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Phenotypic and Molecular Assessment for Genetic Diversity of Egyptian Wheat Varieties

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GENETIC diversity among plant species is important for improving plant traits. Its estimation is very essential to help selection of genetic resources in breeding programs. Moreover, it is the base for effective and successful crop enhancement and can be assessed by several methods i.e. using morphological trait (resistance to leaf rust) and molecular markers. In this study, genetic diversity was assessed among the 53 tested wheat varieties using 15 leaf rust pathotypes under greenhouse condition at seedling stage. In addition, the genetic diversity was assessed also using 10 molecular markers (microsatellite markers) linked to rust resistant genes. The cluster analysis indicated three sub-clusters based on phenotypic and molecular data. In general, low level (r= 0.15) of correlation was obtained between the phenotypic and the genotypic data, however, the molecular analysis is more efficient for estimating genetic diversity. Molecular analysis is an efficient method because it's not affected by environment, fast, more accurate and doesn't need earlier pedigree information which can improve the efficacy of molecular breeding practices.

Keywords: Genetic diversity, Leaf rust resistance, Molecular markers, Wheat.

Introduction

Wheat crop (*Triticum aestivum* L.) is one of the highly stable cereal's worldwide, which provides one-fifth of the protein and calories to more than 4.5 billion people (Shiferaw et al., 2013). Wheat production is affected by not only climate change, but also the emergence of new and important virulent pathotypes.

Leaf rust of wheat caused by the fungus *Puccinia triticina*, is one of the most wide spread diseases in Egypt and worldwide. Several diseases and insect pests, including leaf rust, threaten wheat production sustainability in major areas growing in the world (Huerta-Espino et al., 2011). Leaf rust affected damage on kernels by lowered kernel weight (Bolton et al., 2018) and yield losses become critical if wheat is infected early, and may reach epidemic levels in susceptible wheat varieties under favorable conditions (Gill et al., 2019). Yield losses attributed to leaf rust reached higher than 60% in highly susceptible varieties (Strzembicka et al., 2013; Shahin & El-Orabey, 2016; El-Orabey et al., 2017; El-Orabey & Elkot, 2020.).

Breeding for wheat rust resistance, especially leaf rust, is one of the most effective schemes to control leaf rust disease. Wheat breeding programs all over the world are mainly depended on deploying highly effective rust resistance genes in new released cultivars (Gill et al., 2019). The *P. triticina* populations in Egypt were found to be highly variable (El-Orabey et al., 2015; El-Orabey, 2018; El-Orabey et al., 2018).

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Genetic diversity in crops, especially wheat, is important in breeding program to improve these crops and release genotypes capable to resist biotic and abiotic stress conditions. Also, a successful breeding program for wheat depends mainly on the available types and the extent of genetic variability in wheat genotypes. Genetic variability on wheat genotypes acts as the most important natural resource of supplying the required traits to improve new cultivars (Kamaludddin et al., 2014). Genetic similarity/distance estimates between wheat varieties are very effective in selection process of the parents that will be used for breeding program (Kamaludddin et al., 2014).

Information of wheat genetic diversity between selected adapted genotypes helps wheat breeders in selecting wheat parents for production with greatest heterosis and combining effective genes in adapted genetic genotypes. The narrow genetic base is a huge problem and limits plant breeding to adapt for different abiotic and biotic stresses. However, wheat cultivars developed, including larger genetic base, are very effective in improving yield under several agro-climatic environments and also to resist the continued spread of diseases in newly released cultivars (Asif et al., 2005).

Great efforts have been made recently to study genetic diversity in crop populations (Fu, 2015). Genetic diversity in crops can be measured by morphological (phenotypic) traits, pedigree analysis and by using molecular markers (Kim & Ward, 2000). Genetic diversity depends upon pedigree analysis has been found to be impractical and unrealistic (Maric et al., 2004). Morphological characters (phenotypic characters) are usually used to study genetic diversity which provides a simple way of assessing genetic variation under normal growing environments. However, genetic diversity that depends on morphological characters suffers from many problems, such characters are low in number and affected by the environment and also may be controlled by epistatic and pleiotropic gene effect (Maric et al., 2004). In spite of all these limits, morphological characters have been effectively used in studies of genetic diversity and cultivar development.

