraheliotropism or by leaf rolling as observed in AEC81-0819. Leaf rolling helps plant to survive better in dry condition by two ways (1) Damage caused by increased leaf temperature due to solar radiation can be minimize by reducing the effective leaf area so that less radiation is intercepted by leaf tissue (Corlett *et al.*, 1994); (2) Transpiration rate can be reduced by leaf rolling there by conserving stress water resources (Oppenheimer 1960). Early and late leaf rolling can be due to the action of a single major gene (Singh & Mackill, 1991). Two genes for roll leaf rl-1 and rl-4 on chromosome 1 have been reported in rice (Khush & Kinoshita, 1991) and similar gene has been reported in sugarcane (Carson & Botha, 2000). Steward (1960), reported that sugarcane variety POJ-2725 has a mark feature of leaf rolling to combat the drought conditions.

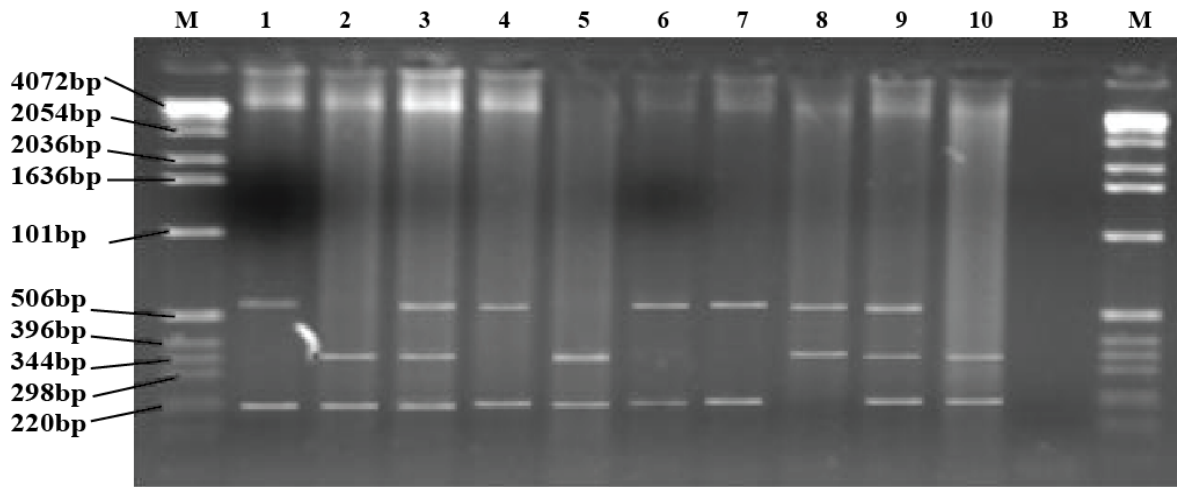


Fig. 1. TRAP profile of sugarcane genotype using surose synthase; M=DNA marker, 1= AEC82-1026, 2= GT-11, 3= AEC92-105, 4= AEC71-2011, 5= Thatta-10, 6= AEC82-223, 7= AEC81-0819, 8= NIA-2004, 9= AEC86-328, 10= L116, B= Blank

