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Research Article

Molecular Assessment of Cultivar Diversity for Water Deficit Stress Tolerance in Saccharum Officinarum using SSR Markers

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Abstract

Eight SSR markers were utilized for assessing the genetic diversity among the 23 sugarcane cultivars. Based on the grouping pattern, the genetic relatedness among sugarcane cultivars was identified. UPGMA based dendrogram analysis grouped 23 sugarcane cultivars into two different clusters. Cluster I consist of two groups. Group I has four cultivars Co 09004, Co 14011, Co 95020 and Co 13003, which were the good cultivars with respect to water stress tolerance. Subsequently, group II has both good cultivar Co 93009 and moderately susceptible Co 0303, Co 92013, Co 12007 and Co 98017 cultivars. Cluster II has been bifurcated into Group III, and group IV. Group III consist of sensitive cultivars Co 92020, Co 13006, Co 86032, Co 92002 and Co 90003, moderately susceptible cultivar Co 85019 Co 98008, Co 94005, Co 08020 and Co 05001 as tolerant cultivar. Subsequently, group IV has both tolerant cultivars Co 10033 and CoC 671and sensitive cultivars Co 06015, and Co 07015. Cultivar Co 10033 and CoC 671 showed highest water stress tolerance and yield. The results revealed that they can be used as parental lines for further breeding. These parental lines inturn serve for backcrossing with good cultivars such as Co 09004, Co 14011, Co 95020 and Co 13003 to improve the genetic potential of sugarcane.

Keywords: Molecular Diversity; UPGMA; (SSR); Jaccard's Similarity Index; Sugarcane

Introduction

Sugarcane is one of the most important commercial crops cultivated for sugar, ethanol and other industrial products. Growth and productivity of sugarcane is adversely affected by various biotic and abiotic factors but the key factor among them is drought. Drought is the main abiotic stress influencing the sugarcane productivity by approximately 30% [14]. It is one of the complex phenomenons than other stresses such as salinity, pests and diseases as it occurs at any crop production period. In India, sugarcane is mostly grown in area where assured irrigation is available. However in parts of peninsular zone, sugarcane do experiences drought or moisture stress during summer months (April-May) which coincides with the formative stage of sugarcane crop [50]. Breeding for water deficit stress is a complicated task as it involves a sequencing of regulatory elements and genes responsible for the drought tolerance traits and their incorporation into the susceptible varieties through hybridization [44]. Drought is known to play a key role in regulating physiological, morphological and molecular changes in sugarcane plants. Under drought conditions net photosynthetic rate decreases, which leads to increased stomatal resistance, low expression of ATP synthase and limited supply of ATP in C_3 plants. Whereas, in C_4 plants the stomatal conductance is less affected due to the mechanism involving carbon-dioxide concentration [23,35,26]. The growth and development of sugarcane crop is severely limited by the occurrence of drought. As sugarcane is water demanding crop, it requires high water during the formative phase (60-150 days), inadequate water supply during this phase affects

the production of crop. Imposing drought at this period would be useful in identifying the drought tolerant varieties [23]. Response to drought in plants occurs by altering the expression of stress inducible gene and as a result the changes will appear at the physiological level. As per the documentation, sugarcane productivity would increase greatly by developing drought tolerant varieties through the integration of traits from its wild relatives through conventional or molecular approaches [12]. Conventional breeding approaches require 12-15 years of time for release a new variety with high capital and labor requirement for selection of parents, hybridization and screening of clones under different stages for phenotypic traits to select an elite clone [1,38]. The laborious method would results in slow rate of development in sugarcane for yield, quality and resistance to biotic and abiotic traits [46]. Therefore, conventional breeding methods coupled with molecular techniques would fasten the process of development of drought tolerant sugarcane varieties [28]. In 1992, an integrated breeding approach involving the molecular and conventional breeding approaches for the development of sugarcane elite clones was envisaged by [8].

One of the important tools for breeders to meet the production demand is by developing molecular breeding method that allows faster development of higher yielding varieties with better adaptability. A molecular marker helps the breeders to track the genetic loci by reducing the need for extensive field trails over space and time [23]. Several types of molecular markers such as random amplified polymorphic DNA (RAPD), single nucleotide polymorphisms (SNP), amplified fragment length polymorphisms (AFLP) and simple sequence repeats (SSR) can be used. SSR markers are also called as microsatellite DNA markers have a monotonous repetition of 1-6 short nucleotide motif showing good polymorphism and co-dominance. It can detect a high number of alleles per locus and dispersed uniformly throughout the genome by providing accurate, economic and efficient way to assess genetic diversity. Phylogenetic relationship of germplasm can be analyzed and it is also advantageous in construction of genetic linkage maps [1,10,26,36,41,46]. These markers with their hyper variability among the related organism make them an excellent choice of marker for genetic marking having associated with gene of known function by tagging itself in a more efficient manner [10,11,26].

