GENETIC VARIABILITY BASED RESPONSE OF SUGARCANE LINES RESISTANT AND SUSCEPTIBLE AGAINST WHIP SMUT

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ABSTRACT: Study was aimed to access genetic relationships and response for whip smut among 103 sugarcane lines/varieties through DNA genotyping. Touchdown PCR amplification based genotyping of four tailed (M-13) and 26 (FAM-labelled) SSRs markers was performed on Licor 4300 DNA Analyzer and ABI Genetic Analyzer 3730, respectively. Using different softwares, 314 alleles were scored, averaging 10.46 alleles per marker. Polymorphism information content (PIC) values ranged from 0.67 to 0.93. DNAMAN generated homology tree revealed 66-88% genetic similarity among the studied sugarcane lines which indicated their narrow genetic base. The markers mSSCIR-19 and mSSCIR-43 were able to distinguish between all the sugarcane lines resistant and susceptible to whip smut. The grouping of sugarcane lines showing different responses to whip smut within the same cluster indicates that many genes with little effects are involved in smut resistance. The results may help sugarcane breeders in variety identification and designing crosses for developing whip smut resistant cultivars.

Keywords; Sugarcane, genetic diversity, homology tree, PIC values.

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INTRODUCTION

Sugarcane is a major crop in the tropical and sub-tropical areas of the world. It has dramatic economic importance in respect to sugar and bio-fuel production. Its breeding programs necessitate the use of more diverse parents in crosses to improve genetic diversity. Sugarcane has a complex poly-aneuploidy genome ranging 100-120 chromosomes (D'Hont *et al*., 1996). Modern sugarcane hybrids were originated from the cross between *Sacharrum officinarum* L. (noble cane 2n=80) and *S. spontaneum* L. (2n=40-128) with minor contributions of *S. barberi* Jeswiet, *S. Sinense* Roxb., and *S. robustam* Brandes (Tew, 2000; Linneus, 1771). Technical difficulties encountered in sugarcane breeding include inflorescence, dipping in hot water (Heinz and Tew, 1987; Divinagracia, 1980), alcohol and low atmospheric temperature (Soeprijanto, 1989). Mixed populations of hybrids and lines badly affected genome purity making breeding more difficult. Understanding the level of genetic diversity present in the sugarcane germplasm is necessary for efficient utilization of germplasm in designing breeding programs. Agro-morphological characters are prone to environmental changes, so more emphasis should be on the molecular markers for assessing the genetic diversity (Gepts, 1993). Conventional breeding programs relied much on anatomical and morphological characters which are greatly influenced by environmental factors that made pedigree information unreliable. These programs usually took nearly 12 years before releasing new cultivar (Heinz, 1987**,** Skinner, 1972). This necessitates the use of molecular data in variety identification (Pan *et al*., 2006). Hence, sugarcane breeders are focusing more on using both traits and species specific molecular markers in designing breeding programs (Govindaraj *et al*., 2005). Among these molecular markers, the Simple Sequence Repeats (SSRs) markers carry much significance. The SSRs microsatellites contain the variable number of 1-6 bp repeat units (Edwards *et al*., 1991; Polymeropoulos *et al*., 1991). DNA finger printing based upon these SSR markers is quite stable and is not affected by any geographical location or environmental changes (Jahangir *et al.*, 2014; Sindhu *et al*., 2011). They are quite abundant, show co-dominance inheritance, highly reproducible (Cordeiro and Henry, 2001; Cordeiro *et al*., 2000) with multi allelic nature and are widely distributed within the entire genome. These are quite helpful in paternity testing, genome mapping and marker assisted selection (Parker *et al*., 2002; Aitken *et al*., 2005). Particularly in sugarcane, SSRs markers are used for genotyping of USA sugarcane lines (Glynn *et al*., 2009), Australian sugarcane clones (Piperidis *et al*., 2001), genetic diversity (Cordeiro *et al*., 2003), useful genes mapping (Singh *et al*., 2005), cultivar identification (Nair *et al*., 2006), phylogenetic relationship among different sugarcane species (Brown *et al*., 2007) and marker assisted selection (Pinto *et al*., 2011). With the advent of molecular marker techniques, it has now become possible

to determine genomic purity and variety identification more accurately (Sindhu *et al*., 2011).

Ustilago scitaminae is the causative agent of whip smut in sugarcane. Smut causes huge losses and badly affects sugarcane quality (Heinz, 1987). Australian sugarcane clones were screened and rated for smut tolerance in Indonesia and it was found that nearly 70% of Australian lines were smut susceptible. So, in Australian sugarcane breeding programs more emphasis is on developing smut resistant cultivars (Skinner 1972). The susceptibility level of variety to different smut races is different (Edwards *et al*., 1991; Polymeropoulos *et al*., 1991).

The objectives of this research work were: 1) Screening of 103 sugarcane lines resistant and susceptible to whip smut. 2) Genetic relationships among sugarcane lines based upon their molecular identification profiles developed by amplification of SSR markers.

MATERIALS AND METHODS

Selection of sugarcane lines: One hundred and three sugarcane lines that varied greatly in their response to whip smut based on two years field results were selected. The response to whip smut of each cultivar was categorized on the basis of scale (Rao *et al*., 1996).

DNA purification: Genomic DNA was extracted from disease free young tender leaves using CTAB method (Doyle and Doyle, 1990). DNA concentration and purity was checked on the Nano Drop Spectrophotometer (ND^{-} 1000) and agarose gel. Final concentrations of 30-50ng/µl were used in PCR reaction mixtures.

Primers synthesis and PCR amplification: Thirty (30) Simple Sequence Repeats (SSRs) markers were used for genotyping of 103 sugarcane lines. All reactions were singleplex. The first set of four primers (i.e mSSCIR14, SMC 179SA, SMC 222CG and SMC 1493CL) were M-13 tailed primers with two separate dyes (IRD-700 and IRD-800) attached having absorbance of wavelengths 700nm and 800nm. These markers were amplified using touch down protocol on S^{-1000} TM thermal cycler (Bio Rad). The PCR amplification conditions were as follow: 6 cycles of 94^{o}C for 45 seconds, 68 ^{o}C for 30 seconds, decreasing 2^{o}C each cycle and 72^{o}C for 1 minute: 8 cycles of 94^{o}C for 45 seconds, 58 ^{o}C for 30 seconds, decreasing 1^{o}C each cycle and 72^{o}C for 30 seconds: 24 cycles of 94^{o}C for 45 seconds, 50^{o}C for 30 seconds and 72^{o}C for 30 sec., followed by final extension at 72^{o}C for 7 min. with infinite hold at 8^{o}C. The PCR volume was 10µl with 2µl of 30-50ng/µl DNA, 2µl X5 clear buffer, 0.6µ$MgCl_2$ (25mM), 0.06µl dNTPs (25mM), 0.5µl each of forward and reverse primer (1µM), 0.1µl Taq polymerase 5U/µl and 4.05 µl distilled water.

Second set of 26 primer pairs were labelled with fluorescent phosphoramidite dye (FAM) at the 5'end of the forward primers. All these primers (except SCC-89-R & SCC-82) are reported by Pan (2006). However, the primers SCC-89 and SCC-82 are reported by Silva, (2012). All these markers were selected keeping in view their high Polymorphism information content (PIC) values. These primers were also amplified using touch down protocol varying the annealing temperatures and number of cycles.

Genotyping: The electrophoresis based size separation of SSR amplified products was performed on two different genotyping machines as optimum for the primer pairs. The PCR products of M13 tailed primers were first denatured at 95^{o}C for 3 min. and then loaded on the Polyacrylamide Gel (PAG) prepared and fixed for Licor 4300 DNA Analyzer. Ladder was run on the first and the last lane of the gel. PCR products were size separated and bands appeared as orange or green in color depending upon the IR-dye attached to the tailed primers. However, the PCR products of FAM-labelled SSR markers after denaturation at 95^{o}C for 3 min. were genotyped on ABI Genetic Analyzer 3130 which revealed electropherograms. All reactions were singleplex with Gene Scan Liz-500 size standard inserted in each well. Gene Scan files were automatically recorded based upon Capillary electrophoresis CE-based separation process.