Finally, studying genetic diversity based on molecular markers overcomes many of the limitations of morphological characters and pedigree analysis (Gupta et al., 1999). It is an efficient method because it's not affected by environment, is plentiful and doesn't need previous pedigree information which can improve the efficacy of molecular breeding practices (Motawei et al., 2007). Today, several kinds of molecular markers analysis have been established for estimation genetic diversity of wheat populations (Khan et al., 2014; El-Orabey et al., 2019). Moreover, genetic diversity using molecular markers played an important role in genomic structure composition, which identified important genes for specific characters, and preserved the genetic materials for future use in breeding programs (Khan et al., 2015). Molecular markers, depends on sequence repeats (SSRs) are frequently used and suitable markers for analyzing genetic diversity particularly in cereals and appear to be more informative in wheat than any other marker technique. These markers show high polymorphism, co-dominant inheritance and good reproducibility (Bryan et al., 1997).

In the present study, phenotypic and molecular analyses were used to estimate the diversity among the tested wheat varieties. This information on the genetic diversity within and among different populations can be effectively used by wheat breeders for the production of genetically diverse wheat cultivars. In this study we try to assess the genetic diversity of 53 Egyptian wheat varieties to leaf rust under greenhouse condition at seedling stage by using phenotypic and molecular using SSR marker linked to resistant genes

Materials and Methods

Plant materials

A total of 53 Egyptian wheat varieties; including 44 bread wheat varieties and nine durum wheat varieties (Table 1) were used to study their genetic diversity. These varieties were produced from Wheat Research Department, Field Crops Institute, ARC, Giza, Egypt.

Leaf rust pathotypes

Uredinispores of fifteen leaf rust pathotypes; CTTTT, FTSSS, KTSPT, NKTSS, NTKTS, NPTNK, NTTPT, PHTTT, PKTPT, PKTST, PTTNS, PTTPT, PTTSS, PTTST and STTTK which of the most frequent during 2016/17 (El-Oraby et al., 2018) were collected and used for evaluation of the tested varieties under greenhouse condition at seedling stage. The spores of these pathotypes were produced by Wheat Diseases Dept., Plant Pathology Inst., ARC, Giza, Egypt.

TABLE 1. Wheat varieties used in this study; pedigree and year of release

No.	Variety	Pedigree	Year of release
		a- Bread wheat varieties	
1	Sids 1	HD2172/PAVON"S"//1158.574"S". SD46-4SD-2SD-1SD-0SD.	1996
2	Sids 2	HD2206/HORK"S3/"/NAP063/INIA66//WERN"S".	1996
3	Sids 3	SAKHA69/GIZA155. SD723-7SD-1SD-1SD-0SD.	1996
4	Sids 5	MAYA"S"/MON"S"//CMH74A.592/3/GIZA158*2. SD10001-7SD-4SD -2SD-0SD.	1996
5	Sids 6	MAYA"S"/MON"S"//CMH74A.592/3/SAKHA8*2. SD10002-4SD-3SD -1SD-0SD.	1996
6	Sids 7	MAYA"S"/MON"S"//CMH74A.592/3/SAKHA8*2. SD10002-8SD-1SD -1SD-0SD.	1996
7	Sids 8	MAYA"S"/MON"S"//CMH74A.592/3/SAKHA8*2.SD10002-14SD-3SD -1SD-0SD.	1996
8	Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160-147/3/BB/GLL/4/CHAT"S"/6/MAYA/ VUL-4SD-1SD-1SD-0SD.	2007
9	Sids 13	KAUZ "S"//TSI/SNB"S". ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD.	2010
10	Sids 14	SW8488*2/KUKUNACGSS01Y00081T-099M-099Y-099M-099B-9Y-0B-0SD.	2018
11	Giza 139	HINDI90/KENYA256G.	1947
12	Giza 144	REGENT/2*GIZA139.	1958
13	Giza 150	MIDA-CADET/2*GIZA139.	1960
14	Giza 155	REGENT/2*GIZA139//MIDA-CADET/2*HINDI62.	1968
15	Giza 156	RIO-NEGRO/2*MENAATANE//KENYA/3/*2GIZA135/LINE950.	1972
16	Giza 157	GIZA155//PIT62/RL64/3/TZPP/KNOTT.	1977
17	Giza 160	CHENAB/GIZA155.	1982
18	Giza 162	Vcm//Cno 67/7C/3/Kal/Bb CM8399-D-4M-3Y-1M-1Y-1M-0Y	1987
19	Giza 163	F-61-70/Bon//Cno /7C CM33009-F-15M-4Y-2M-1M-1M-1Y-0M	1988
20	Giza 165	0MCno/Mfd//Mon "S" CM43339-C-1Y-1M-2Y-1M-2Y-0B	1991
21	Giza 167	Au/UP301//G11/SX/Pew"S"/4/Mai"S"/May"S"//Pew"S".CM67245-C-1M-2Y-1M-7Y-1M-0Y	1995
22	Giza 168	MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B	1999
23	Giza 171	Sakha 93 / Gemmeiza 9 S.6-1GZ-4GZ-1GZ-2GZ-0S	2013
24	Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR. CMSSOYO1881T-050M-030Y-O3OM-030WGY-33M-0Y-0S.	2010
25	Misr 2	SKAUZ/BAV92. CMSS96M0361S-1M-010SY-010M-010SY-8M -0Y-0S.	2011
26	Misr 3	ATTILA*2/ABW65*2/KACHU CMSS06Y00258 2T-099TOPM-099Y-099ZTM-099Y-099M-10WGY-0B-0EGY	2018
27	Shandweel 1	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC. CMSS93B00567S-72Y-010M-010Y-010M-0HTY-0SH	2011
28	Gemmeiza 1	MAYA74/ON//1160-147/3/BB/GALL/4/CHAT"S". CM58924-1GM-0GM.	1991
29	Gemmeiza 3	BB/7C*2//$Y50_E$/KAL*3//SAKHA8/4/PRV/WW15/3/BG"S"//ON. GM4024-1GM-13GM-2GM-0GM.	1997
30	Gemmeiza 5	VEE"S"/SWM6525. GM4017-1GM-6GM-3GM-0GM.	1998
31	Gemmeiza 7	CMH74A.630/SX//SER182/3/AGENT. GM4611-2GM-3GM-1GM -0GM.	1999
32	Gemmeiza 9	ALD"S"/HUAC"S"//CMH74A.630/SX. GM4583-5GM-1GM-0GM.	1999
33	Gemmeiza 10	MAYA74"S"/0N//160-147/3/BB/GLL/4/CHAT"S"/5/CROW"S". GM5820-3GM-1GM-2GM-0GM.	2004

