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PROBING GENETIC DIVERSITY TO CHARACTERIZE RED ROT RESISTANCE IN SUGARCANE

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Abstract

Genetic diversity was assessed in a set of twelve sugarcane genotypes using Random Amplified Polymorphic DNA (RAPD). A total of thirty-two oligo-primers were employed, sixteen of them revealed amplification at 149 loci, out of which 136 were polymorphic. The genotype SPSG-26 showed the highest number of polymorphic loci, followed by CSSG-668 and HSF-242. Pairwise genetic similarity ranged from 67.2% to 83.3%. The UPGMA cluster analysis resolved most of the accessions in two groups. The clustering pattern did not place all resistant varieties in one or related group which depict diverse resistance source in the present set of sugarcane varieties. Ten primers revealed genotype specific bands among which four primers (K07, H02, K10 and F01) produced multiple genotype specific bands that aid genotype identification especially those with red rot resistance. The present study not only provided information on the genetic diversity among the genotypes but also revealed the potential of RAPD-PCR markers for genotype identification and therefore could be utilized in marker assisted selection for red rot resistance in sugarcane.

Introduction

Sugarcane (or *Saccharum hybrid*) is an economically important crop. It provides a rich source of sucrose, alcohol and organic matter waste which is utilized as fertilizer. Sugarcane was cultivated vegetatively as noble canes until the end of 20th century. Today sugarcane is known as an aneuploid hybrid crop derived from a cross between *Saccharum officinarum* L. and a wild relative *Saccharum spontaneum*, through the process of nobilization (Arcenueaux, 1965). The resulting hybrid was repeatedly backcrossed to *S. officinarum* restoring the original sucrose content. Since then sugarcane breeding has advanced by intercrossing few original 'nobilized' clones and their derived progeny consequently reducing its genetic diversity.

The average annual production of sugarcane in Pakistan is approximately 53.4 million tones with a yield per hectare of ~50 tones (Anonymous, 2006). With increasing population needs, sugarcane production requires enhancement, however, various diseases are associated with it. A major production constraint for sugarcane has been the red rot disease rendering yield losses as high as 30% (Alexander & Vishwanathan, 1996). The situation warrants genetic improvement not only through developing resistant varieties but also by improving its genetic base. Genetic diversity is indispensable in a sustainable breeding program. A clear understanding of the diversity level held in the germplasm is fundamental to the development of breeding programs and for efficient management of the gene pool. Sugarcane breeders have traditionally relied on agro-morphological traits and pedigree information for planning their crosses. Today it is well established that morphological characters are influenced by the environmental conditions and thus not preferred for gauging diversity.

In recent years complementary methods have been developed such as DNA-based molecular markers. These markers provide a reliable source (Gepts, 1993) for assessing genetic diversity, for example in cotton (Mumtaz *et al*., 2010) and consequently crop improvement (Andersen & Lubberstedt, 2003). So far, sugarcane diversity has been studied using ribosomal DNA (Glaszmann *et al*., 1990), random fragment length polymorphism (Coto *et al*., 2002); simple sequence repeat (Cordeiro *et al*., 2000); amplified fragment length polymorphism (Butterfield *et al*., 2001) and random amplified polymorphic DNA (Alvi *et al*., 2008). These studies have provided an understanding of the complex genetic structure of sugarcane. Furthermore most of the genetic diversity found in modern sugarcane has been attributed to *S. spontaneum*.

Generally, RAPD is a simpler PCR-based method and thus employed increasingly (Williams *et al*., 1990; Welsh & McClelland, 1990) to assess genetic diversity among cultivars (Leon *et al*., 2001), species identification and parentage testing (Halward *et al*., 1991; Keil & Griffin, 1994; Kindiger & Dewald, 1996; Levi & Rowland, 1997). The present study based on random amplified polymorphic DNA method, aims at: a) characterizing the genetic diversity associated with selected genotypes of sugarcane; b) identify and develop RAPD markers to differentiate the red rot resistant and susceptible genotypes; and c) assess the information generated to develop a pre-breeding program for sugarcane.

Materials and Methods

Twelve sugarcane genotypes were received from the University of Punjab germplasm collection. The accession numbers and the pedigree information on these genotypes have been provided in Table 1. Genomic DNA was extracted using modified CTAB method (Doyle & Doyle, 1990) and quantified using spectrophotometer at O.D. 260nm. The PCR was performed with a total volume of 50µl containing 100ng of DNA, 10x PCR buffer, 2.5mM $MgCl_2$, 4mM dNTP, 100µg/µl primer and 2.5U of Taq polymerase. The reaction conditions included initial denaturation at 94°C for 5 min followed by 40 cycles of 94°C for 1 min, annealing (primer specific) for 30 sec,

extension at 72°C for 2 min and a final extension at 72°C for 7 min. The reaction was carried out in a GeneAmp PCR System 2700 (Applied Biosystems, USA). The PCR was resolved on 1% agarose gel stained with ethidium bromide and observed under UV. The bands were scored as present (1) or absent (0) and their sizes were estimated using 50bp DNA ladder (Fermentas). The genetic diversity estimations were made through cluster analysis using the program Minitab (ver. 13.0 for windows).