In the present study, an attempt was made to understand the genetic relatedness of the sugarcane clones and to determine SSR markers linked to drought tolerance traits and their association with phenotypic traits in terms of their genetic relatedness. Data obtained here is expected to contribute towards marker assisted breeding in sugarcane cultivars for water stress tolerance under induced drought conditions.

Materials and Methods

Plant Materials

In the present study, 23 sugarcane cultivars were planted in a randomized block design along with standards at S. Nijalingappa Sugar Research Institute, Belagavi, Karnataka. The trial was planted during fourth week of January 2019. Each clone was planted in four rows of 0.9 meter width and 6 meter length. The recommended agronomic and prophylactic practices were followed to raise a healthy crop used for the analysis of water deficit stress tolerant sugarcane cultivars which were grown across Northern Karnataka, India. Drought treatment was imposed by withholding the irrigation water during formative phase (60-150 days after planting). Data was recorded at pre-drought, during drought and post-drought stage on all the traits directly or indirectly play role in drought response of the cultivar.

Genomic DNA extraction

Genomic DNA was extracted following the protocol Doyle and Doyle (1987) [16]. The DNA quality was confirmed by agarose gel electrophoresis (0.8%) and quantified with the aid of Nanodrop spectrophotometer (ND-1000, version 3.1.1. USA). The DNA samples were diluted to 20 µl for further polymerase chain reaction (PCR) amplification. In the present study, SSR markers were employed to evaluate the water deficit stress tolerance in 23 sugarcane cultivars of northern Karnataka in order to identify and to select the tolerant cultivars for further hybridization programme.

Simple sequence repeats marker analysis

Eight microsatellite markers table 2 selected based on the literature review, related to drought in sugarcane and DNA was extracted for further analysis. PCR reactions was improved from the protocol of [39] and carried out in a 25µL reaction volume comprising genomic DNA (25-40ng), forward primer (10pM), reverse primer (10pM), dNTP (10 µM), *Taq* buffer (2×), *Taq*DNA polymerase (5U) and finally making the volume to 25 µl using Nanopure water. The PCR amplification which is initial step of was performed using Mastercycler gradient (Eppendorf) with the following conditions: the initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 94°C for 20sec primer annealing condition was set depending on the standardized annealing temperature at 50° for 35 sec and extension at 72° for 45 sec, followed by an extended elongation step at 72° for 45 sec. Samples of 10µL PCR product were mixed with 3µL of 6× loading buffer and spin briefly in a microcentrifuge before loading. The amplification product was

analysed on 2% agarose gel in 1× TAE buffer running at 80v for 50 minutes followed by visualizing under UV light.

Statistical analysis

Genetic diversity of 23 sugarcane cultivars was analyzed using NTSYS software, using Jaccard's coefficient. Similarity matrix coefficient generated of all eight SSR primers based on the NTSYS analysis were used in identifying cultivars based on the genetic diversity. Binary matrix was scored as 1 for the presence of band and 0 for absence of band. Unweighted Pair Group Method with Arithmetic mean (UPGMA), the most straightforward method for tree construction, was used to visualize the cluster pattern of these 23 cultivars.

Results and Discussion

SSR markers are recognized by their informative nature such as the frequency of allele, polymorphism, heterozygosity, effective number of allele, discriminating power and Shannon diversity. These markers are faithfully transmitted from generation to generation and not subjected to environmental influences. In the present study, 23 sugarcane cultivars with significance of high yield, disease resistance and water stress tolerant have been used for the study of genetic diversity to identify the cultivars relatedness (Table 1). The relatedness was evaluated for those 23 cultivars grown under stress and non stress conditions.

