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MOLECULAR ANALYSIS OF RUST RESISTANCE IN SELECTED SUGARCANE CULTIVARS

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ABSTRACT

The present study aimed at to identify rust resistance based on DNA marker linkage among commercially sugarcane **cultivar** of Pakistan. Therefore primer were designed by using reported sequences of Resistance Gene analogues DNA and also universal Random Amplified Polymorphic DNA marker were select/picked from literature, initially, In selected rust resistant and susceptible sugarcane cultivar The Resistance Gene analogous marker shown 52 trackable loci and 76.92% polymorphism and the size of loci are between 100-200bp.While the Random Amplified Polymorphic DNA primer shown 100% polymorphism and 50 trackable loci were recorded and the size of loci are between 200-3000bp, out of eleven primers seven primer produced fourteen specific loci in three sugarcane genotypes, and the Random Amplified Polymorphic DNA Polymerase chain Reaction is important use for identifying *Saccharum*. Spp. By exploiting bivariant data of two different kind of markers (RGA and RAPD), the homology tree was constructed using DNAMAN 5.2.2 software based on Nei's genetic distance and two major discrete groups with different minor groups were recorded for six genotypes having different disease behavior. This research work will be helpful for identifying resistance and susceptible sugarcane cultivars.

Key word: Rust, RAPD, RGA, PCR

INTRODUCTION

Sugarcane is on important commercial crop and belong to tribe Andropogoneae, genus Saccharum and family Poaceae (Daniels & Roach 1987). Sugarcane cultivated in all over the world approximately in 105 countries of the planet earth Pakistan is on 6th number an area and in production they are in 8th while in yield Pakistan is on 60th position. Although Pakistan is on 6th position in the world while it is lowest in production. Rendering to initial report which was estimated in MY 2016/17 the sugarcane production in Pakistan was record 71.5 million tons, this is aggregate to nine percent from prev-ious report 2015/16 which is based on official data. In Pakistan the sugarcane is mostly grown area away from Pakistan's major rivers, now the formers shifting and growing the sugarcane cultivars with high sugar content and recovery and continue to im-prove, increasing to an estimated 17 percent over the past 10 years. (Gain report 2017).

Now days the molecular marker provide a key rule for investigation of complex genome and Mendelian inheritance of sugarcane (Aitken et al., 2005). Due to molecular techniques and increasing use of molecular marker in sugarcane crops, now it has become possible to identify and locate the desirable genes and characters which is difficult in traditional methods of plant breading. (Casu et al., 2005). Sele-ction of phenotypes in crops and to facilitate traditional breading methods DNA Markers/ profiling are used on the basis of presence/ absence in a marker assisted selection (Ribaut et al., 1997). A comparative study was conducted to find out the association of molecular marker. I.e. (SSR) simple sequence repeats, (RAPD) Random amplified polymerphic DNA and (AFLP) amplified fragment length polymorphism PCR, with disease response in sugarcane (Asnaghi et al., 2004; Muhammad et al., 2010). Le Cunff et al., (2008) have reported map-based cloning and isolation of rust resistance gene Bru1in sugarcane French cultivar R 570.

Even though the marketable importance and economic value of sugarcane a very little research investent are developed in sugarcane due to complex genome makeup, but recently various molecular studies and techniques were conducted and developed in sugarcane breading (Grivet and Arruda, 2002). To achieve desire goals and explanation of gene pool is limited in traditional breading programmes, so now the selective breading practices has been used to achieves desire goals (Mariotti, 2002). But due to complexity of genome structure of sugarcane it is difficult to find out genes which are responsible for resistance to stress, it's most important to developed new varieties of sugarcane so therefore the researcher is used new approaches and techniques to develop new sugarcane cultivars which are more resistant to stress.

Recently, identification and isolation of disease resistance genes and sequence identification of resistance genes are most important goals and much study has been conducted (Martin *et al.*, 2003). Various resistance genes from monocotyledon and dicotyledon plants related species allocate common structural features although the single feature of these genes have also been identified (McDowell and Simon, 2006). Degenerate primers based on the

amino-acid sequence of domains nucleotide binding site (NBS) and a carboxy-terminal leucine rich repeat (LRR) domain have meanwhile allowed successful PCR amplification of several RGAs from various plant species with significant homology to known plant disease R genes. An important component for resistance mechanism is the presence of resistance genes in plants (Flor, 1956).

RAPD and RGAs identification is a powerful tool for the discovery of new closely linked DNA markers associated with diseases resistance loci for MAS (marker assisted selection) and cloning of these diseases resistance genes on map-based, moreover there are fifty four different Resistance gene analogues sequence (RGA) were isolated, characterized and were used in sugarcane and sorghum for identification of molecular marker which are linked to major diseases resistance loci (Mclntyre et al., 2005).