Data analysis: Different softwares were used to analyze the data generated by both the genotyping machines. The tiff image files generated by the Licor 4300 Genetic Analyzer were first converted into JPG format and then opened in Gimp 2.0 software which made scoring of bands much easier. The bands were manually scored twice to avoid any error. The cross checker software was also used to verify scoring and estimating band sizes against the size standard.

Capillary electropherograms were revealed from the individual Gene Scan files with the help of Peak Scanner software v1.0 which computed the size of DNA fragments against the GeneScan Liz-500 size standard.

Genotyping analysis: Each allele appeared has a peak on electropherogram and as band on autoradiogram like tiff image file generated by Licor 4300 Genetic Analyzer. Only measurable fluorescence peaks and distinct bands were considered. Each allele was manually scored twice to avoid any error. The presence of allele was designated as "A" while absence as "C". Thus genotyping file of each cultivar was constituted against all the 314 alleles amplified of 30 SSR markers in an affixed sequence order as described by Pan *et al*., (2006).

Polymorphism information content and resolving power values: The potential of each SSR marker for being used in genetic diversity studies was also calculated. Polymorphism information content (PIC) of each SSR marker calculated with the formula of Smith *et al*., (1997).

PIC= 1-$\sum Pi^2$

Where P*i* is the frequency of the *i*th allele

However, resolving power (RP) values of each primer pair was calculated with the formula of Prevost and Wilkinson (1999).

RP=$\sum$lb

Where lb=1-(2×0.5-M)

The "lb" is the allele information while "M" is the proportion of total 103 sugarcane cultivars containing the allele. All calculations were made using Microsoft Excel 2013.

Genetic diversity analysis: Both frequent and trace alleles (with less than 5% presence) showing measurable peaks were scored. However the stutter, dinosaur tails, pull ups and minus-Adenine peaks were not scored (Tew and Pan 2010). The genotyping files of 103 sugarcane lines/varieties were constituted against all the 314 amplified alleles. The resulting genotyping files were aligned using multiple sequence alignment program of DNAMAN software (Lynnon Biosoft, Vaudreuil, Quebec, Canada) to generate homology and phylogenetic tree. All the sugarcane lines/varieties were labelled with numerical values in the homology tree. The names of the sugarcane lines 1-103 were given in the table 2 and 3. We treated the SSR markers as dominant markers in DNAMAN software because in high polyploidy genomes like that of sugarcane, it was difficult to distinguish between the alleles of homologous chromosomes as being heterozygous at particular locus (Oliveira *et al*., 2009; Cordeiro *et al*., 2003). Grouping pattern and diversity among sugarcane clones/varieties were analyzed using homology tree (Chen *et al*., 2009).

RESULTS AND DISCUSSION

Genomic DNA extraction: The concentration and purity of the extracted DNA of all the sugarcane lines/varieties was checked on 0.8% agarose gel and compared with the ladder (Fig. 1). The DNA samples showing compact shining bands were processed for further molecular studies.

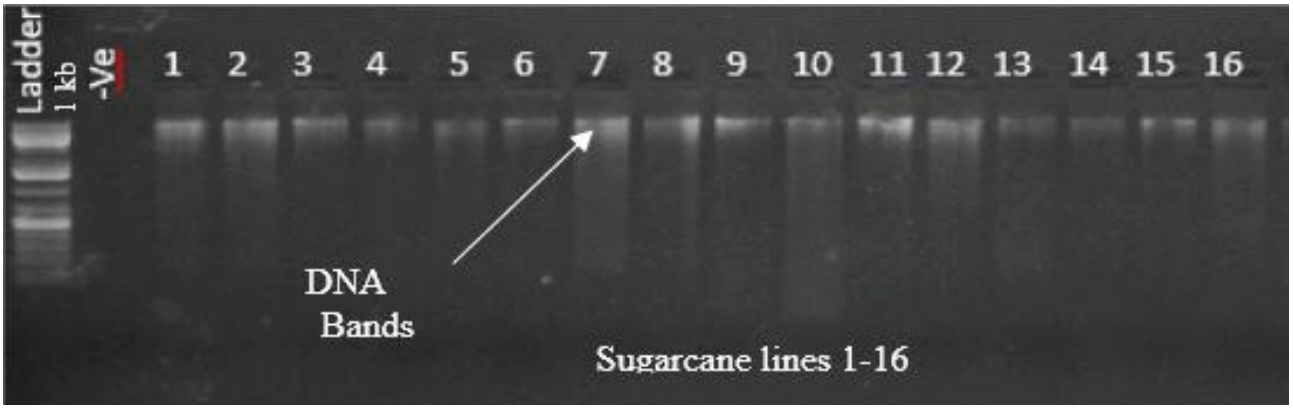


Figure 1: DNA purification of sugarcane samples

PCR amplifications: The extracted DNA samples of all the 103 sugarcane lines/varieties were used as template in PCR amplifications of the 30 SSRs markers. The amplified PCR products of each primer pair were first confirmed by resolving them on 1.8% agarose gel and compared with 100bp ladder. The PCR amplified products of the primer mSSCIR-24 against eighteen (18) sugarcane lines may be seen in figure 2.

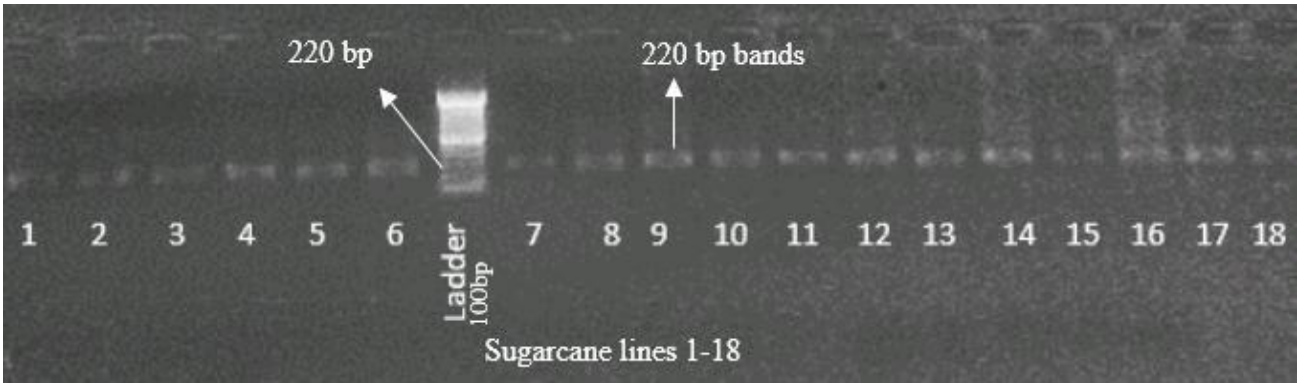


Figure 2: PCR amplification of mSSCIR-24

Genotyping on Licor 4300 DNA Analyzer: The amplified PCR products of M-13 tailed markers were resolved on PAGE gel prepared and fixed for Licor 4300 DNA Analyzer which revealed autoradiograms. Each band represented an allele while shadow bands represented stutters (Fig. 3).

