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Sources of genetic resistance in maize to *Fusarium* stalk rot and their variations at molecular level

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Abstract: Identifying the resistant genotypes is one of the vital strategies to control *Fusarium* stalk rot disease in maize. Fifty accessions of maize germplasm were evaluated for resistance to stalk rot caused by *Fusarium verticillioides* at the Maize and Millet Research Institute, Yousafwala, Pakistan, during the spring and autumn of 2010, and their genetic variations were also studied at the molecular level to avoid environmental effects in the Department of Medicinal Chemistry, University of Minnesota, USA. Disease severity was calculated in the field using a severity scale (SS) and direct estimation of stalk discoloration (SD) by artificial inoculation method. Both SS and SD results were highly significant ($P < 0.01$) in both seasons and maize accessions significantly differed in SS ($P < 0.01$) and SD ($P < 0.01$). Disease assessments based on SS and SD were significantly correlated ($r = 0.983, 0.974$; $P < 0.01$) in spring and autumn, respectively. Two genotypes, EL7 and Y11, showed highly resistant response in both growing seasons. Variations among the lines were also exploited by using 14 simple sequence repeat primer sets. A total of 535 alleles were amplified with an average of 10.7 alleles per genotype. The highest number of alleles per locus was seen with the xp-umc1186 primer, exhibiting 128 alleles with an average of 2.5 alleles per genotype. Resistance genotypes can be utilized in hybridization programs for the improvement of local high-yielding varieties.

Key words: *Fusarium* stalk rot, maize, resistance, alleles

1. Introduction

Maize germplasm has the potential to exhibit many genetic variations that help it to survive in a wide range of environments (Paterniani et al., 2000). Genetic diversity is desirable in all breeding programs and landraces are an important source for tolerant or resistant genes against biotic and abiotic stresses (Araújo and Nass, 2002). Landraces are continuously replaced by high-yielding cultivars having narrower genetic variations (Pollack, 2003), while genetic enhancement of germplasm pools is only possible by exploring wider genetic resources (Rajaram and Van Ginkel 1996).

Stalk rot disease of maize, caused by *Fusarium verticillioides*, is one of the most widespread and damaging diseases throughout the world, resulting in serious yield losses (Shurtleff, 1980; Burgess et al., 1981; Neish et al., 1983; Afolabi et al., 2008). In Pakistan, this disease causes most destructive yield losses in corn (Ahmad et al., 2006). The disease is usually differentiated by disintegration of the pith tissue at or near the base of the stalk and is linked with senescence of stalk pith cells. Stalk rot promotes early maturity and dryness, which causes plant lodging and reduction in grain yield (Sibale et al., 1992).

Use of resistant maize cultivars is thought to be a vital approach to minimize the damage associated with *Fusarium* stalk rot (Munkvold, 1996). Artificial inoculation method for the field evaluation of the breeding materials is the best strategy (Hooker and Draganic, 1980), but it requires a long period of time.

Under such circumstances, molecular techniques have solved the problem of exploring the large amount of germplasm in a short time frame. Molecular markers can provide more detailed information about genetic resources (Popi et al., 2000; Shah et al., 2000; Xia and Achar, 2001), and their great potential helps breeders to identify more reliable combinations for the desired trait in shorter time frames instead for waiting the whole growing season (Borner et al., 2000). Simple sequence repeats (SSRs) are the most suitable markers (Hammer, 2000; Li et al., 2000) and are intensively utilized to depict genetic diversity in advanced breeding materials (Dreisigacker et al., 2004). These microsatellites, or SSRs, are PCR-based

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DNA markers that exhibit codominant inheritance and are consistently scattered throughout the genome (Senior et al., 1998). SSRs are simple in use and require low amounts of genomic DNA for amplification (Roder et al., 2002). DNA-based technologies have been recognized as a vital source for genetic diversity studies. However, marker-based results may lead to inaccurate findings if not validated with field trial data. Therefore, the specific objectives of this study were to evaluate maize germplasm diversity for resistance to *Fusarium* stalk rot under artificial infection, to evaluate disease assessments based on a severity rating scale (SS) and direct estimation of stalk discoloration (SD), and to study genetic variations among the lines with SSRs markers.

2. Materials and methods

Fifty germplasm accessions were collected from the Maize and Millet Research Institute and planted in its field to evaluate their resistance to *Fusarium verticillioides* during two growing seasons (spring and autumn) of 2010. The pathogen was separated and inoculated as described by Qureshi et al. (2015). The experimental accessions were sown in a randomized complete block design (RCBD) with three replications having row to row distances of 75 cm and plant to plant distances of 20 cm. Standard agronomic practices were adopted. SS and SD were calculated as elaborated by Qureshi et al. (2015). In February 2014, these lines were brought to Dr Shier's laboratory in the Department of Medicinal Chemistry of the University of Minnesota, USA, to study genetic variations among the lines using DNA-based makers (SSRs). The plants were grown in the environmental room of the department using the paper towel method.

