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Full Length Research Paper

Assessment of genetic diversity among wheat somaclonal variants lines using morphological traits and molecular markers

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The objectives of this study were to compare the application and utility of inter simple sequence repeats (ISSR) and randomly amplified polymorphic DNA (RAPD) techniques for analysis of genetic diversity among the somaclonal variants derived from the *in vitro* selection procedures and their parental plants, and to compare genetic diversity estimated using molecular markers with morpho-agronomic traits. 27 bread wheat genotypes comprised two parental varieties (Gemmisa-1 and Sakha-69), six somaclonal variant lines derived from the parental cultivar (Gemmisa-1) and 17 somaclonal variant lines derived from the parental cultivar (Sakha-69) and two local check varieties were used in this study. They were evaluated phenotypically and with molecular markers. All the dendrograms generated from standardized morpho-agronomic, ISSR, RAPD and the combined dendrograms (ISSR + RAPD + morpho-agronomic) data separated the 27 wheat genotypes into two main groups which diverged at similarity index of an average 0.479, 0.488, 0.501 and 0.282, respectively. In this study, positive moderate correlation (0.588 and 0.483) was found between agronomical traits and the two molecular markers (RAPD and ISSR markers, respectively) and very weak correlations were found between the other matrices.

Key words: Morpho-agronomic traits, wheat, *Triticum aestivum* L, molecular markers, ISSR, RAPD, genetic diversity, somaclonal variant lines.

INTRODUCTION

Wheat (Triticum aestivum L.) is one of the most important and widely cultivated crops in the world, used mainly for human consumption and support nearly 35% of the world population. Nearly 95% of wheat grown today is hexaploid, used for the preparation of bread and other baked products (Debasis and Khurana, 2001). One of the main objectives of wheat improvement program is to generate genetically diverse germplasm that has high yield potential, wide adaptation, and durable resistance to important diseases such as the rusts. Conventional breeding would probably be more efficient if aided by modern tools such as somaclonal variation and molecular markers. Knowledge of genetic diversity in a crop species is fundamental to its improvement. Evaluation of genetic diversity levels among adapted, wheat germplasm can provide predictive estimates of genetic variation among segregating progeny for pure-line cultivar development (Manjarrez-Sandoval et al., 1997). A number of methods have been used to analyze the genetic diversity of crops, typically utilizing pedigree records (Van Hintum and Haalman, 1994), agronomic and morphological data (Jain et al., 1975; Porceddu, 1976; Peccetti and Annicchiarico, 1993), seed storage proteins (Van Hintum and Elings, 1991; Moragues et al., 2006) and, more recently, DNA markers (Autrique et al., 1996; Soleimani et al., 2002). The most common techniques include isozymes and PCR-based assays such as Randomly Amplified Polymorphic DNA (RAPD; Williams et al., 1990), Simple Sequence Repeats (SSR; Akkaya et al., 1992), Amplified Fragment Length Polymorphism (AFLP; Vos et al., 1995) and Inter Simple Sequence Repeats (ISSR; Zietkiewicz et al., 1994). RAPD markers have been shown to be an effective method for detecting polymorphism in wheat (Sun et al., 1998; Vierling and Nguyen, 1992) and various other crops (Virk et al., 1995; Penner, 1996). Factors such as speed, efficiency and amenability to automation

make RAPD one of the most suitable methods for germplasm management with respect to estimating diversity, monitoring genetic erosion and removing duplicates from germplasm collections (Penner, 1996). The ISSR are a kind of molecular marker involving PCR amplification of DNA by a single primer 16-18 bp long composed of a repeated sequence anchored at the 3' or 5' end by 2-4 arbitrary nucleotides (Zietkiewicz et al., 1994). They are easy to handle, highly informative and repeatable. Since repeated sequences are abundant throughout the genome, SSR primers anneal in several regions typically giving a complex amplification pattern in which fragments are often polymorphic between different individuals. The objectives of this study were to (i) Use ISSR, RAPD and morphological characters for the characterization and assessment of the genetic diversity among wheat parental cultivars and its variant lines. (ii) To compare genetic diversity estimated using molecular markers with agronomic traits to establish the degree of association between these techniques.

MATERIALS AND METHODS

Plant material

This investigation was carried out at two experimental farms Sakha agriculture research. Agriculture Research Center (ARC). Egypt and Nubari Research Station, Egypt during the two wheat successive growing seasons, 2006 to 2007 and 2007 to 2008. Two wheat varieties (Gemmisa-1 and Sakha-69) and their somaclonal variant lines obtained through *in vitro* selection for yellow rust disease among cells under different levels of p-fluorophenylalanine (PFP) (Abouzied, 2004) as well as two check varieties (Sakha-94 and Giza-168) were used to establish the experimental materials for this investigation. All wheat varieties, along with their variant lines are listed in Table 1.

Evaluation of morphological characters

Randomized complete block design, with 3 replications was used with two rows as experimental plot 3m long with a spacing of 20 cm among rows.

Plants were selected at random for 8 morphological traits measurements: plant height (PH), Severity of leaf rust (LR), grain yield (GY), harvest index(HI), spike number / m^2(NS/m^2), kernels / spike (NG/S), 1000-kernel weight (KW), biological yield (BY). Grain yield (GY) was determined from the guarded area of the two rows and converted to grain yield / ha. Kernel / spike (NG/S) was determined in heads of 10 randomly tillers. The severity of leaf rust (LR) spreader and rows of susceptible wheat varieties were planted among plots and around the experiments to initiate leaf rust infection. In each plot, six plants were randomly selected to collect the data of leaf rust disease as follows: (i) the severity was recorded by using the percent of leaf rust area affected (stubbs *et al*., 1986). (ii) the reaction was estimated for each genotype as : (R= resistant; small uredia often surrounded by a necrosis, MR= moderately resistant; small to medium uredia often surrounded by chlororsis or necrosis, X= intermediate; random distribution of variable-sized uredia, MS= moderately susceptible; medium-sized uredia might be associated with chlorosis or rarely necrosis and S= susceptible; large uredia without chlorosis or necrosis). The scale of reaction had a constant value; i.e., R= 0.2, MR= 0.4, X=0.6, MS= 0.8 and S=1.0. The constant value was multiplied with severity score of each genotype to obtain average coefficient of infection (ACI) (Stubbs et al., 1986; and Woldead et al., 2007). ACI was calculated. For example: 20MR= 20 *0.4=8.