TABLE 1. Cont.

No.	Variety	Pedigree	Year of release
34	Gemmeiza 11	B0W"S"/KVZ"S"//7C/SERI82/3/GIZA168/SAKHA61. GM7892-2GM-1GM-2GM-1GM-0GM.	2011
35	Gemmeiza 12	OTUS/3/SARA/THB//VEE. CCMSS97Y00227S-5Y-010M-010Y -010M-2Y-1M-0Y-0GM	2017
36	Sakha 8	Indus 66 x Norteno "S"-Pk 348	1979
37	Sakha 61	INIA/RL4220//7CYR"S". CM15430-2S-2S-0S-0S.	1980
38	Sakha 62	WE – Gto X KAL - Bb	1980
39	Sakha 69	Inia/RL 4220//7C/Yr "S" CM 15430-25-65-0S-0S	1980
40	Sakha 88	WS-1877-7C Bb (INIA·s/SON 64 – Tzpp-Y_{54})	1985
41	Sakha 92	NNAP063/INA66//WERN"S". S.1551-1S-1S-1S-0S.	1987
42	Sakha 93	Sakha 92/TR 810328 S 8871-1S-2S-1S-0S	1999
43	Sakha 94	OPATA/RAYON//KAUZ. CMBW90Y3280-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S.	2004
44	Sakha 95	PASTOR//SITE/MO/3/CHEN/AEGILOPS SQUARROSA(TAUS)//BCN /4/ WBLL1CMSA01Y00158S-040P0Y-040M-030ZTM-040SY-26M-0Y-0SY-0S	2018
		b- Durum wheat varieties	
45	Bani Swif 1	JO"S"/AA"S"//FG"S". CD9799-126M-1M-5Y-0M-0SD.	1987
46	Bani Swif 4	AUSL/5/CANDO/4/BY*2/TACE//II27655/3/TME//ZB/W*2. ICD88-1120-ABL-0TR-1BR-0TR-6AP-OSD.	2007
47	Bani Swif 5	DIPPERZ/BUSHEN3. CDSS92B128-1M-0Y-0M-0Y-3B-0Y-0SD.	2007
48	Bani Swif 6	BOOMER-21/BUSCA-3. CDSS95Y001185-8Y-0M-0Y-0B-1Y-0B0SD	2010
49	Bani Swif 7		
50	Sohag 1	GDOVZ469/JOS"S"//61.130.LSD.	1977
51	Sohag 3	MEXI"S"/MGHA/51792//DURUM6. CD21831-25H-1SH-0SH.	1991
52	Sohag 4	Ajaia-16//Hora/Jro/3/Gan/4/Zar/5/Suok-7/6/Stot//Altar84/Ald CDSS99B00778S-OTOPY-0M-0Y-129Y-0M-0Y-1B-0SH	2016
53	Sohag 5	TRN//21563/AA/3/BD2080/4/BD2339/5/Rascon 37// Tarro 2// Rascon 3/6/Auk/ Gull//GreenCDSS00B00364T-0T0PB-0B-2Y-0M-0Y-1B-0Y-0SH	2016

