UDC 575.
https://doi.org/10.2298/GENSR1903877N
Original scientific paper

IDENTIFICATION OF NOVEL QTLS CONTROLLING SUGARCANE SMUT RESISTANCE AND YIELD TRAITS

Zaheer Ahmad NAZAR[1*], Hafiz Muhammad Walayat Ali KHAN[2], Qurban ALI[1,3*] and Idrees Ahmad NASIR[1]

[1]Center of Excellence in Molecular Biology, University of the Punjab, 87-West Canal Road, Lahore, Pakistan
[2]Sugarcane Research Institute, Ayub Agriculture Research Center, Faisalabad, Pakistan
[3]Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan

Nazar A. Z., H. M. W. Ali Khan, Q. Ali, I. A. Nasir (2019): *Identification of novel QTLs controlling sugarcane smut resistance and yield traits*.- Genetika, Vol 51, No.3, 877-894.
Sugarcane yield depends upon various agro-morphological traits, *viz*., sugar recovery, stalk number, cane girth, cane height and smut resistance. Identification of Quantitative Traits Loci (QTLs) controlling these traits could greatly help sugarcane breeders in marker-assisted selection of sugarcane lines for various breeding programs. Structure and TASSEL software based integration of genotypic and phenotypic data of 103 sugarcane genotypes was resulted in the identification of eighty seven (87) highly associated alleles ($p \leq 0.05$), 34 alleles with smut resistance: 27 alleles with sugar recovery: 13 alleles with cane weight and 20 alleles with each of cane girth and height. The phenotypic variance (R^2-values) explained by these linked alleles ranged 3.1-24.6% for smut resistance, 2.67-22.5% for sugar recovery, 2.81-23.46% for cane height, 2.9-14.34% of cane weight and 1.75-12.8% for cane girth. The varying proportions of phenotypic variance explained by these linked alleles indicated that these traits were controlled by additive genetic effects of multiple genes. It also shows that these traits are the genuine quantitative traits. Moreover, the alleles depicting maximum degree of association for sugar recovery (51-

Corresponding author: Zaheer Ahmad Nazar,Center of Excellence in Molecular Biology, University of the Punjab, 87-West Canal Road, Lahore, Pakistan, email: saim1692@gmail.com, zaheerahmadnazar@gmail.com

131), cane girth (82-184), cane height (52-121), cane weight and smut resistance (51-145 & 51-146) could help in marker assisted selection of sugarcane lines for these traits.

Keywords: association mapping, alleles, sugarcane, QTLs, whip smut, yield traits

INTRODUCTION

Sugarcane belongs to the family Poaceae and genus *Saccharum*. It is consisted of six main species, to be specific, *S. sinense*, *S. officinarum*, *S. robustum*, *S. beriberi* and *S. edule* (D'HONT *et al.*, 1998). Sugarcane has a complex poly-aneuploidy genome with chromosomes ranging from 100 to 130 (ROACH, 1969; NAZAR *et al.*, 2017; NAZAR, 2018). The majority of its attributes are controlled by numerous alleles and inherited quantitatively which make its breeding and gene cloning troublesome for breeders and geneticists (BUTTERFIELD *et al.*, 2001). However, in very recent years an effective endeavors have been made in mapping of its genome and progenitors (AITKEN *et al.*, 2005). With the appearance of PCR (Polymerase Chain Reaction) based innovation, DNA typing based upon molecular markers has now become possible (KORZUN 2002). Broad research on utilizing these molecular markers is being led everywhere throughout the world. These molecular markers included ribosomal DNA (GLASZMANN *et al.*, 1990; SCHOCH *et al.*, 2012) random amplified polymorphic DNA (RAPD) (BURNER *et al.*, 1997; PAN *et al.*, 2001; PAN *et al.*, 2005; BIBI and MUSTAFA, 2015; FAROOQ *et al.*, 2017) amplified fragment length polymorphism (AFLP) (BESSE *et al.*, 1998; BUTTERFIELD *et al.*, 2001) restriction fragment length polymorphism (RFLP) (GRIVET *et al.*, 1996; MING *et al.*, 1998; ALI *et al.*, 2011; SALISU *et al.*, 2017), variable number of tandem repeats (VNTRs) (JEFFREYS *et al.*, 1985), 5 Sr RNA ITS marker (D'HONT *et al.*, 1995; PAN *et al.*, 2001; PIPERIDIS *et al.*, 2001; DEVLIN *et al.*, 2004; HAFEEZ *et al.*, 2015), simple sequence repeats (SSRs) (WEBER and MAY, 1989; EDWARDS *et al.*, 1991), single nucleotide polymorphism (SNPs), targeted region amplified products (TRAP) (CORDEIRO *et al.*, 2001; CORDEIRO and HENRY, 2001; DA SILVA, 2001; PAN *et al.*, 2003; AITKEN *et al.*, 2005; ALWALA *et al.*, 2006) and QTL analysis for yield components (HOARAU *et al.*, 2002). The molecular markers have been utilized in sugarcane projects mainly for assessing genetic variability (BESSE *et al.*, 1998; DA SILVA, 2001; PAN *et al.*, 2001), linkage maps construction (BURNQUIST, 1991; GRIVET *et al.*, 1996; MING *et al.*, 1998; CHUTIMANITSAKUN *et al.*, 2011; HAFEEZ *et al.*, 2019) and marker based selection (D'HONT *et al.*, 1995; PAN *et al.*, 2001; PAN, 2006). Markers labelled with fluorescent dyes are more favorable for genotyping using automated sequencers when contrasted with conventional auto-radiographs or silver staining procedures (COBURN *et al.*, 2002). Genotyping based on semi-automated methods help in better use of markers for DNA fingerprinting of cultivars (HOFFMAN *et al.*, 1995; ALI *et al.*, 2011; FAROOQ *et al.*, 2017), high throughput genotyping (RHODES *et al.*, 1998; PONCE *et al.*, 1999; NAZAR *et al.*, 2017) and genetic diversity studies with more precise genotyping (DIWAN and CREGAN, 1997; MACAULAY *et al.*, 2001; AHMAD *et al.*, 2015).

The identification of QTLs (Quantitative Trait Loci) controlling the important morphological and yield traits in crops are the recent advances in the field of genomics (SELVI *et al.*, 2006; TUBEROSA and SALVI, 2006; COLLINS *et al.*, 2008; COOPER *et al.*, 2009). In various plant species, QTL mapping assisted in recognizing loci connected with complex traits (MAURICIO, 2001; HOLLAND, 2007; HALL *et al.*, 2010). Association mapping is based upon linkage disequilibrium (LD) of alleles in natural populations which have been used to recognize markers with noteworthy allelic variations (OCHIENG *et al.*, 2007). It can be classified into two

categories i) genome wide association mapping, which utilize allelic variation and their association for complex traits in entire genome and ii) candidate gene association mapping which helps to find out selected candidate genes controlling phenotypic variations for specific traits (RISCH and MERIKANGAS, 1996). Association mapping was broadly used in medical genetics to diagnose diseases like Alzheimer (LANDER and SCHORK, 1994; RISCH, 2000) but now its applications have switched to other fields including plant genetics (OCHIENG *et al.,* 2007). In spite of this, still its applications are restricted in plant species due to their population structure which may result spurious associations (PRITCHARD *et al.,* 2010).

The genotypes usually show family relationships and population structure because of their breeding history, geographical origins, and local adaptations (YU and BUCKLER, 2006). Statistical approaches such as structured association (SA) (FALUSH *et al.,* 2003) mixed model approach (YU and BUCKLER, 2006) genomic control (GC) (DEVLIN *et al.,* 2004) and principal component analysis (PRICE *et al.,* 2006) should be adopted to interpret results of association tests. In other case, the association tests without considering the population structure shall be viewed with skepticism. Using the statistical methodologies, the issues of false positives posed by population structure may be resolved (PRICE *et al.,* 2006; YU *et al.,* 2006). The Whip Smut disease caused by *Ustilago scitiminae* in sugarcane was first reported in 1877 in South Africa followed by Asia in later decades (GRIVET and ARRUDA, 2002). However, in Australia, it was first observed on July 1998 at its western Irrigation area of Ordo river. Smut resistance in sugarcane germplasm is probably the result of several characteristics and controlled by a number of genes (LLOYD and NAIDOO, 1983; HÉCTOR *et al.,* 1995; MAJID *et al.,* 2017; NAZAR *et al.,* 2017; NAZAR, 2018).

This study was aimed to find out the alleles associated with various yield traits of sugarcane including its weight, girth, height, sugar recovery and resistance to whip smut.

MATERIALS AND METHODS

Cultivation of sugarcane promising lines and inoculation of whip smut pathogen

In total, hundred and three (103) promising lines of sugarcane were selected and cultivated in the experimental area of Sugarcane Research Institute, Faisalabad in randomized complete block design having two replications of each variety. Under artificially inoculated conditions of whip smut pathogen, phenotypic data of two successive cropping years for whip smut resistance and yield traits was generated.

Leaf sampling for molecular studies

For genomic DNA extraction, young tender leaves were taken, labelled, packaged in aluminum foil and dipped in the liquid nitrogen to prevent nucleic acid degradation. Samples were grinded in liquid nitrogen and finally DNA was extracted using the CTAB method (DOYLE, 1990). Its purity was confirmed both on Nano Drop Spectrophotometer ND^{-}1000 and on agarose gel. Only the DNA samples with absorbance ratio of ~1.8 at 260/280 nm and showing compact distinct bands on agarose gel were processed for further molecular studies.