Molecular characterization

DNA extraction

Genomic DNA was extracted from fresh leaves of wheat genotypes using the Saghai-Maroof et al. (1984) CTAB method. RNA was removed from the DNA preparation by adding 10 µl of RNAase (10 mg/ml) and then incubated for 30 min at 37°C. DNA sample concentration was quantified by using a spectrophotometer (Beckman Du-65). The reagents were obtained from Pharmacia Biotech (Amersham Phar- macia Biotech Limited, UK).

ISSR analysis

Ten ISSR primers (Table 2), obtained from Pharmacia Biotech. (Amersham Pharmacia Biotech., UK Limited, HP 79NA, England), were tested in the present experiment, to amplify the templated DNA of the selected lines and their parents. The ISSR-PCR method was carried out, according to Nagaoka and Ogihara (1997). Amplification was carried out in 25 µl reaction volumes, containing 1 x Taq polymerase buffer (50 mM KCl, 10 mMTris, pH 7.5, 1.5 mM MgCl2) and 1 unit of Taq polymerase (Pharmacia Biotech, Germany), supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 50 pmol of ISSR primers and 50 ng of total genomic DNA. Amplification was performed in a thermal cycler (Thermolyne Amplitron) programmed for 1 cycle of 2 min at 94˚C, and 35 cycles of 30 s at 94˚C, 45 s at 44-60˚C-, and 1.3 min at 72˚C, followed by 20 min at 72˚C. Amplification products were visualized with a 100 bp. Ladder (pharmacia, Germany) as the size standard on 1.6% agarose gel with 1x TBE buffer and detected by staining with an ethidium bromide solution for 45 min. Gels were then distained in deionized water for 10 min and photographed on gel documentation system.

RAPD analysis

Ten RAPD primers (Table 2), obtained from Pharmacia Biotech. (Amersham Pharmacia Biotech UK Limited, England HP79 NA), were tested in this experiment to amplify the templated DNA. Amplification reaction volumes were 25 µl, each containing 1 x PCR buffer, with $MgCl_2$ [50 mM KCl, 10 mM Tris-HCl (pH = 9.0), 2 mM $MgCl_2$ and 1% trition x-100], 200 µM each of 2'-deoxyadenosine triphosphate (dATP) 2'-deoxycytidine 5'-triphosphated (dCTP), guanosine-5'-triphosphate (dGTP) and deoxyribonucleotides monophosphates (dTTP), 50 pmol primer, 50 ng template DNA and 1.5 µl of tag polymerase. Reaction mixtures were exposed to the following conditions: 94°C for 3 min, followed by 45 cycles of 1 min at 94°C, 1 min at 38°C, 2 min at 72°C and a final 7 min extension at 72°C. After completion of PCR, samples were cooled immediately to 10°C and stored at 4°C until gel separation. Amplification products were visualized as described for RAPD analysis.

Statistical analysis

All the measured traits (agronomic traits) were used to determine adata matrix of pair wise similarities between genotypes, the standardized traits mean values (mean of each traits was subtracted from the data values and the result divided by the

Table 1. Wheat entries used for morphological traits and molecular marker analyses.

S/N	Entry	Pedigree
1	Gemmisa-1	Maya 74/On//1160.147/3/Bb/1991 Gall/4/Chat"S"1991
2	Gemmisa-V1	Somaclonal variant line derived from Gemmisa-1
3	Gemmisa-V2	Somaclonal variant line derived from Gemmsa-1
4	Gemmisa-V3	Somaclonal variant line derived from Gemmsa-1
5	Gemmisa-V4	Somaclonal variant line derived from Gemmisa-1
6	Gemmisa-V5	Somaclonal variant line derived from Gemmisa-1
7	Gemmisa-V6	Somaclonal variant line derived from Gemmisa-1
8	Sakha-69	INIA/RL4220//7C/YR"s"CM15430-2S-1S-0S
9	Sakha-V1	Somaclonal variant line derived from Sakha-69
10	Sakha-V2	Somaclonal variant line derived from Sakha-69
11	Sakha-V3	Somaclonal variant line derived from Sakha-69
12	Sakha-V4	Somaclonal variant line derived from Sakha-69
13	Sakha-V5	Somaclonal variant line derived from Sakha-69
14	Sakha-V6	Somaclonal variant line derived from Sakha-69
15	Sakha-V7	Somaclonal variant line derived from Sakha-69
16	Sakha-V8	Somaclonal variant line derived from Sakha-69
17	Sakha-V9	Somaclonal variant line derived from Sakha-69
18	Sakha-V10	Somaclonal variant line derived from Sakha-69
19	Sakha-V11	Somaclonal variant line derived from Sakha-69
20	Sakha-V12	Somaclonal variant line derived from Sakha-69
21	Sakha-V13	Somaclonal variant line derived from Sakha-69
22	Sakha-V14	Somaclonal variant line derived from Sakha-69
23	Sakha-V15	Somaclonal variant line derived from Sakha-69
24	Sakha-V16	Somaclonal variant line derived from Sakha-69
25	Sakha-V17	Somaclonal variant line derived from Sakha-69
26	Sakha-94 [a]	OPATA/RAYON//KAUZ
27	GIZA 168 [a]	MRL/BUC//SERI

[a] Check variety.

Table 2. ISSR and RAPD Primer sequences used to detect polymorphism between somaclones and their parents.