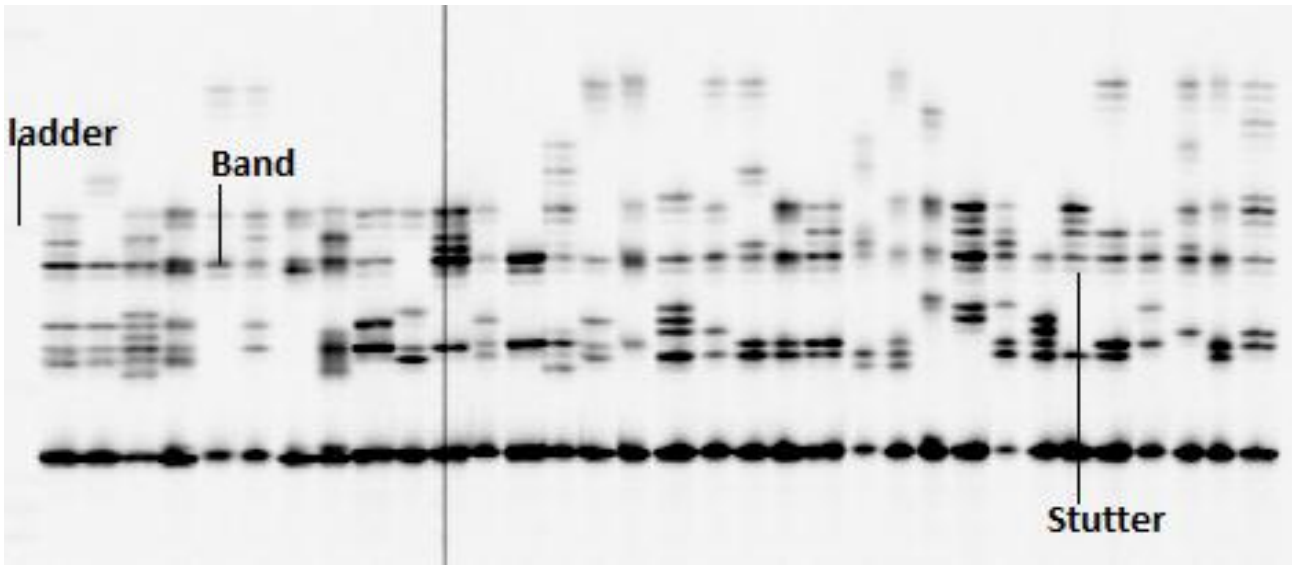


Figure 3: Autoradiogram revealed by Licor 4300 DNA analyzer against SMC 1493 CL

Genotyping on ABI Genetic Analyzer 3730: The PCR amplified products of FAM labelled SSRs markers were first denatured and then genotyped on ABI Genetic Analyzer 3730 which revealed electropherograms. Each larger peak represented an allele while the smaller peaks represented the stutter peaks (Fig. 4). The stutter peaks were normally 1/6th of the real peak in size and one repeat unit larger or smaller than the real peak.

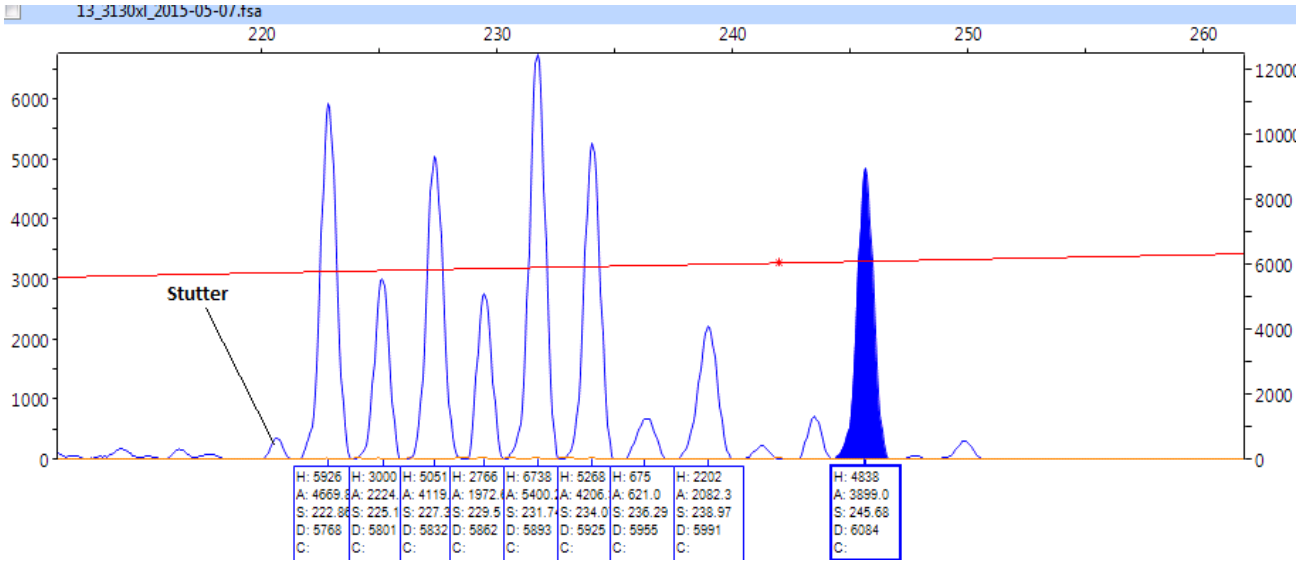


Figure 4: Electropherogram revealed by ABI 3730 Genetic Analyzer against mSSCIR-43

Polymorphic potential of SSR markers: The locus specificity and polymorphic nature of SSRs markers made them highly suitable for genetic diversity studies of sugarcane lines (Glynn *et al*., 2009; Cordeiro *et al*., 2000). Polymorphism information content (PIC), resolving power (RP) values and the number of amplified alleles determines the marker effectiveness for use in genetic diversity studies (Prevost and Wilkinson, 1999; Korkovelos *et al*., 2008; Smith *et al*., 1997). In this study, the number of amplified alleles ranged from 4 (mSSCIR-4) to 19 (SMC640 CS & SMC 2017-FL) with a total of 314 alleles, averaging 10.46 alleles per marker. Three

hundred and eleven (99%) alleles were found polymorphic while only three (1%) alleles were monomorphic in all sugarcane lines/varieties. This indicated high polyploidy and heterozygous nature of sugarcane germplasm. The amplification of so many alleles and their polymorphic nature also revealed the potential of these markers for use in genetic diversity studies. The size range of bands amplified from SMC 1604 SA (i.e. 107-425) was maximum among all the markers.

Polymorphism information content and resolving power values: The ability of SSR primer pair in distinguishing the number of sugarcane clones depends upon the PIC, RP values and the number of detectable alleles. PIC values ranged from 0.67 (mSSCIR-4) to 0.93 (SMC 640 CS) with an average of 0.845 per marker. This indicated high polymorphic potential of these markers. PIC values of any marker merely serve as a reference for its ability to detect genetic variability and it is not necessary for them to be constant. With the change in detection systems, amplification protocols, number and genetic identity of the tested samples, PIC values expected to change accordingly (Pan, 2006). Linear relationship between the PIC values and the number of alleles amplified was observed for each marker.

The RP values based upon the distribution of alleles within the genotypes. These values ranged from 3.68 (SCC-89) to 16.54 (SMC 545 MS) with an average of 9.12 per marker. Prevost and Wilkinson (1999) observed strong linear relationship between discrimination power and resolving power of a marker. Like PIC values, RP values also need not to be constant and may change depending upon the number and nature of the sugarcane germplasm being tested. The SSR markers with their forward and reverse sequences, their PIC values, RP values, number of amplified alleles and their size ranges are given in table 1.

Genetic diversity among 103 sugarcane lines: Homology tree showed genetic similarity of 66-88% among all the tested sugarcane lines indicative of their narrow genetic base (Fig. 5). Hameed *et al*. (2012) reported genetic similarity of 58-79% among 20 sugarcane lines resistant and susceptible to red rot using 21 SSR markers while Alvi *et al*. (2008) observed 67.2-83.3% genetic similarity among 12 sugarcane accessions and mapped them for red rot resistance using 32 RAPD markers. Similarly, 78.9% genetic similarity was found by Afghan *et al*. (2005), while Harvey *et al*. (1994) and later Harvey and Botha (1996) found genetic similarity nearly 80%. Mumtaz *et al*. (2011) also reported mean genetic similarity of 86.3%. The sugarcane clones derived from S. *officinarum* was 80% genetically similar while those derived from S. *spontanium*, were 69.7% genetically similar, indicating their more diverse nature (Selvi *et al.,* 2003). Genetic similarity observed in this study was found slightly higher than what was reported earlier. Such high degree of genetic similarity may be resulted due to frequent self-pollination and cross between closely related genotypes (Shinwari, 2011).

The phylogenetic tree grouped all the 103 sugarcane lines into nine major clusters based upon their evolutionary relationships and indicated their common origin (Fig. 6). The branch lengths determined the amount of change that was occurred between the two sugarcane lines since they had common ancestry.

Response of 103 sugarcane lines against whip smut: Maximum 88% genetic similarity was found among SL-96-278 and SL-96-234 cultivars. Both of these were found moderately resistant to whip smut. Based upon the relationships among all the sugarcane cultivars, these were grouped into eighteen clusters in roman numbering (Table 2 and 3). Maximum thirteen sugarcane lines were grouped in the cluster V while minimum two sugarcane lines (SPSG-24 & S. 2006-US-384) grouped in cluster XII. The varietal response to whip smut in each cluster was also screened out (Table 2 and 3).