Synthesis of SSR microsatellites

We used 30 SSR markers for genotyping of sugarcane lines **(**Table 1**)**. These primers were selected based upon their potential for being used in genetic diversity studies. Their

sequences and Polymorphism Information Content values were already reported. Four of these markers namely SMC 222CG, mSSCIR14, SMC 1493CL and SMC 179SA were provided by USDA Sugarcane Production Research, Florida. These were M-13 tailed primers labelled with two fluorescent dyes (IRDye 700 and IRDye 800), showing absorbance at 700nm and 800nm respectively. The forward sequences of the rest of the primer pairs were labelled with fluorescent phosphoramidite (FAM) dye. The potential of these primers (except SCC-82 & SCC-89) was already categorized based upon their polymorphism Information Content values calculated (PAN 2006) while PIC values of the primer pairs SCC-82 and SCC-89 was also reported (SILVA *et al.* 2012).

Table 1: Names of the Primer Pairs and their Sequences

Sr.#	Primer	Primer Seq - Fwd 5'--> 3'	Primer Seq - Rev 3' --> 5'
1	mSSCIR14	GAT TGT TTT TCC CCC ACT A	CAC CTT GTT CTT GCT TTA CTC
2	SMC179SA	CAT TTG ACC AAC CAT GCA CAG C	GGC TTG GCA GGA TTG GAA AC
3	SMC 222 CG	TTT CAC GAA CAC CCC ACC TA	AGG GAC TAG CAC ACA TTA TTG TG
4	SMC 1493 CL	CGA TGA GTA AAT GGG CAG C	GAT ATA GAG GAA GGG ATT GAA GG
5	SMC 668 CS	ACG CTT GCG TGC TCC ATT	CCA ATC GTG CCA CTG TAG TAA G
6	mSSCIR-1	CTT GTG GAT TGG ATT GGA T	AGG AAA TGG ATT GCT CAG G
7	mSSCIR-4	TTC CAG CAG CAG CAT CAA T	CCC ACT AGG AGA AGC AAT AAC T
8	mSSCIR-17	AGC ATA GTT TTT GTG GAC	AGT TCT TTT CGT TCT CTG G
9	mSSCIR-19	GGT TCC AAA ATA CAC AAA	CAA TCT TAT CTA CGC ACT T
10	mSSCIR-24	AGA TGA ACC CAA AAA CTT A	TTA CTC CGC CTC TTT ACT
11	mSSCIR-43	ATT CAA CGA TTT TCA CGA G	AAC CTA GCA ATT TAC AAG AG
12	mSSCIR-52	ACA AGG GAA GAC AAA TCA G	ACC AAA CCA CAA AGC AAA
13	SCC-89	AGT GTT GCG AGA AGC AGC AG	CCC ATG GAT CAC ATG ACA GA
14	SCC-82	CTA TCC CAT CCC GGA AAA A	CCG ACT TGA ACA CCA CCA G
15	SMC 7 CUQ	GCC AAA GCA AGG GTC ACT AGA	AGC TCT ATC AGT TGA AAC CGA
16	SMC 25 DUQ	GCT TCC TAA TCC ATT GTT ATT CTT	GCC ACT CCA TCT GCT AGT GTT C
17	SMC-39BUQ	CGT CTG GCG GAT GAA ATT GAG	CCT ATC GGC ATC AAA TGG TCG
18	SMC 334 BS	CAA TTC TGA CCG TGC AAA GAT	CGA TGA GCT TGA TTG CGA ATG
19	SMC 336 BS	ATT CTA GTG CCA ATC CAT CTC A	CAT GCC AAC TTC CAA ACA GAC
20	SMC-545 MS	AGG CTA CAT GCT TAC AGC CAT	TGG TCT ATC ACT TAA TCA GCC AC
21	SMC 569 CS	GCG ATG GTT CCT ATG CAA CTT	TTC GTG GCT GAG ATT CAC ACT A
22	SMC 597 CS	GCA CAC CAC TCG AAT AAC GGA T	AGT ATA TCG TCC CTG GCA TTC A
23	SMC 640 CS	TTA AGA GAC CCG CCT TTG GAA	TGC CAG AAG TGG TTG TGC TCA
24	SMC 703 BS	GCC TTT CTC CAA ACC AAT TAG T	GTT GTT TAT GGA ATG GTG AGG A
25	SMC 766 BS	TTA CTC GGC TGG GTT TTG TTC	TAA GAA TCG TTC GCT CCA GC
26	SMC 851 MS	ACT AAA ATG GCA AGG GTG GT	CGT GAG CCC ACA TAT CAT GC
27	SMC 1282FL	CGG TGA CCT TAG GCT ACC AT	TGG GAG AAT CTA GCT TGA CAA C
28	SMC 1604 SA	AGG GAA AAG GTA GCC TTG G	TTC CAA CAG ACT TGG GTG G
29	SMC 1751 CL	GCC ATG CCC ATG CTA AAG AT	ACG TTG GTC CCG GAA CCG
30	SMC 2017 FL	CAC AAG TGA AGA TAA TAG TGT CCC T	GAT CCC AAA TCC CTT GAT CTC

PCR amplification protocols

Touchdown PCR was adopted for the amplification of SSR markers. The FAM-labelled primer pairs were amplified following the program of 15 cycles of 94ºC for 30 sec., 65ºC for 30 sec., decreasing 1ºC each cycle and 72ºC for 1 min. followed by 25 cycles of 94ºC for 30 sec., 50ºC for 30 sec., and 72ºC for 1 min and final extension of 7minutes at 72ºC with infinite hold at 8ºC. The annealing temperatures, number of cycles, the degree of decreasing temperature with each cycle and final extension time were slightly modified to achieve better amplification of all the primer pairs. The thermo cycler used for the amplification of FAM- labelled markers was *iCycler* of Bio Rad. The amplification protocol was slightly modified (PAN, 2006), who used standard amplification protocol not the touchdown protocol as we did. The reaction volume of PCR mixture was 10µl with 2µl being 10x PCR buffer, 0.3µl 50mM $MgCl_2$, 0.75µl 2mM dNTPs, 0.3µl each of 1µM Primer (F+R) and 0.3µl 5U/µl Taq polymerase, 4.05µl deionized water and the remaining 2µl 30-50ng/µl purified DNA of each sugarcane line. The quality of amplified products was first checked on 1.8% agarose gel before being processed for genotyping (Figure 1).

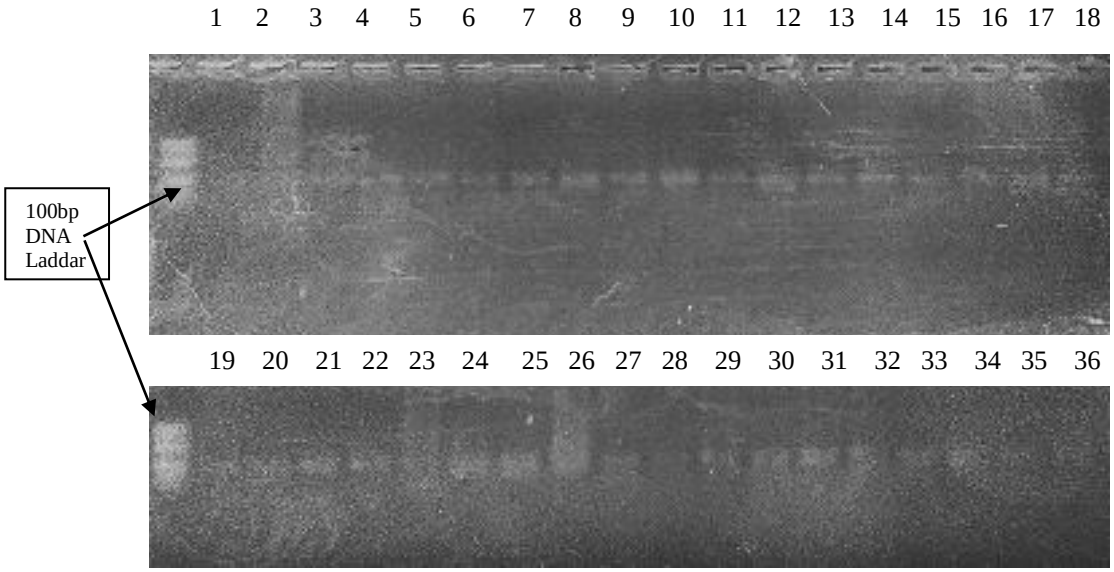

Figure 1. PCR amplification of mSSCIR-43 (100) for 36 sugarcane lines

Genotyping of sugarcane lines

The amplified products of M-13 tailed primers and FAM- labelled primer pairs were genotyped on Licor-4300 DNA Analyzer and ABI Genetic Analyzer 3730 respectively. The SSR amplified products of tailed primer pairs were size separated on Licor-4300 DNA analyzer used at Sugarcane Production Research, USDA, Florida. The samples were run on the freshly prepared PAG gel used in Licor-4300 DNA analyzer and were allowed to run for 50 minutes.

The bands representing the alleles were size sorted based upon the ladder which was run on the first and the last lane of the gel. The autoradiograms were saved as tiff image files which were then converted into JPG format for scoring the alleles **(**Figure 2). Capillary based electrophoresis of the PCR amplified products of FAM labelled SSR primer pairs was performed on ABI Genetic Analyzer 3130. The Peak Scanner software v1.0 was used to reveal electropherograms. The software computed size of each peak against Gene Scan Liz-500 size standard inserted in each well **(**Figure 3). The measurable fluorescence peaks on electropherograms and distinct bands on autoradiograms were considered for allele scoring. The dinosaur tails, stutters, minus-Adenine peaks and pull ups were not scored (PAN *et al.*, 2003).