Primer name/number	Nucleotide sequence (5-3)	Number of amplification B	Number of polymorphic a	Polymorphism b/a (%)
ISSR				
AD2	(AGC)6G	2	2	100
AD3	(ACC)6G	7	7	100
AD8	(AGC)6GC	2	2	100
M-1	(AC)8CG	4	4	100
Pr-1	AGA CAG ACA GAC AGA CGC	3	3	100
Total		18	18	
Mean		3.6	3.6	100%
RAPD				
1	GGGTAACGCC	4	2	50
2	TCGGCGATAG	3	3	100
3	GACCGCTTGT	5	4	80
4	AGGTGACCGT	3	2	66.6
5	CAAACGTCGG	4	4	100
Total		19	15	
Mean		3.8	3	79.32

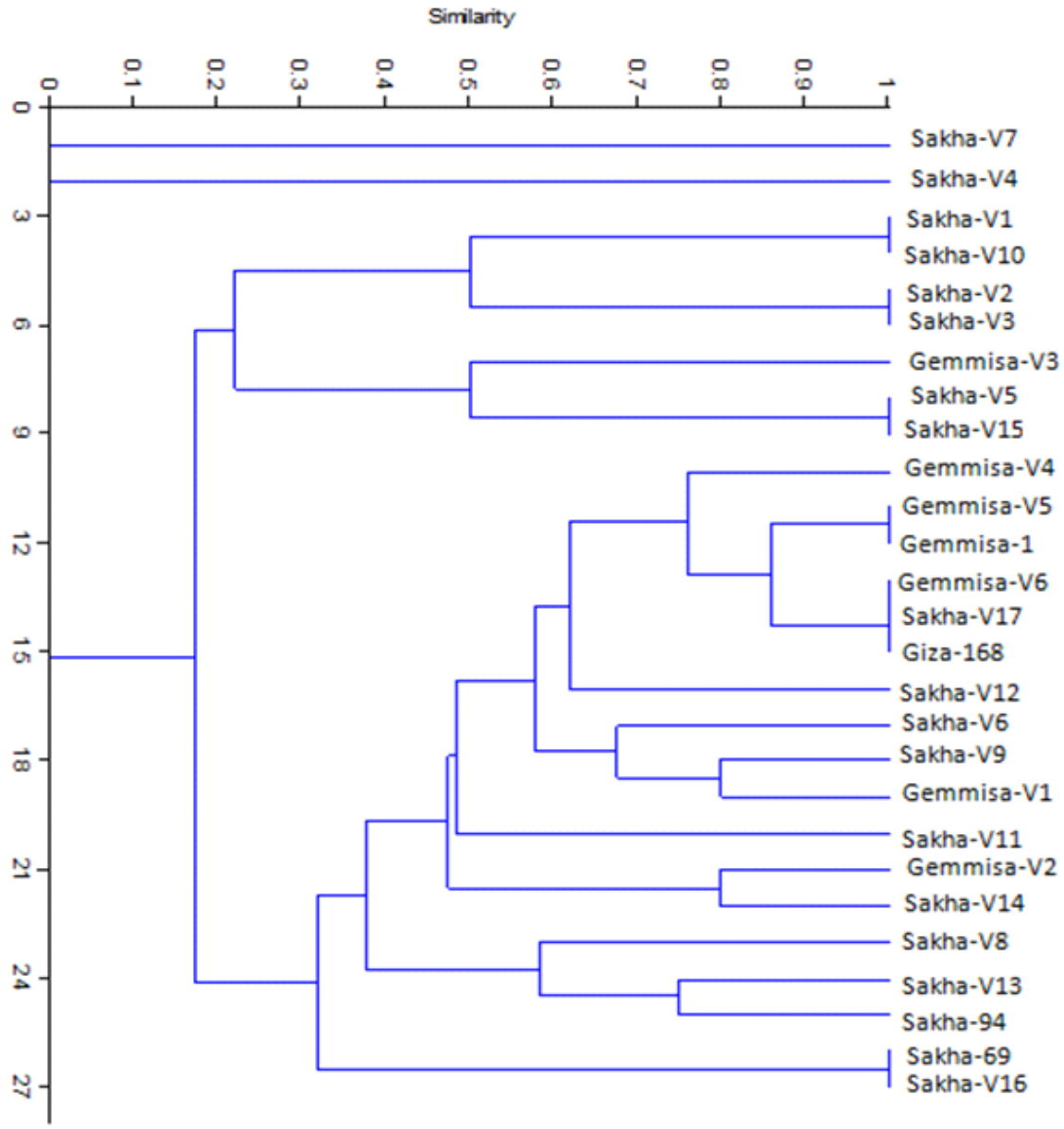

Figure 1. Dendrogram based on Jaccard similarity coefficient of twenty seven wheat genotypes, generated by eight agronomic traits over two seasons.

standard deviation) were used, according to Jaccard coefficient (Jaccard, 1908). ISSR and RAPD data were scored for presence (1), absence (0) or as a missing observation and each band was regarded as a locus. Two matrices, one for each marker, were generated. Pair wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to determine a data matrix of pair wise similarities between cultivars, according to Jaccard coefficient. All matrices (based on agronomic traits and molecular markers) were used to obtain the respective dendrograms using the algorithm UPGMA (Unweighed Pair Group Method with Arithmetic Average) (Sokal and Michene, 1958) employed the SAHN (Aequential, Agglomerative, Hierarchical, and Nested clustering) from the software NTSYS.pc (Numercial Taxonomy and Multivariate Analysis System, version 1.80 (Applied Biostatics Program (Rohlf, 1993).

The correlation coefficients between the Jaccard distance matrix based on agronomic traits and genetic distance matrix obtained with molecular markers were analyzed according to Mantel (Mantel, 1967) using NTSYS-pc.