Molecular marker study for finding RGAs and RAPD marker using BSA techniques for identification of rust resistance and susceptible cultivars of Pakistan has not been conducted, so therefore this research study was conducted to find out rust resistant and susceptible cultivars of Pakistan based on molecular work

MATERIAL AND METHOD

Plant material: For present research work six rust resistance/ rust susceptible sugarcane cultivars grown in Punjab were collected (Table 1) from Shakarganj Research Institute (SRI), Jhang, Pakistan, selected cultivar was grown in research field department of genetics Hazara University Mansehra, Pakistan.

DNA Extraction: Genomic DNA were extracted from sugarcane, by modified CTAB method (Dolye & Dolye 1987) the protocol was described as follow.

Table: 1: Trait Specific Characteristic and Parentage of Sugarcane cultivars.

Cultivars	Parentage	Rust Responses
CSSG-668	81-N289C×P74-2005	R
SPSG-26	SP73-5368XSP70-1143	R
NSG-555	CP63-588 × MO/F	R
COJ-84	Co-1148XNot known	S
SPF-234	SP71-8210 × SP71-6180	S
CPSG-2453	MQ87-1215 × 86A3626	S

0.5 gm of plant tissue was crushed in liquid nitrogen with help of mortar and festal then the grinded tissue were transfer into fresh Eppendorf tube, and added 900µl 2% CTAB Buffer (CTAB 2gm, EDTA 1.6 gm, PVP 2.4 gm. Tris 2.4gm. Nacl 16.4 gm. dH2O 100 ml. 200ul of beta mercaptoethanol) and heated the solution) in each tube containing tissue, the samples were then incubate for 24 hours at 56^0C in incubator after incubation 200 µl of Chloroform: Iso Amyl Alcohol (24:1) were added to each sample, then the samples were centrifuge at 10000rpm for 20 mints After centrifugation the supernatant was transfer to fresh Eppendorf tube and added 500 µl of cold iso-propanol to each tube and centrifuge the samples for 15 mints at 8000 rpm After centrifugation the supernatant were discorded and the pellet was washed with 70% ethanol and centrifuge the samples at 8000 rpm for 5 mints After centrifugation the ethanol were discorded and the samples were kept at room temperature for overnight After drying 60 µl of dd H2O were added to each tube.

Gel electrophoresis of Extracted DNA: The quality of extracted DNA were checked on 1% agarose gel, the preparation of agarose gel consist of taking 98ml DH_2O and 1 gram of agarose powder, 2 ml of 50xTAE were mixed and heated for several minutes after heating the solution was kept at room temperature for few mints then added 25ul of ethidium bromide(10mg/ml), the solution was shacked well and transfer into gel tray were combs' were fixed, when the gel was solidified then comb were removed and gel was placed in gel tank consist of 1xTBE solution current were passed from the solution for several mints, the gel was then examined in UV apparatus and photograph was taken. (Fig.1.)

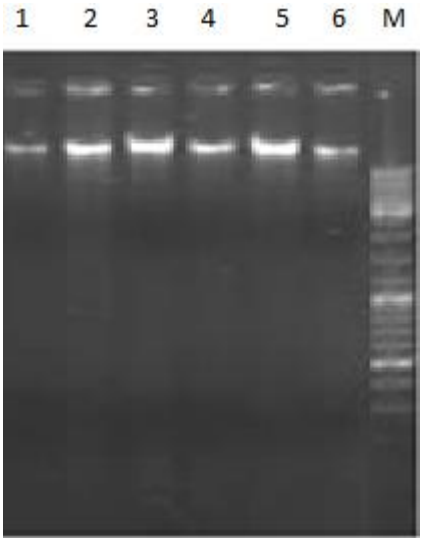

Fig. 1: gel Photograph of extracted DNA from sugar cane genotypes

RGA Primers: Important features of RGA is a specific single primer use to amplify the targeted loci without knowing the target loci in template DNA, for present nine RGA marker were design although long chain marker was screen out in selected sugarcane genotypes for Rust resistance/ rust susceptible and those markers were selected for

further study which shown bands and give polymerphism (Table 2).