2.1. DNA extraction

Fresh leaves of 8–10 cm in length were collected from the plants and stored at –80 °C in the laboratory. Isolation of DNA was performed with Sigma's GenElute Plant Genomic DNA Miniprep Kit. Three to four leaves were put in a mortar and pestle and liquid nitrogen was added to it. Leaves were then crushed to a fine powder. The powder (100 mg) was transferred to a microcentrifuge tube and 350 µL of lysis solution (A) or 50 µL of lysis solution (B) was added to the tube along with 15 µL of RNase enzyme. Material in tube was mixed thoroughly by vortexing and inverting until a homogeneous mixture was formed. Eppendorf tubes were then placed in a water bath at 65 °C for 10 min, and 130 µL of precipitation solution was added to mixture. Tubes were centrifuged at 13,000 relative centrifugal force (RCF) for 5 min to pellet the cellular debris, proteins, and polysaccharides. Liquid from the Eppendorf tube was taken very carefully into the GenElute filtration column and again centrifuged for 1 min at the speed of 21,000 RCF. This removed the cellular debris not removed in the previous step. The filtration column was discarded and collection tubes were retained, and 700 µL of binding solution was added to collection tubes and mixed thoroughly by inversion. GenElute Miniprep Binding Column tubes were then taken and 500 µL of column preparation solution was added. These tubes were centrifuged at 12,000 RCF for 1 min. Flow-through liquid was discarded and 700 µL from the binding solution (previously collected in collection tubes) was added carefully to binding column tubes and centrifuged at 21,000 RCF for 1 min. Flow-through liquid was again discarded and collection tubes were again retained. The same step was repeated with the remaining binding solution. The binding column was placed into a fresh 2-mL collection tube and 500 µL of the diluted wash solution was applied. Collection tubes were centrifuged at maximum for 1 min. Flow-through liquid was again discarded and collection tubes were retained. The same step was repeated again, except that centrifugation was performed for 3 min. In the last step, the binding column was transferred to a fresh 2-mL collection tube, 100 µL of prewarmed (65 °C) elute solution was applied to the column, and the tube was centrifuged at maximum speed for 1 min. This process was repeated again, and then the entire DNA was collected from the liquid of the collection tube and stored at –20 °C in a refrigerator. Concentration of DNA was checked by putting 2 µL of extracted liquid on a U-cuvette G 1.0 and placing it into a Biophotometer.

2.2. SSR primers and PCR amplification

Fourteen pairs of microsatellite/STS primers (p-umc1715, xp-umc1154, p-umc1354, xp-umc1354, xp-mmc0411, p-umc1472, xp-umc1184, xp-umc1824, xp-umc1984, xp-umc1186, xp-umc1325, xp-umc1586, xp-umc2281 (direct and reverse)) obtained from the BioMedical Genomics Center at the University of Minnesota were exploited to find out the genetic variations among the selected lines. PCR amplifications of the SSRs were executed in 50-µL reactions containing 20 ng of genomic DNA, 1 µL of each primer (forward and reverse), 1 µL of dNTPs, 5 µL of 10X buffer for Taq polymerase, and 2.5 U of Taq DNA polymerase; the remaining volume was completed with the addition of autoclave water. Amplification was carried out in a 1000 Touch Thermocycler programmed for a first denaturation step of 4 min at 94 °C (initial denaturation), followed by 40 cycles of 1 min at 94 °C (denaturation), 54 °C for 1 min (primer annealing), and 72 °C for 120 s (extension).

2.3. Gel electrophoresis and imaging

Three grams of agarose was added to 0.5X TBE solutions to form 1.5% agarose gel. Ethidium bromide solution was added to the agarose gel for staining purpose. About 3 µL of the PCR products were loaded after mixing with 7 µL of gel loading dye (bromophenol blue) in a reaction volume

of 10 µL. PCR products of SSRs were run over 1.5% agarose/TBE gels for 1 h by maintaining the current below 50 A. After electrophoresis the gel was isolated from the plates and placed on a UV tray for imaging with the Gel Doc EZ Imager apparatus using Image Lab 4.1 software provided by Bio-Rad.

2.4. Statistical analysis and scoring of SSR data

Data on SS and SD of field trials were evaluated to separate accessions into various levels of stalk rot resistance. Data recorded about SS and SD in the RCBD in both growing seasons were analyzed using ASSISTAT software (Silva and Azevedo, 2009). In the statistical model, it was checked whether accessions differed from each other in their response to stalk rot disease through analysis of variance technique as described by Steel et al. (1997). Differences between accessions were further determined using Tukey's test. Simple linear correlation was used to determine the relationship between SS and SD. Linear correlations were performed separately for each season using ASSISTAT software. In the case of the molecular technique, every visualized band was scored as a single locus/allele for the analysis. Presence (1) or absence (0) of the band was considered for the scoring of the locus in the genotypes. Genetic distances were computed by using the unweighted pair group method with arithmetic mean (UPGMA) from a bivariate (1-0) data matrix (Nei and Li, 1979):

$$GD = 1 - dxy/dx + dy - dxy,$$

where GD is the genetic distance between two genotypes, dxy is the total number of common loci in two genotypes, dx is the total number of loci (bands) in one genotype, and dy is the total number of loci (bands) in the second genotype.

3. Results

Fusarium stalk rot was assessed on 50 accessions following artificial inoculation during two seasons (spring and autumn) in 2010. During spring, season stalk rot severity (SS) ranged from 1.0 to 9.0 with a mean of 4.7, and the corresponding levels of disease severity based on stalk rot discoloration (SD) ranged from 2.0% to 15.33% with a mean value of 8.5%. In autumn, the mean SS and SD was 5.2 (range: 1.3 to 9.6) and 9.3% (2.0% to 17.6%), respectively (Table 1). Table 1 shows that Y11 and EL7 had highly resistant responses in both growing seasons. Combined analysis of variance (ANOVA) in Table 2 shows highly significant results ($P < 0.01$) for treatment and treatment × season interaction for SS and SD. The significant interaction (treatment × season) described the different behaviors of treatments under different environmental conditions. Separate ANOVA was performed for each measuring scale after observing significant interaction studies. SS and SD explained highly significant ($P < 0.01$) results for the treatments in spring and autumn, respectively (Table 3). Accessions EL7 and Y11 exhibited highly resistant responses (1.0) to SS, while DR69 showed a highly susceptible response (9.0). The data further revealed that Y11 showed less stalk discoloration (1.98%), while DR69 had the highest SD (16.0%).

Mean SS and SD were significantly higher in autumn than in spring. The genotypes Y12 and Y13 appeared to be resistant in both seasons. The genotypes Y2, Y3, Y6, Y93, and Y95 showed resistant responses in spring, but in autumn Y2, Y3, and Y6 appeared to be mildly resistant whereas Y93 and Y95 showed mild susceptibility towards *Fusarium* stalk rot disease. In addition, disease assessments based on SS were significantly linearly correlated with assessments based on SD in spring ($r = 0.983$) and in autumn ($r = 0.974$) (Figure 1).