Screening of wheat varieties to leaf rust at seedling stage

Evaluation of the tested wheat varieties using 15 leaf rust pathotypes was conducted in the greenhouse of Wheat Diseases Res. Dept., Plant Pathology Res. Inst., ARC, Giza, Egypt at seedling stage during 2019/2020 growing season.

A total of six seeds of each of variety were grown in 6 cm diameter plastic pots singly in each corner in clockwise order. Each pot contains a mixture of peat-moss and soil in a ratio of 1:1 (v:v). Seedlings of seven-days-old were inoculated by brushing with urediniospores of the 15 pathotypes during 2019/2020 growing season. After inoculation, the seedlings were incubated in a dew chamber for 24 hours at 18-20ºC to permit spore germination and infection. The inoculated seedlings were transferred to greenhouse benches where the daily temperature was fixed at 20-24ºC and 70-80 % RH with 12 h photoperiod (Kolmer et al., 2005).

Infection types (IT) for each variety was recorded 10-12 days after inoculation using a 0-4 scale (Roelfs et al., 1992; Kolmer et al., 2005).

Molecular marker

DNA isolation

DNA isolation and PCR procedure were performed at ICARDA Biotechnology Lab, at the Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt. The total genomic DNA was isolated from young leaves following the procedure described by Rogers & Bendich (1994). The DNA was dissolved and preserved in TE (1 X) buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) at a concentration of 50ng/µL. The quality of DNA was estimated by electrophoresis using agarose gel (0.8%).

DNA amplification

A total of 12 microsatellite markers linked to 12 different genes were used for molecular characterization (Table 2). These markers already applied as marker assisted selection (MAS) in the wheat breeding program at ICARDA. Amplification was performed using a thermocycler (Multigene optimax.), in a total volume of 20µL consisting of 5ng DNA template, 10 picomol of forward primer, 10 picomol of reverse primer, 0.1 U of *Taq* DNA polymerase, Bioline GmbH, Germany, 0.6mM of $MgCl_2$, 0.2mM dNTPs and 1 X PCR buffer in 96 well micro titer plates using Applied bio system thermal cycler, made in Singapore. For the SSR markers, PCR program was used to amplify DNA fragments: initial denaturation was 2 min at 95°C. For *Yr*10, *Yr*18, *Yr*45, *Sr*22, *Sr*24, *Sr*25, *Lr*9, *Lr*19 , *Lr*34, *Lr*46, *Lr*67 and *Lr*68 this was followed by initial 35 cycles of denaturation for 20sec at 94°C, annealing for 20sec at 55, 55, 55, 51, 59, 55, 55, 55, 55, 60, 60 and 48^{O}C for each primer, and 30sec at 72°C. Subsequently, 7min final extension at 72°C. PCR products were electrophoresed through agarose gel (2.5 %) for all the markers except *Lr* 9 and *Lr* 19 where the agarose gel was (1.5%). Electrophoresis was applied at 100 V constant for around 90min and a 1 X TAE buffer was used during electrophoresis. Fragment sizes were estimated by comparison with 100 bp DNA ladder (Vivantis). The amplified product was visualized under UV light on a gel documentation system from BIorad Company after staining the gel with 5µL Ethidium bromide.

TABLE 2.Wheat Microsatellite marker name, the name of the linked genes, primer sequences and the annealing temperature