Table 2: List of RGA primer used in the present study

Primer name	Sequence	GC content (%)	Tm (0C)
K01F	GGGGAAGACGACTTTGGC	52.2	56.6
K01R	GGCCACCACACATACGGAC	55.4	69.2
K11F	CGATGTCTGGCCGAACGCT	55.4	59.2
K11R	CTGTCCCTCAATTCATCCAAG	46.8	55.9
K15F	GACTACTCTTGCGAGGGC	52.6	56.6
K15R	GCTAGGGGGAGGCCATCAC	54.9	61.4
K35F	GCGTCGGTAAGACCACACTC	53.8	50.3
K35R	CCAATGATCTACAGTAAGTCAG	55.9	56.3
K41F	GAAGAGGGCGCAGTAGAT	50.3	54.3
K41R	GTGTGGCAGTCAGACACATGG	56.3	59.8

RAPD decamer Primers: The importance of RAPD single 10-oligonuclitide random primer is to target loci without knowing prior knowledge of the targeted loci. Basic criteria suggested by Williams et al. (1990) were used and a list of Random Amplified DNA markers were synthesized and after screening in Genome of selected genotype of sugarcane those primers were selected which are shown polymorphic band. (Table.3).

Table 3: List of RAPD primer used in the present study (provided by BIO NEER)

Primer Name	Sequence	TB	PB	MB	PP	Range of bp
K01F	GGGGAAGACGACTTTGGC	3	3	0	100	500-800
K01R	GGCCACCACACATACGGAC	6	5	1	83.3	100-800
K11F	CGATGTCTGGCCGAACGCT	6	5	1	83.3	400-1000
K11R	CTGTCCCTCAATTCATCCAAG	6	4	2	66.6	150-800
K15F	GACTACTCTTGCGAGGGC	8	7	1	87.5	600-1300
K35F	GCGTCGGTAAGACCACACTC	6	6	0	100	400-1500
K35R	CCAATGATCTACAGTAAGTCAG	4	4	0	100	1000-2000
K41F	GAAGAGGGCGCAGTAGAT	6	3	3	50	300-1000
K41R	GTGTGGCAGTCAGACACATGG	7	3	4	42.8	150-900
Total		52				

PCR components and amplification of RGAs and RAPD Markers: Various PCR condition were amplified for RGAs and RAPD primers in genomic DNA of selected cultivars of sugarcane, 25ul of PCR mixture was prepared in 0.2ml of PCR tube, the mixture consist of 10x buffer, 2.5 ul, $MgCl_2$ 3ul, dNTPs 3ul, Primer 2ul, *Taq*-polymerase 0.5ul, template DNA 2ul and PCR water 11ul. The PCR cond-ition for RGAs are initial denaturation 94^{o}C for 5 mints, Denaturation 94^{0}C for I mints, annealing 37 ^{0}C for 1 mint, elongation 72^{0}C for 1 mints number of cycle 40 and final elongation 72^{0}C for 10 mints, while the PCR condition for RAPD are same as for RGAs except annealing which are 34^{0}C for 1 mints.

Gel electrophoresis of PCR amplified product of RGA and RAPD Markers: The PCR amplified product were further confirmed by 1.5% gel and the band size was compared with 1Kb DNA marker. The gel was checked in UV apparatus and photograph was taken. As shown in (Fig. 2 and Fig. 3)