To compare the treatment means based on SS and SD values, Tukey's test was employed (Table 1). Means in Table 1 with same letter are not significantly different. Accessions with different letters showed different responses to *Fusarium* stalk rot disease in the two seasons. The genotypes that showed similar patterns of response to stalk rot disease are placed in one group. Accessions EL7, EL7, Y5, Y9, Y11, Y12, Y13, Y2, Y3, Y6, Y93, and Y95 are placed together in the resistant group.

For the assessment of environmental factors affecting the development of disease, the average monthly maximum and minimum temperature, relative humidity, and rainfall were calculated during the experimental periods of spring and autumn. Generally, mean monthly maximum and minimum temperatures were similar across the two seasons within a range of 20–44 °C, whereas relative humidity was lower in spring (27%) than in autumn (43%). Similarly, there was more rainfall in autumn (17 mm) than in spring (2 mm) (Figure 2).

Using 14 SSR markers, a total of 535 alleles were identified among 50 maize genotypes. Primers exhibited high polymorphism with an average amplification of 10.7 per genotype. The highest number of alleles per locus was seen with the xp-umc1186 primer, exhibiting 128 alleles with an average of 2.5 alleles per genotype (Figure 3). Primers xp-mmc0411, p-umc1715, and p-umc1472 showed fewer than 35 alleles. Tivang et al. (1994) reported that 20 SSR loci were enough to examine the genotypes with accuracy. Primer xp-umc1184, xp-umc1824, and p-umc1354 did not show any amplification with the genomic DNA, which may be due to error in PCR reactions or absence of recognition sites in the genotypes under study. Mean genetic distance varied from 0% to 89% (Table 4) among the genotypes. Maximum genetic distance was seen between lines DR36 and Y13, which appeared to be susceptible and resistant genotypes, respectively. On the other hand, minimum average distance was observed between lines DR33 and DR32, which confirmed the field

Table 1. *Fusarium* stalk rot intensity means for maize accessions artificially inoculated with *Fusarium verticillioides* in spring and autumn of 2010 and Tukey's test to compare treatment means.

Accessions	Means					
	Spring			Autumn		
	SS	SD (%)	Scoring	SS	SD (%)	Scoring
EL7	1.0 i	2.0 l	HR	1.33 i	2.0 o	HR
Y5	2.0 hi	4.0 ijl	R	2.0 i	4.0 mno	R
Y9	2.0 hi	3.0 jl	R	2.0 i	3.0 no	R
Y11	1.0 i	2.0 l	HR	1.21 i	1.98 o	HR
Y12	2.0 hi	4.0 ijl	R	2.0 i	4.0 mno	R
Y13	2.0 hi	3.0 jl	R	2.0 i	3.0 no	R
Y2	2.0 hi	4.0 ijl	R	3.0 hi	6.0 jlmn	MR
Y3	2.0 hi	4.0 ijl	R	3.0 hi	6.0 jlmn	MR
Y6	2.0 hi	4.0 ijl	R	3.0 hi	5.0 lmno	MR
Y93	2.0 hi	4.0 ijl	R	4.0 gh	8.0 hijl	MS
Y95	2.0 hi	4.0 ijl	R	4.0 gh	8.0 hijl	MS
Y97	3.0 gh	6.0 ghi	MR	4.0 gh	7.0 ijlm	MS
Y83	3.0 gh	5.0 hij	MR	4.0 gh	6.0 jlmn	MS
DR5	3.0 gh	5.0 hij	MR	4.0 gh	7.0 ijlm	MS
DR74	3.0 gh	5.0 hij	MR	4.0 gh	6.0 jlmn	MS
DR9	4.0 fg	6.0 ghi	MS	4.0 gh	6.0 jlmn	MS
DR27	4.0 fg	7.0 fgh	MS	4.0 gh	7.0 ijlm	MS
DR35	4.0 fg	7.0 fgh	MS	4.0 gh	7.0 ijlm	MS
DR40	4.0 fg	7.0 fgh	MS	4.0 gh	7.0 ijlm	MS
DR44	4.0 fg	7.0 fgh	MS	4.0 gh	7.0 ijlm	MS
DR61	4.0 fg	7.0 fgh	MS	4.0 gh	7.0 ijlm	MS
DR70	4.0 fg	7.0 fgh	MS	4.0 gh	7.0 ijlm	MS
Y81	4.0 fg	8.0 fg	MS	5.0 fg	10.0 fghi	S
Y85	4.0 fg	7.0 fgh	MS	5.0 fg	9.0 ghij	S
DR17	4.0 fg	7.0 fgh	MS	5.0 fg	9.0 ghij	S
DR19	4.0 fg	7.0 fgh	MS	5.0 fg	8.0 hijl	S
DR31	4.0 fg	6.0 ghi	MS	5.0 fg	8.0 hijl	S
DR36	4.0 fg	8.0 fg	MS	5.0 fg	10.0 fghi	S
DR38	4.0 fg	8.0 fg	MS	5.0 fg	10.0 fghi	S
DR14	5.0 ef	9.0 ef	S	7.3 bcde	12.6 cdef	HS
DR16	5.0 ef	11.0 de	S	8.3 abc	17.6 a	HS
DR20	5.0 ef	9.0 ef	S	8.6 ab	16.3 ab	HS
DR26	6.3 de	12.6 bcd	HS	7.0 bcde	14.0 bcd	HS
DR30	7.3 bcd	12.6 bcd	HS	7.0 bcde	12.0 defg	HS
DR32	8.3 abc	13.6 bcd	HS	6.6 cdef	11.0 defgh	HS
DR33	6.6 d	11.0 de	HS	6.3 def	10.6 efgh	HS
DR41	8.6 ab	15.3 b	HS	7.3 bcde	13.3 bcde	HS
DR43	6.3 de	12.6 bcd	HS	5.6 efg	11.3 defg	HS
DR48	7.0 cd	13.0 bcd	HS	6.6 cdef	13.0 cdef	HS
DR49	6.3 de	12.6 bcd	HS	7.0 bcde	13.6 bcde	HS
DR50	7.3 bcd	13.6 abcd	HS	6.6 cdef	12.3 cdef	HS
DR51	8.3 abc	13.6 abcd	HS	7.6 bcd	12.3 cdef	HS
DR52	7.6 abcd	13.3 abcd	HS	7.3 bcde	12.6 cdef	HS
DR54	7.0 cd	12.6 bcd	HS	6.6 cdef	12.3 cdef	HS
DR55	7.6 abcd	13.0 bcd	HS	6.3 def	11.0 defgh	HS
DR56	8.6 ab	15.3 ab	HS	8.6 ab	15.3 abc	HS
DR57	7.3 bcd	11.6 de	HS	9.6 a	15.3 abc	HS
DR58	7.6 abcd	12.0 cd	HS	7.3 bcde	11.6 defg	HS
DR59	8.6 ab	14.6 abc	HS	7.0 bcde	12.3 cdef	HS
DR69	9.0 a	16.0 a	HS	8.6 ab	15.3 abc	HS