The grouping patterns of all the sugarcane lines and their response to smut can be categorized in two major types of clusters. The sugarcane lines in most of the clusters were found resistant to whip smut while in others differential response to whip smut was observed. The clustering of whip smut resistant sugarcane lines together in the several clusters (i.e. cluster I, III, IV, V, VI, VII, X, XI, XII, XIV & XVIII) indicated the presence of genomic regions responsible for inducing whip smut resistance among these sugarcane lines. The germplasm of sugarcane lines within these clusters could be used in future breeding programs for developing whip smut resistant cultivars.

However despite sharing a certain amount of genetic similarity, whip smut resistant, moderately resistant, moderately susceptible and susceptible sugarcane lines were observed in cluster VIII. Presence of whip smut resistant and susceptible sugarcane lines together in the same clusters (IV, VIII, IX and XV) indicate that whip smut resistance is neither restricted to particular sub-populations nor governed by genes with large effects but instead is a genuine quantitative trait. Differential response to whip smut also indicates that whip smut resistance is controlled by genes not linked with one another. Numerous genes control smut resistance in sugarcane (Hector, *et al.,* 1995; Lioyd and Naidoo, 1983). Ten chitinase genes were found differentially expressed in defense response against whip smut (Su *et al.,* 2015). Differences in susceptibility of varieties to different smut races have been observed and the resistance does not follow strict gene for gene pattern as observed in some host-pathogen interactions (Grisham, 2001; Gillaspie, *et al.,* 1983). This study helped to find out markers associated with the whip smut.

The sugarcane variety HSF-240 was resistant to the whip smut at the time of its approval in 2002 for general cultivation in Pakistan but now due to change in climatic conditions it has turned into whip smut susceptible cultivar. During the last 7-8 years the weather conditions have been altered to great extent that winter season become too short and summer has become too long with dry season followed by unscheduled raining which is highly favorable for the development of whip smut. Due to forth mentioned reasons, the variety HSF-240 has become highly susceptible to whip smut. This variety was the cross of CP43-33 which also had tendency to whip smut.

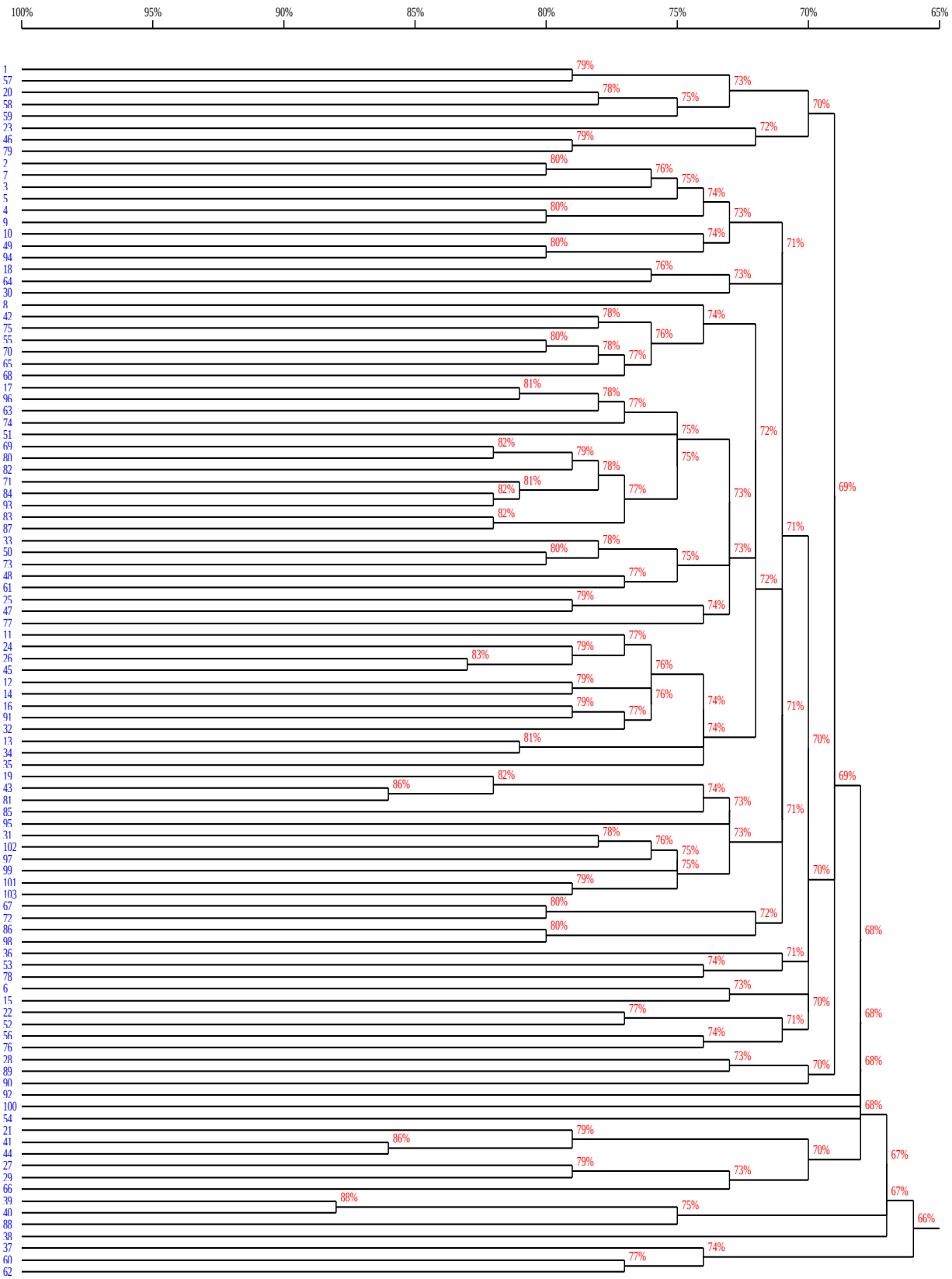
Molecular identification profiles of 103 sugarcane lines: Genotyping files of 103 sugarcane lines against 29 amplified alleles of mSSCIR-19 and mSSCIR-43 markers produced unique binary sequences which were able to distinguish between all the sugarcane lines resistant and susceptible to whip smut (Table 4 and 5). For example, the molecular identification profile of the sugarcane lines HOSG-31 may be read as CACCCACCCAAACACCAAAACAACAACAC.

Molecular identification profiles of five elite sugarcane clones against amplified alleles of various SSR markers may greatly help cane breeders in sugarcane germplasm evaluation and variety identification (Pan, 2006)

Table 1. Primers, their sequences, number and size of amplified alleles with polymorphic alleles in parenthesis, PIC and RP values.