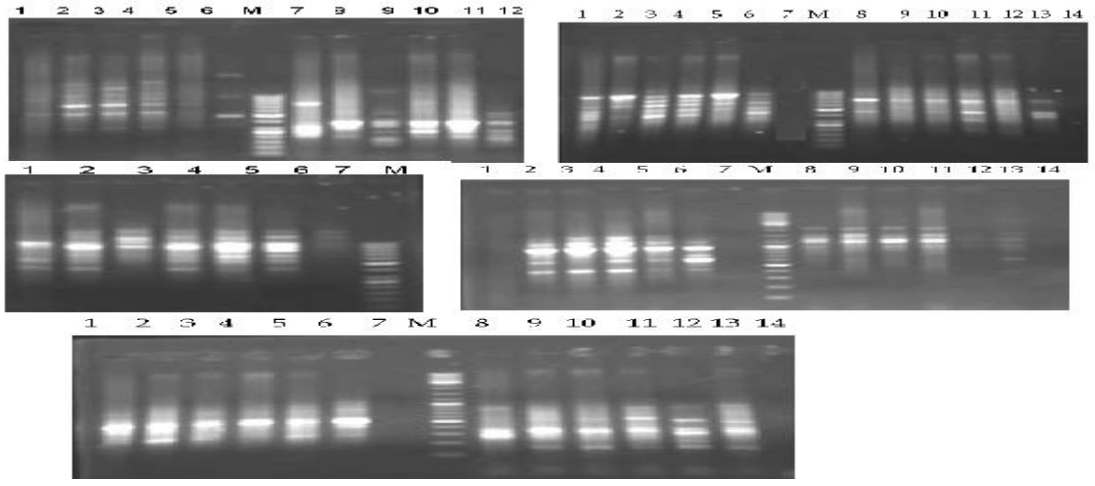

Fig. 2. Gel photograph of PCR amplified product six RGA primers M= Marker ladder mix (Fermentas; cat# SM0373), Lanes: 1-3 represent rust resistant cultivars of sugarcane CSSG-668, NSG-555 and SPSG-26 respectively and Lanes: 4-7 represent rust susceptible cultivars of sugarcane Coj-84, SPF-234, CPSG 2453 and–Ve (water) respectively. Similarly, lanes 8-10 represent rust resistant cultivars of sugarcane CSSG-668, NSG-555 and SPSG-26 respectively and lanes 11-14 represent Coj-84, SPF-234, CPSG 2453 and –Ve (water) respectively.

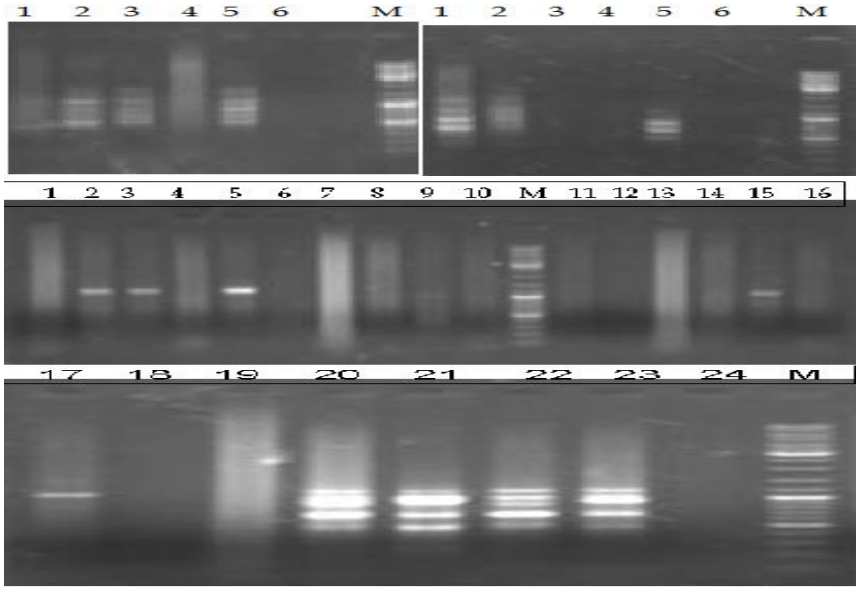

Fig. 3. Agarose gel photograph of PCR amplified product of RAPD marker M= Marker ladder mix (Fermentas; cat# SM0331), Lanes: 1-3 represent rust resistant cultivars of sugarcane CSSG-668, NSG-555 and SPSG-26 respectively and Lanes: 4-6 represent rust susceptible cultivars of sugarcane Coj-84, SPF-234 and CPSG 2453 respectively 7-9 and 13-15 represent rust resistant cultivars of sugarcane CSSG-668, NSG-555 and SPSG-26 respectively and Lanes: 4-6, 10-12 represent rust susceptible cultivars of sugarcane CoJ-84, SPF-234 and CPSG-2453 respectively and lane 16 represents CoJ-84. Lanes: 19-21 represent rust resistant cultivars of sugarcane CSSG-668, NSG-555 and SPSG-26 respectively and Lanes: 22-23 represent rust susceptible cultivars of sugarcane Coj-84, SPF-234 and CPSG-2453 respectively

Data analysis: The PCR amplified product of all RGA and RAPD primer were analysed, the amplification of RGA and RAPD PCR was done twice. The band size of amplified RAPD and RGA primer were compared with standard known DNA Marker. The fragment presence and absence were recorded in the samples as either 0 (absent) or 1 (presence) for further process the binary data was recorded on excel sheet. The DNAMAN 5.2.2.0 software were used to draw dendogram based on UPGMA.

RESULT AND DISCUSSION

Amplification of RGA primers: The total genomic DNA was isolated from six sugarcane rust susceptible/rust resistant genotypes were used for RGA PCR amplification, nine primers were selected and polymerase chain reaction was carried out 52 loci were recorded and amplified in genomic DNA of selected sugarcane genotypes for further analysis of Data the DNAMAN 5.2.2.0 software were used on the basis of some parameters i.e. monomorphic bands (MP), polymorphic Bands (PP), total Bands(TB) and rust resistance and rust susceptible (R and S) specificity

Polymorphism: To distinguish the genetic basis of trait in crops the DNA marker is play important rule and the Polymorphism is the important application of DNA markers. In our DNA samples the selected nine RGA marker produced 52 loci with the mean of 5.77 loci per primer (Table. 4). Approximately Three to eight loci with size ranging from 100 to 200bp were amplified and generated seventy-six points ninety-two percentage of polymorphism. And the observed polymorphic loci could be used to evaluate rust resistance and rust susceptible sugarcane cultivars on the basis of presence and absence of specific locus. Least number of bands/loci i.e. pri-mer K01F amplified three bands while primer K15-F amplified eight bands/loci. (Table 4).