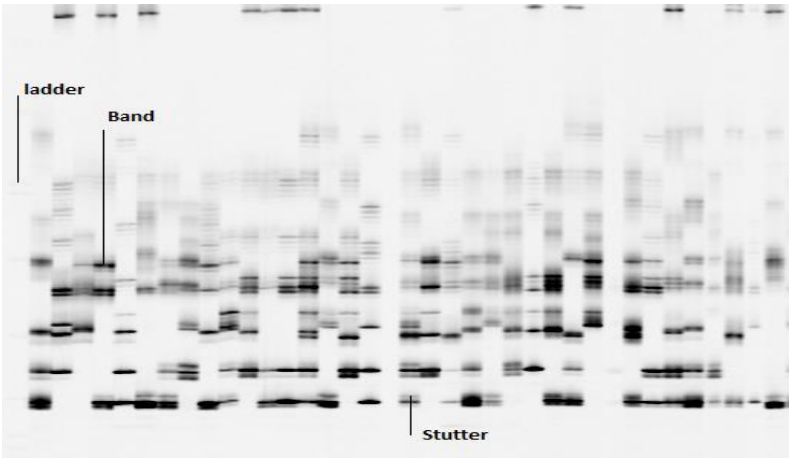

Figure 2. Autoradiogram generated by licor-4300 DNA analyzer against 179Sa-700 marker

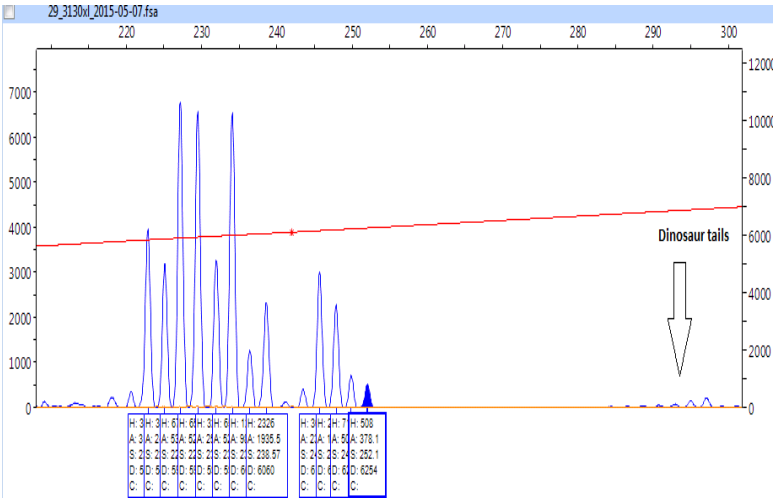

Figure 3. Electropherogram generated by Genetic analyzer 3130

Allele Designation

Each allele was designated by prefix number representing a particular marker followed by allele size in base pairs. The prefix number representing their respective markers are14=mSSCIR-14,79=SMC179SA,22=SMC222CG, 93=SMC1493CL,68=SMC668CS,mSSCIR1,4=mSSCIR4,17=mSSCIR17,19=mSSCIR19,24=mSSCIR24,43=mSSCIR43,52=mSSCIR52, 89=SCC89, 82=SCC82, 7=SMC7CUQ, 25=SMC25DUQ, 39=SMC39BUQ, 34=SMC334 BS, 36=SMC336BS, 45=SMC545MS, 69=SMC569CS, 97=SMC597CS, 40=SMC640CS, 03-SMC703BS, 66=SMC766 BS, 51=SMC851MS, 82=SMC1282FL, 04=SMC1604 SA, 51=SMC1751CL and 17=SMC2017FL. The amplified alleles against each marker are mentioned in the Table 2.

Table 2. Names of the 30 markers and their amplified alleles

Marker	mSSCIR-14						SMC179SA						SMC222CG							SMC1493CL							
Alleles	14-245	14-237	14-231	14-222	14-220	14-215	79-262	79-173	79-162	79-145	79-128	79-118	22-209	22-200	22-196	22-183	22-170	22-165	22-150	93-108	93-124	93-126	93-129	93-139	93-143	93-147	93-166
Marker	SMC668CS						mSSCIR1													mSSCIR4				mSSCIR17			
Alleles	68-213	68-219	68-222	68-225	68-230	68-233	68-239	1-127	1-130	1-142	1-144	1-146	1-150	1-156	1-158	1-167	1-172	1-183	1-187	4-240	4-245	4-249	4-259	17-226	17-230	17-232	17-234
Marker	mSSCIR17							mSSCIR19																mSSCIR24			
Alleles	17-236	17-238	17-240	17-242	17-244	17-245	17-246	17-256	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	24-218	24-233	24-235	24-240
Marker	mSSCIR24						mSSCIR43														mSSCIR52						
Alleles	24-242	24-244	24-246	24-248	24-250	24-252	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	52-121	52-127	52-131	52-132	52-133	52-135	52-137
Marker	mSSCIR52			SCC89					SCC82											SMC7CUQ							
Alleles	52-139	52-142	52-144	89-183	89-197	89-201	89-206	89-222	82-154	82-162	82-172	82-180	82-184	82-188	82-192	82-195	82-197	82-198	7-154	7-156	7-158	7-160	7-162	7-164	7-166	7-168	7-170
Marker	SMC25DUQ							SMC39BUQ									SMC334 BS										
Alleles	25-212	25-214	25-215	25-216	25-218	25-225	25-227	25-232	39-128	39-131	39-132	39-135	39-140	39-143	39-145	39-149	34-135	34-140	34-144	34-148	34-151	34-153	34-155	34-159	34-161	36-133	36-140
Marker	SMC336BS												SMC545MS												SMC569 CS		
Alleles	36-144	36-149	36-153	36-160	36-162	36-165	36-166	36-168	36-170	36-174	36-176	36-182	45-113	45-117	45-120	45-123	45-126	45-127	45-130	45-132	45-135	45-138	45-142	45-145	69-157	69-158	69-165
Marker	SMC569CS						SMC597CS												SMC640CS								
Alleles	69-207	69-210	69-211	69-215	69-217	69-220	97-142	97-144	97-147	97-150	97-151	97-153	97-156	97-160	97-163	97-164	97-167	97-177	40-216	40-217	40-219	40-221	40-224	40-226	40-227	40-228	40-230

Marker	SMC640CS										SMC703BS										SMC766 BS							
Alleles	40-232	40-236	40-238	40-242	40-244	40-245	40-249	40-251	40-255	40-257	03-193	03-194	03-199	03-203	03-207	03-209	03-211	03-213	03-215	03-218	66-177	66-179	66-181	66-184	66-188	66-190	66-192	
Marker	SMC766BS								SMC851MS											SMC1282FL								
Alleles	66-197	66-199	66-201	66-203	66-205	66-207	66-211	66-216	51-125	51-126	51-127	51-128	51-129	51-131	51-133	51-135	51-139	51-142	51-144	82-340	82-348	82-351	82-354	82-357	82-360	82-365	82-372	
Marker	SMC1282FL							SMC1604 SA														SMC1751CL						
Alleles	82-374	82-383	82-386	82-389	82-392	82-395	82-411	04-107	04-110	04-113	04-116	04-119	04-121	04-128	04-413	04-414	04-416	04-417	04-419	04-421	04-425	51-139	51-142	51-145	51-146	51-148	51-149	
Marker		SMC2017FL																										
Alleles	51-152	17-211	17-214	17-217	17-218	17-221	17-223	17-226	17-227	17-229	17-230	17-232	17-233	17-236	17-239	17-242	17-245	17-249	17-252	17-255								

Each allele is designated by the prefix number representing marker followed by allele size in base pairs (14=mSSCIR-14, 79=SMC179SA, 22=SMC222CG, 93=SMC1493CL, 68=SMC668CS, mSSCIR1, 4=mSSCIR4, 17=mSSCIR17, 19=mSSCIR19, 24=mSSCIR24, 43=mSSCIR43, 52=mSSCIR52, 89=SCC89, 82=SCC82, 7=SMC7CUQ, 25=SMC25DUQ, 39=SMC39BUQ, 34=SMC334 BS, 36=SMC336BS, 45=SMC545MS, 69=SMC569CS, 97=SMC597CS, 40=SMC640CS, 03-SMC703BS, 66=SMC766 BS, 51=SMC851MS, 82=SMC1282FL, 04=SMC1604 SA, 51=SMC1751CL, 17=SMC2017FL

Population Structure and Association Mapping

Association mapping was performed using two widely used statistical software, Structure 2.3.4 (PRITCHARD *et al.*, 2010) and TASSEL (BUCKLER *et al.*, 2009). The genotyping data of all sugarcane lines were processed through Structure 2.3.4 software which used model based clustering method to infer population structure. Allele frequencies were correlated using the admixture model. The parameters set by using the values of the Burnin period (100,000) and Markov Chain Monte Carlo (MCMC) repeats after Burnin (100,000) to get reliable convergence. Simulations were run using the values of k ranging from 1-10 with the number of replications (iterations) set at 5. The simulation summary was saved. The graph between the values of k and average values of LnP(D) against each k was plotted on excel sheet to determine the value of optimum k. The value of optimum k was used to infer population structure in the form of inferred ancestry (Q-matrix) of genotypes**.** The inferred ancestry coefficients (Q- matrix) of sugarcane lines across the sub-populations were used as covariate in Trait Analysis by Association, Evolution and Linkage (TASSEL) to avoid spurious marker-trait associations. Appropriate formats of genotyping data, phenotype data and inferred ancestry coefficient data were created to perform Linkage Disequilibrium (LD) based association mapping on TASSEL version 5.0 standalone using General Linear Model (GLM) algorithm. The marker-trait associations with p-values ≤ 0.05 were considered as significant.

RESULTS

Inference of Population Structure

The value of optimum k was found to be six (6) by plotting the graph between average values of LnP(D) vs k ranging from 1-10 **(**Figure 4**)**. The population structure was inferred in the form of inferred incestory (Q- matrix) of genotypes **(**Table 3). The graphic representation in the form of bar plot structure of all the 103 genotypes has been shown in Figure 5 and 6.