RESULTS

Diversity analysis based on morpho-agronomic traits

A dendrogram generated from the standardized morpho-agronomic data is presented in Figure 1. The UPGMA dendrogram separated the 27 wheat genotypes into two clusters. The first cluster consist of six variant lines

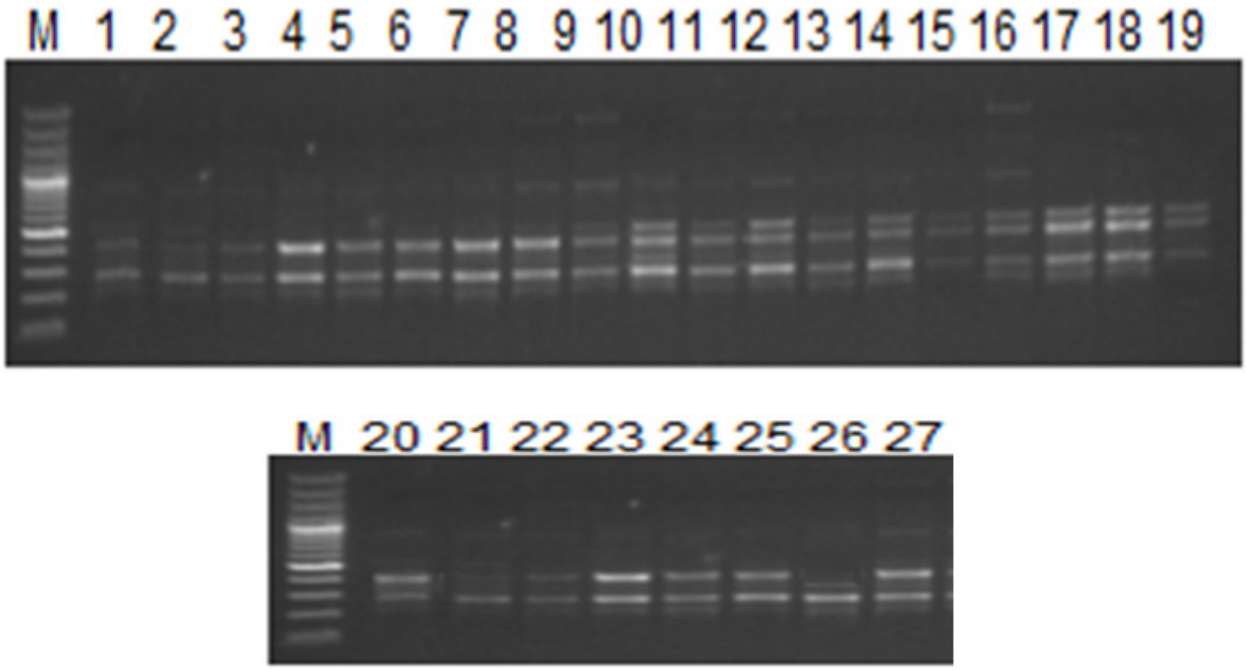

Figure 2. ISSR marker profiles of 27 wheat genotypes generated by primer M-1**.**

derived from Sakha-69 and one variant line (Gemmisa-V3) derived from Gemmisa-1. The second cluster was divided into many sub-groups as example one sub-group included the parent cultivar sakha-69 and only one of its variant line Sakha-V16, an another sub-group contained the check variety (Giza-168) together with the parental cultivar Gemmisa-1 and three from its variants lines (Gemmisa-V4, Gemmisa-V5 and Gemmisa-V6), also another sub-group lies on the same cluster consisted of the check variety Sakha-94 and the variant line Sakha-V13 (Figure 1). The two variants lines (Sakha-V7 and Sakha-V4) derived from Sakha-69 were not included in any cluster (Figure 1). The mean morphological similarity among the twenty seven wheat genotypes was 0.479 with value ranging from 0.17 to 0.88. The variant lines sakha-V14 and Gemmisa-V2 genotypes showed a very high degree of similarity (0.98) indicating that these two genotypes had similar agronomic traits. On the other hand, Sakha-V10 and Sakha- V16 genotypes showed a low degree of similarity (0.17) indicated that this pair is highly different from each other and had different agronomic traits. In comparison for the degree of similarity between the parental cultivar (Gemmisa-1) and its variant lines, the variant line (Gemmisa-V5) showed the highest degree of similarity (0.74) while variant line (Gemmisa-V3) had the lowest degree of similarity (0.47). Similarly in comparison between the parental cultivar Sakha-69 and its variant lines, the most closely variant to Sakha-69 was Sakha-V16 (0.69), while both of the two variant lines Sakha-V4 and Sakha-V7 showed the lowest degree of similarity (0.38).

Identification and evaluation of ISSR and RAPD markers for diversity estimates

Initially, ten ISSR and ten RAPD primers were screened against genomic DNA of 27 wheat genotypes for their ability to amplify DNA fragments. Out of the 10 ISSR primers, three produced no distinct bands on a smeary background and two resulted in very faint bands upon a highly smeared background. The remaining five primers (Table 2) produced clear amplification patterns. The number of amplified DNA fragments ranged from 2.0 to 7.0 depending on the primer and the DNA sample with a mean value of 3.6 bands per primer (Table 2). A total of 18 fragments were produced by the five primers and all of these were 100% polymorphic. As an example, the pattern obtained for each genotype with ISSR primers is shown in Figure 2. RAPD used to detect the genetic diversity and the genetic relationships among wheat genotypes using 10 primers. Among the 10 RAPD primers, five were chosen for their good amplification products. Each of the remaining varied in their ability to resolve variability among cultivars. A total of 19 reproducible and scorable amplification products were generated across 27 genotypes (Table 2). As an example, the pattern obtained for each genotype with RAPD primers is shown in Figure 3. The number of ampli-fication products is generated by each primer varied from 3 to 5 with an average of 3.8 bands. A total of 15 polymorphic bands were observed ranging from 2 to 4 fragments. The polymorphism parentage was ranged from 50 to 100% within average 79.32%.