Table. 4. The detail of Polymorphic and Monomorphic bands produced by nine long chain RGA Primers in six Sugarcane Genotypes

Primer name	Sequence	Bands/ Loci(bp)	Specific to	R/S
K01F	GGGGAAGACGACTTTGGC	500	CPSG-2453	S
K01R	GGCCACCACACATACGGAC	100	NSG-555	R
K11F	CGATGTCTGGCCGAACGCT	800	CSSG-668	R
K11R	CTGTCCCTCAATTCATCCAAG	150	COJ-84	S
K35F	GCGTCGGTAAGACCACACTC	350	COJ-84	S
K35R	CCAATGATCTACAGTAAGTCAG	400	SPF-234	S
K15F	GACTACTCTTGCGAGGGC	1100	NSG-555	R
K41F	GAAGAGGGCGCAGTAGAT	1000	CPSG-2453	S
K41R	GTGTGGCAGTCAGACACATGG	250	SPF-234	S

Specificity of RGA marker: In present research work we have three rust resistance genotypes as R, while three rust susceptible genotypes as S (Table 1). In selected RGA primers a few primers produced specific loci in genomic DNA as shown (table 4). Nine loci were recorded and produced by six RGA marker with size ranging from 100-1100bp.

Primer K01F produced 500bp band, in the rust susceptible cultivar CPSG-2453. Primer K01R produced 100bp and 800bp, in rust resistant cultivars NSG-555 and CSSG-668 respectively. Primer K11R produced 3 loci i.e. 150bp and 350bp, in rust susceptible cultivars COJ-84 and 400 bands in SPF-234. Primer K15F produced 1100bp band, in rust resistant cultivar NSG-555. Primer K41F also produced 1000bp band, in rust susceptible cultivar CPSG-2453. Primer K41R generated 250bp locus against SPF-234 (Table 5).

Table 5: Selective RGA primers produced specific loci against Rust Resistant (R) and Rust susceptible (S) groups of sugarcane cultivars.

Primer name	Sequence	GC conten (%)	Tm (0C)
B-20	GGACCCTTAC	60.0%	32.0
I-16	TCTCCGCCCT	70.0%	34.0
M-20	AGGTCTTGGG	60.0%	32.0
G-O1	CTACGGAGGA	60.0%	32.0
E-13	CCCGATTCGG	70.0%	34.0
D-11	AGCGCCATTG	60.0%	32.0
K-15	CTCCTGCCAA	60%	32.0
E-03	CCAGATGCAC	60%	32.0
C-18	TGAGTGGGTG	60%	32.0
J-13	CCACACTACC	60%	32.0
H-07	CTGCATCGCA	60%	32.0

Abbreviations: TB = Total Bands; MB = Monomorphic Bands; PB = Polymorphic Bands; PP = % of polymorphism. Overall total loci per primer = 5.77: Overall P.B loci per primer = 4.44

Amplification of RAPD primers: Eleven RAPD primer were selected from the long chain RAPD markers screening in genomic DNA of selected sugarcane genotypes. Fifty loci were produced by eleven RAPD marker and the result were further analysed by DNAMAN 5.2.2.0 software on the basis of some parameters i.e. total bands (TB), polymorphic bands (PB), monomorphic bands (MP), percentage of polymorphism (PP) and rust resistant/susceptible cultivar (R and S) specificity.

Polymorphism: Polymorphism is the main application of molecular marker which are used to distinguish the genetic basis of trait in crops. The selected eleven RAPD primer produced fifty loci in our sample with mean of 4.55 loci per primer (Table 6.). From one to eight loci with size range between 200-3000bp was amplified with 100% polymorphic loci in selected sugarcane genotypes and this polymorphic locus are could be used for identification of rust resistance and rust susceptible on the basis of presence and absence of specific locus. The least number of bands/loci i.e. one was amplified with primers E13, while maximum number of loci i.e. 8 was amplified with primer B-20 and M-20 (Table 6).