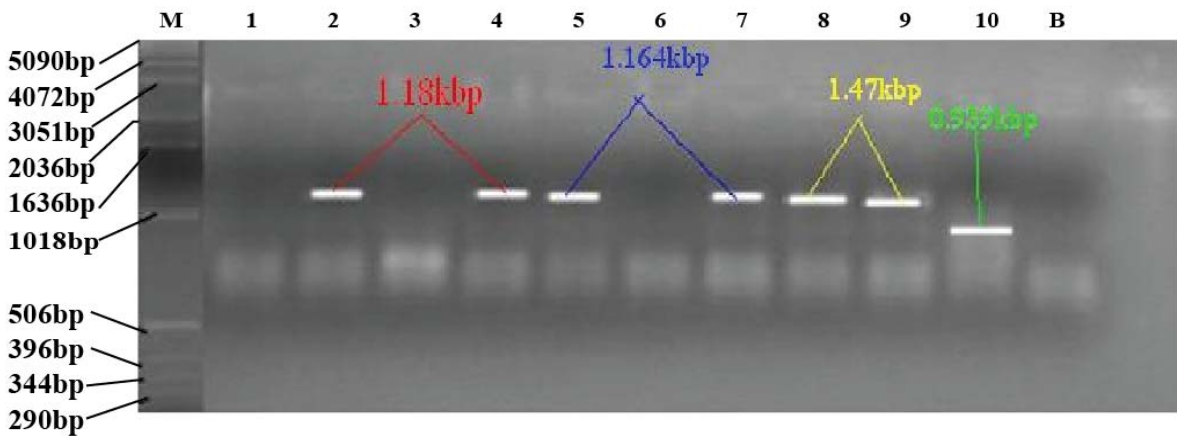


Fig. 2. STS profile of sugarcane genotype using DREB sequence; M=DNA marker, 1= AEC82-1026, 2= GT-11, 3= AEC92-105, 4= AEC71-2011, 5= Thatta-10, 6= AEC82-223, 7= AEC81-0819, 8= NIA-2004, 9= AEC86-328, 10= L116, B= Blank

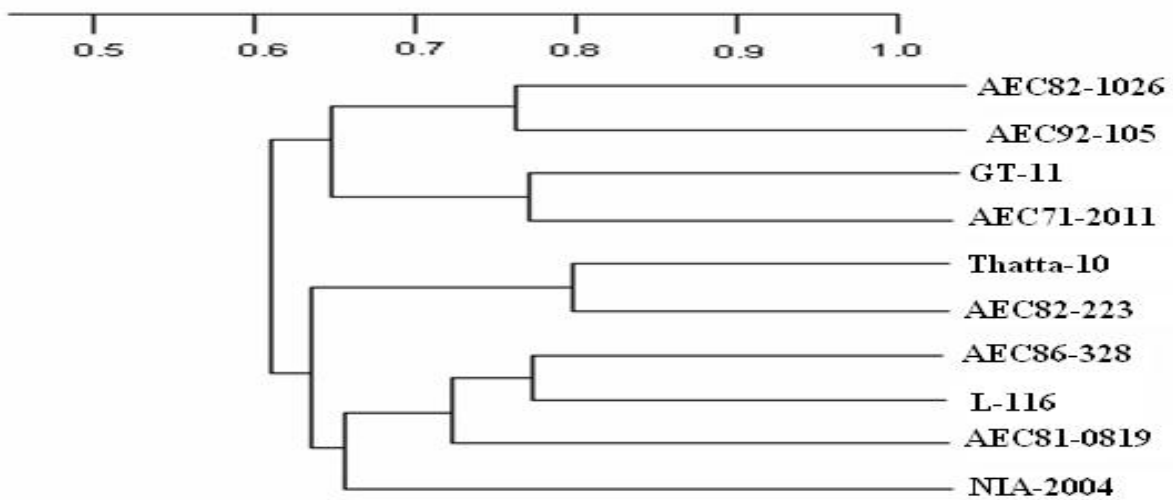


Fig. 3. The dendrogram of genetic distance among all the tested sugarcane varieties generated by RAPD-PCR amplification.

Table 3. The similarity coefficient values among all sugarcane varieties generated by RAPD-PCR amplification.

	GT11	AEC71-2011	AEC82-1026	AEC81-0819	NIA-2004	AEC86-328	L116	Thatta-10	AEC92-105	AEC82-223
GT11	1									
AEC71-2011	62.5	1								
AEC82-1026	76.6	66.5	1							
AEC81-0819	61.3	76.7	71.6	1						
NIA-2004	61.9	49.8	68.5	70.1	1					
AEC86-328	53.8	52.8	63.4	66.5	67.5	1				
L116	67.1	56.2	54.8	59.6	74.9	53.6	1			
Thatta-10	50.4	62.1	65.3	72.3	64	52.7	71.4	1		
AEC92-105	51.1	55.5	62.2	55.9	66.6	75	76.2	66.4	1	
AEC82-223	55.4	70.2	65.9	62.5	57.9	68.8	69.5	80.4	79.3	1

