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ABA dependent candidate gene identification and diversity analysis through SSR in sugarcane (*Saccharum officinarum* L.) under salinity stress

Ranjana Singh and RS Sengar

Abstract
Eleven (11) abscisic acid (ABA) dependent gene specific primer and twenty five (25) simple sequence repeats (SSR) primer pairs were chosen to detect the presence of gene responding under salinity stress and polymorphism to the 10 sugarcane accessions respectively. Out of 11 only 6 ABA denendent gene primer got amplified. The highest PIC (polymorphism information content) 0.764 for the primer RGS1 and lowest PIC for primer CDPK 0.22 was recorded. The resolving power were obtained in the range of 0.80 to 1.60. In case of SSR out of 25 primer only 19 were got amplified and generated 67 reproducible bands, to which 52 were polymorphic and 13 were monomorphic. The average number of polymorphic bands was 2.73 per primer, polymorphism range from 66.67% to 100%. The highest PIC 0.99 was recorded for the primer UGSM507 while lowest PIC 0.16 was recorded for the primer UGSM695, with resolving power ranging from 0.20 to 1.80. Similarity value for all the 10 genotypes ranged from 0.58 to 1.00. The minimum similarity exhibited by genotype Co 0238, Co 8279 and Co 0118, whereas the maximum similarity was shown by the genotype Co 5011 and CoS 7250.

Keywords: ABA, SSR, sugarcane, PIC, polymorphism, genotypes

Introduction
Sugarcane crop (*Saccharum officinarum* L.) of andropogoneae tribe and poaceae family, widely known for its multipurpose uses grown in both tropical and subtropical regions of world. *S. officinarum* are the result of interspecific crosses made in between S. *officinarum* and *S. spontaneum*, *S. officinarum* is high in sugar while *S. spontaneum* were resistant to several pest and abiotic stress [1, 2] Sugarcane hybrid show high ploidy and aneuploid level with an average of 100-120 chromosome and 10,000 Mb of somatic cell size. Chromosome number can be different in different cultivar with size range of genome from 760 to 926 Mb which is similar to that of sorghum and double to rice (389 Mb)^3. Sugarcane besides producing sugar well known for its importance in generating empowerment in various allied fields like production of bioethanol, acetic acid, paper, industrial enzymes and feed for animals [4, 5]. To enhance the commercial character it is the need of current scenario to breed and develop high quality canes with improved agronomic values.
Microsatellites or simple sequence repeats (SSRs) derived from both coding (eSSR) and non-coding (gSSR) regions of the genome are highly informative and reproducible markers. The hyper- variability of SSRs among related organisms makes them an informative and excellent choice of markers for a wide range of applications in sugarcane, that include high-density genetic mapping [6], gene tagging, identification of genotype, genetic diversity analysis, determination of paternity and phenotype mapping along with marker assisted selection [7, 8]. Its ubiquitous distribution makes the marker highly polymorphic and reliable [9].
Salinity changes the soil texture and making it non productive day by day due to malpractice of irrigation, inappropriate drainage system and overdose of chemicals and fertilizer. Salinity reduces the plant growth by lowering the osmotic potential of soil, misbalance of nutrition present in it and specific ion effect. As a result of these changes, plants get suffered at genetic level also, in addition to, physio-biochemical activity of plants also get hampered [10, 11]. Sugarcane genotype differs with each other with respect to its behaviour and stage towards salinity [12-14]. Drought and salinity both stresses affect the formative and grand growth phases of sugarcane that are critical stages of plant growth [15]. As salinity creates physiological drought condition in plant, the relationship in between water and respective physiological

behaviour can better discriminate the genotypes for tolerance and sensitiveness [16, 17].

Abscisic acid is considered as a stress hormone that regulate the expression of genes and act as a signal molecule for the initiation of processes to be involved in adaptation and mitigation of stress. Abscisic acid has been reported to induce the expression of various water and salt stress related genes such as ABF2, ABF3, ATHB7, CIPK 14, CDPK 18, LEA 3, MYB 2, RD 28, RGS1, SNRK 2.5, TPS 2 (see abbreviations) etc. [18] Under stress response, transcription factors undergo reversible phosphorylation of transcription factors, phosphorylation allows transcription factors to switch rapidly and accurately, [19] that plant on exposure to stress behave accordingly.