Table 6. The detail of Polymorphic and Monomorphic bands produced by eleven RAPD Primers in six Sugarcane Genotypes

Primer Name	Sequence	TB	PB	MB	PP	Range of bp
B-20	GGACCCTTAC	8	8	0	100	500-2000
M-20	AGGTCTTGGG	8	8	0	100	600-2500
G-01	CTACGGAGGA	4	4	0	100	500-1000
E-13	CCCGATTCGG	1	1	0	100	600
D-11	AGCGCCATTG	3	3	0	100	600-3000
I-16	TCTCCGCCCT	6	6	0	100	400-1500
H-07	CTGCATCGCA	5	5	0	100	500-2000
K-15	CTCCTGCCAA	3	3	0	100	800-1300
E-03	CCAGATGCAC	4	4	0	100	300-1000
C-18	TGAGTGGGTG	2	2	0	100	900-1000
J-13	CCACACTACC	6	6	0	100	200-1200
Total		50				

Abbreviations: TB = Total Bands; MB = Monomorphic Bands; PB = Polymorphic Bands; PP = % of polymorphism. Overall total loci per primer = 4.55: Overall P.B loci per primer = 4.55

Specificity of RAPD marker: In present research work we have three rust resistance genotypes as R, while three rust susceptible genotypes as S, (Table 7). In selected RAPD primers a few primers produced specific loci in genomic DNA as shown (Table 7). Fourteen loci were recorded and produced by seven RAPD marker with size ranging from 400 -3000bp. Primer B-20 produced 900bp and 800bp band, in the rust susceptible cultivar COJ-84 and CPSG-2453 respectively. Primer M-20 produced 2500bp, 1500bp, 800bp, 600bp band, in rust resistant cultivar CSSG-668 and similarly M-20 produced 900bp band, in rust susceptible cultivar COJ-84. Primer E-13 produced 600bp band, in rust susceptible cultivar COJ-84. Primer D-11 produced 3000bp band, in rust resistant cultivar CSSG-668. Primer D-11 also produced 700bp band, in rust susceptible cultivar CPSG-2453. Primer I-16 and H-07 generated 400bp and 500bp loci against CSS-668 respectively (Table 7).

Table 7: Selective RAPD primers produced specific loci against Rust Resistant (R) and Rust susceptible (S) groups of sugarcane cultivars.

Primer name	Sequence	Bands/ Loci(bp)	Specific to	R/S
B-20	GGACCCTTAC	900	COJ-84	S
B-20	GGACCCTTAC	800	CPSG-2453	S
M-20	AGGTCTTGGG	2500	CSSG-668	R
M-20	AGGTCTTGGG	1500	CSSG-668	R
M-20	AGGTCTTGGG	1100	CSSG-668	R
M-20	AGGTCTTGGG	900	COJ-84	S
M-20	AGGTCTTGGG	800	CSSG-668	R
M-20	AGGTCTTGGG	600	CSSG-668	R
E-13	CCCGATTCGG	600	COJ-84	S
D-11	AGCGCCATTG	3000	CSSG-668	R
D-11	AGCGCCATTG	700	CPSG-2453	S
I-16	TCTCCGCCCT	400	CSSG-668	R
H-07	CTGCATCGCA	500	CSSG-668	R
H-07	CTGCATCGCA	2000	CSSG-668	R

Cluster analysis: For specific amplification of RGA and RAPD primer was recorded and scored as for presence of band (1 scored) while for absence of band (0 scored), while the data were further analyzed by using statistical software DNAMAN 5.2.2.0. Dendogram was constructed on the base of divergence matrix and (NJ) neighbor joining method, the dendogram showing the fifty-two binary characters of the amplicon absence-presence, and similarities between the six objects as determined with eleven primers (Saitou and Nei, 1987).

The six sugarcane cultivars classified into two major group on the base of 57% homology by the Nei's (1978) genetic distance based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Moreover, the two major group are further divided the group I are classified into Ia group and Ib group while the major group II are divided into IIa group and IIb group (Figure 4).

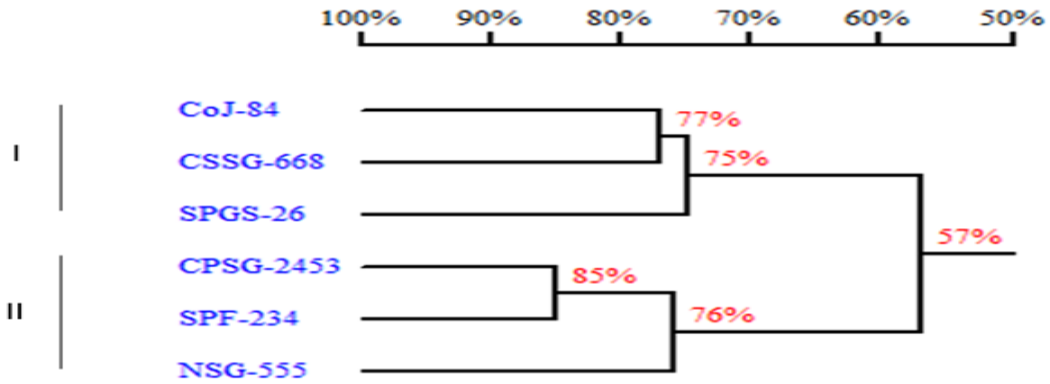

Fig. 4: Homology dendrogram constructed showing the genetic similarity among six sugarcane cultivars from Pakistan by DNAMAN 5.2.2.0 version based on Nei's (1978) identities/distances.