SS = Severity score, SD = stalk discoloration, HR = highly resistant, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, HS = highly susceptible. Means with same letters do not differ significantly.

Table 2. Combined ANOVA for stalk rot severity (SS).

SOV	DF	SS	MS	*F*	P-value
T	49	1059.65	21.6255	1677.77	0.001**
S	1	0.08	0.0774	6.01	0.015*
T × S	49	450.69	9.1977	713.58	0.001**
Combined ANOVA for stalk discoloration (SD)					
T	49	4324.92	88.2637	107.27	0.001**
S	1	49.61	49.6133	60.30	0.002 **
T × S	49	299.05	6.1031	7.42	0.010**

*: Significant at P ≤ 0.05, **: significant at P ≤ 0.01, ns = nonsignificant (P ≥ 0 .05).
T = Treatment, S = season, T × S = treatment × season.

Table 3. ANOVA for stalk rot severity (SS) in spring.

SOV	DF	SS	MS	*F*	P-value
Blocks	2	0.0133	0.00667	0.0297	0.050 ns
Treatments	49	819.18	16.718	74.51	0.001**
ANOVA for stalk discoloration (SD) in spring season					
Blocks	2	0.0133	0.00667	0.0095	0.019*
Treatments	49	2450.66	50.013	71.39	0.001**
ANOVA for stalk rot severity (SS) in autumn season					
Blocks	2	0.41333	0.20667	0.6412	0.050 ns
Treatments	49	642.29	13.1080	40.6686	0.001**
ANOVA for stalk discoloration (SD) in autumn season					
Blocks	2	0.69333	0.34667	0.3615	0.050 ns
Treatments	49	2173.30	44.35	46.25	0.001**

*: Significant at P ≤ 0.05, **: significant at P ≤ 0.01, ns = nonsignificant (P ≥ 0 .05).

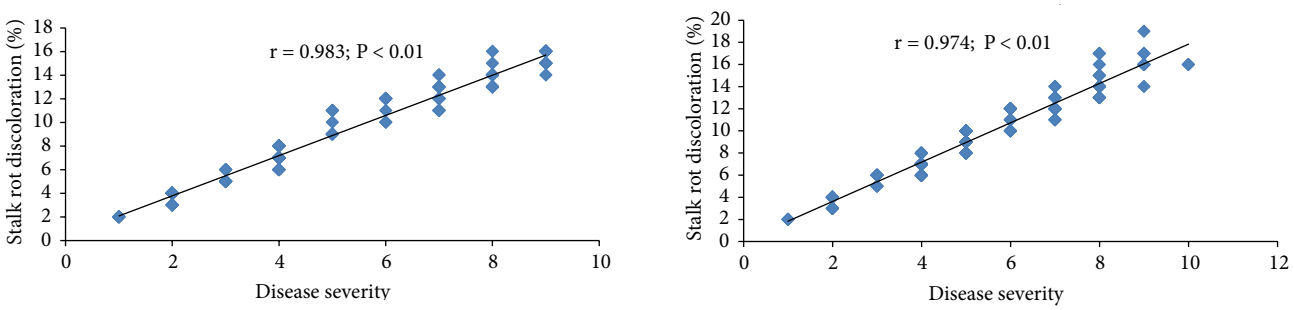

Figure 1. Linear correlation of *Fusarium* stalk rot assessments based on severity scale (SS) rating and assessment of stalk discoloration (SD %) following artificial inoculation in spring and autumn.

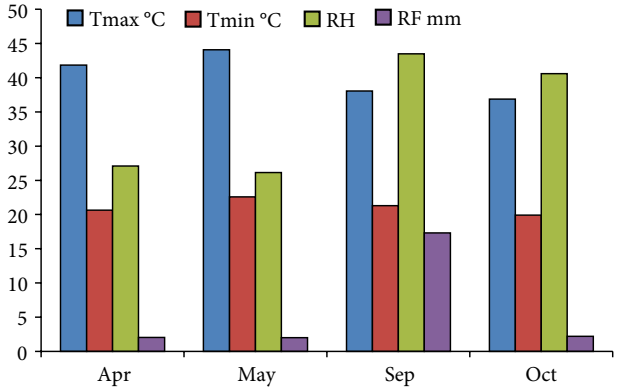

Figure 2. Average monthly maximum and minimum temperature, relative humidity, and rainfall during the experimental periods of two seasons in 2010.

results of this study. Amplified fragment length for all genomic DNA by SSR primers ranged from 100 to 750 bp. Maximum size of amplified fragments was observed with primers xp-umc1154, xp-umc1354, and xp-umc1586, while minimum size was observed with xp-umc1984 and xp-umc1325.