Material and Methods

Plant material, SSR marker and ABA dependent primers

Ten genotypes of sugarcane Co 0118, Co 0238, Co 5009, Co 5011, CoS 7250, CoPant 97222, CoSe 8457, CoS 8279, Co 98014 and CoLk 99270 comprising of released varieties from different part of agro-climatic zone of India were selected from the Field laboratory and Experiment station, Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture & Technology (SVPUA&T) Meerut, India. Artificial salt stress condition were created by irrigating the plants through an ECiw of 10 dS/m and ECiw 20 dS/m at their formative phase (40-150 DAP), and fresh smples for DNA isolation [20] were taken at grand growth phase (160-240 DAP). The quality and quantity of the genomic DNA was checked on 0.8% (w/v) agarose gel and diluted appropriately for working concentration of 25ng/µl. Purity and concentration of the extracted DNA for each sample was also checked by UV spectrophotometer (Perkin Elmer, Lambda 25).

Some microsatellite (SSR) primers (Table. 1) reported in different studies were used in ten genotypes of sugarcane for genetic divergence study [21, 22]. sequences were synthesized from Bangalore Genei, these primers were diluted in 0.1X Tris- EDTA buffer solution for making primers stocks and later further diluted to appropriate working concentrations using ion-free double distilled water. Simultaneously eleven ABA dependent gene specific primer [18] (Table. 2) also synthesised by Bangalore Genei and were diluted in 0.1X Tris- EDTA buffer solution. These were used for the assessment of their presence and absence in the sugarcane genotypes with respect to salinity stress. Both the diversity analysis and gene specificity for salinity tolerance were done simultaneously to assess any correlation present if any, under the influence of salt.

Polymerase chain reaction analysis

Each PCR was performed in a volume of 20µl in S-1000 thermal cycler (Biorad- German), consisting of 2.0 µl of 10X PCR buffer, 0.8 µl of dNTPs (2.5mM each dNTP) (Banglore Genei) and 1 µl each of forward and reverse primers (Bangalore Genei), 1.0 unit of *Taq* DNA polymerase (Bangalore Genei, India) and 60 ng of DNA. Different primers run accordingly with their specific annealing temperature ranging from 52 °C to 62 °C for 1 minute (Table. 3). After the final extension (polymerization) step, the samples were transferred to 4 °C or to -20 °C if they were not going to be used immediately.

Agarose gel electrophoresis

The amplified PCR products for SSR and gene specific markers were resolved on to 1.2% agarose gel in 1X TAE buffer. To each PCR product, 3 µl of 6X loading dye was added, mixed well and loaded in the wells of the gel. 100 bp and 1 kb DNA ladder (Bangalore Genei) was used as molecular weight marker. The gel was run for 45 minutes to 60 minutes at 75 volts using Power-pack 3000 (Bio-Rad, USA).

Data Analysis

Amplified product generated through SSR and ABA dependent gene specific primers were analyzed by pair wise comparisons of the genotype using UPGMA, [23] also the genetic distance matrix was constructed by applying this UPGMA and a dendrogram showing genetic relatedness were form. DNA bands were scored for presence (1) or absence (0) in all 10 genotypes under investigation and the data were used to calculate the Jaccard's similarity coefficient (JSCs) by NTSYS-pc software[24] While in case of gene specific primers we have given only the calculative values PIC (polymorphism information content), (resolving power) RP, polymorphism percentage and number of amplified band (NAB) obtained through presence (1) and absence (0) of bands in the selected ten varieties.

PIC was calculated for each primer using the formula

$$PjCi = 1 - \sum_{i=1}^{n} p^2\, ij$$

P_{ij} is the frequency of i^{th} allele for marker j and the summation extends over an alleles [25] Resolving power[26] was obtained according to formula:

$$RP = \Sigma\, Ib$$

where Ib is the band informativeness that takes the value of

$$1- [2 \times (0.5-P_i)]$$

P_i = proportion of genotype containing the i^{th} band.