Table 1. The accession number and pedigree information of the genotypes used and their field response to red rot and mosaic disease.

Genotypes	Male parent	Female parent	Disease	
			Red rot	Mosaic
CO-1148	CO-301	P-4383	S	NK
CSSG-676	ROC-1	CP74-2005	R	R
CPF-237	86P-19	CP70-1133	R	R
SPSG-79	SP70-1143	SP73-5368	R	NK
NSG-555	CP63-588	MO/F	R	NK
CSSG-668	81-N289	CP74-2005	R	NK
HSF-240	CP43-33	Open pollination	R	S
SPSG-26	SP73-5368	SP70-1143	R	S
HSF-242	SPSH89-2085	Poly cross	S	NK
NSG-39	84F-2753	MO/F	R	NK
SPSG-114	N5679	SP70-1143	S	NK
CP-77/400	Not known	Not known	R	NK

Note back: S = Susceptible, R = Resistant and NK = Not known.

Results and Discussion

A set of thirty two primers were employed to probe genetic diversity in sugarcane. A subset of only sixteen (50%) primers successfully revealed amplifications at 149 loci with 139 being polymorphic. Thus each primer amplified an average of 9.31 loci. Among this subset, only five (31.5%) primers amplified more than the average polymorphism, for example OPK10, OPH02 and OPK07 revealed 16, 15 and 14 polymorphic loci, respectively. Most of the depicted variation among these loci has been contributed by the genotypes: SPSG-26, CSSG-668 and HSF-242 (Table 2). On the contrary, several primers did not reveal any polymorphism, such a pattern was revealed in case of OPF01 oligo. Alvi *et al*., (2008) described the propagation practices for sugarcane as the probable reason for the lack of variation. However, our observations further suggest a careful selection of oligos to assess the genomic diversity in sugarcane.

Table 2. The amplified (A) and polymorphic (P) loci in sugarcane genotypes. The numbers in bold highlight the difference in amplified and polymorphic loci.

Primer		A	D		E	F	H	K					L	M			
		10	3	16	4	1	2	1	2	7	9	10	4	5	10	12	13
CO-1148	A	3	**6**	**6**	2	**2**	3	**4**	2	**2**	**5**	2	6	2	2	3	2
	P	3	**2**	**2**	2	**1**	3	**2**	2	**1**	**4**	2	6	2	2	3	2
CSSG-676	A	3	**6**	**6**	2	**3**	1	**4**	2	**4**	**6**	3	3	1	2	3	1
	P	3	**2**	**2**	2	**2**	1	**2**	2	**3**	**5**	3	3	1	2	3	1
CPF-237	A	3	**6**	**6**	2	**3**	4	**4**	5	**2**	**4**	4	3	1	1	1	1
	P	3	**2**	**2**	2	**2**	4	**2**	5	**1**	**3**	4	3	1	1	1	1
SPSG-79	A	4	**6**	**7**	2	**3**	7	**4**	4	**5**	**4**	5	2	2	2	1	1
	P	4	**2**	**3**	2	**2**	7	**2**	4	**4**	**3**	5	2	2	2	1	1
NSG-555	A	4	**7**	**6**	2	**1**	2	**3**	3	**7**	**3**	4	7	2	1	2	2
	P	4	**3**	**2**	2	**0**	2	**1**	3	**6**	**2**	4	7	2	1	2	2
CSSG-668	A	4	**6**	**5**	2	**5**	5	**3**	5	**7**	**6**	3	6	1	2	2	1
	P	4	**2**	**1**	2	**4**	5	**1**	5	**6**	**5**	3	6	1	2	2	1
HSF-240	A	3	**6**	**6**	2	**2**	5	**5**	1	**7**	**2**	4	7	1	1	2	1
	P	3	**3**	**2**	2	**1**	5	**3**	1	**6**	**1**	4	7	1	1	2	1
SPSG-26	A	4	**7**	**7**	2	**4**	6	**5**	2	**7**	**2**	7	6	3	2	2	1
	P	4	**2**	**3**	2	**3**	6	**3**	2	**6**	**1**	7	6	3	2	2	1
HSF-242	A	4	**6**	**6**	1	**3**	6	**5**	2	**4**	**2**	6	2	3	2	5	3
	P	4	**2**	**2**	1	**2**	6	**3**	2	**3**	**1**	6	2	3	2	5	3
NSG-39	A	2	**6**	**6**	1	**1**	4	**5**	3	**8**	**1**	4	1	1	3	3	3
	P	2	**2**	**2**	1	**0**	4	**3**	3	**7**	**0**	4	1	1	3	3	3
SPSG-114	A	3	**6**	**7**	1	**1**	4	**6**	3	**8**	**2**	6	1	2	3	2	2
	P	3	**2**	**3**	1	**0**	4	**4**	3	**7**	**1**	6	1	2	3	2	2
CP-77/400	A	2	**6**	**5**	1	**2**	4	**6**	3	**8**	**2**	7	1	1	3	1	3
	P	2	**0**	**1**	1	**1**	4	**4**	3	**7**	**1**	7	1	1	3	1	3