4. Discussion

Identification of the various sources of resistance is an important issue among breeders for developing disease-free hybrids and varieties to reduce the inoculum load, prolong the life of hybrids, and reduce the cost of cultivation. In the present experiment, maize accessions were evaluated in two different seasons to identify the accessions that are resistant to *Fusarium* stalk rot. Accessions identified in this study can be useful sources of resistance for breeding maize with resistance to *Fusarium* stalk rot disease. Genotypes exhibited significant differences to *Fusarium* stalk rot in both seasons, with higher disease levels in autumn than in spring. This difference in the disease development or disease severity may be due to the effect of the environment. The environment in autumn was hot and humid, most suitable for infection by *Fusarium* stalk rot (Reid et al., 1999). The disease is more severe when the temperature is warm with low rainfall (Dodd, 1983; Schneider and Pendery, 1983; Afolabi et al., 2008). High levels of stalk rot were observed in autumn as compared to spring due to more humid conditions. Ahmad et al. (1997) also found the same results of stalk rot severity explained by the greater impact of hot and humid environments on disease severity. Among the differences in disease severity between the two seasons, genotypes EL7 and Y11 had consistently low levels of SS (1, 1) and SD (2%, 1.98%) respectively and thus appeared to be highly resistant. Accessions Y5, Y9, Y12, and Y13 showed consistently low SS and SD values and appeared to be resistant to stalk rot disease in both seasons. These genotypes would be good material for developing maize varieties resistant to *Fusarium* stalk rot disease.

Stalk rot in maize is mostly analyzed by severity scale (Gilbertson et al., 1985; Bohra et al., 2001; Ledencan et al., 2003) due to the ease of the procedure. However, direct estimation gives more precise results (Forbes and Korva, 1994) than the rating scales. However, simple linear correlation between SS and SD in both seasons supported the idea of rating by two methods. For the selection of resistant genotypes, disease assessment procedure is very important. Several studies (Hooker and Draganic, 1980) have supported the procedure of artificial inoculation to get desirable outcomes of resistant genotypes in maize against *Fusarium* stalk rot. An artificial inoculation technique was utilized in the experiment to create more inoculum pressure that helps to differentiate between the levels of genotypes' resistance.

Genetic variations among the accessions are necessary to bring desirable changes and knowledge of this plays a vital role in the improvement of crop plants (Franco et al., 2001). Diversity is the estimation of the average divergence between two accessions for a particular locus (Huang et al., 2002). Breeders try to produce variation by utilizing exotic resources (Zohary et al., 1969) or by crossing the adopted varieties and making intensive selections in them (Ceccarelli et al., 1987). Different types of diversity can be estimated with respect to breeding programs and latent diversity is determined through molecular measurements; it eliminates the effect of environment as in the field (Cox et al., 1985; Maric et al., 1998). Different morphological, biochemical, and molecular markers are utilized by the breeders to identify the genetic diversity among the accessions (Pejic et al., 1998; Bauer et al., 2005). However, a highly informative, cost- and time-saving technique (molecular genetic markers) has replaced all