No.	Marker	Gene	Primer sequences	Annealing temp. (ºC)
1	CsLV34	*Yr* 18	F: GTTGGTTAAGACTGGTGATGG R: TGCTTGCTATTGCTGAATAGT	55
2	Yr10	*Yr* 10	F: GCAGACCTGTGTCATTGGTC R: GATATAGTGGCAGCAGGATAC	55
3	Xwgp118	*Yr* 45	F: AGTGTCTTGTAGGGTATC R: ACACTGGTCCATGAGGTT	55
4	CFA2123	*Sr* 22	F :CGGTCTTTGTTTGCTCTAAAC R: ACCGGCCATCTATGATGAAG	51
5	Sr24#12	*Sr* 24	F: CACCCGTGACATGCTCGTA R: AACAGGAAATGAGCAACGATGT	59
6	Gb	*Sr* 25	F: CATCCTTGGGGACCT R: CCAGCTCGCATACATCCA	55
7	J13	*Lr* 9	F: TCCTTTTATTCCGCACGCCGG R: CCACACTACCCCAAAGAGAG	55
8	Scs123	*Lr* 19	F: CCTGATCACCAATGACGATT R: CCTGATCACCTTGCTACAGA	55
9	CsLv343D	*Lr* 34	F: CGA AAG TAA CAG CGC AGT GA R: GTTGGT TAAGAC TGG TGATGG	55
10	Xgwm259	*Lr* 46	F: AGGGAAAAG ACATCT TTT TTT TC R: CGACCGACTTCGGGT TC	60
11	Cfd71	*Lr* 67	F: CAATAAGTAGGCCGG GACAA R: TGTGCCAGTTGAGTTTGCTC	60
12	CSG5	*Lr* 68	F: AAGATTGTTCACAGATCCATG TCA R: GAGTATTCCGGCTCAAAA AGG	48

Data analysis

Phenotypic data analysis

About 10-14 days after inoculation, the infection types (IT·s) was scored for all tested wheat varieties using 0-4 scale (Roelfs et al., 1992; Kolmer et al., 2005).

The IT data was converted into a binary code of 0 for resistant genotype and 1 for susceptible genotype of the tested wheat varieties. Moreover, a binary data matrix was generated for all SSR markers based on the presence (1) or absence (0) of amplification products. A matrix cluster of both virulence and molecular data were derived with GenAlex 6 (Peakall & Smouse, 2006). Correlation between the SSR marker data and morphological data was determined by power marker software (Liu & Muse, 2005).

A histogram illustrating the reaction of each genotype against each of the genotypes under investigation was done by using excel application (Microsoft office 365).

Genetic similarity estimation and cluster analysis

The SSR profiles were converted into a binary matrix where the presence of the band at a precise level is scored as 1 and its absence is scored as 0 and then the data matrix was prepared for analyses. A pair-wise similarity matrix was produced with the GenAlex software (Rohlf, 1998). Phylogenetic tree was done by using Past software (Hammer et al., 2001) by using the Jaccard equation.

$$Djk = M / (M+N).$$

Marker polymorphism

To evaluate the polymorphism of the used primers of the SSR, the polymorphism information content (PIC) for each marker was calculated by power marker software (Liu & Muse, 2005) using the formula:

$$PIC = 1 - Pi^2$$

where k is the total number of alleles detected for a locus of a marker and *P* the *i* frequency of the *i* the allele in the set of 53 wheat varieties investigated.

Population structure

In order to assess the population structure of the Egyptian varieties under investigation, three different statistical methods were adopted and compared. First, a clustering approach based on the Bayesian model was applied to estimate the real number of subpopulations (K) using the admixture model of STRUCTURE software 2.3.4 with correlated allele frequencies (Pritchard et al., 2000). Three independent runs were performed for each hypothetical number of subpopulations (K) from one to seven applying a burn-in period of 100,000 iterations followed by 100,000 Markov Chain Monte Carlo iterations to obtain a precise parameter estimate. The most probable number of subpopulations was determined by means of the ΔK method using STRUCTURE HARVESTER software (Evanno et al., 2005).

Results

Evaluation of wheat varieties against 15 leaf rust races under greenhouse condition

Seedling reaction of the 53 wheat varieties against the most aggressive and frequent leaf rust races (CTTTT, FTSSS, KTSPT, NKTSS, NPTNK, NTKTS, NTTPT, PHTTT, PKTPT, PKTST, PTTNS, PTTPT, PTTSS, PTTST and STTTK) are given in Table 3. Out of 53 tested wheat varieties, only six wheat varieties (Bani Swif 1, Bani Swif 4, Bani Swif 5, Bani Swif 6, Bani Swif 7 and Sohag 4) were resistant to all tested pathotypes and showed low infection type, followed by Sohag 3 which was resistant to most tested pathotypes except NKTSS and Sohag 5 which was resistant to all tested pathotypes except the three pathotypes (CTTTT, KTSPT and NKTSS). While, the 27 wheat varieties (Sids 6, Sids 7, Sids 8, Sids 13, Sids 14, Giza 150, Giza 155, Giza 156, Giza 157, Giza 160, Giza 162, Giza 163, Giza 165, Giza 167, Giza 168, Giza 171, Misr 3, Shandweel 1, Gemmeiza 1, Gemmeiza 3, Gemmeiza 5, Gemmeiza 9, Gemmeiza 10, Sakha 8, Sakha 69, Sakha 88 and Giza 139) were highly susceptible against all of the tested pathotypes.