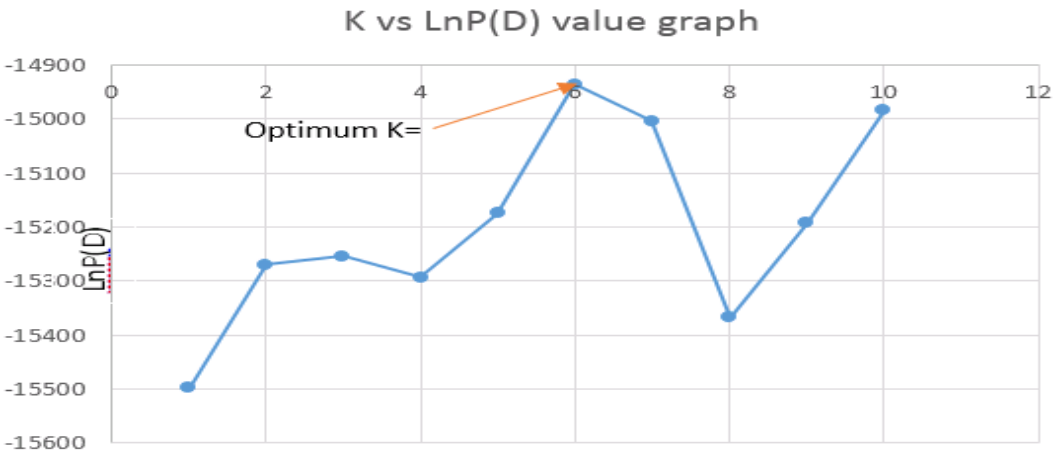

Figure 4. Inference of correct number of sub-populations

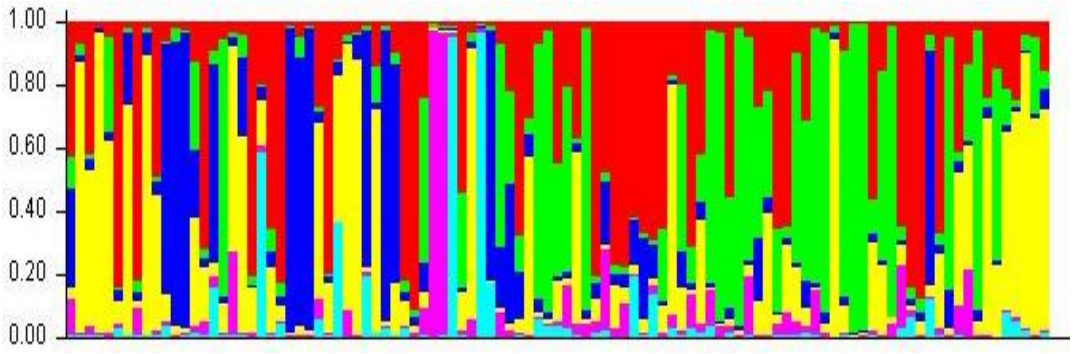

Figure 5. Bar Plot Structure of 103 Sugarcane lines

Table 3. Inferred ancestry of individuals (Q- Matrix)

Label	(%Miss) :	Inferred clusters	Label	(%Miss) :	Inferred clusters
1 GENO1	(0)	: 0.423 0.103 0.315 0.036 0.115 0.008	53 GENO53	(0)	: 0.207 0.582 0.022 0.023 0.131 0.035
2 GENO2	(0)	: 0.068 0.036 0.020 0.859 0.008 0.010	54 GENO54	(0)	: 0.369 0.016 0.028 0.542 0.037 0.009
3 GENO3	(0)	: 0.418 0.012 0.040 0.494 0.024 0.012	55 GENO55	(0)	: 0.015 0.899 0.027 0.011 0.041 0.007
4 GENO4	(0)	: 0.017 0.004 0.010 0.950 0.005 0.014	56 GENO56	(0)	: 0.801 0.024 0.053 0.065 0.036 0.021
5 GENO5	(0)	: 0.050 0.297 0.029 0.605 0.013 0.006	57 GENO57	(0)	: 0.475 0.030 0.199 0.015 0.257 0.024
6 GENO6	(0)	: 0.839 0.011 0.029 0.074 0.017 0.030	58 GENO58	(0)	: 0.770 0.027 0.037 0.133 0.027 0.006
7 GENO7	(0)	: 0.019 0.011 0.232 0.724 0.006 0.008	59 GENO59	(0)	: 0.775 0.024 0.042 0.048 0.105 0.006
8 GENO8	(0)	: 0.820 0.037 0.025 0.022 0.085 0.011	60 GENO60	(0)	: 0.616 0.006 0.144 0.029 0.009 0.195
9 GENO9	(0)	: 0.016 0.013 0.078 0.881 0.003 0.010	61 GENO61	(0)	: 0.667 0.015 0.254 0.049 0.005 0.010
10 GENO10	(0)	: 0.488 0.016 0.041 0.433 0.012 0.010	62 GENO62	(0)	: 0.689 0.016 0.104 0.020 0.034 0.137
11 GENO11	(0)	: 0.060 0.010 0.794 0.080 0.020 0.035	63 GENO63	(0)	: 0.652 0.233 0.010 0.090 0.010 0.004
12 GENO12	(0)	: 0.021 0.038 0.902 0.026 0.007 0.006	64 GENO64	(0)	: 0.169 0.015 0.010 0.731 0.045 0.031
13 GENO13	(0)	: 0.025 0.006 0.934 0.017 0.006 0.011	65 GENO65	(0)	: 0.200 0.526 0.114 0.132 0.014 0.015
14 GENO14	(0)	: 0.124 0.281 0.210 0.348 0.022 0.014	66 GENO66	(0)	: 0.714 0.109 0.023 0.018 0.107 0.028
15 GENO15	(0)	: 0.718 0.029 0.029 0.170 0.041 0.013	67 GENO67	(0)	: 0.419 0.148 0.055 0.334 0.027 0.017
16 GENO16	(0)	: 0.087 0.044 0.632 0.044 0.032 0.161	68 GENO68	(0)	: 0.023 0.805 0.008 0.010 0.113 0.040
17 GENO17	(0)	: 0.053 0.814 0.022 0.096 0.004 0.011	69 GENO69	(0)	: 0.031 0.916 0.005 0.010 0.031 0.006
18 GENO18	(0)	: 0.030 0.019 0.025 0.651 0.267 0.008	70 GENO70	(0)	: 0.555 0.348 0.035 0.021 0.025 0.016
19 GENO19	(0)	: 0.036 0.072 0.255 0.619 0.008 0.010	71 GENO71	(0)	: 0.016 0.948 0.011 0.014 0.006 0.005
20 GENO20	(0)	: 0.807 0.026 0.008 0.143 0.011 0.004	72 GENO72	(0)	: 0.047 0.705 0.013 0.041 0.184 0.010
21 GENO21	(0)	: 0.197 0.010 0.036 0.148 0.021 0.589	73 GENO73	(0)	: 0.265 0.414 0.204 0.108 0.005 0.004
22 GENO22	(0)	: 0.655 0.073 0.048 0.205 0.009 0.011	74 GENO74	(0)	: 0.215 0.342 0.048 0.386 0.006 0.004
23 GENO23	(0)	: 0.825 0.044 0.031 0.044 0.010 0.045	75 GENO75	(0)	: 0.651 0.264 0.013 0.023 0.040 0.009
24 GENO24	(0)	: 0.010 0.007 0.964 0.010 0.005 0.004	76 GENO76	(0)	: 0.645 0.037 0.024 0.214 0.067 0.013
25 GENO25	(0)	: 0.044 0.066 0.854 0.026 0.005 0.005	77 GENO77	(0)	: 0.094 0.667 0.011 0.176 0.044 0.007
26 GENO26	(0)	: 0.008 0.007 0.958 0.017 0.003 0.006	78 GENO78	(0)	: 0.309 0.510 0.083 0.056 0.027 0.014
27 GENO27	(0)	: 0.269 0.011 0.040 0.557 0.063 0.060	78 GENO78	(0)	: 0.309 0.510 0.083 0.056 0.027 0.014
28 GENO28	(0)	: 0.795 0.011 0.022 0.156 0.006 0.009	79 GENO79	(0)	: 0.018 0.805 0.019 0.007 0.142 0.009
29 GENO29	(0)	: 0.118 0.009 0.042 0.461 0.005 0.365	80 GENO80	(0)	: 0.033 0.887 0.033 0.029 0.011 0.007
30 GENO30	(0)	: 0.040 0.018 0.010 0.845 0.081 0.007	81 GENO81	(0)	: 0.013 0.018 0.020 0.943 0.003 0.003
31 GENO31	(0)	: 0.030 0.012 0.076 0.869 0.006 0.007	82 GENO82	(0)	: 0.090 0.779 0.031 0.085 0.007 0.008
32 GENO32	(0)	: 0.014 0.008 0.753 0.014 0.014 0.197	83 GENO83	(0)	: 0.006 0.978 0.006 0.006 0.003 0.002
33 GENO33	(0)	: 0.138 0.114 0.022 0.705 0.004 0.017	84 GENO84	(0)	: 0.007 0.965 0.007 0.013 0.005 0.002
34 GENO34	(0)	: 0.013 0.009 0.925 0.016 0.007 0.030	85 GENO85	(0)	: 0.564 0.102 0.030 0.279 0.016 0.008
35 GENO35	(0)	: 0.099 0.030 0.697 0.161 0.005 0.007	86 GENO86	(0)	: 0.156 0.600 0.015 0.217 0.008 0.005
36 GENO36	(0)	: 0.820 0.033 0.026 0.080 0.008 0.033	87 GENO87	(0)	: 0.010 0.920 0.005 0.015 0.047 0.003
37 GENO37	(0)	: 0.909 0.023 0.031 0.018 0.013 0.006	88 GENO88	(0)	: 0.648 0.038 0.018 0.066 0.199 0.031
38 GENO38	(0)	: 0.239 0.523 0.090 0.050 0.089 0.009	89 GENO89	(0)	: 0.840 0.034 0.022 0.013 0.026 0.064
39 GENO39	(0)	: 0.006 0.009 0.006 0.006 0.970 0.004	90 GENO90	(0)	: 0.878 0.037 0.030 0.043 0.005 0.006
40 GENO40	(0)	: 0.009 0.012 0.006 0.007 0.955 0.012	91 GENO91	(0)	: 0.037 0.051 0.747 0.030 0.007 0.128
41 GENO41	(0)	: 0.011 0.006 0.006 0.009 0.017 0.950	92 GENO92	(0)	: 0.669 0.031 0.032 0.238 0.020 0.011
42 GENO42	(0)	: 0.539 0.303 0.012 0.121 0.012 0.012	93 GENO93	(0)	: 0.044 0.804 0.118 0.022 0.010 0.002
43 GENO43	(0)	: 0.030 0.030 0.019 0.860 0.056 0.004	94 GENO94	(0)	: 0.412 0.027 0.035 0.423 0.091 0.012
44 GENO44	(0)	: 0.006 0.004 0.006 0.004 0.015 0.965	95 GENO95	(0)	: 0.129 0.244 0.017 0.394 0.212 0.005
45 GENO45	(0)	: 0.010 0.018 0.788 0.008 0.002 0.173	96 GENO96	(0)	: 0.025 0.884 0.034 0.045 0.006 0.006
46 GENO46	(0)	: 0.070 0.644 0.190 0.015 0.075 0.006	97 GENO97	(0)	: 0.236 0.029 0.040 0.682 0.006 0.007
47 GENO47	(0)	: 0.217 0.297 0.443 0.017 0.019 0.007	98 GENO98	(0)	: 0.145 0.607 0.014 0.227 0.003 0.004
48 GENO48	(0)	: 0.674 0.116 0.147 0.048 0.005 0.009	99 GENO99	(0)	: 0.210 0.116 0.021 0.566 0.005 0.082
49 GENO49	(0)	: 0.303 0.051 0.074 0.560 0.008 0.005	100 GENO100	(0)	: 0.247 0.019 0.015 0.653 0.009 0.058
50 GENO50	(0)	: 0.065 0.812 0.039 0.019 0.005 0.060	101 GENO101	(0)	: 0.038 0.052 0.009 0.870 0.003 0.028
51 GENO51	(0)	: 0.023 0.898 0.015 0.014 0.009 0.040	102 GENO102	(0)	: 0.047 0.241 0.014 0.688 0.005 0.005
52 GENO52	(0)	: 0.444 0.363 0.013 0.129 0.012 0.039	103 GENO103	(0)	: 0.155 0.054 0.065 0.701 0.004 0.022