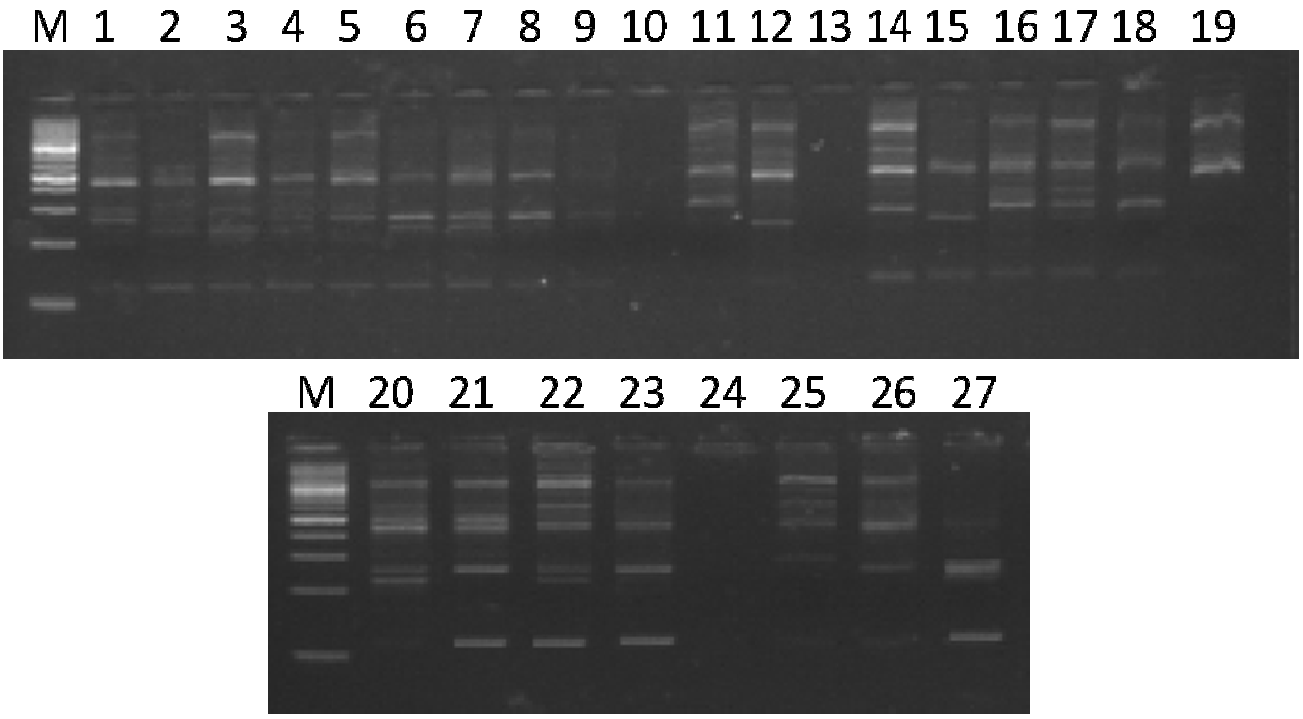

Figure 3. RAPD marker profiles of 27 wheat genotypes generated by primer sequence CAAACGTCGG.

Genetic diversity of molecular markers

The relationships among the 27 wheat genotypes were estimated by a UPGMA cluster analysis of genetic similarity matrices. The composition of clusters obtained using ISSR markers alone were shown in (Figure 4), RAPD markers alone (Figure 5). Cluster analysis using ISSR data grouped the 27 wheat genotypes into two main clusters with Jaccard's similarity coefficient varied between 0.00 to 1.00 with an average 0.488 (Figure 4). The highest similarity was found between the wheat variant lines (Gemmisa-V2, Gemmisa-V4, and Sakha-V15, Sakha-16 (1.00) and the lowest were between Sakha-V10 and Sakha-V14, Sakha-V15 and Sakha-V16 (0.00). The first cluster included the two check varieties (Sakha-94 and Giza 168) in addition to the variant lines (Sakha-V12, Sakha-V13, Sakha-V14, Sakha-V15, Sakha-V16 and Sakha-V17) while the second cluster included the two parental cultivars (Gemmisa-1 and Sakha-69) as well as the rest of the other variants line. The dendrogram generated from RAPD data clearly indicated two main clusters (Figure 4). The Jaccard similarity coefficient ranged from 0.08 to 1.00 with mean 0,501. Maximum similarity was found between the two variant lines Sakha-V14 and the check variety Sakha-94 (1.00). Lowest similarity was found between the two variant lines Sakha-V2 and Sakha-V15 (0.08). The first cluster included two variant lines Sakha-V5 and Sakha-V16. The second cluster included all the other wheat genotypes separated into several sub-groups.

Combined dendrogram and correlation between morpho-agronomic traits and molecular markers

ISSR and RAPD and morpho-agronomic data were combined to produce a dendrogram. The average of similarity coefficient among the wheat genotypes was 0.282 varied between 0.05 to 0.66 with the highest being between the two variant lines Gemmisa-V2 and Gemmisa-V4 and the lowest being between Sakha-V2 and Sakha-V15. Cluster analysis revealed there were two clusters (Figure 6). The first cluster contained Sakha-V15 and Sakha-V16. The second cluster consisted of the wheat cultivars as well as the rest of all the variant lines in many sub groups. Results of Mantel's test are presented in Table 3. The test showed weak correlation among all the examined distance matrixes except for the two distance matrixes (ISSR + Agro-morphological and RAPD + Agro- morphological) they showed moderate correlation 0.483 and 0.588, respectively.