Genetic diversity evaluation and cluster analysis based on host pathogen interaction (phenotypic data)

The dendrogram produced based on the phenotypic data of the response of the 53 tested wheat varieties against 15 leaf rust races reveals two main clusters (Fig. 1). The first main cluster contained 13 varieties from Upper Egypt named as Sohag and Bani Swif. The second main cluster comprised 40 most of them from Delta region (Gemmeiza, Giza and Sakha) except 9 varieties named as Sids from upper Egypt.

TABLE 3. Response of 53 wheat varieties against 15 leaf rust races at seedling stage under greenhouse condition during 2019/20 growing season.

No.	Variety	Leaf rust pathotypes/ infection type														
		CTTTT	FTSSS	KTSPT	NKTSS	NPTNK	NTKTS	NTTPT	PHTTT	PKTPT	PKTST	PTTNS	PTTPT	PTTSS	PTTST	STTTK
1	Sids 1	0	4	3	4	0;	4	3	0	4	3	3	4	4	3	3
2	Sids 2	4	3	3	3	0	3	3	4	4	4	3	4	3	4	3
3	Sids 3	4	4	3	3	0;	4	3	3	4	3	4	3	4	4	3
4	Sids 5	0	3	4	4	0;	4	3	4	3	4	3	4	3	3	3
5	Sids 6	4	3	3	4	3	3	4	4	4	4	3	4	3	3	4
6	Sids 7	4	4	4	4	4	3	3	4	4	4	4	3	4	3	4
7	Sids 8	4	4	3	4	4	3	3	4	3	4	3	4	4	3	4
8	Sids 12	0	4	3	4	3	4	0	4	0	2	1	3	4	4	3
9	Sids 13	4	4	3	4	3	4	3	3	3	3	4	3	3	3	3
10	Sids 14	4	4	3	3	3	4	3	3	4	3	4	4	4	4	3
11	Giza 144	3	3	4	3	0	4	0	3	4	4	4	3	2	3	3
12	Giza 150	4	3	4	3	3	4	3	3	4	3	4	4	3	4	4
13	Giza 155	3	3	4	4	4	3	4	4	4	4	4	3	3	4	4
14	Giza 156	3	4	4	3	4	3	3	4	3	4	4	4	3	3	3
15	Giza 157	3	4	3	4	3	3	4	4	4	4	4	4	4	3	3
16	Giza 160	4	4	4	3	3	3	4	4	3	3	4	4	3	4	3
17	Giza 162	4	3	4	3	4	4	4	2	4	4	3	4	4	3	3
18	Giza 163	3	3	4	3	4	4	4	4	4	4	3	3	3	4	3
19	Giza 165	4	3	4	3	3	3	3	4	4	4	4	3	4	3	4
20	Giza 167	4	3	3	4	3	3	4	4	3	4	4	4	4	4	4
21	Giza 168	4	3	4	4	3	3	4	4	4	3	4	4	4	4	3
22	Giza 171	4	3	4	4	3	4	4	4	3	3	3	4	3	4	4
23	Misr 1	0	4	4	4	0	1	4	0;	0;	1	2	1	3	0	3
24	Misr 2	0;	4	3	4	0,	0,	3	4	2	0;	2	3	3	3	4
25	Misr 3	4	3	4	4	3	3	4	4	3	4	4	4	4	4	3
26	Shandweel 1	4	3	4	3	4	3	4	4	3	3	3	4	4	4	4
27	Gemmeiza 1	4	4	4	4	4	4	3	4	4	3	4	4	3	3	4
28	Gemmeiza 3	3	4	3	4	3	4	4	4	4	3	3	4	4	3	3
29	Gemmeiza 5	3	4	3	4	3	4	3	4	3	3	4	3	3	4	4
30	Gemmeiza 7	4	4	3	3	3	4	4	0	3	3	3	3	4	1	3
31	Gemmeiza 9	4	4	3	4	4	3	4	4	3	4	3	3	4	3	3
32	Gemmeiza 10	4	4	4	4	3	3	4	3	4	3	4	3	4	3	4
33	Gemmeiza 11	3	2	4	3	4	4	4	4	1	2	4	0;	3	4	0
34	Gemmeiza 12	4	0;	3	4	4	3	3	3	4	3	3	3	4	3	2
35	Sakha 8	4	3	4	4	3	3	4	4	4	4	3	4	4	3	3
36	Sakha 61	4	4	3	4	4	4	3	4	0;	1	3	4	3	4	4
37	Sakha 62	1	1	0;	0;	0	0;	4	4	3	0;	2	0	4	3	2
38	Sakha 69	3	4	3	4	4	4	4	3	4	3	4	4	3	4	4
39	Sakha 88	4	4	3	3	4	3	3	3	4	3	3	3	3	4	4
40	Sakha 92	4	0;	4	4	4	3	4	3	4	3	4	4	4	4	0