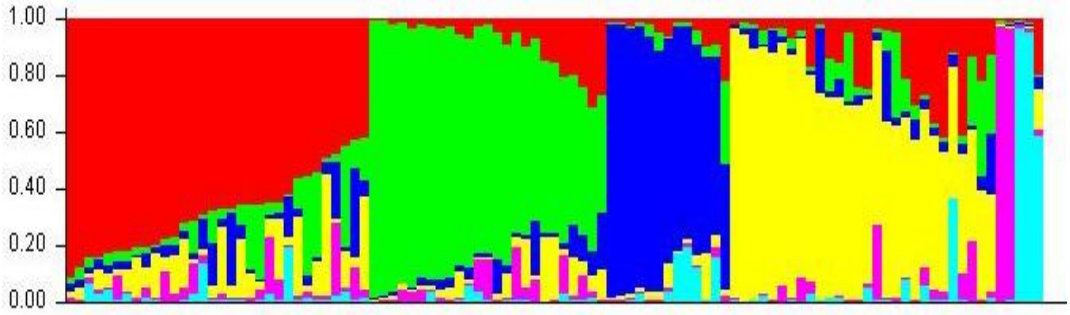

Figure 6. Bar Plot Structure (Sort by Q)

Marker-Trait Associations

The markers associated with different agro-morphological traits and their explained proportion of phenotypic variance are listed in the Table 4. Based upon the value of $p \leq 0.05$, eighty seven (87) alleles were found significantly associated with different agro-morphological traits namely, whip smut resistance, cane weight, height, girth, and sugar recovery. The twenty seven (27) alleles showed associations with more than one phenotypic trait. The alleles 43-252 and 36-160 were found associated with a maximum of three phenotypic traits. The maximum of 34 alleles was found associated with whip smut resistance followed by 27 alleles with sugar recovery while 20 alleles were found associated with each of cane height and girth. The least number of 13 alleles was found associated with cane weight. The marker SMC 2017-FL got the top position with 7 alleles showing associations with different phenotypic traits while mSSCIR-52 stood at second position with 6 alleles showing associations. The five (5) alleles of each of mSSCIR-19, mSSCIR-24, SMC 640 CS and SCC 82 SSR markers were found associated with different agro-morphological traits. The phenotypic variance (R^2-values) explained by the linked alleles ranged 2.81% to 7.82% for cane height, 1.75% to 4.3% for cane girth, 2.9% to 4.78% of cane weight, 2.67% to 7.5% for sugar recovery and 3.1% to 8.2% for whip smut.

The robustness of association of each allele was determined by p-value and R^2-value. Fourteen out of eighty seven (87) alleles stood prominent among all of the alleles associated with the yield traits. Among these, 5 alleles namely, 03-207 ($p=0.017$, $R^2=0.04525$), 66-199 ($p=0.0149$, $R^2=0.04715$), 25-218 ($p=0.1828$, $R^2=0.04435$), 51-145 ($p=0.01414$, $R^2=0.04784$) and 51-146 ($p=0.01414$, $R^2=0.04784$) showed a maximum degree of association with cane weight. The amplified alleles (51-145 and 51-146) of the marker SMC 1751 CL ranked at top position controlling maximum of 4.8% of phenotypic variation for cane weight. Maximum degree of association for cane height was displayed by the allele 52-121 ($p=0.00105$, $R^2=0.07815$) followed by the allele 82-195 ($p=0.00322$, $R^2=0.06383$). For cane girth, 4 alleles namely, 93-129 ($p=0.00382$, $R^2=0.03896$), 82-184 ($p=0.00233$, $R^2=0.04253$), 45-127 ($p=0.00381$, $R^2=0.0386$) and 51-144($p=0.00369$, $R^2=0.03885$) depicted a maximum degree of association. Similarly, for sugar recovery, three alleles namely, 19-148 ($p=0.00616$, $R^2=0.05471$), 34-161 ($p=0.00543$, $R^2=0.05574$) and 51-131($p=0.0012$, $R^2=0.07461$) were found highly associated. Five out of thirty four (34) alleles associated ($p \leq 0.05$) with whip smut resistance *viz.*, 7-154 ($p=0.00233$, $R^2=0.07542$), 25-215 ($p=0.0026$, $R^2=0.07315$), 36-170 ($p=0.0261$, $R^2=0.07383$), 51-145

(p=0.00137, R^2=0.0821) and 51-145 (p=0.00137, R^2=0.0821) each explained more than 7% of phenotypic variation.

Table 4. Marker-Trait associations

SSR Markers	Allele sizes	Whip Smut		Weight		Cane height		Girth		Sugar Recovery	
		p-value	R^2value	p-value	R^2value	p-value	R^2value	p-value	R^2value	p-value	R^2value
mSSCIR-14	14-215	0.00828	0.05684	-	-	-	-	-	-	-	-
mSSCIR-14	14-222	-	-	-	-	-	-	-	-	0.03335	0.03319
179 Sa -700	79-162	-	-	-	-	-	-	0.03252	0.02149	-	-
179 Sa -700	79-128	0.04507	0.03324	-	-	-	-	-	-	-	-
179 Sa -700	79-118	0.01407	0.04989	-	-	-	-	0.04136	0.0198	-	-
222 CG-700	22-183	-	-	-	-	0.00876	0.05205	-	-	-	-
222 CG-700	22-165	-	-	-	-	-	-	-	-	0.00901	0.04942
1493CL-700	93-124	-	-	-	-	-	-	-	-	0.05387	0.02736
1493CL-700	93-129	-	-	-	-	-	-	0.00382	0.03896	-	-
SMC-668-CS	68-219	0.04863	0.0322	-	-	0.05153	0.02859	0.05367	0.01758	-	-
mSSCIR-1	1-142	-	-	-	-	0.00719	0.05353	-	-	-	-
mSSCIR-1	1-156	0.05455	0.03064	-	-	0.0211	0.03979	-	-	-	-
mSSCIR-4	4-249	0.01649	0.04717	-	-	-	-	-	-	0.03406	0.03293
mSSCIR-17	17-232	0.01005	0.05357	-	-	-	-	-	-	0.02762	0.03515
mSSCIR-19	19-131	-	-	-	-	0.05159	0.02887	-	-	0.02239	0.03849
mSSCIR-19	19-132	0.03675	0.03604	-	-	-	-	-	-	-	-
mSSCIR-19	19-135	0.02766	0.03996	-	-	0.03412	0.03373	-	-	-	-
mSSCIR-19	19-148	-	-	-	-	-	-	-	-	0.00616	0.05471
mSSCIR-19	19-153	-	-	-	-	-	-	-	-	0.00862	0.04997
mSSCIR-24	24-218	-	-	-	-	0.0158	0.04347	-	-	-	-
mSSCIR-24	24-242	-	-	-	-	-	-	-	-	0.0234	0.03756
mSSCIR-24	24-244	-	-	-	-	-	-	-	-	0.0511	0.028
mSSCIR-24	24-246	0.02628	0.04067	-	-	-	-	-	-	-	-
mSSCIR-24	24-248	0.00697	0.05926	-	-	0.02462	0.03784	-	-	-	-
mSSCIR-43	43-222	-	-	-	-	-	-	-	-	0.01051	0.04707
mSSCIR-43	43-245	0.01035	0.05371	-	-	-	-	-	-	-	-
mSSCIR-43	43-247	0.01918	0.04461	-	-	0.04828	0.0291	-	-	-	-
mSSCIR-43	43-252	-	-	0.0402	0.03375	0.03082	0.03501	-	-	0.05491	0.02713
SMC 640 CS	40-216	-	-	-	-	-	-	-	-	0.02754	0.03555
SMC 640 CS	40-217	-	-	-	-	-	-	-	-	0.03542	0.03246
SMC 640 CS	40-227	0.01512	0.04839	-	-	-	-	-	-	-	-
SMC 640 CS	40-249	0.03441	0.03732	-	-	-	-	-	-	0.01125	0.04712
SMC 640 CS	40-255	-	-	-	-	-	-	0.01411	0.02839	-	-
SMC 703 BS	03-194	-	-	-	-	-	-	0.05379	0.01756	-	-
SMC 703 BS	03-207	-	-	0.0171	0.04525	-	-	-	-	-	-
SMC 703 BS	03-213	-	-	-	-	-	-	-	-	0.03848	0.03177
SMC 766 BS	66-181	-	-	-	-	0.00594	0.05653	0.05133	0.01811	-	-
SMC 766 BS	66-188	0.04839	0.03227	-	-	0.04932	0.02913	-	-	-	-
SMC 766 BS	66-199	-	-	0.0149	0.04715	-	-	-	-	-	-
SMC 766 BS	66-203	-	-	-	-	-	-	0.03303	0.02136	-	-
mSSCIR-52	52-121	-	-	-	-	0.00105	0.07815	-	-	-	-