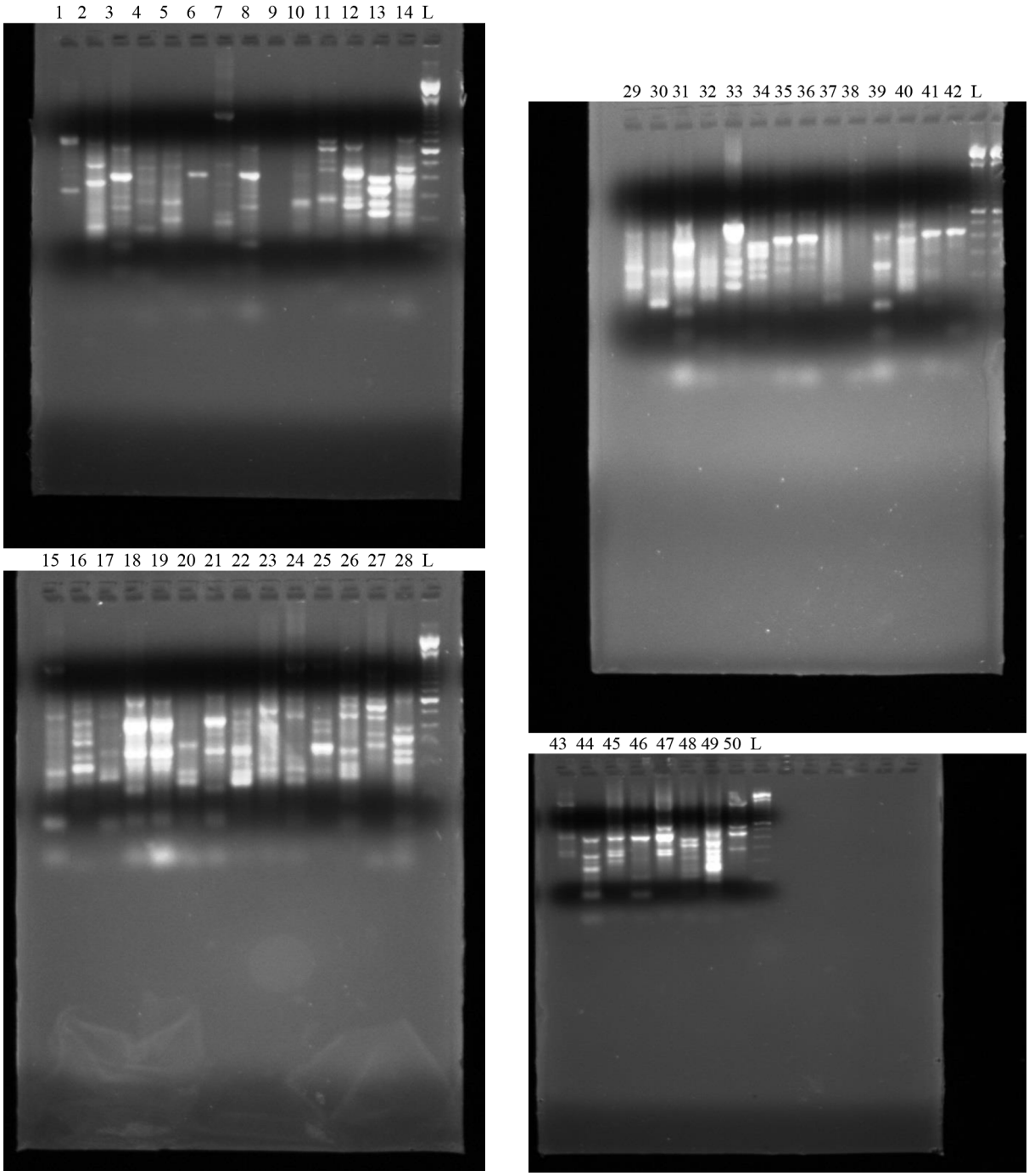

Figure 3. Bands of 50 maize accessions with xp-umc1186 SSRs marker by PCR reactions. L = 1-kbp ladder, 1 = EL7, 2 = Y5, 3 = Y9, 4 = Y11, 5 = Y12, 6 = Y13, 7 = Y2, 8 = Y3, 9 = Y6, 10 = Y93, 11 = Y95, 12 = Y97, 13 = Y83, 14 = DR5, 15 = DR74, 16 = DR9, 17 = DR27, 18 = DR35, 19 = DR40, 20 = DR44, 21 = DR61, 22 = DR70, 23 = Y81, 24 = Y85, 25 = DR17, 26 = DR19, 27 = DR31, 28 = DR36, 29 = DR38, 30 = DR14, 31 = DR16, 32 = DR20, 33 = DR26, 34 = DR30, 35 = DR32, 36 = DR33, 37 = DR41, 38 = DR43, 39 = DR48, 40 = DR49, 41 = DR50, 42 = DR51, 43 = DR52, 44 = DR54, 45 = DR55, 46 = DR56, 47 = DR57, 48 = DR58, 49 = DR59, 50 = DR69.