TABLE 3. Cont.

No.	Variety	Leaf rust pathotypes/ infection type														
		CTTTT	FTSSS	KTSPT	NKTSS	NPTNK	NTKTS	NTTPT	PHTTT	PKTPT	PKTST	PTTNS	PTTPT	PTTSS	PTTST	STTTK
41	Sakha 93	4	3	3	4	4	3	4	4	0;	4	3	3	3	3	3
42	Sakha 94	4	4	4	4	3	4	4	4	0;	3	4	3	4	4	4
43	Sakha 95	3	4	4	4	3	3	3	3	0;	3	3	4	4	3	4
44	Bani Swif 1	1	0	0	2	;0	1	0;	1	0;	1	2	0;	2	0	1
45	Bani Swif 4	0;	1	1	2	0;	2	2	0;	2	2	1	2	0	2	0
46	Bani Swif 5	0;	0;	0;	1	1	0;	0	1	0;	0;	1	1	0;	0;	0
47	Bani Swif 6	0;	1	0;	2	0;	1	0	1	1	0;	0	2	0;	2	0;
48	Bani Swif 7	0;	1	0;	2	0;	1	0	0;	1	0	0;	0	2	2	1
49	Sohag 1	3	0	4	4	4	0;	1	0	0	0;	3	2	2	1	0;
50	Sohag 3	0;	0;	0;	4	2	0;	0;	0	0;	0;	1	0;	1	1	2
51	Sohag 4	0	0;	1	0;	1	1	0	0;	1	2	2	0	1	0	2
52	Sohag 5	3	0	4	4	2	0;	0	0	0;	0;	0	1	1	0;	1
53	Giza 139	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4

Genetic diversity evaluation and cluster analysis based on molecular markers

The consensus dendrogram based on molecular markers indicated four clusters named as A, B, C and D. The first main cluster "A" included four varieties from Bani Swif and all are resistant to all the pathotypes used. The cluster "B" comprised six varieties from Sakha except one form Sids and all these races were susceptible to most of all races except Sakha 62. Cluster "C" comprised varieties with more diverse reaction to the rust pathotypes, while cluster "D" included varieties with susceptibility to all the pathotypes (Fig. 2).

Correlation between the tested pathotypes based on virulence analysis and molecular characterization

The relationship between the phenotypic and molecular data among the 53 wheat varieties was illustrated in Fig. 3. The correlation between phenotypic and molecular data was very low (R^2= 0.15).

Diversity based on molecular marker pattern:

The diversity and genetic distance among the tested wheat varieties based on molecular marker pattern data were constructed by using past software (Hammer et al., 2001). The similarity was calculated according to Jaccard equation. Results of molecular analysis of variance showed that the genetic variation within varieties is 93% and difference among verities is 7% and very high gene flows among varieties exist (Table 4, Fig. 4).

Population structure for the genotypic data:

Population structure (Porras-Hurtado et al., 2013) was done by using structure software to cluster the genotypes under investigation as shown in Fig. 4. It was found that the genotypes under investigation form three populations according to the genotypic data.

The population structure demonstrated three populations (Fig. 5). The first population (in red) comprised seventeen varieties (Bani swif 1, Bani swif 4, Bani swif 5, Bani swif 6, Gemmeiza 3, Gemmeiza 5 , Gemmeiza 9, Sakha 8, Sakha 62, Sakha 69, Sakha 88, Sakha 93, Sakha 94, Sakha 95, Sids 13, Sids 14). While, the second populations (in green) comprised twenty varieties (Bani swif 6, Gemmeiza 1, Giza 150, Giza 157, Giza 162, Giza 163, Giza 165, Giza 167, Giza 171, Misr 1, Misr 2, Sakha 92, Sids 1, Sids 2, Sids 3, Sids 8, Sohag 1, Sohag 3, Sohag 4 and Sohag. The third population (in blue) comprised 16 varieties (Gemmeiza 7, Gemmeiza 10, Gemmeiza 11, Gemmeiza 12, Giza 139, Giza 144, Giza 155, Giza 156, Giza 160, Giza 168, Sakha 61, Shandaweel 1, Sids 5, Sids 6, Sids 7 and Sids 12).