mSSCIR-52	52-131	-	-	0.0538	0.02991	-	-	-	-	-	-
mSSCIR-52	52-133	-	-	-	-	-	-	-	-	0.02359	0.03784
mSSCIR-52	52-139	-	-	0.02553	0.03984	-	-	-	-	-	-
mSSCIR-52	52-142	0.03006	0.03881	-	-	-	-	-	-	-	-
mSSCIR-52	52-144	0.04962	0.03193	-	-	-	-	0.04989	0.01814	-	-
SCC-89	89-197	-	-	-	-	-	-	-	-	0.01348	0.04484
SCC-82	82-154	-	-	-	-	0.00541	0.05662	0.05165	0.01769	-	-
SCC-82	82-162	0.01897	0.04476	-	-	0.02112	0.03938	-	-	-	-
SCC-82	82-184	-	-	-	-	-	-	0.00233	0.04253	-	-
SCC-82	82-192	-	-	-	-	-	-	0.03144	0.02175	-	-
SCC-82	82-195	-	-	-	-	0.00322	0.06383	-	-	-	-
SMC 7 CUQ	7-154	0.00233	0.07542	-	-	0.02983	0.03578	-	-	-	-
SMC 7 CUQ	7-156	0.01976	0.0451	-	-	-	-	-	-	-	-
SMC 7 CUQ	7-166	0.01703	0.04625	-	-	-	-	-	-	-	-
SMC 25 DUQ	25-214	0.05179	0.03135	-	-	-	-	-	-	0.04525	0.02947
SMC 25 DUQ	25-215	0.0026	0.07315	-	-	-	-	-	-	-	-
SMC 25 DUQ	25-218	-	-	0.01828	0.04435	-	-	-	-	-	-
SMC-39BUQ	39-132	-	-	-	-	-	-	0.03931	0.02	-	-
SMC-39BUQ	39-135	0.0127	0.05083	-	-	-	-	-	-	-	-
SMC-39BUQ	39-149	-	-	-	-	-	-	-	-	0.05587	0.02665
SMC 334 BS	34-148	-	-	-	-	0.05356	0.02811	-	-	-	-
SMC 334 BS	34-155	-	-	0.05561	0.02947	-	-	-	-	-	-
SMC 334 BS	34-161	-	-	-	-	-	-	-	-	0.00543	0.05574
SMC 336 BS	36-160	0.02436	0.04215	-	-	0.04771	0.02985	-	-	0.03109	0.0344
SMC 336 BS	36-170	0.00261	0.07383	-	-	-	-	-	-	-	-
SMC 336 BS	36-174	-	-	-	-	-	-	-	-	0.01478	0.04369
SMC-545 MS	45-127	-	-	-	-	-	-	0.00381	0.0386	0.01808	0.04075
SMC 569 CS	69-158	-	-	-	-	-	-	0.0428	0.01934	-	-
SMC 569 CS	69-207	-	-	-	-	-	-	0.03342	0.02149	-	-
SMC 597 CS	97-147	-	-	-	-	-	-	0.05355	0.01759	-	-
SMC 851 MS	51-125	-	-	-	-	-	-	-	-	0.02929	0.03514
SMC 851 MS	51-131	-	-	-	-	-	-	-	-	0.0012	0.07461
SMC 851 MS	51-139	0.00752	0.05877	-	-	-	-	-	-	-	-
SMC 851 MS	51-144	-	-	-	-	-	-	0.00369	0.03885	-	-
SMC 1282FL	82-360	0.05028	0.03175	-	-	-	-	-	-	-	-
SMC 1604SA	04-107	-	-	-	-	0.04521	0.03021	-	-	-	-
SMC 1751CL	51-145	0.00137	0.0821	0.01414	0.04784	-	-	-	-	-	-
SMC 1751CL	51-146	0.00137	0.0821	0.01414	0.04784	-	-	-	-	-	-
SMC 1751CL	51-148	-	-	0.03053	0.03744	-	-	-	-	-	-
SMC 2017FL	17-211	-	-	0.03423	0.03591	-	-	0.05435	0.01748	-	-
SMC 2017FL	17-214	-	-	0.02805	0.03858	-	-	-	-	-	-
SMC 2017FL	17-236	0.01274	0.0513	-	-	-	-	0.05001	0.01831	-	-
SMC 2017FL	17-242	-	-	0.05318	-	-	-	-	-	-	-
SMC 2017FL	17-249	0.04631	0.03321	-	-	-	-	-	-	-	-
SMC 2017FL	17-252	0.01769	0.04619	-	-	-	-	-	-	-	-
SMC 2017FL	17-255	-	-	-	-	-	-	-	-	0.04051	0.03145

DISCUSSION

The SSR markers were amplified using Touchdown PCR amplification protocol, which offered increased sensitivity, specificity and high yield without the need of lengthy optimizations and redesigning of primers (KORBIE and MATTICK, 2008). However the number of PCR cycles and extent of decrease in temperature across each cycle was varied for better amplifications. TD-PCR amplification greatly helped in avoiding spurious smaller fragments which often resulted in target gene amplification of complex genomes like sugarcane during the PCR cycles (DON *et al.*, 1991; QAMAR *et al.,* 2015; NAZAR *et al.,* 2017; QAMAR *et al.,* 2017; AWAIS *et al.,* 2019). Sugarcane yield depends upon various agro-morphological traits, *viz*., stalk number, cane height, cane girth and sugar recovery. Detection of Quantitative Traits Loci (QTLs) linked with these traits could greatly help in marker-assisted selection of sugarcane lines in various breeding programs. Phenotypic variation explained by associated alleles with cane weight, height, girth and sugar recovery ranged from 1.75% to 7.82% which indicated that these traits may be controlled by the additive effect of multiple genes not by a single gene and that these are the genuine quantitative traits. These alleles could be selected in marker-assisted selection of sugarcane lines for yield traits. Linkage disequilibrium based detection of QTLs among modern sugarcane cultivars was the result of past breeding programs (WEI *et al.*, 2006). In similar studies, 102 QTLs were found significantly associated with sugar content, polarity, number of tillers, cane weight and fibre content (MING *et al.,* 2002). While 41 QTLs were reported for yield parameters: 20 for cane height, 15 for stalk number and 6 for girth (PINTO *et al.,* 2011). Numerous QTLs were also found to be associated with cane height, diameter, stalk number and brix explaining 3- 7% of phenotypic variation (HOARAU *et al.,* 2002). Many DArT markers were found associated with sugar content and cane yield while taking into account the population structure (WEI *et al.,* 2010). Many positive QTLs were found associated with plants with much sugar yield. In an another study 27 genomic regions were identified as being significantly associated with cane weight, diameter, height, biomass and number of tillers explaining 4 to 10% of phenotypic variance (AITKEN *et al.*, 2008).

Marker-trait associations were also performed to analyze genetic determinism for sugarcane smut resistance. Thirty four alleles were found to be significantly associated with whip smut resistance explaining 3.1 to 8.2% of phenotypic variance. The alleles 51-145 and 51-146 were found top ranked associated alleles for both whip smut resistance and cane weight. Their explained portion of phenotypic variance was 8.2% for whip smut and 4.8% of cane weight. The detection of various linked alleles with little effects on smut resistance indicated complex genetic determinism for smut resistance (RABOIN *et al.,* 2003). This also indicated that whip smut resistance was controlled by multiple genes with little effects (LLOYD and NAIDOO, 1983; HÉCTOR *et al.,* 1995; NAZAR *et al.,* 2017). More genotyping data could be used for genome wide association mapping while taking into account the family relationship and population structure for identifying QTLs with widespread effect on smut resistance (GOUY *et al.,* 2015). These results were further endorsed by (WEI *et al.,* 2006), who reported 11 markers being associated with whip smut with all showing a total of 59% phenotypic variance.

CONCLUSIONS

The positive results of our study would encourage researchers to apply these protocols for genetic determinism of complex agro-morphological and disease resistance traits of various other

crops which we have used for sugarcane studies. These results are also the first report on identification of QTLs controlling sugarcane yield traits and smut resistance among promising sugarcane lines/varieties being cultivated in Pakistan. These QTLs will be helpful for marker assisted selection of sugarcane lines in future breeding programs.