Results and discussion

Microsatellite or SSR markers are interspersed repetitive elements present in the coding and non coding region of eukaryotic DNA. Molecular markers based on PCR methods, such as SSR or microsatellite, have become important genetic markers in wide range of crops, including sugarcane [22]. SSR are excellent molecular marker system with an advantage of being co-dominant, high genomic coverage, and show higher level of polymorphism than other genetic markers. The results of this study indicate that relatively large number of SSR primers could be used to distinguish genetic variation among sugarcane genotypes. Variation in the banding pattern of the amplicon occurs because of variation in the length of DNA sequence flanked by primers. Among 25 primers taken for the study only 19 were shown polymorphism with the ten sugarcane genotypes.

A total of 25 SSR primers generate 67 reproducible and clearly scorable bands across the ten genotype with an average of 3.52 bands per primer. UGSM562 and UGSM394 primer gave the highest number of amplified alleles i.e. 6, followed by primer UGSM667, UGSM573, UGSM513, UGSM681 and SOMS118 that showed total amplified alleles i.e 5, further primer UGSM358 gave 4 alleles per primer and UGSM452, SCM32, UGSM 354, UGSM445, SCM14, SCM4 and SCM16 with 3 alleles per primer followed by UGCM565, UGSM507, UGSM585, UGSM575, UGSM576 with 2 alleles per primer across the studied sugarcane genotypes (Fig 3). Out of 67 amplified bands, 13 were monomorphic and 52

were polymorphic. The average number of polymorphic bands was 2.73 per primer. Among the tested SSR primers, nineteen amplified polymorphic SSR loci, polymorphism range from 66.67% to 100%. The size of amplified alleles varies from 2000 bp to 100 bp. The approximate size of the largest DNA fragment produced was 2000 bp for UGSM562, UGSM513, SOMS118 primer while many primers registered the smallest amplitude of amplified fragments size of 100bp.

Polymorphism information content (PIC) and resolving power

Polymorphism information content (PIC) was calculated for nineteen polymorphic SSR primers that amplified during PCR. A PIC value was found to be varied from 0.19 to 0.99 with mean PIC value for SSR primers was 0.473. The highest PIC 0.99 was recorded for the primer UGSM507. On the other hand the lowest PIC 0.16 was recorded with the primer UGSM695. The higher mean PIC value indicated the informativeness of the primers pairs in detecting genetic diversity. Hence, the primer pairs UGCM667, UGSM507, UGSM513, UGSM445, UGCM681, SCM15, SOMS118, SCM4, SCM16 and UGSM571 seems to be more informative as they shown the PIC value higher than 0.473 and can be used in future studies in the field of taxonomical and genetic resource management. Lower PIC value may be the result of closely related genotypes. SSR have been found as efficient marker for the sugarcane diversity analysis based on the PIC values obtained [27, 28]. PIC value of 0.9445 were found when evaluating 27 sugarcane genotype [29].

The resolving power (RP) of primer explains the ability of primers to resolve the studied germplasm, it indicates the discriminatory potential of the primers chosen. The resolving power of 19 polymorphic SSR primers varies between 0.20 to 1.80 with an average value of 1.26, (Table. 4) and (Fig. 3, a and b). The primer UGSM507 showed the lowest resolving power, while UGSM394 showed maximum resolving power. On the basis of resolving power the primer UGSM562, UGSM452, UGCM565, UGSM358, SCM32, UGSM394, UGSM354, UGSM585 and UGSM575 are seems to be good in resolving the studied genotypes as they shown more than 1.26 resolving power. Thus, the significant value of resolving power indicated the ability of primers to resolve the different closely related genotypes of sugarcane.