Cultivars	Significance	References
Co 09004	Tall cane, high tillering, high sugar content, early maturation and drought tolerant	[31]
Co14011	Higher yield	[4]
Co 0303	Medium canes, resistant to red rot	[4]
Co 13003	Early cane forming and early maturing, medium thick canes	[31]
Co 98017	Cane yield, juice quality and high ratoonability	[31]
Co 95020	Higher rationing performance	[20]
Co 93009	Resistance towards red rot	[3]
Co 92013	Medium thick and erect canes, resistant to red rot	[3]
Co 12007	Lower cane yield	[49]
Co 07015	Moderate cane yield, more sucrose % and susceptible to smut disease	[5]
Co 08020	Medium thick canes, more Number of Millable Canes (NMC) and uniform crop stand, moderately tolerant to drought stress	[31]
Co 85019	High sucrose and early maturing and performed well under limited irrigation	[31]
Co 86032	High sucrose, early maturing and good rationing	[22]
Co 90003	Thick and tall canes, moderate thickness, good donor parent for cane thickness	[31]
Co 13006	High sucrose and early maturing and performed well under limited irrigation	[31]
Co 92002	Medium yield	[30]
Co 06015	Developed from wild relative (*Erianthus*spp.), erect and medium thick canes, moderate sucrose %, tolerant drought stress	[5]
Co 92020	Medium to lower cane yield and lower ratoon yield	[31]
Co 94005	Medium thick canes, high (NMC), sparse flowering	[31]
Co 98008	Thick canes and short canes, tolerant to drought stress	[7]
Co 05001	Thick green yellow to purple cane with cylindrical solid internodes having small corky patches and feeble ivory marking	[6]
Co 10033	Uniform and thick canes, high NMC and higher cane yield, moderate sucrose %	[6]
CoC 671	Early maturing and thick cane	[48]

Table 1: List of sugarcane cultivars used in the present study and their significant features.

Molecular profiles were generated using eight SSR primers (Table 2). These primers were selected based on their high polymorphism for distinguishing cultivars with higher yield. Due to their relative molecular profile of genetic resource these DNA based markers have gained better understanding of genetic variation in sugarcane crop. The molecular profile of primers NKS7, NKS9, NKS21, NKS30, NKS31, NKS49, NKS57 and NKS27 was showed (Figure 1 and 2). An unweighted neighbor-joining tree, using Jaccard similarity index of eight SSR markers, showed cultivar Co 09004 and Co 14011 has 86% of similarity; these unique genotypes could be used further as potential donor for hybridization with the recurrent parents for abiotic stress tolerance. Similar observation regarding similarity was noticed by [51].

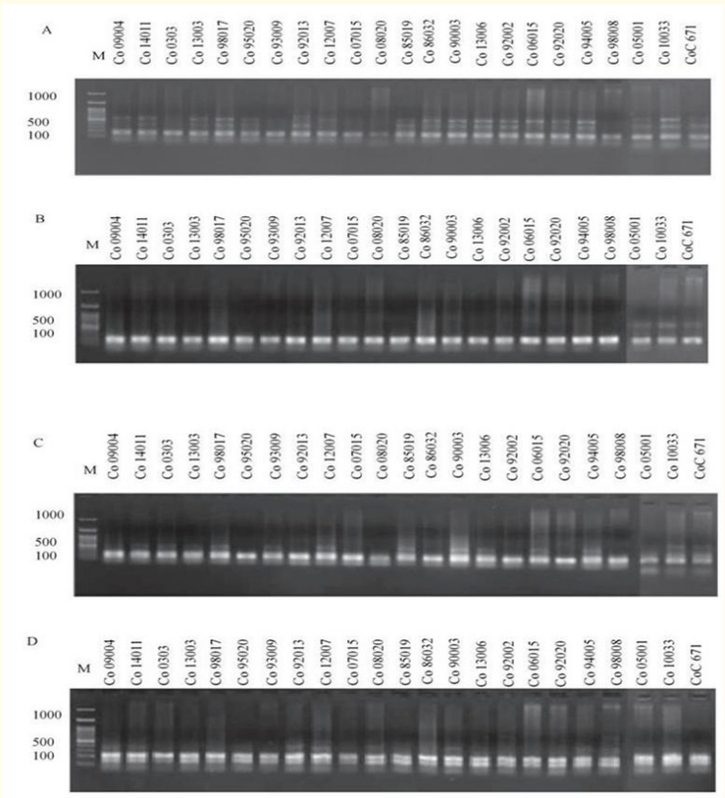

Figure 1: PCR profile of 23 sugarcane cultivars using SSR primer (A-NKS7, B-NKS9, C- NKS21 and D-NKS30.