Sr.#	Primer	PIC values	RP values	# of alleles	Size range	Primer Seq - Fwd 5'--> 3'	Primer Seq - Rev 3' --> 5'
1	mSSCIR14	0.83	8.69	6(6)	215-245	GAT TGT TTT TCC CCC ACT A	CAC CTT GTT CTT GCT TTA CTC
2	SMC179SA	0.81	8.25	6(6)	118-262	CAT TTG ACC AAC CAT GCA CAG C	GGC TTG GCA GGA TTG GAA AC
3	SMC 222 CG	0.8	6.87	7(7)	150-209	TTT CAC GAA CAC CCC ACC TA	AGG GAC TAG CAC ACA TTA TTG TG
4	SMC 1493 CL	0.87	10.79	8(8)	108-166	CGA TGA GTA AAT GGG CAG C	GAT ATA GAG GAA GGG ATT GAA GG
5	SMC 668 CS	0.78	6.97	7(6)	213-239	ACG CTT GCG TGC TCC ATT	CCA ATC GTG CCA CTG TAG TAA G
6	mSSCIR-1	0.89	5.61	12(12)	127-187	CTT GTG GAT TGG ATT GGA T	AGG AAA TGG ATT GCT CAG G
7	mSSCIR-4	0.67	4.66	4(3)	240-259	TTC CAG CAG CAG CAT CAA T	CCC ACT AGG AGA AGC AAT AAC T
8	mSSCIR-17	0.88	12.6	12(12)	226-256	AGC ATA GTT TTT GTG GAC	AGT TCT TTT CGT TCT CTG G
9	mSSCIR-19	0.89	11.84	15(15)	120-153	GGT TCC AAA ATA CAC AAA	CAA TCT TAT CTA CGC ACT T
10	mSSCIR-24	0.82	5.49	10(10)	218-252	AGA TGA ACC CAA AAA CTT A	TTA CTC CGC CTC TTT ACT
11	mSSCIR-43	0.92	14.4	14(14)	222-252	ATT CAA CGA TTT TCA CGA G	AAC CTA GCA ATT TAC AAG AG
12	mSSCIR-52	0.84	8.62	10(10)	121-144	ACA AGG GAA GAC AAA TCA G	ACC AAA CCA CAA AGC AAA
13	SCC-89	0.69	3.68	5(5)	183-222	AGT GTT GCG AGA AGC AGC AG	CCC ATG GAT CAC ATG ACA GA
14	SCC-82	0.88	8.99	10(10)	154-198	CTA TCC CAT CCC GGA AAA A	CCG ACT TGA ACA CCA CCA G
15	SMC 7 CUQ	0.85	9.67	9(9)	154-170	GCC AAA GCA AGG GTC ACT AGA	AGC TCT ATC AGT TGA AAC CGA
16	SMC 25 DUQ	0.78	5.61	8(8)	212-232	GCT TCC TAA TCC ATT GTT ATT CTT	GCC ACT CCA TCT GCT AGT GTT C
17	SMC-39BUQ	0.84	9.13	8(7)	128-149	CGT CTG GCG GAT GAA ATT GAG	CCT ATC GGC ATC AAA TGG TCG
18	SMC 334 BS	0.85	8.14	9(9)	135-161	CAA TTC TGA CCG TGC AAA GAT	CGA TGA GCT TGA TTG CGA ATG
19	SMC 336 BS	0.88	8.66	14(14)	133-182	ATT CTA GTG CCA ATC CAT CTC A	CAT GCC AAC TTC CAA ACA GAC
20	SMC-545 MS	0.91	16.54	9(8)	113-145	AGG CTA CAT GCT TAC AGC CAT	TGG TCT ATC ACT TAA TCA GCC AC
21	SMC 569 CS	0.83	6.87	9(9)	157-220	GCG ATG GTT CCT ATG CAA CTT	TTC GTG GCT GAG ATT CAC ACT A
22	SMC 597 CS	0.88	9.96	12(12)	142-177	GCA CAC CAC TCG AAT AAC GGA T	AGT ATA TCG TCC CTG GCA TTC A
23	SMC 640 CS	0.93	14.83	19(19)	216-257	TTA AGA GAC CCG CCT TTG GAA	TGC CAG AAG TGG TTG TGC TCA
24	SMC 703 BS	0.87	11.84	10(10)	193-218	GCC TTT CTC CAA ACC AAT TAG T	GTT GTT TAT GGA ATG GTG AGG A
25	SMC 766 BS	0.9	9.17	15(15)	177-216	TTA CTC GGC TGG GTT TTG TTC	TAA GAA TCG TTC GCT CCA GC
26	SMC 851 MS	0.86	7.44	11(11)	125-144	ACT AAA ATG GCA AGG GTG GT	CGT GAG CCC ACA TAT CAT GC
27	SMC 1282FL	0.8	6.16	15(15)	340-411	CGG TGA CCT TAG GCT ACC AT	TGG GAG AAT CTA GCT TGA CAA C
28	SMC 1604 SA	0.9	14.9	14(14)	107-425	AGG GAA AAG GTA GCC TTG G	TTC CAA CAG ACT TGG GTG G
29	SMC 1751 CL	0.79	7.76	7(7)	139-152	GCC ATG CCC ATG CTA AAG AT	ACG TTG GTC CCG GAA CCG
30	SMC 2017 FL	0.91	9.36	19(19)	211-255	CAC AAG TGA AGA TAA TAG TGT CCC T	GAT CCC AAA TCC CTT GAT CTC
Mean values		0.85	9.12				
Standard Deviation		±0.06	±3.15				

PIC= Polymorphism Information Content
RP= Resolving Power.



The names of the sugarcane lines 1-103 may be seen in table 2 and 3.

Figure 5. Homology tree with numerical values representing 103 sugarcane lines

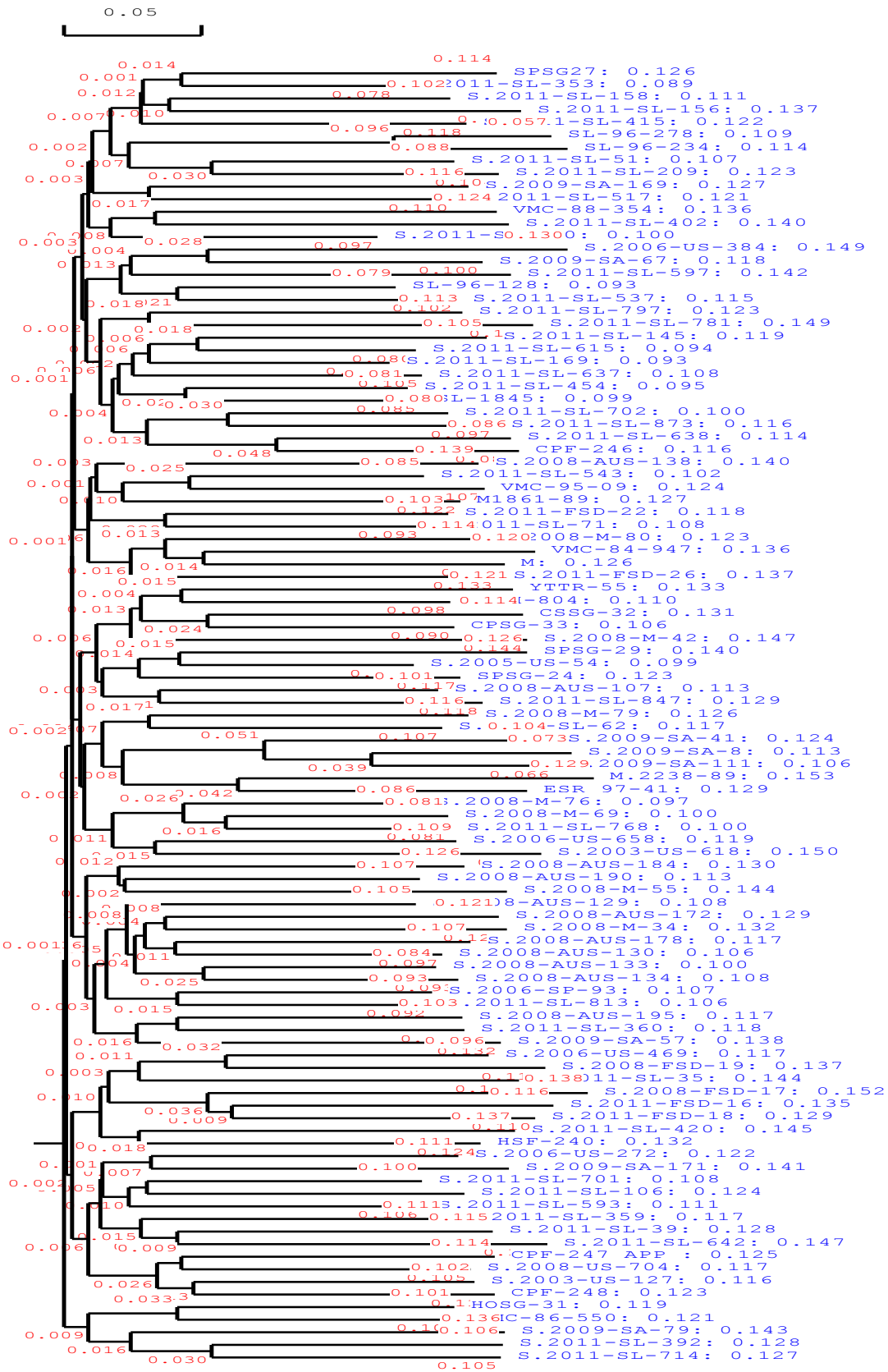


Figure 6. Phylogenetic tree of 103 sugarcane lines

Table2. Varietal response to whip smut, assigned numerical values and observed clusters in homology tree.