Genetic variation within Sugarcane accessions: Similarity vs. dissimilarity analysis

SSR data were used to make pair wise comparison of the accessions based on shared and unique amplification products to generate a similarity matrix with NTSYS-PC (version 2.02.e). Dendrogram was drawn through UPGMA method. Similarity value for all the 10 genotypes ranged from 0.58 to 1.00. The minimum similarity exhibited by genotype Co 0238 and Co 8279 to Co 0118. Whereas the maximum similarity was shown by genotype Co 5011 and CoS 7250 (Table. 5). 62 DNA fragments were found using 30 SSR primer among 17 sugarcane accessions [5]. While assessing 20 sugarcane cultivar by 21 SSR markers identified about 144 alleles with 3-11 alleles per marker [30]. While doing the diversity analysis of *Saccharum* and other Indian cultivars of sugarcane through SSR, the similarity coefficient were found ranging from 0.347 (CoS 96268 and Calcutta) to 0.865 (CoS 95255 and CoS 767) [22]. During the analysis of genetic diversity of 9 sugarcane cultivar through SSR under salinity level of 8.3 dS/m, found 54 alleles with an average of 2.8 alleles/ locus, PIC value for the genotype ranged from 0.10 to 1.80 with similarity coefficient 0.25 to 1.00 [31].

Cluster analysis

The cluster analysis based on the unweighted paired group method of arithmetic means (UPGMA) with 19 SSR primers allowed the discrimination of cultivars. The UPGMA based clustering grouped 10 sugarcane genotypes in to three major group's i.e. I, II and III groups (Fig. 1). The group I includes 2 genotypes namely Co 0118 and Co 5009. The group II includes 06 genotypes namely Co 5011, CoS 7250, CoPant 97222, CoLk 99270, CoSe 8457 and Co 98014. Sugarcane genotypes Co 5011 and CoS 7250 showed high similarity to each other. The group III includes 2 genotypes namely Co 0238 and CoS 8279.

Molecular characterisation of different sugarcane genotypes through gene specific markers

Eleven ABA dependent gene specific primer were used in this study. [18] Table. 2) and were studied for the presence (1) and absence (0) of band with respect to salinity stress tolerance. Out of eleven primers, only six were amplified and shown the presence of specific bands. All the ten genes amplified in *Saccharum* and *Erianthus* species except *ATHB7* [18]. Their presence only in the tolerant species clones implicating those as the repository of ten genes to be used in breeding program for stress tolerance. However no such reports are seen in case of sugarcane under salinity stress yet ours result indicate that some of genes that express in drought also express in salinity.

As under, for the sustainable performance primers were measured using the PIC [25] and RP [26] values obtained based on the gel images (Fig. 2, a-f). The reproducibility of bands were calculated and presented as number of amplified bands, number of polymorphic band and percentage of polymorphic bands (Table. 6). The PIC value of primers varies from one to another as, 0.5275 for ABF2, 0.59 for MYB, 0.22 for CDPK, 0.764 for RGS 1, 0.511 for CIPK and 0.36 for LEA. The highest PIC 0.764 was recorded for the primer RGS1. On the other hand the lowest PIC 0.22 was recorded for the primer CDPK. High PIC value indicates the informativeness of the primer pairs. Lower PIC value may be the result of closely related genotypes. The resolving power of primer explains the ability of primers to resolve the studied germplasm it indicates the discriminatory potential of the primers chosen. The resolving power of six gene specific primers varies between 0.80 to 1.60. The primer RGS 1 showed the lowest resolving power, while the LEA gene showed maximum resolving power of 1.60. The approximate size of amplification with respect to the 100 bp ladder is shown in gel images respectively. However, such type of work is not done in case of salinity specific gene identification of sugarcane before. Though salinity creates a condition of physiological drought in plant, it is evident that the result obtained here are in correlation with the gene specificity for salinity tolerant as found in earlier studies [18] for drought tolerance of *Saccharum* and *Erianthus* species of sugarcane. Presence of CIPK 14 and other gene in *A. thaliana* conferred salinity and drought tolerant of the plant simultaneously, as suggested by earlier report [32].

Conclusion

Genetic diversity study of sugarcane through SSR were done on the ten sugarcane genotypes essential for developing better breeding strategies. SSR being co-dominant and biallelic is an effective marker used for the genetic diversity assessment that efficiently categorized the varieties in different cluster group. Among eleven, only six ABA dependent gene were amplified well in the sugarcane varieties that somehow represent that

varieties are able to stand in the salinity prone zone. Though, in case of variety Co 0118 only CDPK candidate gene amplified effectively. Further studies on expression level can better clarify the potential of plant in resisting the salinity stress and can efficiently pave the way for developing salt tolerant cultivars. Through SSR breeders can easily select the genotype for crosses in between and within the group so as to recombine the beneficial traits of high potential.