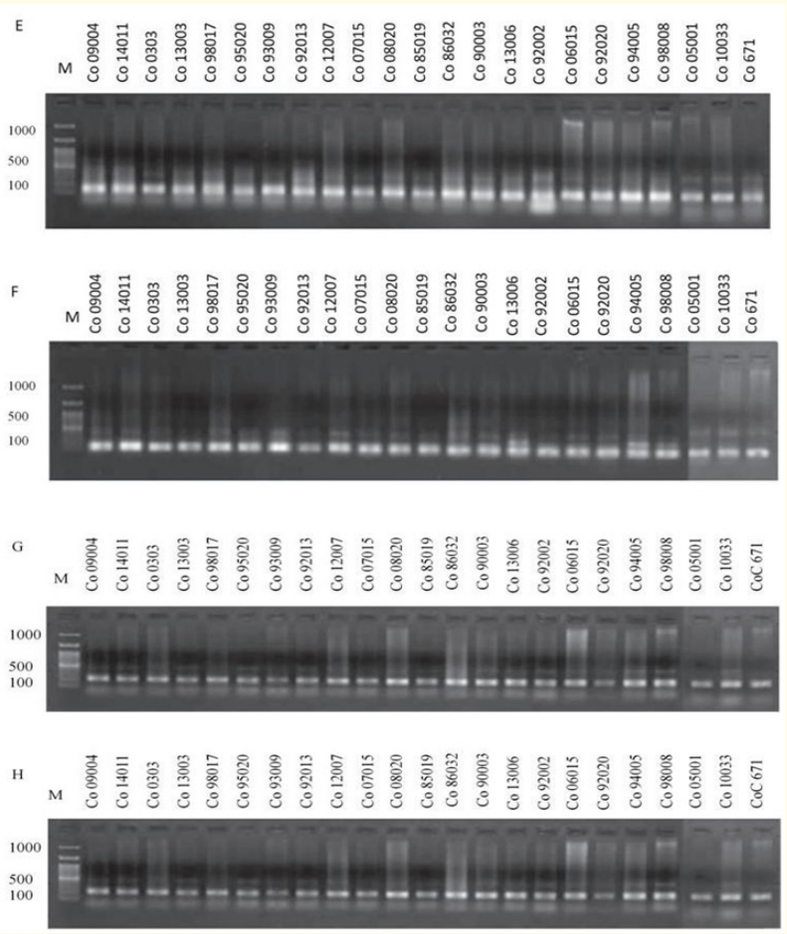

Figure 2: PCR profile of 23 sugarcane cultivars using SSR primer (E-NKS31, F-NKS49, G-NKS57 and H-NKS27).

The compiled dendrogram of all 8 makers showed, Jaccard similarity index ranged from 2.8 to 6.2 with an average of 3.9, indicating moderate level of genetic diversity present in cultivars. The cultivars Co 90003 showed highest genetic similarity with Co 0303 (6.2), followed by Co 86032 with Co 95020 (5.8), subsequently, Co 92020 with Co 98017 (5.6), Co 05001 with Co 0303 (5.5) and Co 85019 with Co 92013 (5.3). Clustering based on UPGMA provided a clear resolution of relationships among all the 23 sugarcane cultivars. The genetic similarity coefficient between the two clusters was observed to be 0.15.

Markers	Forward sequence (5'-3')	Reverse sequence (3'-5')	Fragment size (bp)	SSR motif
NKS7	TTACAGCCTGGAGCTCGTTT	CGAAGCCTCTCCTCTCCTC	179-325	(cgg)9
NKS9	CTTTCAGTGGCCATCTCCAT	GAATGCGCAGGGATAGGATA	142-1260	(cgc)6
NKS21	TAAGCCATTGGGAAGAGGTG	CTGATGCCTGGGAATCTTTC	190-682	(ga)20
NKS30	CTCCTTCTCCTTCGCATCCT	CACCTTTCTGGAGCACGTTA	149-520	(cgg)7
NKS31	AACCACCACTCATCGTCCTC	CACCGAGTTCCCATTGTTCT	200-522	(cgg)8
NKS49	CTCACGTCCTGTTGGTGCTA	TACATGGGACACATGCTTGC	92-670	(tg)19
NKS57	CGAGCCTCCCTCCATAGATT	ACCACCACCAACCTCATCTC	105-490	(ga)21
NKS27	TGGATTTGGGTAAGGATGGA	TAATGCCTCTGGGCTCAAAT	174-461	(ga)20

Table 2: List of SSR primers used to screen 23 sugarcane cultivars against water stress tolerance.
*(adapted from Hemaprabha and Simon, 2012).