Table 4. Mean genetic distance among 50 maize accessions at molecular level by SSRs markers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	-																								
2	0.35																								
3	0.27	0.45	-																						
4	0.18	0.62	0.46	-																					
5	0.20	0.65	0.67	0.26	-																				
6	0.34	0.61	0.80	0.18	0.22	-																			
7	0.31	0.35	0.65	0.34	0.32	0.41	-																		
8	0.33	0.42	0.16	0.40	0.42	0.45	0.66	-																	
9	0.25	0.49	0.14	0.46	0.18	0.65	0.41	0.34	-																
10	0.15	0.30	0.45	0.25	0.22	0.63	0.80	0.50	0.61	-															
11	0.25	0.28	0.56	0.31	0.52	0.61	0.30	0.51	0.35	0.51	-														
12	0.30	0.02	0.67	0.21	0.32	0.23	0.25	0.67	0.56	0.34	0.20	-													
13	0.45	0.25	0.73	0.27	0.15	0.45	0.43	0.61	0.59	0.73	0.23	0.82	-												
14	0.35	0.27	0.29	0.29	0.22	0.41	0.56	0.45	0.71	0.31	0.30	0.34	0.45	-											
15	0.31	0.38	0.17	0.43	0.42	0.09	0.61	0.56	0.38	0.23	0.37	0.56	0.67	0.34	-										
16	0.33	0.38	0.33	0.46	0.52	0.51	0.45	0.41	0.75	0.70	0.34	0.76	0.58	0.65	0.23	-									
17	0.40	0.54	0.35	0.26	0.38	0.48	0.51	0.48	0.44	0.55	0.29	0.62	0.32	0.78	0.56	0.34	-								
18	0.42	0.38	0.36	0.19	0.35	0.43	0.54	0.61	0.55	0.531	0.45	0.39	0.21	0.76	0.87	0.32	0.36	-							
19	0.41	0.29	0.29	0.23	0.30	0.26	0.34	0.30	0.34	0.54	0.65	0.20	0.34	0.65	0.56	0.45	0.52	0.15	-						
20	0.23	0.25	0.80	0.18	0.29	0.56	0.61	0.18	0.39	0.54	0.70	0.34	0.56	0.54	0.43	0.65	0.50	0.67	0.23	-					
21	0.15	0.34	0.34	0.24	0.37	0.32	0.51	0.25	0.51	0.52	0.24	0.27	0.76	0.34	0.23	0.45	0.55	0.87	0.56	0.22	-				
22	0.37	0.29	0.56	0.17	0.26	0.31	0.34	0.63	0.28	0.58	0.35	0.56	0.43	0.27	0.56	0.65	0.23	0.45	0.37	0.34	0.52	-			
23	0.36	0.35	0.29	0.28	0.36	0.45	0.61	0.54	0.37	0.34	0.45	0.37	0.43	0.23	0.25	0.75	0.42	0.43	0.43	0.56	0.25	0.34	-		
24	0.45	0.45	0.27	0.27	0.27	0.78	0.54	0.57	0.61	0.34	0.56	0.08	0.45	0.45	0.23	0.33	0.43	0.58	0.21	0.78	0.64	0.47	0.20	-	
25	0.47	0.63	0.36	0.46	0.35	0.81	0.42	0.45	0.53	0.50	0.25	0.34	0.67	0.56	0.45	0.29	0.67	0.34	0.45	0.54	0.65	0.50	0.34	0.29	-
26	0.33	0.60	0.82	0.32	0.31	0.32	0.49	0.55	0.53	0.45	0.34	0.55	0.86	0.57	0.65	0.36	0.51	0.67	0.56	0.34	0.87	0.55	0.65	0.32	0.44
27	0.31	0.30	0.55	0.33	0.36	0.43	0.46	0.31	0.76	0.46	0.45	0.52	0.54	0.81	0.43	0.34	0.30	0.60	0.38	0.34	0.54	0.44	0.54	0.36	0.34
28	0.38	0.45	0.82	0.32	0.31	0.89	0.51	0.39	0.53	0.74	0.70	0.67	0.34	0.43	0.56	0.23	0.21	0.34	0.34	0.56	0.33	0.34	0.34	0.48	0.76
29	0.42	0.57	0.61	0.25	0.34	0.56	0.31	0.61	0.38	0.63	0.28	0.78	0.21	0.23	0.32	0.54	0.27	0.56	0.56	0.43	0.21	0.56	0.47	0.41	0.45
30	0.50	0.49	0.44	0.18	0.35	0.76	0.59	0.52	0.62	0.56	0.43	0.32	0.34	0.45	0.45	0.05	0.56	0.89	0.71	0.32	0.35	0.76	0.49	0.45	0.19
31	0.30	0.56	0.23	0.26	0.37	0.47	0.65	0.73	0.78	0.58	0.40	0.31	0.56	0.33	0.65	0.72	0.43	0.19	0.34	0.45	0.56	0.51	0.44	0.56	0.48
32	0.38	0.34	0.39	0.20	0.36	0.23	0.20	0.64	0.64	0.63	0.52	0.20	0.67	0.44	0.54	0.36	0.67	0.34	0.65	0.67	0.37	0.23	0.65	0.29	0.63
33	0.30	0.23	0.51	0.25	0.45	0.51	0.42	0.39	0.65	0.52	0.34	0.29	0.43	0.34	0.56	0.39	0.32	0.56	0.67	0.21	0.43	0.35	0.67	0.34	0.41
34	0.35	0.35	0.42	0.31	0.41	0.43	0.60	0.64	0.63	0.50	0.54	0.24	0.19	0.33	0.76	0.41	0.31	0.32	0.87	0.45	0.32	0.67	0.61	0.54	0.39
35	0.24	0.20	0.40	0.30	0.28	0.44	0.53	0.48	0.67	0.53	0.29	0.25	0.34	0.56	0.45	0.42	0.27	0.46	0.54	0.60	0.48	0.78	0.34	0.87	0.48
36	0.31	0.39	0.39	0.16	0.29	0.51	0.40	0.58	0.32	0.55	0.27	0.56	0.56	0.76	0.56	0.48	0.56	0.78	0.32	0.23	0.44	0.43	0.45	0.54	0.65
37	0.27	0.36	0.27	0.32	0.31	0.44	0.47	0.54	0.41	0.78	0.67	0.67	0.87	0.43	0.67	0.49	0.72	0.32	0.38	0.45	0.56	0.32	0.47	0.32	0.49
38	0.31	0.46	0.15	0.17	0.32	0.67	0.43	0.77	0.33	0.62	0.34	0.43	0.65	0.33	0.43	0.50	0.27	0.67	0.23	0.67	0.78	0.34	0.32	0.25	0.39
39	0.33	0.46	0.28	0.31	0.27	0.61	0.37	0.58	0.63	0.54	0.48	0.34	0.34	0.23	0.23	0.62	0.23	0.44	0.45	0.43	0.32	0.45	0.67	0.25	0.31
40	0.41	0.39	0.28	0.33	0.42	0.34	0.30	0.53	0.73	0.45	0.56	0.45	0.23	0.56	0.68	0.49	0.32	0.47	0.47	0.44	0.34	0.67	0.54	0.56	0.22
41	0.49	0.29	0.21	0.32	0.37	0.23	0.53	0.64	0.49	0.48	0.38	0.45	0.46	0.76	0.45	0.62	0.35	0.45	0.65	0.56	0.55	0.55	0.78	0.76	0.67
42	0.52	0.35	0.29	0.23	0.52	0.32	0.56	0.53	0.65	0.45	0.45	0.78	0.27	0.54	0.72	0.58	0.56	0.34	0.41	0.66	0.45	0.28	0.31	0.32	0.55
43	0.25	0.46	0.36	0.26	0.35	0.41	0.47	0.39	0.71	0.65	0.47	0.54	0.26	0.43	0.52	0.82	0.34	0.21	0.44	0.65	0.67	0.55	0.23	0.87	0.73
44	0.32	0.78	0.38	0.23	0.24	0.48	0.61	0.61	0.34	0.84	0.39	0.23	0.28	0.25	0.23	0.47	0.27	0.67	0.49	0.44	0.34	0.76	0.34	0.34	0.28
45	0.33	0.52	0.35	0.31	0.29	0.37	0.39	0.45	0.18	0.35	0.72	0.24	0.54	0.28	0.43	0.44	0.67	0.81	0.34	0.32	0.23	0.23	0.38	0.29	0.39
46	0.31	0.29	0.81	0.54	0.31	0.45	0.19	0.67	0.23	0.55	0.39	0.29	0.37	0.22	0.45	0.34	0.24	0.34	0.55	0.56	0.24	0.56	0.35	0.18	0.17
47	0.30	0.76	0.38	0.41	0.65	0.23	0.18	0.80	0.28	0.54	0.51	0.45	0.65	0.42	0.34	0.45	0.25	0.56	0.32	0.78	0.56	0.48	0.46	0.29	0.34
48	0.37	0.36	0.31	0.42	0.43	0.26	0.17	0.31	0.36	0.35	0.34	0.34	0.54	0.36	0.56	0.67	0.34	0.65	0.43	0.54	0.34	0.69	0.87	0.34	0.49
49	0.39	0.29	0.20	0.40	0.50	0.20	0.20	0.59	0.37	0.55	0.51	0.56	0.34	0.47	0.76	0.87	0.56	0.44	0.19	0.55	0.35	0.83	0.54	0.67	0.38
50	0.32	0.33	0.25	0.36	0.51	0.23	0.17	0.50	0.56	0.65	0.28	0.44	0.23	0.64	0.45	0.65	0.62	0.56	0.20	0.32	0.65	0.32	0.23	0.35	0.35

Table 4. (Continued).