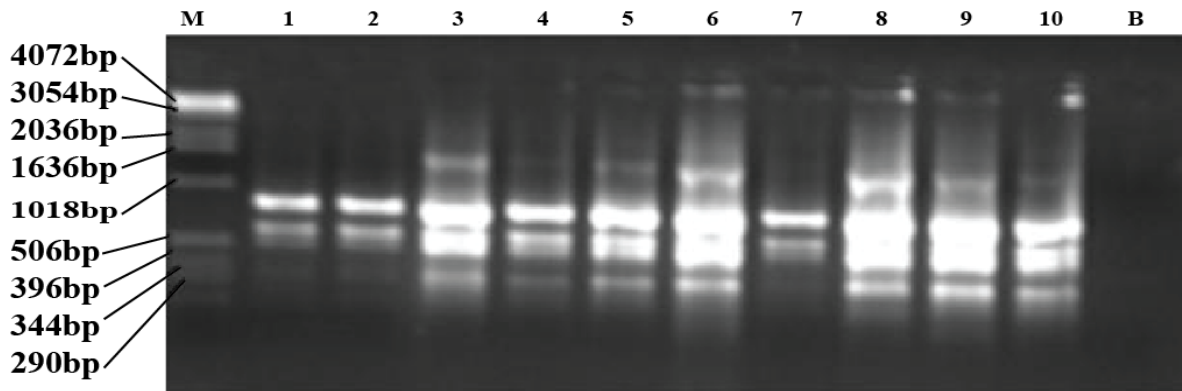


Fig. 4. Results of RAPD-PCR with primer C-02; M=DNA marker, 1=GT-11, 2=AEC71-2011, 3=AEC82-1026, 4=AEC81-0819, 5=NIA-2004, 6=AEC86-328, 7=L116, 8=Thatta-10, 9=AEC92-105, 10=AEC82-223, B= Blank

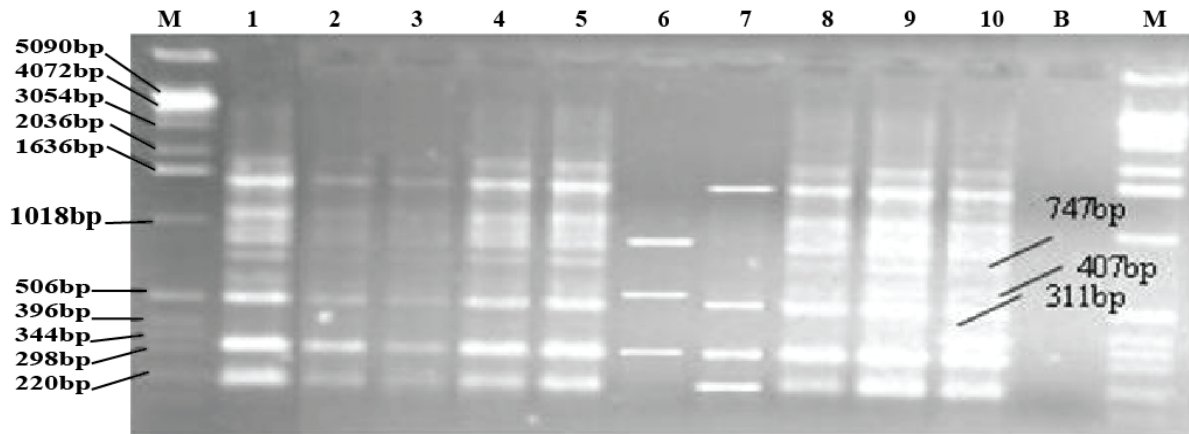


Fig. 5. Results of RAPD-PCR with primer B-02; M=DNA marker, 1=GT-11, 2=AEC71-2011, 3=AEC82-1026, 4=AEC81-0819, 5=NIA-2004, 6=AEC86-328, 7=L116, 8=Thatta-10, 9=AEC92-105, 10=AEC82-223, B= Blank

Genetic markers RAPD are usually considered as dominant markers but several issues relating to their use have been recorded; such as the inability of the marker to distinguish between homology of fragments that run at the same band size, and mutations in the binding region of primers resulting in the loss of the PCR product (null-alleles) (Hu, 1993; Callen *et al.*, 1993; Pemberton *et al.*, 1999) These

issues are compounded in the highly polyploid sugarcane genome, particularly where the difficulty in distinguishing alleles from homoeologous chromosomes makes it difficult to determine heterozygosity at any particular locus. Besides, all limitations RAPDs have been considered as dominant markers and good source of calculating genetic diversity in the pool.