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Table 1: Summary of SSR primer used in this investigation

S. No.	Primer	Forward primer (5′–3′)	Reverse primer (5′–3′)
1.	SCM 32	GATGAAGCCGACACCGAC	AGTTGCCTGTTCCCATTT
2.	SCM 16	GTGCGAGAGGAACTGTGT	AGCCCTGCCTAACAAGGA
3.	UGCM 667	CTATCCTCTTGTTGGGTCCT	TCCGCACCTCCGTTCACC
4.	UGCM 681	ACACATCGCTTTCCCACA	GCATACCTGTCGTCGTCT
5.	SOMS 118	GAGGAAGCCAAGAAGGTG	TAGAGCGAGGAGCGAAGG
6.	SCM 15	GGAGATGTTTGAGAGGGAA	AGAGTAGCATAAAGGAGGCAG
7.	UGSM 565	ACCTCCACCTCCACCTCAGTTC	CGTTCAGCTTCAGGGTGTCGAT
8.	SCM4	CATTGTTCTGTGCCTGCT	CCGTTTCCCTTCCTTCCC
9.	UGSM 575	CTGTTTCCTTCCTTCTCGT	CAATCATAGCCCAGACACC
10.	UGSM 585	GAAGAGGAGGAGAGGAGAAG	TGGGATGGTTGTTGACTG
11.	UGSM 358	ACCTTCCCATTCCCATC	CTCCAGGTTCGCCACCAC
12.	UGSM 354	ACTGACACACACGCACAC	TGGAAGTGAATGAAGCGA
13.	UGSM 445	GCGAGAGAGATAGAGGGAGAGA	AGGTGCCGTTCATGAGGTAGT
14.	UGSM 394	ACTCCTCCCGCCTCCACTAC	CTCACCGAAGCAATCAAG
15.	UGSM 452	CACCGCAGCCTGACACAGAACC	AGGAACTCAGCATACTCGTGAC
16.	UGSM 507	CCTCCAACTCTCTTCTTGTCT	AGGGAACCATCCTCAATCT
17.	UGSM 513	GAACCACGGAACCCACTC	CTACGACCACCAGTCACAC
18.	UGSM 562	CCATCAGAGAGGAGTAGCAG	ATTCCAAGTTCACAAGTTCA
19.	UGSM 571	GTTCCAAGTTACAGACCAGAA	GATAAGAAAGATGTCGTCGCT
20.	UGSM 632	CGTTCGTCTCTCTCTCCTC	ATTTACAGGTCATCCCAAAC
21.	UGSM 634	GCTCTCCTCCTCCTCTCC	GCCACTTTATCATCCTCAGTT
22.	UGSM 644	TCCACAAACAGAAACAGTCC	CTACCGTGAGAAGCACCA
23.	UGSM 649	CAGGACTACAGGGAACAATAA	GAAATACCAGGCTCACTTCA
24.	UGSM 674	GCATCTATCGGTCTTCTGG	ATCCAATCCTTCATCTTCTTC
25.	UGSM 682	ACTGCTGCTCACCGACTT	AAGAATAATAACAACCGCACA

Table 2: List of gene specific primers used in this investigation

Sr. No.	Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Transcription factors–regulation			
1.	ABF 2	GGCTCAGGCTCAGAGTCAGA	GCTCCTGCGACGATGAACTT
2.	ABF 3	GCAACAAACGCTGGGAGAGA	CATCCCATTCCCCATGGCTG
3.	LEA 3	AACCAGAACCAGGCCAGCTA	AAATCAAGCGCGAACGATGC
4.	MYB 2	CAGCAATTCGTACCGGCTACG	ATTGGCATCACGCACGAATG
5.	ATHB-7	TTAGTCTCTGAGTTGCAGAGG	AACGAGTCATTTGCTGGACC
Protein kinases			
6.	CIPK 14	GTGGAATTTCCGCCGGAGAA	GGGATGCGAAATTCCCCCTT
7.	CDPK18	CGCTCTCGGTTCCTCCAAGA	GGCGCCACATAGTATGCACT
8.	SNRK2.5	TACGCTCAAGTGTGGGAGCA	CACAGGACCAAACATCCGCA
Membrane associated proteins and receptors			
9.	RD 28	CCGGTATCTCAGGTGGTCAC	TCGTGGCTAAGTTTAAACGTTG
10.	RGS 1	CTGTTGACCCAAACGAGCCA	AAACCAAAATCCGAACCGGACA
	Enzyme		
11.	TPS 2	TCGCGATCTAGACTATTGCGG	TGCCACCCTTCATCTCTCGT