Clusters	Varieties	Num. Val.	Response to Whip Smut	Shared Homology	Clusters	Varieties	Num. Val.	Response to Whip Smut	Shared Homology
1	HOSG-31	1	Resistant	70%	V	S.2011-SL-145	69	Resistant	75%
	VMC-86-550	57	Resistant			S.2011-SL-615	80	Resistant	
	S.2008-M-80	20	Resistant			S.2011-SL-637	82	Resistant	
	VMC-84-947	58	Resistant			S.2011-SL-169	71	Resistant	
	M70-89	59	Resistant			S.2011-SL-454	84	Mod. Resistant	
	S.2009-SA-79	23	Resistant			S.2011-SL-1845	93	Resistant	
	S.2011-SL-392	46	Resistant			S.2011-SL-702	83	Resistant	
	S.2011-SL-714	79	Resistant			S.2011-SL-873	87	Resistant	
II	YTTR-55	2	Resistant	73%	VI	S.2006-US-272	33	Mod. Resistant	75%
	CPSG-33	7	Resistant			S.2011-SL-701	50	Resistant	
	CSSG-32	3	Mod. Resistant			S.2011-SL-106	73	Resistant	
	BPTH-804	5	Resistant			S.2011-SL-359	48	Resistant	
	SPSG-29	4	Mod. Susceptible			S.2011-FD-16	61	Resistant	
	S.2005-US-54	9	Resistant						
	S.2008-AUS-138	10	Mod. Resistant						
	S.2011-SL-543	49	Resistant						
	VMC-95-09	94	Resistant						
III	S.2008-M-79	18	Mod. Resistant	73%	VII	S.2008-AUS-195	25	Resistant	74%
	S.2011-SL-62	64	Resistant			S.2011-SL-360	47	Resistant	
	S.2008-M-42	30	Resistant			S.2011-SL-517	77	Resistant	
IV	SPSG-27	8	Resistant	74%	VIII	S.2008-AUS-184	11	Resistant	74%
	S.2009-SA-169	42	Resistant			S.2008-AUS-178	24	Mod. Susceptible	
	S.2011-SL-156	75	Resistant			S.2008-AUS-133	26	Resistant	
	S.2011-SL-353	55	Resistant			S.2008-AUS-134	45	Mod. Susceptible	
	S.2011-SL-158	70	Resistant			S.2008-AUS-129	12	Mod. Resistant	
	S.2011-SL-430	65	Resistant			S.2008-AUS-190	14	Resistant	
	S.2011-SL-415	68	Resistant			S.2006-SP-93	16	Mod. Resistant	
						S.2011-SL-813	91	Resistant	
						S.2008-AUS-130	32	Resistant	
						S.2008-AUS-172	13	Mod. Resistant	
						S.2008-M-34	34	Susceptible	
						S.2009-SA-57	35	Resistant	
V	SL-96-128	7	Resistant	75%	IX	S.2008-M-76	19	Mod. Resistant	73%
	S.2011-SL-593	96	Resistant			S.2008-M-69	43	Resistant	
	S.2011-FD-22	63	Resistant			S.2011-SL-768	81	Resistant	
	S.2011-SL-71	74	Resistant			S.2011-SL-847	85	Resistant	
	S.2011-SL-797	51	Resistant						

Num. Val. = Numerical values
Mod. = Moderately

Table 3: Varietal response to whip smut, assigned numerical values and observed clusters in homology tree

Clusters	Varieties	Num. Val.	Response to Whip Smut	Shared Homology	Clusters	Varieties	Num. Val.	Response to Whip Smut	Shared Homology
	M1861-89	95	Resistant			S.2008AUS107	28	Resistant	
	S.2006-US-658	31	Resistant			ESR 97-41	89	Resistant	
	S.2008-US-704	102	Resistant		XIV	S.2011-SL-642	90	Resistant	70%
	CPF-247	97	Resistant			S.2011-FD-26	92	Resistant	
	HSF-240	99	Susceptible			S.2003-US-618	100	Susceptible	
	S.2003-US-127	101	Resistant		XV	S.2011-SL-35	54	Resistant	68%
IX	CPF-248	103	Resistant	73%		S.2009-SA-41	21	Resistant	
	S.2011-SL-51	67	Resistant			S.2009-SA-8	41	Resistant	
	S.2011-SL-209	72	Resistant			S.2009-SA-111	44	Mod. Resistant	
	S.2011-SL-638	86	Resistant			S.2006-US-469	27	Mod. Susceptible	
X	CPF-246	98	Resistant	72%	XVI	S.2008-FD-19	29	Resistant	
	S.2009-SA-171	36	Resistant			S.2011-SL-420	66	Resistant	70%
	S.2011-SL-797	53	Resistant			SL-96-278	39	Mod. Resistant	
XI	S.2011-SL-781	78	Resistant	71%		SL-96-234	40	Mod. Resistant	
	SPSG-24	6	Resistant			M.2238-89	88	Resistant	
XII	S.2006-US-384	15	Mod. Resistant	73%	XVII	S.2008-M-55	38	Mod. Susceptible	75%
	S.2009-SA-67	22	Mod. Susceptible			S.2008-FD-17	37	Resistant	
	S.2011-SL-537	52	Resistant			S.2011-FD-16	60	Resistant	74%
XIII	VMC-88-354	56	Resistant	71%	XVIII	S.2011-FD-18	62	Resistant	
	S.2011-SL-402	76	Resistant						

Num. Val. = Numerical values Mod. = Moderately

Table 4. Genotyping files of sugarcane lines against 29 alleles of mSSCIR-19 and mSSCIR-43.