When individual genotype contributions were assessed, there were only very few accessions with considerable contributions towards polymorphic loci. Majority of the accessions did not reveal even 50% contribution with any of primers. Few varieties for example, SPSG-114 and CP-77/400 revealed 80% polymorphism when probed with the primers OPK01 and OPK02. Similarly, the primer OPM12 depicted 70% diversity in HSF-242. On the contrary, three accessions i.e. NSG-555, NSG-39 and SPSG-114 did not contribute any polymorphic loci. A similar pattern of diversity distribution has been observed in other studies based on sugarcane (Harvey *et al*., 1994; Pan *et al*., 2004) and in case of other crops for example cotton (Khan *et al*., 2010; Mumtaz *et al*., 2010) and rice (Shah *et al*., 2011).

Cluster analysis and genetic diversity in sugarcane: The mean genetic similarity was found to be 86.3%. Alvi *et al*., (2008) reported the similarity range of 67.2 to 83.3% among sugarcane genotypes. The maximum similarity was 95% found between NSG-39 and SPSG-114, therefore our analysis is an overestimation of genetic similarly. A similar range of affinity was observed by Pan *et al*., (2004) for *S. spontaneum*. Similarly, Afghan *et al*., (2005) observed 78.9% similarity among sugarcane genotypes. Harvey *et al*., (1994) and later Harvey & Botha (1996) reported nearly 80% genetic similarity. The extensive similarity assessed has been a result of lack of genetic distance between parental genotypes (Alvi *et al*., 2008) and frequent self-pollination causing a reduction in genetic variation.

On the basis of polymorphic loci, the sugarcane genotypes may be categorized into two groups. In the first group, polymorphic loci were 45 to 55 as in SPSG-26, CSSG-668, HSF-242 and SPSG-79. In the second group, 35 to 44 polymorphic loci were revealed as in SPSG-114, NSG-555, CP-77/400, CO-1148, NSG-39, CSSG-676 and CPF-237. A total of 54 polymorphic loci were found in SPSG-26, while minimum number of polymorphic loci was in CSSG-676 and CPF-237. This indicated that the highest level of polymorphism existed between SPSG-26 and CSSG-676 and also between SPSG-26 and CPF-237 (Fig. 1). Parental diversity did not have any effect on genetic distances among genotypes (Alvi *et al*., 2008). This was because most varieties are often repeated (Nair *et al*., 2002) so they have conserved genome. This is shown by the fact that most of the genotypes with same single or both parents e.g. SPSG-26, SPSG-79 and SPSG-114 with the common parent SP-70/1143 and, CSSG-676 and CSSG-668 with the common parent CP-74/2005 showed less similarity than those with unrelated parents e.g. NSG-39 and SPSG-114.