Cluster I consist of two groups. Group I has four cultivars Co 09004, Co 14011, Co 95020 and Co 13003, which were the good cultivars with respect to water stress tolerance. Subsequently, group II has both good cultivar Co 93009 and moderately susceptible Co 0303, Co 92013, Co 12007 and Co 98017 cultivars. Cluster II has been bifurcated into Group III, and group IV. Group III consist of sensitive cultivars Co 92020, Co 13006, Co 86032, Co 92002 and Co 90003, moderately susceptible cultivar Co 85019 Co 98008, Co 94005, Co 08020 and Co 05001 as tolerant cultivar. Subsequently, group IV has both tolerant cultivars Co 10033 and CoC 671 and sensitive cultivars Co 06015 and Co 07015. As compared to the other markers, SSR markers have been successfully used to determine the genetic diversity, to assess the sugarcane germplasm and to exploit large number of microsatellite loci in commercial cultivars [15,18,23,37,40,44,46] SSRs were also previously used in identifying the drought tolerant cultivars in sugarcane [2,9,19,46,]. Thus cultivars Co 10033 and CoC 671 are unique and they are grouped into separate group having close genetic relatedness by showing 95% similarity between them.

Cultivars CoC 671 and Co 10033 has been grouped in a separate group with good potentiality in terms of quality and vigor and serves as parent in breeding programme. Genetic diversity assessment in germplasm collections is one of the potential approaches for developing new varieties while identification of the parent is intended to be a primary step for designing effective breeding strategy [35,41] A recent study carried out by [39] reported similar observations wherein the study involved selection of SSR marker for drought resistant sugarcane with the aid of physiological traits and agronomical traits. NJ analysis showed cultivar Co 09004 and Co 14011 has 86% and Co 10033 with CoC 671 has 95% of similarity.

Similarly, CoC 671 had unique banding pattern and grouped in separate group (Figure 3). A study made by [27] was in correlation with the present study, wherein primer SSR15 produced one band which was used as drought sensitive and other marker SSR80 showed two bands of different fragment size which could be used as negative and positive marker. The results of SSR marker were in correlation with the physiological and morphological traits as observed by [25,26].

Conclusion

This study demonstrated the performance of 23 sugarcane cultivars due to water stress based on eight SSR markers that are responsible for conferring water stress tolerance. Based on the UPGMA clustering, these twenty three sugarcane cultivars categorized into three major groups depending upon their genetic relatedness. NJ analysis showed cultivar Co 09004 and Co 14011 has 86% and Co 10033 with CoC 671 has 95% of similarity. Results indicated that four elite cultivars from cluster I- Co 09004, Co 14011, Co 95020 and Co 13003 and two cultivars from group IV of cluster II-Co 10033 and CoC 671 showing higher water stress tolerance, which can be used as a potential source of genes conferring water stress tolerance and also could be used as potential donor for fur-

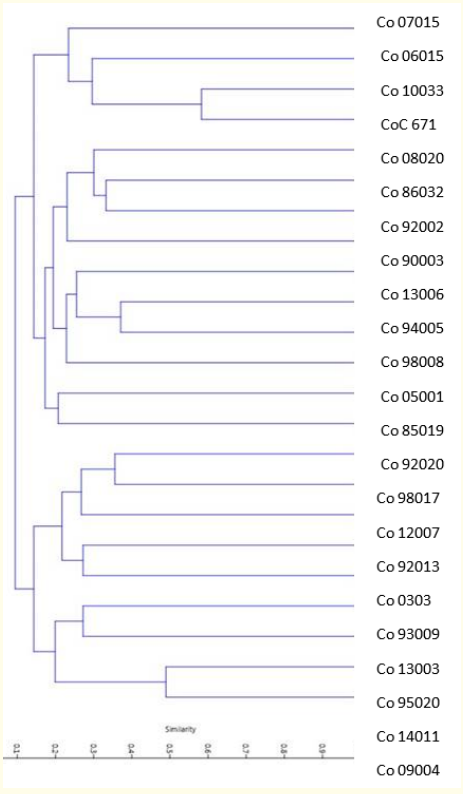

Figure 3: Dendro gram showing clustering of 23 sugarcane cultivars constructed using UPGMA based Jaccard'sco efficient obtained from SSR marker analysis.

ther molecular breeding purposes, with improved vigor and yield. Thus, these results may be useful for further breeding programs in selecting parents with higher level of water stress tolerance, while crossing such beneficial cultivars and further combining the genes in new cultivar, so that the genetic potential of sugarcane would be further improved. But still there is need to develop ample of SSR markers which can be used further to identify various kinds of abiotic stress.

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