	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
26	-																								
27	0.04	-																							
28	0.33	0.24	-																						
29	0.37	0.34	0.34	-																					
30	0.38	0.45	0.39	0.33	-																				
31	0.29	0.58	0.40	0.73	0.31	-																			
32	0.20	0.23	0.41	0.37	0.45	0.40	-																		
33	0.22	0.29	0.54	0.28	0.40	0.37	0.65	-																	
34	0.42	0.24	0.43	0.31	0.43	0.42	0.78	0.45	-																
35	0.34	0.28	0.29	0.20	0.42	0.45	0.27	0.64	0.54	-															
36	0.32	0.34	0.20	0.73	0.47	0.40	0.20	0.71	0.73	0.19	-														
37	0.31	0.44	0.23	0.51	0.41	0.63	0.28	0.47	0.67	0.31	0.47	-													
38	0.39	0.76	0.31	0.32	0.30	0.61	0.26	0.54	0.44	0.45	0.54	0.43	-												
39	0.30	0.67	0.28	0.60	0.32	0.43	0.56	0.77	0.49	0.56	0.39	0.56	0.32	-											
40	0.33	0.41	0.39	0.80	0.39	0.09	0.66	0.28	0.56	0.67	0.45	0.76	0.34	0.45	-										
41	0.29	0.32	0.33	0.26	0.29	0.81	0.27	0.54	0.34	0.59	0.38	0.34	0.56	0.23	0.46	-									
42	0.27	0.33	0.18	0.37	0.21	0.30	0.28	0.37	0.56	0.23	0.29	0.56	0.78	0.45	0.54	0.34	-								
43	0.28	0.39	0.20	0.39	0.45	0.32	0.20	0.54	0.45	0.43	0.23	0.32	0.54	0.67	0.67	0.65	0.21	-							
44	0.33	0.33	0.19	0.76	0.29	0.39	0.37	0.34	0.61	0.45	0.45	0.33	0.32	0.87	0.34	0.56	0.56	0.45	-						
45	0.34	0.30	0.29	0.58	0.45	0.37	0.27	0.56	0.63	0.43	0.82	0.45	0.45	0.65	0.21	0.47	0.43	0.34	0.45	-					
46	0.45	0.89	0.20	0.43	0.65	0.19	0.20	0.57	0.66	0.76	0.29	0.44	0.67	0.33	0.78	0.83	0.78	0.23	0.61	0.54	-				
47	0.44	0.22	0.21	0.65	0.82	0.65	0.56	0.56	0.65	0.53	0.34	0.56	0.50	0.34	0.65	0.25	0.65	0.26	0.23	0.34	0.22	-			
48	0.34	0.35	0.32	0.35	0.34	0.41	0.66	0.59	0.65	0.26	0.46	0.81	0.31	0.65	0.34	0.47	0.34	0.43	0.44	0.67	0.23	0.39	-		
49	0.39	0.23	0.29	0.23	0.32	0.71	0.71	0.45	0.63	0.29	0.53	0.43	0.23	0.44	0.32	0.36	0.56	0.45	0.45	0.42	0.45	0.33	0.45	-	
50	0.45	0.51	0.43	0.21	0.23	0.31	0.28	0.49	0.58	0.34	0.59	0.50	0.34	0.34	0.45	0.36	0.71	0.56	0.38	0.32	0.67	0.25	0.20	0.45	-

1 = EL7, 2 = Y5, 3 = Y9, 4 = Y11, 5 = Y12, 6 = Y13, 7 = Y2, 8 = Y3, 9 = Y6, 10 = Y93, 11 = Y95, 12 = Y97, 13 = Y83, 14 = DR5, 15 = DR74, 16 = DR9, 17 = DR27, 18 = DR35, 19 = DR40, 20 = DR44, 21 = DR61, 22 = DR70, 23 = Y81, 24 = Y85, 25 = DR17, 26 = DR19, 27 = DR31, 28 = DR36, 29 = DR38, 30 = DR14, 31 = DR16, 32 = DR20, 33 = DR26, 34 = DR30, 35 = DR32, 36 = DR33, 37 = DR41, 38 = DR43, 39 = DR48, 40 = DR49, 41 = DR50, 42 = DR51, 43 = DR52, 44 = DR54, 45 = DR55, 46 = DR56, 47 = DR57, 48 = DR58, 49 = DR59, 50 = DR69.

other techniques (Roder et al., 1998; Carvalho et al., 2004; Bruel et al., 2007). Among these markers, SSRs have more importance because of their codominant characteristics (Pejic et al., 1998; Tautz, 1989).

The polymorphism obtained in this study was monomorphic as well as polymorphic. Our results of polymorphism were in accordance with the results of Sun et al. (2001). Variation generated in the level of polymorphism may be due to assessment of a specific region by the markers for amplification. Eschholz et al. (2008) reported an average of 10.8 alleles per locus amplification with 10 primers in Swiss flint maize germplasm, which also supports our results. SSRs elaborated 0% to 89% of genetic dissimilarities among the lines, which confirmed the findings of Laborda et al. (2005) and Deloose and Gheysen (1995), who stated that the SSR technique produced more productive and diverse information than other any other technique.

Among the various DNA-based markers currently available, the set of microsatellite markers exploited in the present study allowed a positive assessment of the ability of SSR markers in producing unique DNA profiles and establishing unique identities of maize genotypes, which was otherwise difficult on the basis of morphological traits only. Maize accessions with high levels of resistance to *Fusarium* stalk rot in the field, after verifying their variations at molecular level, can be exploited for the development of hybrids with good levels of resistance to stalk rot and sources of resistance genes in maize breeding programs.

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