DISCUSSION

The molecular markers is a useful tool for assessing genetic variations; one of the goals of the present study was to investigate the efficiency of ISSR and RAPD markers in determining, accurately, the genetic relationship between wheat somaclones and their parents. In this study, morphological data analysis of the bread wheat genotypes was coupled with molecular analyses (ISSR

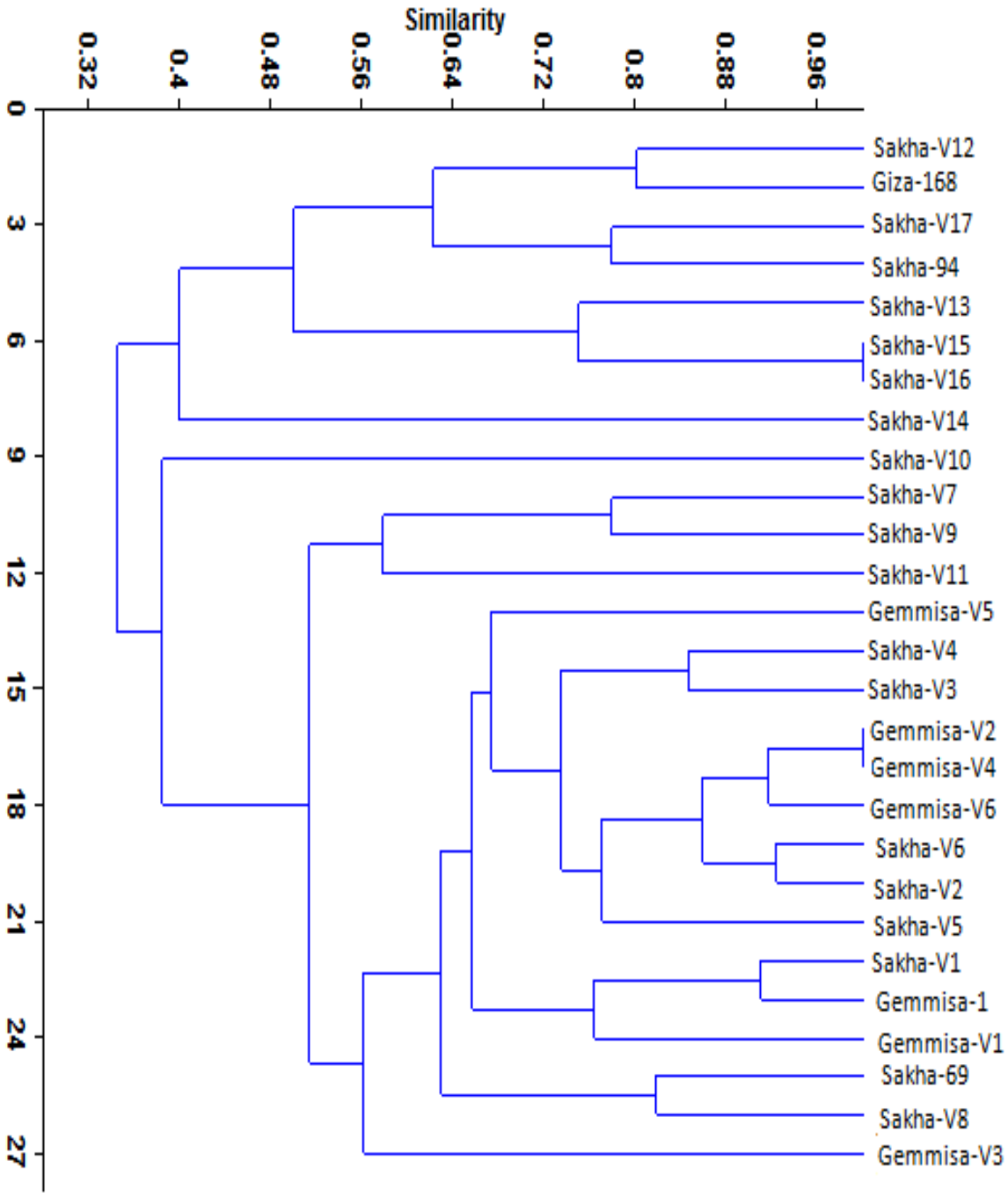

Figure 4. Dendrogram based on Jaccard similarity coefficient of twenty seven wheat genotypes, generated using ISSR markers.

and RAPD markers) to investigate the genetic relationships among 2 parental Egyptian bread wheat varieties and their twenty three somaclonal variant lines as well as two bread wheat check varieties. The genotypes showed diverse morphological traits and distinct ISSR and RAPD markers patterns. ISSRs have been successfully used to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of crop species which include rice (Joshi et al., 2000), wheat (Nagaoka and Ogihara, 1997), fingermillet (Salimath et al., 1995), *Vigna* (Ajibade et al., 2000), sweet potato (Huang and Sun, 2000) and *Plantago* (Wolff and Morgan-Richards, 1998). Superiority of ISSR-PCR over other marker techniques has been brought out in such investigations by various workers. In this study, the average percentage of amplified and polymorphic bands per each primer