The group Ia included the rust susceptible cultivars CoJ-84 and rust resistant cultivar CSSG-668 having 77% homology. The subgroup Ib included the rust resistant cultivar SPSG-26 with sharing 75% homology. The subgroup IIa included the rust susceptible cultivars CPSG-2453 and SPF-234 having 85% homology. The subgroup IIb included rust resistant cultivar NSG-555 with sharing 76% homology. The homology 56% between group I and II was observed during present study of RAPD primers (Fig 5).

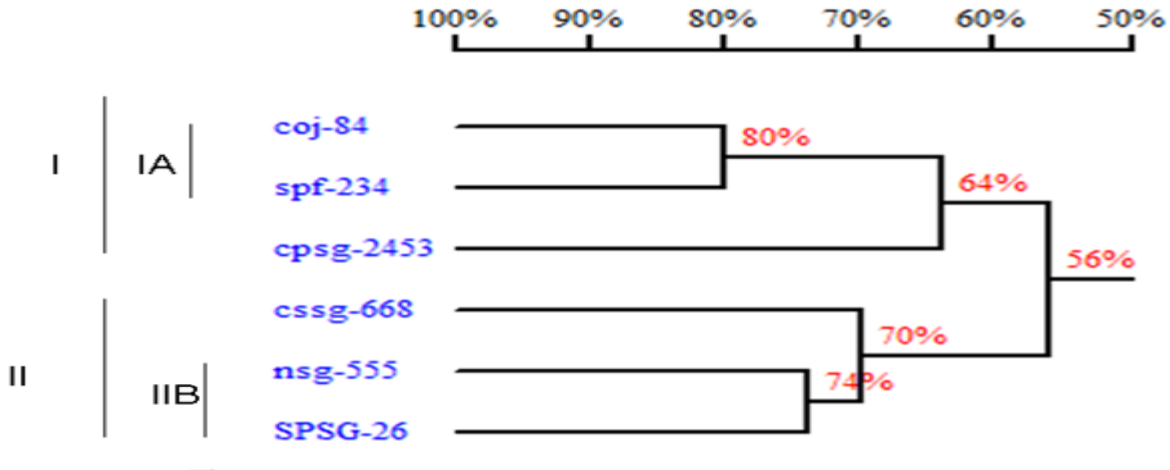

Fig. 5. Homology dendrogram constructed showing the genetic similarity among six sugarcane cultivars from Pakistan by DNAMAN 5.2.2.0 version based on Nei's (1978) identities/distances.

The group Ia included the rust susceptible cultivars SPF-234 and CoJ-84 having 80% homology. The subgroup Ib included the rust susceptible cultivar CPSG-2453 with sharing 64% homology. The subgroup IIa included the rust resistant cultivar CSSG-668 having 70% homology with IIb. The subgroup IIb included two rust resistant cultivar NSG-555 and SPSG-26 having 74% homology with subgroup IIa. The homology 56% between group I and II was observed during present study. Different pattern were showed according the relationship of genetic distance and polymorphisms of RGAs and RAPD primers by genetic diversity among these six sugarcane cultivars.

DISCUSSION

Sugarcane is on important commercial crop and belong to family Poaceae, genus Saccharum and tribe Andropogoneae (Daniels & Roach 1987). Sugarcane cultivated in all over the world approximately in 105 countries of the planet earth Pakistan is on 6th number an area and in production they are in 8th while in yield Pakistan is on 60th position. Although Pakistan is on 6th position in the world while it is lowest in production. Rendering to initial report which was estimated in MY 2016/17 the sugarcane production in Pakistan was record 71.5 million tons, this is aggregate to nine percent from previous report 2015/16 which is based on official data. In Pakistan the sugarcane is mostly grown area away from Pakistan's major rivers, now the formers shifting and growing the sugarcane cultivars with high sugar content and recovery and continue to improve, increasing to an estimated 17 percent over the past 10 years. (Gain report 2017). Ratoon crop of sugarcane is considered economical for the farming communities of Pakistan because production cost is 25-30% less than plant crop along with saving of seed material (Aamer et al., 2017) Isolation and identification of region in genomic DNA or gene related with desirable trait through Marker associated assortment using molecular marker technology have important impact (Sial et al., 2006) DNA marker and the progress in DNA marker technology have remarkable value in Positional cloning of gene and phylogenetic analysis (Karim et al., 2004)