Sr. No.	Markers	mSSCIR-19																mSSCIR-43												
	Alleles Varieties	19-120	19-127	19-131	19-132	19-135	19-137	19-139	19-141	19-142	19-144	19-146	19-148	19-150	19-152	19-153	43-222	43-225	43-227	43-229	43-231	43-234	43-236	43-238	43-241	43-243	43-245	43-247	43-249	43-252
1	HOSG-31	C	A	C	C	C	A	C	C	C	A	A	A	C	A	C	C	A	A	A	A	C	A	A	C	A	A	C	A	C
2	YTTR-55	C	C	C	C	A	A	C	C	C	A	A	C	A	A	C	C	A	C	A	C	A	C	C	C	A	A	C	C	C
3	CSSG-32	C	C	C	C	A	C	C	C	C	A	A	A	C	C	A	A	A	A	A	A	A	A	A	C	A	A	A	A	C
4	SPSG-29	C	C	C	C	C	C	C	C	C	A	A	A	A	A	C	A	C	A	C	A	C	C	C	C	A	A	A	C	A
5	BPTH-804	C	A	C	C	C	C	C	C	C	A	A	A	A	A	C	A	C	A	C	A	C	C	C	C	C	A	C	C	C
6	SPSG-24	C	A	C	C	C	C	C	C	C	A	A	A	A	C	C	C	A	A	A	A	C	C	C	C	A	A	A	A	A
7	CPSG-33	C	C	C	C	A	C	C	C	C	A	C	A	C	A	C	C	C	C	A	C	A	C	C	C	A	A	A	C	C
8	SPSG-27	C	A	C	C	C	C	C	C	C	A	A	A	C	C	C	C	C	C	C	A	A	C	C	C	C	A	C	C	C
9	S.2005-US-54	C	A	C	C	C	C	C	C	C	A	A	A	A	A	C	C	A	C	A	A	C	A	C	C	A	A	A	A	C
10	S.2008-AUS-138	C	C	C	C	C	A	C	C	C	A	A	C	A	C	C	A	C	A	C	A	A	A	C	C	A	A	A	A	A
11	S.2008-AUS-184	C	C	C	C	C	C	C	C	C	A	A	A	C	C	C	A	A	A	A	A	C	A	C	C	A	A	A	A	C
12	S.2008-AUS-129	A	A	C	C	C	A	A	A	C	A	A	A	C	C	A	C	C	A	C	A	A	A	A	C	A	A	A	A	A
13	S.2008-AUS-172	C	A	C	C	C	A	C	C	C	A	A	A	C	C	C	A	A	A	A	A	A	A	A	C	A	A	C	A	C
14	S.2008-AUS-190	C	A	C	C	A	A	C	C	C	A	A	A	C	C	C	A	C	A	A	A	C	A	A	C	A	A	A	C	C
15	S.2006-US-384	C	A	C	C	A	C	C	C	C	A	A	C	C	A	C	C	C	A	C	A	C	A	C	C	A	A	A	A	A
16	S.2006-SP-93	C	A	C	C	C	A	C	C	C	A	A	A	C	A	C	C	A	C	A	C	A	A	A	C	A	C	A	C	C
17	SL-96-128	C	A	C	C	C	A	A	A	C	A	A	A	C	C	C	C	C	A	C	A	A	A	A	A	A	A	A	A	A
18	S.2008-M-79	C	A	C	C	C	A	C	C	C	A	A	A	C	A	C	A	C	A	C	A	C	A	A	C	C	A	C	C	C
19	S.2008-M-76	C	A	C	C	C	A	C	C	C	A	A	A	A	C	A	A	A	C	C	C	C	A	A	C	C	A	A	C	C
20	S.2008-M-80	C	A	C	C	C	A	A	C	C	A	A	A	C	C	C	C	A	A	A	C	A	C	A	C	A	A	A	A	C
21	S.2009-SA-41	C	C	C	C	C	A	A	C	C	A	A	C	A	C	C	C	A	C	C	C	A	C	C	C	C	A	A	C	C
22	S.2009-SA-67	C	C	C	C	C	A	A	C	C	A	A	C	C	C	C	C	C	A	C	A	C	C	A	C	A	A	A	A	A
23	S.2009-SA-79	A	A	C	C	C	A	C	A	C	A	A	A	A	A	C	C	A	A	A	A	A	C	A	C	C	C	A	C	A
24	S.2008-AUS-178	A	C	C	C	C	A	A	A	C	A	A	A	C	C	A	C	A	C	A	A	C	C	C	C	A	A	A	A	C
25	S.2008-AUS-195	C	C	C	C	C	A	A	C	C	A	A	A	C	C	A	A	A	A	A	C	A	C	A	C	A	A	A	A	A
26	S.2008-AUS-133	A	A	C	C	C	A	A	C	C	A	A	A	C	A	C	A	A	A	A	A	A	C	A	C	A	A	A	C	C
27	S.2006-US-469	C	C	C	C	C	C	C	C	C	A	A	C	A	A	C	A	C	A	A	A	A	C	A	C	A	C	A	C	C
28	S.2008-AUS-107	C	C	C	C	C	C	C	C	C	C	A	A	C	C	C	C	A	A	A	A	A	C	A	C	A	A	A	A	C
29	S.2008-FSD-19	C	C	C	C	C	C	A	A	C	A	A	A	A	A	C	A	A	A	A	A	A	A	A	C	C	A	A	A	A
30	S.2008-M-42	C	C	C	A	A	C	C	C	C	A	A	A	C	A	C	A	C	C	C	C	A	C	C	C	C	A	A	C	C
31	S.2006-US-658	C	A	C	C	C	A	C	C	C	A	A	A	C	A	C	A	C	C	C	C	A	C	C	C	C	C	A	C	C
32	S.2008-AUS-130	C	C	C	C	C	A	A	C	C	A	A	A	A	C	C	C	A	C	A	A	C	C	C	C	C	A	A	A	A
33	S.2006-US-272	C	C	C	C	C	A	A	C	C	A	A	A	A	C	C	C	C	C	C	C	A	C	A	A	A	A	A	C	C
34	S.2008-M-34	C	C	C	C	C	A	A	A	C	A	A	A	C	C	A	C	A	C	A	C	A	C	A	C	C	C	A	C	A
35	S.2009-SA-57	C	C	C	C	C	A	A	C	C	A	A	A	C	A	C	A	A	A	A	C	C	C	C	C	A	A	A	A	A
36	S.2009-SA-171	C	A	C	C	A	C	C	C	C	A	A	C	A	C	C	C	A	C	A	C	A	C	A	C	A	A	A	A	A
37	S.2008-FSD-17	C	A	A	C	A	A	A	A	C	A	A	C	C	C	C	C	A	C	A	C	C	C	A	A	A	A	A	A	A
38	S.2008-M-55	A	A	C	C	C	A	C	C	C	A	A	C	C	C	C	C	C	A	C	A	C	A	C	C	A	C	A	C	C
39	SL-96-278	C	C	C	C	C	A	C	C	C	A	A	A	C	C	C	A	C	A	C	A	A	C	A	C	C	C	C	C	C

40	SL-96-234	C	C	C	C	C	A	C	C	C	A	A	C	A	C	C	C	C	C	C	A	A	C	C	C	A	A	A	C	C
41	S.2009-SA-8	C	C	C	C	C	A	A	C	C	A	A	A	A	C	C	C	C	A	C	A	C	A	C	C	C	A	A	C	C
42	S.2009-SA-169	C	A	C	A	A	C	A	C	C	C	A	A	C	C	C	C	A	C	C	C	C	C	C	C	A	A	A	C	C
43	S.2008-M-69	C	A	C	C	C	A	A	C	C	A	A	A	C	A	C	C	C	C	C	A	C	A	C	C	A	A	C	C	C
44	S.2009-SA-111	C	C	C	C	C	A	A	C	C	A	A	C	A	C	C	C	A	A	A	A	C	C	C	C	C	C	A	C	A
45	S.2008-AUS-134	A	A	C	C	C	A	A	A	C	A	A	A	A	C	A	A	A	A	A	A	C	C	C	C	A	C	A	C	C
46	S.2011-SL-392	A	A	C	C	C	A	A	C	C	A	A	A	C	C	C	A	A	A	A	A	A	C	A	C	C	C	C	C	C
47	S.2011-SL-360	C	C	C	C	C	C	A	C	C	A	C	A	C	C	A	A	A	A	A	C	A	C	A	C	A	A	A	A	A
48	S.2011-SL-359	C	C	C	A	C	A	A	A	A	A	A	A	C	C	C	C	A	C	A	A	A	C	A	C	C	A	A	A	A
49	S.2011-SL-543	C	A	C	C	C	C	A	C	C	A	A	A	A	C	C	C	C	A	C	A	C	A	C	C	A	A	A	A	A
50	S.2011-SL-701	A	A	C	C	C	A	A	C	C	A	A	A	A	C	C	C	C	A	C	A	C	C	A	C	A	A	A	A	A
51	S.2011-SL-797	C	A	C	C	C	A	A	C	C	A	A	A	A	C	C	C	A	A	A	A	A	A	C	C	C	A	A	A	A
52	S.2011-SL-537	C	C	C	C	C	C	A	C	C	A	A	C	C	C	C	C	C	A	C	A	C	A	A	A	A	C	A	C	A

Table 5. Genotyping Files of sugarcane lines against 29 alleles of mSSCIR-19 and mSSCIR-43.