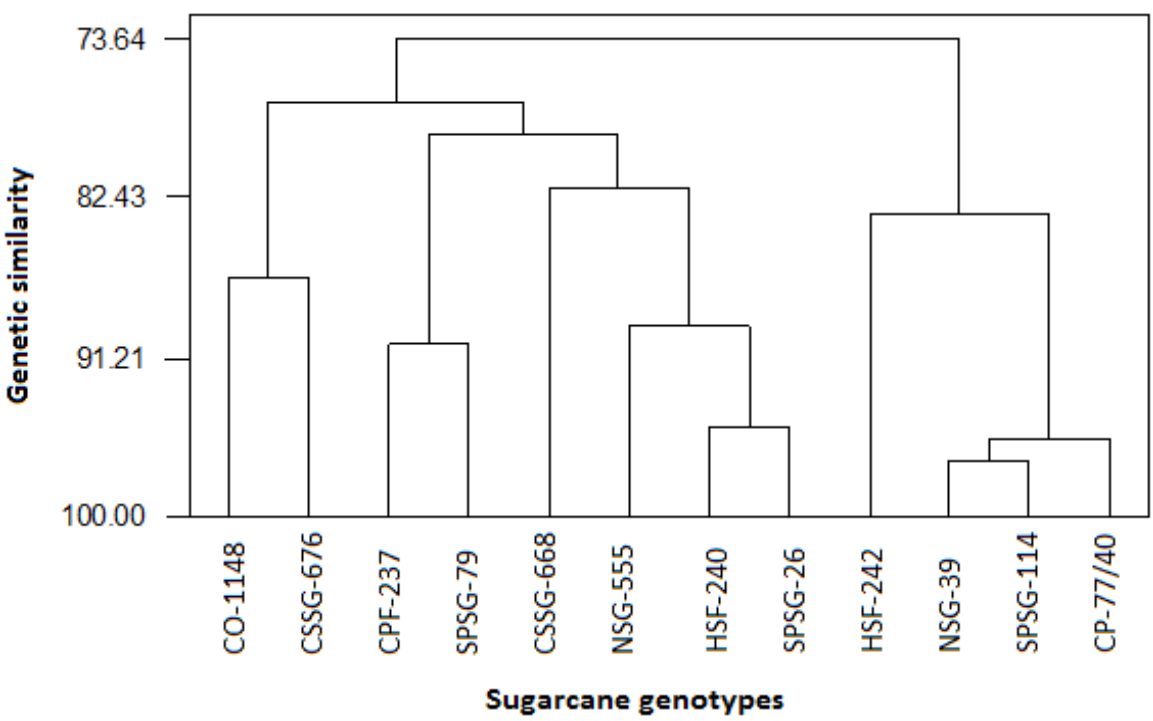

Fig. 1. Dendrogram showing clustering pattern and diversity depicted for each genotype.

Genotype specific bands and their association with red rot in sugarcane: A total of 35 genotype specific bands were produced among genotypes under study (Table 3). Altogether, 10 oligos produced genotype specific bands, among these the oligos OPF01 and OPH02 revealed a maximum of 5 bands followed by OPK07 and OPK10 with 4 bands each. On the other hand, the primers OPA10 and OPK09 produced only one genotype-specific band each. It was further observed that the genotype-specific bands were produced in higher proportion in the red rot resistant genotypes: SPSG-79, SPSG-26 and CPF-237. On the contrary, the genotypes CO-1148 and SPSG-114, being red rot susceptible, revealed only one band. Furthermore, all red rot resistant varieties were susceptible to mosaic disease yet the current data remained insufficient in explaining any possible correlation with the mosaic disease resistance. Hence the presence of genotype-specific bands can only be related to red rot resistance.

Table 3. Genotype specific bands revealed by different oligo primers.

Primer	Sequence (5'-3')	Band size in base pairs	Genotype
OPK-07	AGCGAGCAAG	600	CSSG676
		100	SPSG79
		1350	NSG39
		800	CP77400
OPA-10	GTGATCGCAG	550	CPF237
OPH-02	TCGGACGTGA	1800, 1000	SPSG79
		10,000	NSG555
		500	CSSG668
		200	HSF240
		250	HSF242
OPL-04	GACTGCACAC	1200	CSSG668
		1500	SPSG26
OPM-05	GGGAACGTGT	900	CO1148
		2000	HSF240
		1100	SPSG114
OPM-10	TCTGGCGCAC	1300	CPF237
		200, 1550	SPSG26
OPM-12	GGGACGTTGG	1000	CSSG676
		400, 1200	HSF242
		450	NSG39
OPK-10	GTGCAACGTG	2500	SPSG79
		250	SPSG26
		700	HSF242
		1200, 1050	CP77400
OPF-01	ACGGATCCTG	250	CSSG676
		300, 600	CPF237
		100	SPSG79
		200	CSSG668
		2000	SPSG26
OPK-9	CCCTACCGAC	200	CPF237

Number of genotype-specific bands has been an important character for the identification of genotypes (Chandra *et al*., 2006). The results of earlier studies have shown low number of genotype specific bands, for example Alvi *et al*., (2008) showed only 10 genotype specific bands as opposed to thirty-five bands in the present study. This points to the fact that RAPD markers do have the potential for species identification in the genus *Saccharum* in addition to identifying hybrids, clones and genotypes (Pan *et al*., 2004). The study also highlighted their relevance in marking the resistance and susceptibility in sugarcane though it can only be linked to red rot disease.

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