Received, October 10th, 2018
Accepted August 18th, 2019

REFERENCES

AHMAD, H., M., RAHMAN, F., AZEEM, Q., ALI (2015): QTL mapping for the improvement of drought tolerance in cereal crops: A review. Life Sci. J., *12*: 102-108.

AITKEN, K., S., HERMANN, K., KARNO, G., BONNETT, L., MCINTYRE *et al.* (2008): Genetic control of yield related stalk traits in sugarcane. TAG, *117*: 1191-1203.

AITKEN, K., P., JACKSON, C., MCINTYRE (2005): A combination of AFLP and SSR markers provides extensive map coverage and identification of homo (eo) logous linkage groups in a sugarcane cultivar. TAG, *110*: 789-801.

ALI, Q., M., AHSAN, M.H.N., TAHIR, J., FAROOQ, M., WASEEM *et al.* (2011): Molecular markers and QTLs for Ascochyta rabiei resistance in chickpea. International Journal for Agro Veterinary and Medical Sci., *5*: 249-270.

ALWALA, S., A., SUMAN, J.A., ARRO, J.C., VEREMIS, C.A., KIMBENG (2006): Target region amplification polymorphism (TRAP) for assessing genetic diversity in sugarcane germplasm collections. Crop Sci., *46*: 448-455.

AWAIS, M., M., TARIQ, Q., ALI, A., KHAN, A., ALI *et al.* (2019): Isolation, characterization and association among phosphate solubilizing bacteria from sugarcane rhizosphere. Cytology and Genetics, *53*: 86-95.

BESSE, P., G., TAYLOR, B., CARROLL, N., BERDING, D., BURNER *et al.* (1998): Assessing genetic diversity in a sugarcane germplasm collection using an automated AFLP analysis. Genetica, *104*: 143-153.

BIBI, T., H.S.B.E.-U.-H.S., MUSTAFA, T., MAHMOOD, Q., ALI (2015): Analysis of genetic diversity in linseed using molecular markers. Life Sci J., *12*: 28-37.

BUCKLER, E.S., J.B., HOLLAND, P.J., BRADBURY, C.B., ACHARYA, P.J., BROWN *et al.* (2009): The genetic architecture of maize flowering time. Science, *325*: 714-718.

BURNER, D.M., Y.-B., PAN, R.D., WEBSTER (1997): Genetic diversity of North American and Old World Saccharum assessed by RAPD analysis. Gen. Res. Crop Evol., *44*: 235-240.

BURNQUIST, W.L. (1991): *Development and Application of Restriction Fragment Length Polymorphism Technology in Sugarcane (Saccharum SPP) Breeding*. Cornell University, Jan.

BUTTERFIELD, M., A., D'HONT, N., BERDING (2001): The sugarcane genome: a synthesis of current understanding, and lessons for breeding and biotechnology, Proc. S. Afr. Sug. Technol. Ass. Citeseer, pp. 1-5.

CHUTIMANITSAKUN, Y., R.W., NIPPER, A., CUESTA-MARCOS, L., CISTUÉ, A., COREY *et al.* (2011): Construction and application for QTL analysis of a Restriction Site Associated DNA (RAD) linkage map in barley. BMC genomics, *12*: 4.

COBURN, J.R., S.V., TEMNYKH, E., PAUL, S.R., MCCOUCH (2002): Design and Application of Microsatellite Marker Panels for Semiautomated Genotyping of Rice (L.). Crop Sci., *42*: 2092-2099.

COLLINS, N.C., F., TARDIEU, R., TUBEROSA (2008): Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiology, *147*: 469-486.

COOPER, M., F.A., VAN EEUWIJK, G.L., HAMMER, D.W., PODLICH, C., MESSINA (2009): Modeling QTL for complex traits: detection and context for plant breeding. Current Opinion in Plant Biology, *12*: 231-240.

CORDEIRO, G.M., R., CASU, C.L., MCINTYRE, J.M., MANNERS, R.J., HENRY (2001): Microsatellite markers from sugarcane (Saccharum spp.) ESTs cross transferable to erianthus and sorghum. Plant Sci., *160*: 1115-1123.

CORDEIRO, G.M. and R.J., HENRY (2001): Evaluation of microsatellites (Simple Sequence Repeats) as genetic markers in sugarcane. Proceedings of International Society of Sugar Cane Technologists, 627-629.

D'HONT, A., D., ISON, K., ALIX, C., ROUX, J.C., GLASZMANN (1998): Determination of basic chromosome numbers in the genus Saccharum by physical mapping of ribosomal RNA genes. Genome, *41*: 221-225.

D'HONT, A., P., RAO, P., FELDMANN, L., GRIVET, N., ISLAM-FARIDI *et al.* (1995): Identification and characterisation of sugarcane intergeneric hybrids, Saccharum officinarum x Erianthus arundinaceus, with molecular markers and DNA in situ hybridisation. TAG, *91*: 320-326.

DA SILVA, J.A. (2001): Preliminary analysis of microsatellite markers derived from sugarcane expressed sequence tags (ESTs). Genetics and Molecular Biology, *24*: 155-159.

DEVLIN, B., S.-A., BACANU, K., ROEDER (2004): Genomic control to the extreme. Nature Genetics, *36*: 1129-1130.

DIWAN, N. and P., CREGAN (1997): Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. TAG, *95*: 723-733.

DON, R., P., COX, B., WAINWRIGHT, K., BAKER, J., MATTICK (1991): 'Touchdown'PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res., *19*: 4008.

DOYLE, J. J. (1990): Isolation of plant DNA from fresh tissue. Focus, *12*: 13-15.

EDWARDS, A., A., CIVITELLO, H.A., HAMMOND, C.T., CASKEY (1991): DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. American J. Human Genetics, *49*: 746.

FALUSH, D., M., STEPHENS, J.K., PRITCHARD (2003): Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, *164*: 1567-1587.

FAROOQ, A., I., NASIR, Q., ALI, B., TABASSUM, T., HUSNAIN (2017): Identification and interrelationship of yield related traits through DNA fingerprinting in Zea mays. International Journal of Biology, Pharmacy and Allied Sciences, *6*: 1276-1303.

GLASZMANN, J., Y., LU, C., LANAUD (1990): Variation of nuclear ribosomal DNA in sugarcane. J. Genetics and Breeding, *44*: 191-197.

GOUY, M., Y., ROUSSELLE, A.T., CHANE, A., ANGLADE, S., ROYAERT *et al.* (2015): Genome wide association mapping of agro-morphological and disease resistance traits in sugarcane. Euphytica, *202*: 269-284.

GRIVET, L. and P., ARRUDA (2002): Sugarcane genomics: depicting the complex genome of an important tropical crop. Current Opinion in Plant Biology, *5*: 122-127.

GRIVET, L., A., D'HONT, D., ROQUES, P., FELDMANN, C., LANAUD *et al.* (1996): RFLP mapping in cultivated sugarcane (Saccharum spp.): genome organization in a highly polyploid and aneuploid interspecific hybrid. Genetics, *142*: 987-1000.

HAFEEZ, M., S., AHMAD, M., MAMOON-UR-RASHID, A., ALI, S., SALMAN *et al.* (2019): An overview of enhancing drought tolerance in cotton through manipulating stress resistance genes. Applied Ecology and Environmental Res., *17*: 7003-7025.

HAFEEZ, M., S., SADIQUE, S., HASSAN, M., SARWAR, B., RASHID *et al.* (2015): Physiological, morphological, biochemical and molecular basis of drought tolerance in cotton. International Journal of Biology, Pharmacy and Allied Sciences, *4*: 1091-1112.

HALL, D., C., TEGSTRÖM, P.K., INGVARSSON (2010): Using association mapping to dissect the genetic basis of complex traits in plants. Briefings in Functional Genomics: elp048.

HÉCTOR, E., F.D., PRADA, R., RODRIGUEZ (1995): Experimental evidence for the presence of different smut resistance mechanisms in sugarcane, pp. in *Proceedings XXI Congress of ISSCT, Bangkok (Thailand), 5-14 Mar 1992*.

HOARAU, J.-Y., L., GRIVET, B., OFFMANN, L.-M., RABOIN, J.-P., DIORFLAR *et al.* (2002): Genetic dissection of a modern sugarcane cultivar (Saccharum spp.). II. Detection of QTLs for yield components. TAG, *105*: 1027-1037.

HOFFMAN, S., P., FERNANDEZ-SALGUERO, F., GONZALEZ, H., MOHRENWEISER (1995): Organization and evolution of the cytochrome P450 CYP2A-2B-2F subfamily gene cluster on human chromosome 19. J. Mol. Evol., *41*: 894-900.

HOLLAND, J. B. (2007): Genetic architecture of complex traits in plants. Curr. Opinion Plant Biol., *10*: 156-161.

JEFFREYS, A. J., V., WILSON, S.L., THEIN (1985): Hypervariable'minisatellite' regions in human DNA. Nature, *314*: 67-73.

KORBIE, D.J. and J.S., MATTICK (2008): Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nature protocols, *3*: 1452-1456.

KORZUN, V. (2002): Use of molecular markers in cereal breeding. Cellular and molecular biology letters, *7*: 811-820.

LANDER, E.S. and N.J., SCHORK (1994): Genetic dissection of complex traits. Science-New York then Washington: 2037-2037.

LLOYD, H. and G., NAIDOO (1983): Chemical assay potentially suitable for determination of smut resistance of sugarcane cultivars. Plant disease, *67*: 1103-1105.

MACAULAY, M., L., RAMSAY, W., POWELL, R., WAUGH (2001): A representative, highly informative'genotyping set'of barley SSRs. TAG, *102:* 801-809.