	Markers	**mSSCIR-19**																**mSSCIR-43**												
	Alleles Varieties	**19-120**	**19-127**	**19-131**	**19-132**	**19-135**	**19-137**	**19-139**	**19-141**	**19-142**	**19-144**	**19-146**	**19-148**	**19-150**	**19-152**	**19-153**	**43-222**	**43-225**	**43-227**	**43-229**	**43-231**	**43-234**	**43-236**	**43-238**	**43-241**	**43-243**	**43-245**	**43-247**	**43-249**	**43-252**
53	S.2011-SL-597	C	A	C	C	C	A	A	C	C	A	A	C	A	A	C	C	C	A	C	A	C	A	A	C	A	C	A	C	A
54	S.2011-SL-35	C	C	C	C	C	C	A	C	A	A	A	A	A	A	C	C	C	C	C	A	C	A	A	A	A	A	C	C	C
55	S.2011-SL-353	C	A	C	C	C	C	A	C	C	A	A	C	C	C	C	C	C	C	C	A	C	C	C	C	A	C	C	C	C
56	VMC-88-354	C	C	C	A	A	A	A	C	C	A	A	A	C	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
57	VMC-86-550	C	A	C	C	C	A	C	C	C	A	A	C	C	C	C	C	A	A	A	A	C	A	A	C	A	A	A	A	A
58	VMC-84-947	C	C	C	C	C	A	A	C	C	A	A	A	C	C	C	C	C	A	C	A	C	C	A	A	A	A	A	A	A
59	M70-89	C	C	C	C	C	C	A	C	C	A	A	A	A	C	A	A	A	A	A	A	A	C	A	A	A	A	A	A	C
60	S.2011-FSD-16	C	A	C	C	C	C	A	C	C	C	A	C	C	A	C	C	A	C	A	C	A	C	A	C	A	A	A	A	A
61	S.2011-SL-39	C	A	A	C	A	A	A	C	C	A	A	A	A	C	C	C	A	A	A	A	A	C	A	C	C	C	A	C	A
62	S.2011-FSD-18	C	C	C	C	A	C	A	A	C	C	A	C	C	C	A	C	C	A	C	A	A	C	A	C	A	A	A	A	A
63	S.2011-FSD-22	C	A	C	C	C	A	C	C	C	A	C	C	C	C	C	C	C	A	C	A	A	C	A	C	A	A	A	A	A
64	S.2011-SL-62	C	C	C	A	A	C	C	C	C	A	A	A	C	C	C	C	C	C	C	A	A	C	C	C	C	A	A	C	C
65	S.2011-SL-430	C	A	C	C	C	A	A	C	C	A	A	A	A	C	C	C	A	A	A	C	A	C	A	C	A	A	A	C	C
66	S.2011-SL-420	C	A	C	C	C	C	A	C	C	A	A	A	C	A	C	C	A	A	A	A	A	C	A	A	A	A	A	A	A
67	S.2011-SL-51	C	A	C	C	C	A	A	C	C	A	A	A	A	C	C	A	C	C	C	C	C	C	C	C	C	A	A	C	C
68	S.2011-SL-415	C	A	C	C	C	A	A	C	C	A	A	A	C	C	C	A	C	C	C	C	C	C	C	C	A	A	A	C	C
69	S.2011-SL-145	C	A	C	C	C	A	C	C	C	A	A	C	C	C	C	C	C	A	C	A	C	A	C	A	A	A	A	A	A
70	S.2011-SL-158	C	A	C	C	C	A	C	C	C	A	A	C	C	C	C	C	C	C	C	C	A	A	A	C	A	A	C	C	C
71	S.2011-SL-169	C	A	C	C	C	A	A	C	C	A	A	A	A	C	C	C	C	A	C	A	C	C	A	C	A	A	A	A	A
72	S.2011-SL-209	C	A	C	C	C	A	C	C	C	A	C	A	A	C	C	C	C	A	C	A	A	C	A	C	A	A	A	C	C
73	S.2011-SL-106	C	A	C	C	C	A	A	C	C	A	A	A	A	C	C	C	C	A	C	A	A	C	A	C	A	A	A	C	C
74	S.2011-SL-71	A	A	C	C	C	A	C	C	C	A	A	A	C	C	C	C	C	A	C	A	C	C	C	C	A	A	A	A	A
75	S.2011-SL-156	C	A	C	C	C	A	A	A	C	A	A	A	C	C	C	A	C	C	C	A	A	C	A	C	A	A	C	C	C
76	S.2011-SL-402	C	C	C	C	C	C	A	C	A	A	A	A	C	C	A	C	C	A	C	C	A	C	A	C	C	A	C	C	C
77	S.2011-SL-517	C	A	C	C	A	C	A	C	C	C	A	A	C	C	C	C	C	C	C	A	A	C	C	C	A	A	A	C	C

78	S.2011-SL-781	C	A	C	C	C	A	A	C	C	A	A	A	A	C	A	C	A	A	A	A	A	A	A	C	C	C	A	C	A
79	S.2011-SL-714	A	A	C	C	C	A	A	A	C	A	A	A	C	C	C	C	A	C	A	C	A	C	A	C	A	C	A	C	A
80	S.2011-SL-615	C	A	C	C	C	C	C	C	C	A	A	C	C	C	C	A	C	A	C	A	A	C	A	C	A	A	A	C	A
81	S.2011-SL-768	C	A	C	C	C	C	C	C	C	A	A	A	A	A	C	A	C	C	C	C	C	C	C	C	A	A	A	C	C
82	S.2011-SL-637	C	A	C	A	A	A	A	C	A	A	A	A	C	C	C	C	A	A	A	A	C	C	C	C	C	A	A	A	A
83	S.2011-SL-702	C	A	C	C	C	C	A	C	C	A	A	A	C	C	C	C	C	C	C	C	C	C	A	C	C	C	A	A	C
84	S.2011-SL-454	C	A	C	C	C	A	A	C	C	A	A	A	C	C	C	C	C	A	C	A	C	C	A	C	A	C	A	C	C
85	S.2011-SL-847	C	C	C	C	C	A	C	C	C	A	A	A	A	C	A	C	C	A	C	C	C	C	C	C	A	C	A	C	C
86	S.2011-SL-638	C	C	C	C	A	C	A	C	C	A	A	A	C	C	A	C	C	C	C	A	C	C	C	C	A	C	A	C	C
87	S.2011-SL-873	C	C	C	C	C	A	A	C	C	A	A	A	C	C	A	C	C	C	C	A	C	C	C	A	A	A	C	C	C
88	M.2238-89	C	A	C	C	C	A	A	C	C	A	A	A	C	C	C	C	C	A	C	A	A	A	A	C	C	A	A	A	A
89	ESR 97-41	C	A	C	C	C	A	A	C	C	A	A	A	A	C	C	A	A	A	A	A	A	A	A	C	C	A	A	A	A
90	S.2011-SL-642	C	A	C	C	C	A	A	A	C	A	A	A	A	C	A	A	A	C	A	A	A	C	A	C	C	A	A	A	A
91	S.2011-SL-813	C	C	C	C	C	A	C	C	C	A	A	A	C	C	C	A	A	A	A	A	C	A	A	C	C	A	A	A	A
92	S.2011-FSD-26	C	C	C	C	C	A	A	C	C	A	A	A	C	C	A	C	C	A	C	A	A	A	A	C	A	A	A	A	A
93	S.2011-SL-1845	C	A	C	C	C	C	A	C	C	A	A	A	C	C	A	C	C	A	C	A	A	C	A	C	A	A	A	A	A
94	VMC-95-09	C	A	C	C	C	A	A	C	C	A	A	A	A	A	C	C	C	A	C	A	C	A	A	C	A	A	C	A	C
95	M1861-89	C	A	C	C	C	C	C	C	C	A	A	C	A	A	C	C	C	A	C	A	A	C	A	C	C	C	C	C	C
96	S.2011-SL-593	C	A	C	C	C	A	A	C	C	A	A	C	C	C	C	C	C	A	C	A	A	C	A	C	C	A	A	A	C
97	CPF-247	C	C	C	C	C	C	A	C	C	A	A	C	A	C	C	C	C	A	C	A	C	C	A	C	C	A	A	A	C
98	CPF-246	C	C	A	C	A	C	A	A	C	A	A	A	C	C	A	C	C	A	C	A	A	C	A	C	C	A	A	A	C
99	HSF-240	C	A	C	C	C	C	A	C	C	C	A	A	A	A	C	C	C	C	C	A	A	C	C	C	C	C	A	A	C
100	S.2003-US-618	C	C	C	C	C	A	A	C	C	A	A	A	A	A	C	C	C	C	A	C	C	C	C	A	C	C	C	C	A
101	S.2003-US-127	C	C	C	C	C	A	C	C	C	A	A	C	A	A	C	C	C	C	C	A	C	A	C	C	C	C	A	A	C
102	S.2008-US-704	C	C	C	C	C	A	A	C	C	A	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A
103	CPF-248	C	C	C	A	A	A	A	A	C	A	A	A	A	C	C	C	C	C	C	C	A	C	C	C	C	C	A	A	A

Suggestions: To the best of my knowledge, this is the first report revealing genetic diversity based response of promising sugarcane lines/varieties for response against whip smut. From general perspective, this study would help cane breeders in variety identification and designing crosses for developing whip smut resistant cultivars. More diverse whip smut resistant sugarcane lines could be selected for crosses. This will expand the genetic base of sugarcane and the resulting progenies showing better morphological traits and resistance to whip smut could be selected and propagated for the improvement of sugarcane crop in Pakistan.

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