Genetic diversity in sugarcane is hampered due to lack of hybridization programme in Pakistan, thus, causing the narrowing of the genetic base. Therefore, high genetic similarity as observed in this study. Efforts must be made to ensure a broad and diverse genetic base of sugarcane genotypes by deploying new approaches like genetic engineering. The average genetic similarity was 76.02% among the sugarcane genotypes used in this study. This is almost the same range as given by Pan *et al.*, (2004) for *Saccharum spontaneum* and elite accessions. Harvey & Botha (1996) reported similarities of 77-95% among 20 elite sugarcane varieties whereas, Harvey *et al.*, (1994) reported nearly 80% genetic similarity among most of the 21 South African sugarcane varieties. They also found that a *S. spontaneum* clone and an elite variety were more divergent with almost 30% similarity and suggested hybridization to develop new genetically more diverse commercial varieties. Khan *et al.*, (2009) also observed that genetic distance among the 20 accessions was 39.03% implying that genetic diversity among the genotypes is limited. Parentage of the genotypes did not contribute significantly to the genetic similarity or have any significant effect on the clustering pattern. This probably is due to the repeated use of a few set of varieties which are themselves related as parents (Nair *et al.*, 2002). The diversity in plant types and growing environments suggest numerous adaptive mechanisms against environmental stresses, which enable them to tolerate stress (Steponkus, 1980; Svensson *et al.*, 2002). Higher activity of DREB (transacting element) under stress condition triggers biochemical/ physiological modifications in the plant resulting in the biosynthesis of osmoprotectants and structural/functional protein (Thomashow, 1998).

Sugarcane exhibited polygenic inheritance for most of the characters such as sugar content and water stress (Miller, 1977). The level of tolerance expression in the genotype may be due to differences in the genes coding for this trait or may be the result of variation in environmental condition and the amount of genes passed from parent to offspring (Hogarth, 1981).

It is also important that marker assisted selection for breeder can be more helpful to incorporate genes which are functional for a specific crop, and may induce the functional polymorphism (Anderson & Lubberspedt, 2003; Liu *et al.*, 2004; Alwala *et al.*, 2006). Sequence Tag Sites and TRAP techniques have been used in this study to observe the polymorphism in coding region for specific trait (SuSy-2 and DREB). Dehydrogenase Reductase Enzyme Binding protein is selected for the drought tolerance in sugarcane. Which may be directly involved with the phenotypic expression? Moreover, in our study, specific region of the sugarcane genome related to drought tolerance, rather than the entire genome, were sampled to evaluate the genetic variability of elite sugarcane varieties in figure-2. It was observed that the field performance of tagged varieties is better under water stress condition as compared to susceptible ones. At 18 irrigations reduction in cane yield was recorded as 13 and 18% in AEC81-0819 and Thatta-10 respectively (Table 1). Both genotypes bear the same allele for drought tolerance (Fig. 2) but there is a difference in the sucrose synthase allele (Fig. 1) due to which AEC82-0819 produces more sugar as compare to Thatta-10. Because of different allele, clone AEC81-0819 showed only 5% reduction in sugar yield whereas, 14.5% reduction was observed in Thatta-10 at 18

irrigations. This can be concluded from the morphological and molecular data that genotype having sucrose synthase allele (561bp) when combines with DREB allele 1.164kbp will exhibit minimum sugar yield loss. Terzi *et al.*, (2009) reported that 69 genes were associated with sucrose content under drought conditions. Whereas, Casu *et al.*, (2004) reported that 23 genes responsible for drought tolerance were associated with sucrose content. Khan *et al.*, (2002) reported a negative correlation of cane yield with sucrose %. From our results it can be concluded that sucrose synthase allele (561bp) showed weak negative correlation with cane yield as compared to allele (327bp) which is present in Thatta-10.

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