Now days the molecular marker provide a key rule for investigation of complex genome and Mendelian inheritance of sugarcane (Aitken et al., 2005). To identify and locate the gene of desirable characters are now possible and easy due to navel molecular techniques and molecular marker and their use in sugarcane crops while it is difficult in traditional plant breeding techniques. (Casu et al., 2005). Selection of phenotypes in crops and to facilitate traditional breading methods DNA Markers/profiling are used based on presence/ absence in a marker assisted selection (Ribaut et al., 1997). Many Research study were conducted to find out the association in molecular markers i.e. Random Ampified Polymorphic DNA, Amplified Fragment Length Polymorphism PCR and (SSR) Simple Sequence Repeats in sugarcane response to abiotic and biotic stress (Asnaghi et al., 2004; Muhammad et al., 2010). In French sugarcane cultivars R570 Le Cunffet al., (2008) have reported isolation and map base cloning of rust resistance gene Bru1.

In present research work initially sixty molecular markers were used in the genomic DNA of selected sugarcane cultivars, after screening the elven primers were selected on the basis of reproducibility and polymorphism, fifty trackable loci with 100% polymorphisom and range of 200-3000bp were recorded to distinguish rust resistant and rust susceptible sugarcane cultivars, in eleven primers seven primer generated fourteen specific loci in three sugarcane cultivars, which is a potential use of RAPD PCR for identifying Saccharum spp. Hybrids and clones. Two loci with range of 900bp and 800bp were produced by primer B-20 in the cultivar COJ-84 and CPSG-2453 which are rust susceptible. Six loci with range of 2500bp, 1500bp, 1100bp, 800bp and 600bp are produced by Primer M-20 in cultivars CSSG-668 which are resistant and also in rust susceptible cultivars COJ-84 with range of 900bp. In rust susceptible cultivars COJ-84 the primer E-13 produced only locus of 600bp.two loci are produced by primer D-11 with range of 3000bp in cultivar CSSG-668 which are rust resistant and in rust susceptible cultivar CPSG-2453 700bp loci are produced. Only one locus with range of 800bp in rust susceptible cultivar CPSG-2453 were produced by primer I-16 while in rust resistant cultivar CSSG-668 primer H-07 produced two loci with range of 2000bp and 500bp.

In the genomic DNA of selected sugarcane cultivars nine long chain RGAs Marker were used, after initial study of these RGAs Marker in genomic DNA all the nine markers were selected basis on of reproducibility and polymorphism. Fifty-two trackable loci were recorded by these-marker to distinguish the rust resistant and rust susceptible cultivars of sugarcane with 76.92% polymorphism and with range of 100-2000bp. Six primer in nine RGAs primer produced nine specific loci in five sugarcane cultivars, and it is a potential use of RGA-PCR to identify Saccharum spp. Clones and hybrid. Primer K01F produced one locus i.e. 500bp in the rust susceptible cultivar CPSG-2453. Primer K01R had generated 2 loci i.e. 100bp in rust resistant cultivars NSG-555 and 800bp in rust resistant cultivars CSSG-668. Primer K11R produced 3 loci i.e. 150bp in rust susceptible cultivar COJ-84, 350bp also in rust susceptible cultivar COJ-84 and 400bp in SPF-234. Primer K15F produced one locus i.e. 1100bp in rust resistant cultivar NSG-555. Primer K41F produced only one locus i.e. 1000bp in rust susceptible cultivar CPSG-2453. In rust susceptible cultivar SPF-234 the primer K41R produced one locus with range of 250bp. Software DNAMAN 5.2.2. Was used for constructing homology tree based on Nei's genetic distance and two major discrete groups with different minor groups were recorded for six genotypes having different disease behavior

Conclusion

The selected sugarcane cultivars for this study was highly rust resistant and rust susceptible. In sixty RAPD primers eleven primers showed 100% polymorphism while six RAPD primers showed genotypes specificity to distinguish cultivars to rust resistant and susceptibility. Moreover, in present study the nine-long chain RGA primers showed 76.92% polymorphism. Six RGA markers showed genotype specificity to identify rust resistant and susceptible cultivars. Among the selected six sugarcane cultivars the cluster analysis showed the remarkable relationship with a specific character i.e. rust resistant and rust susceptibility.

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