Table 3: Thermocycler Program for the PCR-based marker amplification

Steps	Temperature	Time	Number of cycles
Initial denaturation	95 °C	3 min	1 (first)
Denaturation	94 °C	1-1.5 min	
Annealing*	52-62 °C	1 min	
Extension	72 °C	30 sec	35 cycles
Final extension	72 °C	7 min	1 cycle
Hold	4 °C	Forever	
*depending on individual primer			

Table 4: A summarized view of the values obtained through SSR analysis

S. No.	Primer Code	Molecular weight range	Amplified bands	Polymorphic bands	Monomorphic band	Polymorphism %	PIC Value	Resolving power
1	UGSM562	400-2000	06	06	0	100.00	0.347	1.60
2	UGSM452	150-600	03	02	01	66.67	0.310	1.52
3	UGCM565	150-300	02	02	0	100.00	0.360	1.60
4	UGCM667	100-3000	05	04	01	80.00	0.660	0.88
5	UGSM507	100-200	02	02	0	100.00	0.990	0.20
6	UGSM358	100-1000	04	04	0	100.00	0.345	1.60
7	UGSM513	150-2000	05	04	01	80.00	0.524	1.20
8	SCM 32	150-700	03	02	01	66.67	0.250	1.66
9	UGSM394	200-1000	06	05	01	83.33	0.190	1.80
10	UGSM354	200-400	03	02	01	66.67	0.240	1.73
11	UGSM445	150-1500	03	02	01	66.67	0.517	1.26
12	UGCM681	150-1500	05	04	01	80.00	0.674	1.12
13	SCM15	150-400	03	02	01	66.67	0.650	0.86
14	UGSM585	150-200	02	01	01	50.00	0.320	1.60
15	UGSM575	200-400	02	01	01	50.00	0.255	1.70
16	SOMS118	150-2000	05	04	01	80.00	0.524	1.20
17	SCM4	150-500	03	03	0	100.00	0.614	1.20
18	SCM16	200-400	03	02	01	66.67	0.530	1.26
19	UGSM571	100-500	02	02	0	100.00	0.695	1.12
	Total		67	52	13			
	Average		3.52	2.73	0.68	79.12	0.473	1.26

Table 5: Jaccard's similarity coefficient (1908) value obtained through NTSYS-PC version 2.02e

	Co 118	Co 0238	Co 5009	Co 5011	CoS 7250	Co Pant 97222	Co Se 8457	Co S 8279	Co 98014	Co Lk 99270
Co 0118	1.00									
Co 0238	0.63	1.00								
Co 5009	0.73	0.73	1.00							
Co 5011	0.78	0.72	0.82	1.00						
CoS 7250	0.73	0.63	0.73	0.88	1.00					
Co Pant 97222	0.78	0.62	0.78	0.83	0.85	1.00				
Co Se 8457	0.75	0.58	0.75	0.77	0.75	0.80	1.00			
Co S 8279	0.58	0.68	0.68	0.67	0.62	0.67	0.57	1.00		
Co 98014	0.68	0.65	0.75	0.77	0.68	0.77	0.80	0.67	1.00	
Co Lk 99270	0.77	0.63	0.73	0.85	0.80	0.82	0.82	0.58	0.82	1.00

Table 6: A summarized view of PCR amplification of ten sugarcane genotypes through gene specific primers