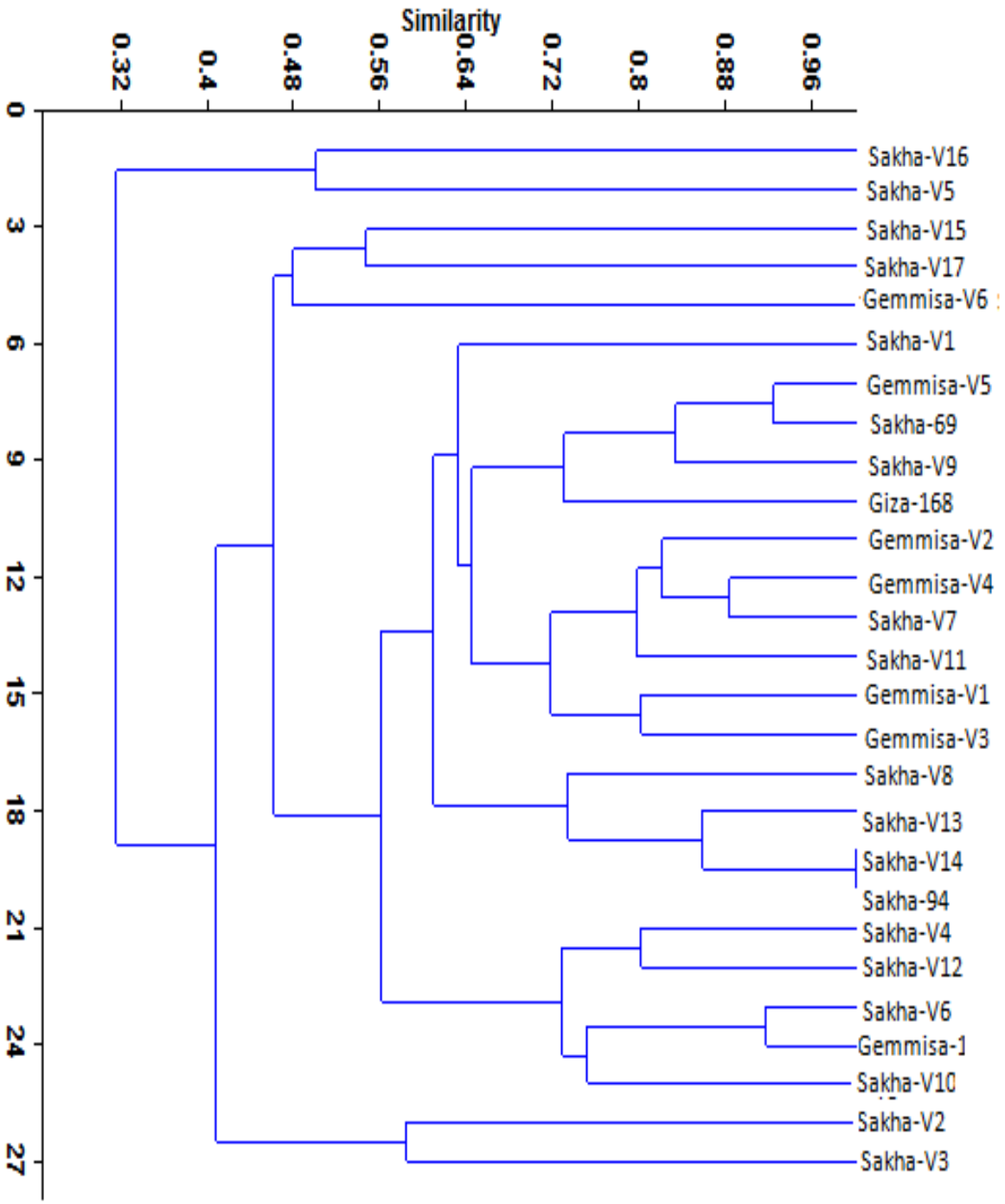

Figure 5. Dendrogram based on Jaccard similarity coefficient of twenty seven wheat genotypes, generated by RAPD.

was 100%. Amplified bands information related to each primer is giving in Table 2. High level of polymorphisms among wheat genotypes using ISSR markers was obtained by (Nagoaka and Ogihara, 1997; Song et al., 2002).

In this investigation, RAPD markers also showed a high level of polymorphism, the average polymorphism across all the 27 wheat genotypes was found to be 79 .32%, which is quite consistent with that (83%) obtained by De Freitis et al. (2000) among 14 genotypes of Brazilian wheat. Gerwal et al. (2007) showed that the average polymorphism across all the studied 20 wheat varieties was found to be 85.49±2.87 Pujar et al. (1999) observed 78.6% polymorphism, whereas Teshale et al. (2003)