MAJID, M.U., M.F., AWAN, K., FATIMA, M.S., TAHIR, Q., ALI *et al.* (2017): Genetic resources of chili pepper (Capsicum annuum L.) against Phytophthora capsici and their induction through various biotic and abiotic factors. Cytology and Genetics, *51*: 296-304.

MAURICIO, R. (2001): Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. Nature Reviews Genetics, *2*: 370-381.

MING, R., S.-C., LIU, Y.-R., LIN, J., DA SILVA, W., WILSON *et al.* (1998): Detailed alignment of Saccharum and Sorghum chromosomes: comparative organization of closely related diploid and polyploid genomes. Genetics, *150*: 1663-1682.

MING, R., Y., WANG, X., DRAYE, P., MOORE, J., IRVINE *et al.* (2002): Molecular dissection of complex traits in autopolyploids: mapping QTLs affecting sugar yield and related traits in sugarcane. TAG, *105*: 332-345.

NAZAR, Z., I., NASIR, H., KHAN, Q., ALI, T., HUSNAIN (2017): Genetic variability based response of sugarcane lines resistant and susceptible against whip smut. Pakistan J. Sci., *69*: 22.

NAZAR, Z. A. (2018): Genetic diversity studies for whip smut tolerance in sugarcane germplasm, pp. University of the Punjab, Lahore.

OCHIENG, J.W., A.W., MUIGAI, G.N., UDE (2007): Localizing genes using linkage disequilibrium in plants: integrating lessons from the medical genetics. African J. Biotech., *6*.

PAN, Y.-B. (2006): Highly polymorphic microsatellite DNA markers for sugarcane germplasm evaluation and variety identity testing. Sugar Tech., *8*: 246-256.

PAN, Y.-B., D., BURNER, B., LEGENDRE, M., GRISHAM, W., WHITE (2005): An assessment of the genetic diversity within a collection ofSaccharum spontaneum L. with RAPD-PCR. Gen. Res. Crop Evol., *51*: 895-903.

PAN, Y., D., BURNER, Q., WEI (2001): Developing species-specific DNA markers to assist in sugarcane breeding. Proc. int. soc. Sugar cane Technol., 337-342

PAN, Y., J., MILLER, R., SCHNELL, E., RICHARD JR, Q., WEI (2003): Application of microsatellite and RAPD fingerprints in Florida sugarcane variety program. Plant and Animal Genome Abstract XI Abstract Book W, *189*: 43.

PINTO, L., D., LEITE, T., FAVERO, M., PASTINA, A., GARCIA *et al.* (2011): Identification of microsatellites markers associated with yield components and quality parameters in sugarcane. International Sugar Journal: 140-144.

PIPERIDIS, G., G., TAYLOR, G., SMITH, D., HOGARTH (2001): A microsatellite marker database for fingerprinting sugarcane clones. International Society of Sugar Cane Technologists. Proceedings of the XXIV Congress, Brisbane, Australia, 17-21 September 2001. Volume 2. Australian Society of Sugar Cane Technologists: 632-633.

PONCE, M., P., ROBLES, J., MICOL (1999): High-throughput genetic mapping in Arabidopsis thaliana. Molecular and General Genetics, *261*: 408-415.

PRICE, A.L., N.J., PATTERSON, R.M., PLENGE, M.E., WEINBLATT, N.A., SHADICK *et al.* (2006): Principal components analysis corrects for stratification in genome-wide association studies. Nature genetics, *38*: 904-909.

PRITCHARD, J., X., WEN, D., FALUSH (2010): Documentation for structure software: Version 2.3. University of Chicago, Chicago, IL.

QAMAR, Z., S., RIAZ, I.A., NASIR, Q., ALI, T., HUSNAIN (2015): Transformation and transgenic expression studies of glyphosate tolerant and cane borer resistance genes in sugarcane (*Saccharum officinarum* L.). Molecular Plant Breeding, *6*.

QAMAR, Z., S., RIAZ, I.A., NASIR, Q., ALI, T., HUSNAIN (2017): Transformation and evaluation of different transgenic lines for Glyphosate tolerance and cane borer resistance genes in sugarcane (*Saccharum officinarum* L.). Cytology and Genetics, *51*: 401-412.

RABOIN, L., J., HOARAU, L., COSTET, H., TELISMART, J., GLASZMANN *et al.* (2003): Progress in genetic mapping of sugarcane smut resistance. Proc S Afr Sug Technol Ass. Citeseer: 134-141.

RHODES, M., R., STRAW, S., FERNANDO, A., EVANS, T., LACEY *et al.* (1998): A high-resolution microsatellite map of the mouse genome. Genome Research, *8*: 531-542.

RISCH, N. and K., MERIKANGAS (1996): The future of genetic studies of complex human diseases. Science, *273*: 1516-1517.

RISCH, N. J. (2000): Searching for genetic determinants in the new millennium. Nature, *405*: 847-856.

ROACH, B. (1969): Cytological studies in Saccharum. chromosome transmission interspecific and intergeneric crosses. Int. Soc. Sugar Cane Technol. Proc. Congr.

SALISU, I.B., A.A., SHAHID, A., YAQOOB, Q., ALI, K.S., BAJWA *et al.* (2017): Molecular approaches for high throughput detection and quantification of genetically modified crops: a review. Frontiers in Plant Sci., *8*: 1670.

SCHOCH, C.L., K.A., SEIFERT, S., HUHNDORF, V., ROBERT, J.L., SPOUGE *et al.* (2012): Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc. Nat. Ac. Sci., *109*: 6241-6246.

SELVI, A., N., NAIR, J., NOYER, N., SINGH, N., BALASUNDARAM *et al.* (2006): AFLP analysis of the phenetic organization and genetic diversity in the sugarcane complex, Saccharum and Erianthus. Gen. Res. Crop Evol., *53*: 831-842.

SILVA, D.C., J., DOS SANTOS, G.V. DE SOUZA BARBOSA, C., ALMEIDA (2012): DNA fingerprinting based on simple sequence repeat (SSR) markers in sugarcane clones from the breeding program RIDESA. African J. Biotech., *11*: 4722-4728.

TUBEROSA, R. and S., SALVI (2006): Genomics-based approaches to improve drought tolerance of crops. Trends in Plant Sci., *11*: 405-412.

WEBER, J.L. and P.E., MAY (1989): Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. American J. Human Genetics, *44*: 388.

WEI, X., P. A., JACKSON, S., HERMANN, A., KILIAN, K., HELLER-USZYNSKA *et al.* (2010): Simultaneously accounting for population structure, genotype by environment interaction, and spatial variation in marker-trait associations in sugarcane This article is one of a selection of papers from the conference "Exploiting Genome-wide Association in Oilseed Brassicas: a model for genetic improvement of major OECD crops for sustainable farming". Genome, *53*: 973-981.

WEI, X., P.A., JACKSON, C.L., MCINTYRE, K.S., AITKEN, B., CROFT (2006): Associations between DNA markers and resistance to diseases in sugarcane and effects of population substructure. TAG, *114*: 155-164.

YU, J. and E.S., BUCKLER (2006): Genetic association mapping and genome organization of maize. Curr. Opinion Biotech., *17*: 155-160.

YU, J., G., PRESSOIR, W.H., BRIGGS, I.V., BI, M., YAMASAKI *et al.* (2006): A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature genetics, *38*: 203-208

IDENTIFIKACIJA NOVIH QTL-OVA KOJI KONTROLIŠU OTPORNOST NA GAR I OSOBINE PRINOSA KOD ŠEĆERNE TRSKE

Zaheer Ahmad NAZAR[1*], Hafiz Muhammad Walayat Ali KHAN[2], Qurban ALI[1,3*]
Idrees Ahmad NASIR[1]

[1]Centar izuzetnih vrednosti u molekularnoj biologiji, Pendžab univerzitet, 87-Put zapadni kanal, Lahore, Pakistan
[2]Istraživački Institut za šećernu trsku, Ayub poljoprivredni istraživački centar, Faisalabad, Pakistan
[3]Institut za molekularnu biologiju i biotehnologiju, Univerzitet u Lahoru, Lahoru, Pakistan

Izvod

Prinos šećerne trske zavisi od različitih agro-morfoloških karakteristika, npr. obnavljanja šećera, broja stabljika, opsega trske, visine trske i otpornosti na gar. Identifikacija QTL-ova koji kontrolišu ove osobine može pomoći oplemenjivačima šećerne trske u odabiru linija šećerne trske uz pomoć marker asistirane selekcije za razne oplemenjivačke programe. Structure i TASSEL softver zasnovani na integraciji genotipskih i fenotipskih podataka 103 genotipa šećerne trske rezultirali su identifikacijom osamdeset sedam (87) visoko povezanih alela ($p \leq 0.05$), 34 alela sa otpornošću na gar: 27 alela za šećer: 13 alela sa težinu trske i 20 alela za obim i visinu trske. Fenotipska varijansa (vrednosti R^2) koja je objašnjena povezanim alelima iznosila je 3,1-24,6% za otpornost na gar, 2,67-22,5% za oporavak šećera, 2,81-23,46% za visinu trske, 2,9-14,34% mase trske i 1,75-12,8% za obim trske. Različite proporcije fenotipske varijanse objašnjene povezanim alelima ukazuju da su ove osobine kontrolisane aditivnim genetskim efektima više gena. Takođe pokazuje da su ove osobine istinske kvantitativne osobine. Štaviše, aleli koji prikazuju maksimalni stepen povezanosti za oporavak šećera (51-131), obim trske (82-184), visina trske (52-121), težina trske i otpornost na gar (51-145 i 51-146) mogli bi pomoći u marker asistiranoj selekciji za izbor linija šećerne trske za ove osobine.

Primljeno 10.X. 2018.
Odobreno 18. VIII. 2019.