S. N	Primer Code	Molecular weight (bp) (approx.)	Amplified bands	Polymorphic bands	Monomorphic band	Polymorphism %	PIC Value	Resolving power
1	ABF 2	502	04	04	0	100.00	0.5275	1.15
2	MYB 2	300	02	02	0	100	0.59	1.00
3	CDPK 18	302	02	01	01	50	0.22	1.5
4	RGS 1	840	05	05	0	100.00	0.764	0.80
5	CIPK 14	530	03	03	0	100.00	0.511	1.20
6	LEA 3	290	01	01	0	100.00	0.36	1.60
	Total		17	16	1			

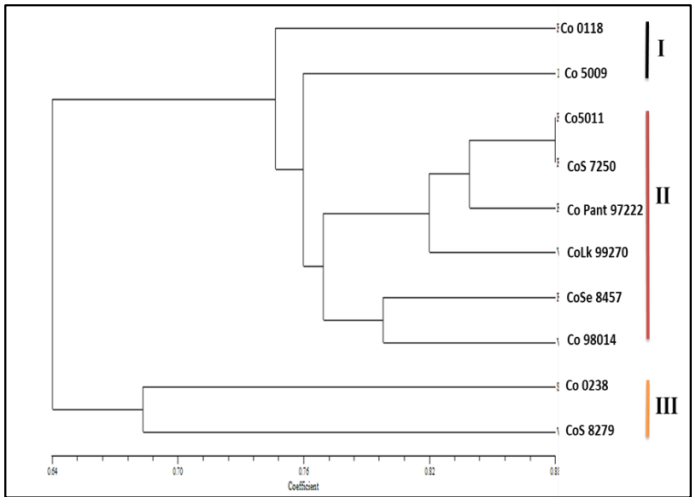
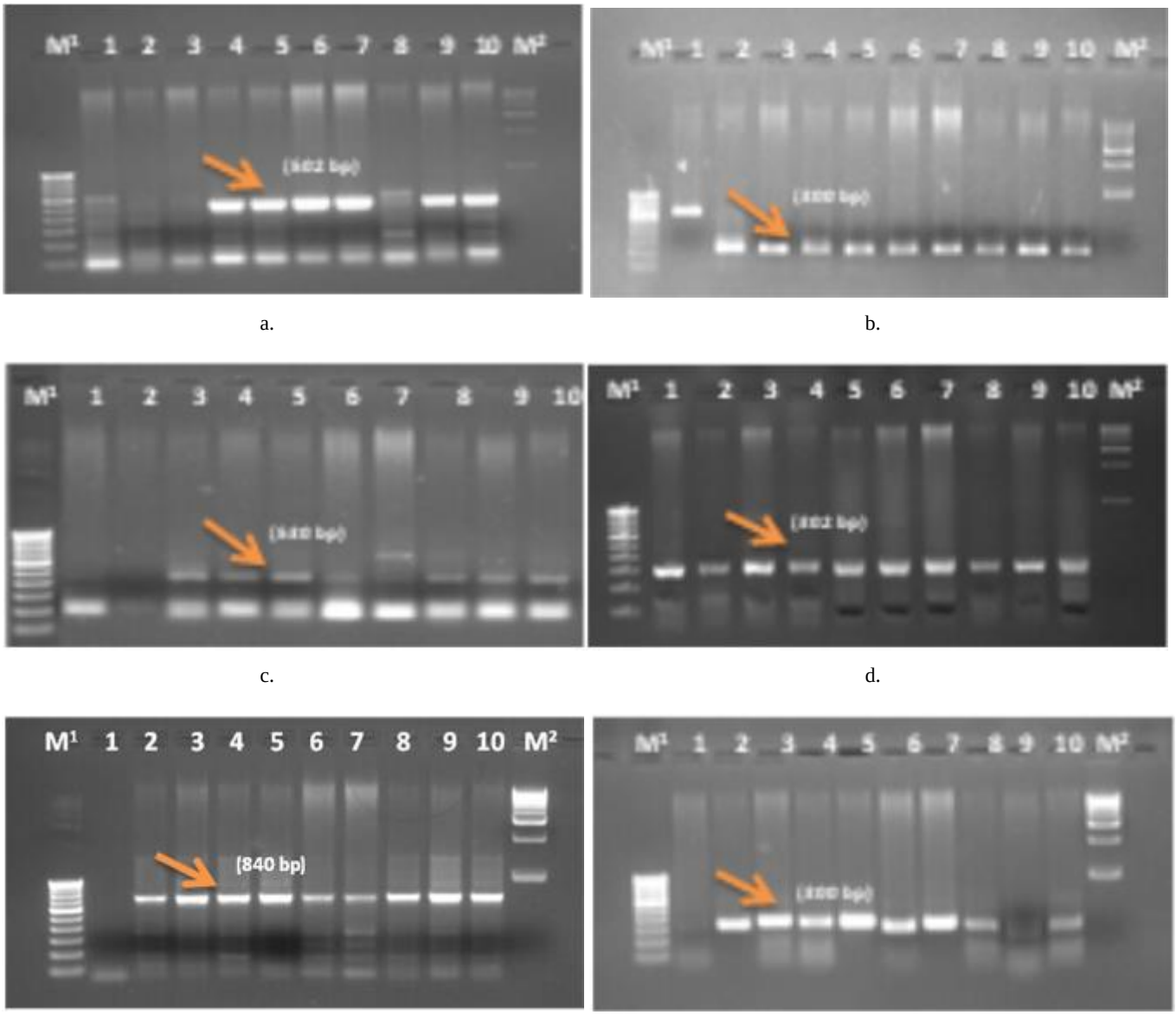