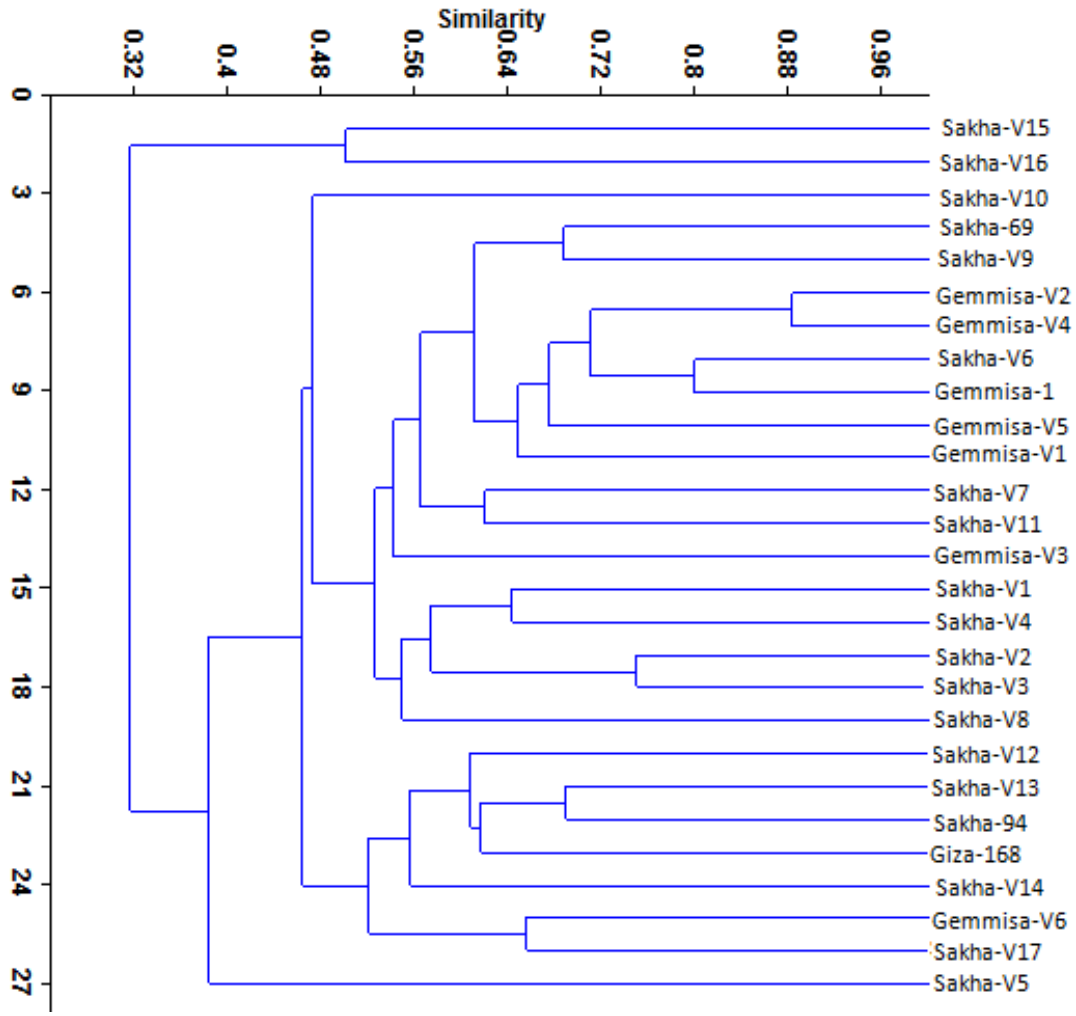

Figure 6. Dendrogram based on Jaccard similarity coefficient of 27 wheat genotypes, generated using combined molecular markers and morpho-agronomic traits.

observed 79.6% polymorphism among the selected genotypes, which is also close to the present values. Both RAPD and ISSR markers have been used to assess somaclonal variation in maize and were found to be highly efficient (Osipova et al., 2003). In rice, Ngezahayo et al. (2007) observed that the RAPD markers have revealed more genetic variation than the ISSR markers. Moreover, Jin et al. (2008) result suggested that 2, 4-D and kinetin hormone combination could induce relative high somaclonal variation and RAPD and SSR markers are useful in detecting somaclonal variation of regenerated cotton plants via somatic embryogenesis. Genetic similarity matrices obtained from morphological traits, ISSR markers and RAPD markers showed that similarity coefficients, ranging from 0.05 to 0.66, 0.00 to 1.0 and 0.08 to 1.00, respectively suggested a broad genetic base for wheat varieties and their somaclonal variant lines derived from tissue culture tecchiques.

The occurrence of genetic changes in plant, derived from tissue culture (somaclonal variations) had been previously reported by (Larkin and Scowcroft, 1981). Somaclonal variation has been intensively studied by using various molecular markers in several plant species, including *Arabidopsis* (Polanco and Ruiz, 2002) and rice (Kim et al., 2003). Nonetheless, few studies have addressed the molecular basis or nature of somaclonal variation (Al-Zahim et al., 1999; Yang et al., 1999), though it was found more than a decade ago that alteration in DNA methylation probably plays a role (Muller et al., 1990). In order to compare the extent of agreement among dendrograms, derived from morphology and molecular markers, a distance matrix was constructed for each assay and compared using the Mantel matrix correspondence test. In this study positive moderate correlation was found between agronomical traits + RAPD markers and agronomical traits + ISSR markers (0.589 and 0.483 respectively). On the other hand, very weak correlations were found between the other matrices. Kumar and Miaja (2007) examined the genetic diversity of 127 wheat genotypes; they found no

Table 3. The correlation coefficients between the Jaccard distance matrix based on agronomic traits and genetic distance matrix obtained with molecular markers analyzed according to Mantel's test.

Distance matrixes	Correlation coefficient
ISSR+ RAPD	0.043
ISSR + Agro- morphological	0.483
RAPD + Agro- morphological	0.588
(ISSR+RAPD + Agro- morphological) + ISSR	0.090
(ISSR+RAPD + Agro- morphological) + RAPD	0.100
(ISSR+RAPD + Agro- morphological) + Agromorphological	0.081

correlation coefficient was significant in their experiment between protein markers and DNA markers. Maric et al. (2004) evaluated usability of RAPD markers for estimation of genetic diversity among wheat cultivars in comparison with morphological traits and pedigree record data they found no significant correlations were observed among the methods tested. The DNA markers and morphological traits will not necessarily gain closely matching results (Vollmann et al., 2005; Mart´nez et al., 2005). Semagn (2002) suggested two reasons for low correlation between DNA markers and morphological as well as protein data: (a) DNA markers cover a larger proportion of the genome, including coding and non coding regions, than the morphological markers and (b) DNA markers are less subjected to artificial selection compared with morphological markers. Mart´nez et al. (2005) believed that the correspondence between different methods might be improved by analysing more morphological characters and DNA markers.

In summary, data showed significant polymorphisms in morphological traits, ISSR and RAPD among wheat genotypes. Morphological and molecular markers could be used to characterized wheat somaclonal variants; this will be of particular importance in the future when dealing with in vitro selection to stress condition.

Abbreviations:

ISSR, Inter simple sequence repeats; **RAPD,** randomly amplified polymorphic **DNA; PCR,** polymerase chain reaction; **SSR,** simple sequence repeats; **AFLP,** amplified fragment length polymorphism; **ARC,** Agriculture Research Center; **PFP,** p-fluorophenylalanine; **PH,** plant height; **LR,** severity of leaf rust; **GY,** grain yield; **HI,** harvest index; **KW,** kernel weight; **BY,** biological yield; **GY,** grain yield; **MR,** moderately resistant; **ACI,** average coefficient of infection; **dNTPs**, deoxynucleotide triphosphates; **dATP**, 2'-deoxyadenosine triphosphate; **dCTP**, 2'-deoxycytidine 5'-triphosphated; **GTP,** guanosine-5'-triphosphate; **dTTP,** deoxyribonucleotides monophosphates; **UPGMA,** unweighed pair group method with arithmetic average.

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