Fig 1: Genetic diversity dendrogram of 10 sugarcane varieties by using UPGMA cluster analysis

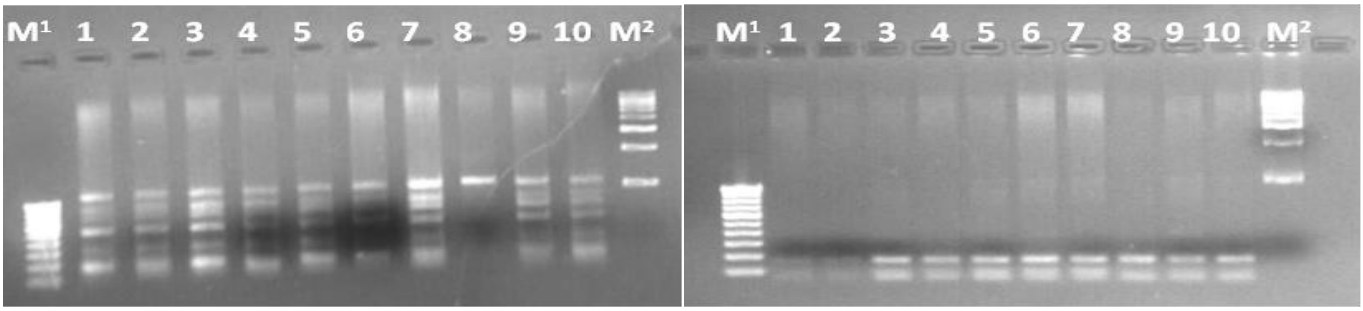


a. b.

c. d.

e. f.

Fig 2: PCR amplification of ABA dependent gene specific primer where, (a) ABF2 (b) MYB 2 (c) CIPK 14 (d) CDPK 18 (e) RGS 1 (f) LEA 3 and lane 1- Co 0118, lane 2- Co 0238, lane 3- Co 5009, lane 4- Co 5011, lane 5- CoS 7250, lane 6- CoPant 97222, lane 7- CoSe 8457, lane 8- CoS 8279, lane 9- Co 98014 and lane 10- CoLk 99270, M1- 100bp ladder and M2- 1 kb DNA ladder



a) UGSM394 b) UGSM507

Fig 3: PCR amplification of 10 sugarcane genotype with SSR primer, where lane 1- Co 0118, lane 2- Co 0238, lane 3- Co 5009, lane 4- Co 5011, lane 5- CoS 7250, lane 6- CoPant 97222, lane 7- CoSe 8457, lane 8- CoS 8279, lane 9- Co 98014 and lane 10- CoLk 99270, M1- 100bp ladder and M2- 1 kb DNA ladder

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