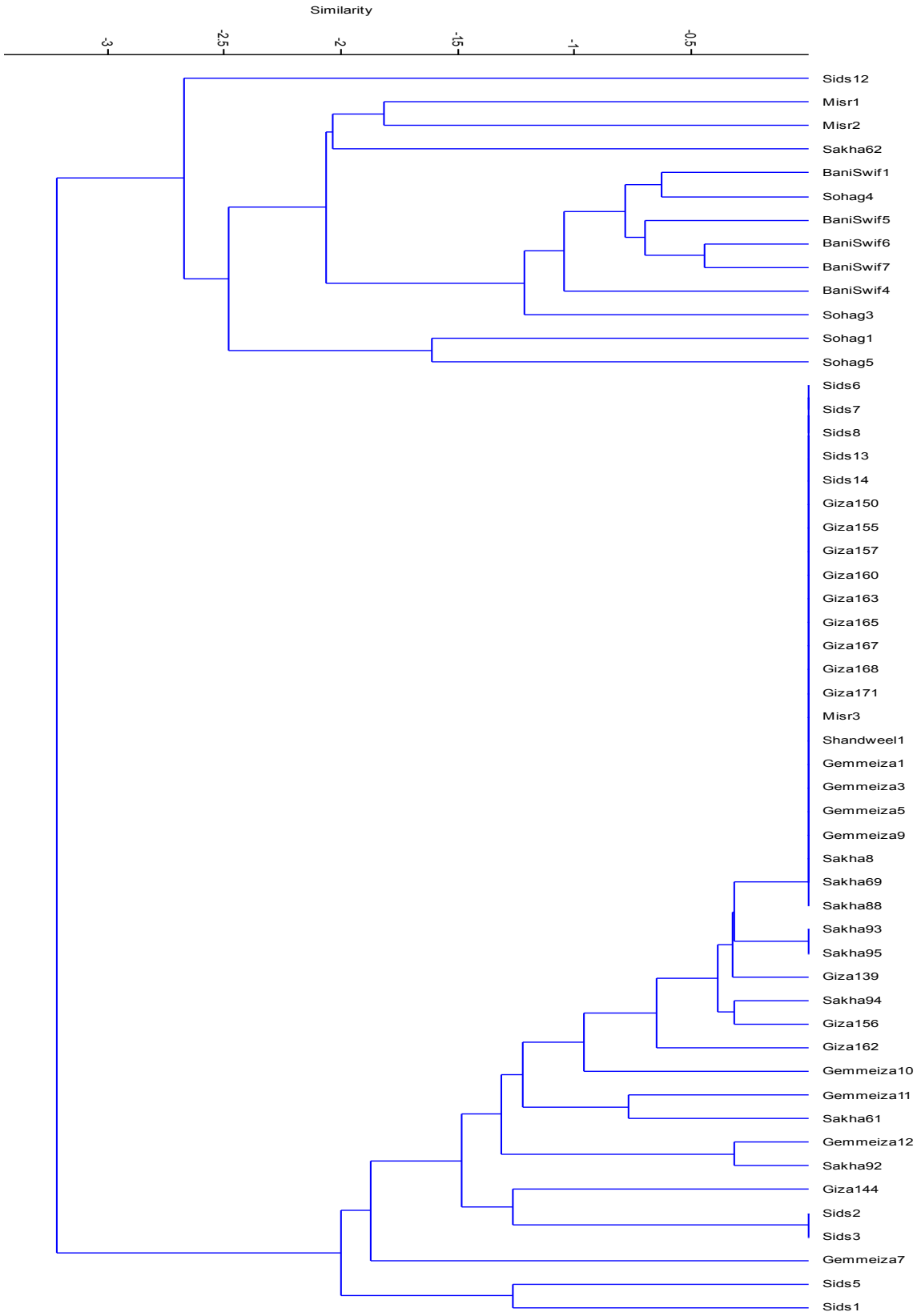

Fig. 1. Dendogram of 53 wheat varieties based on the seedling response against 15 leaf rust pathotypes

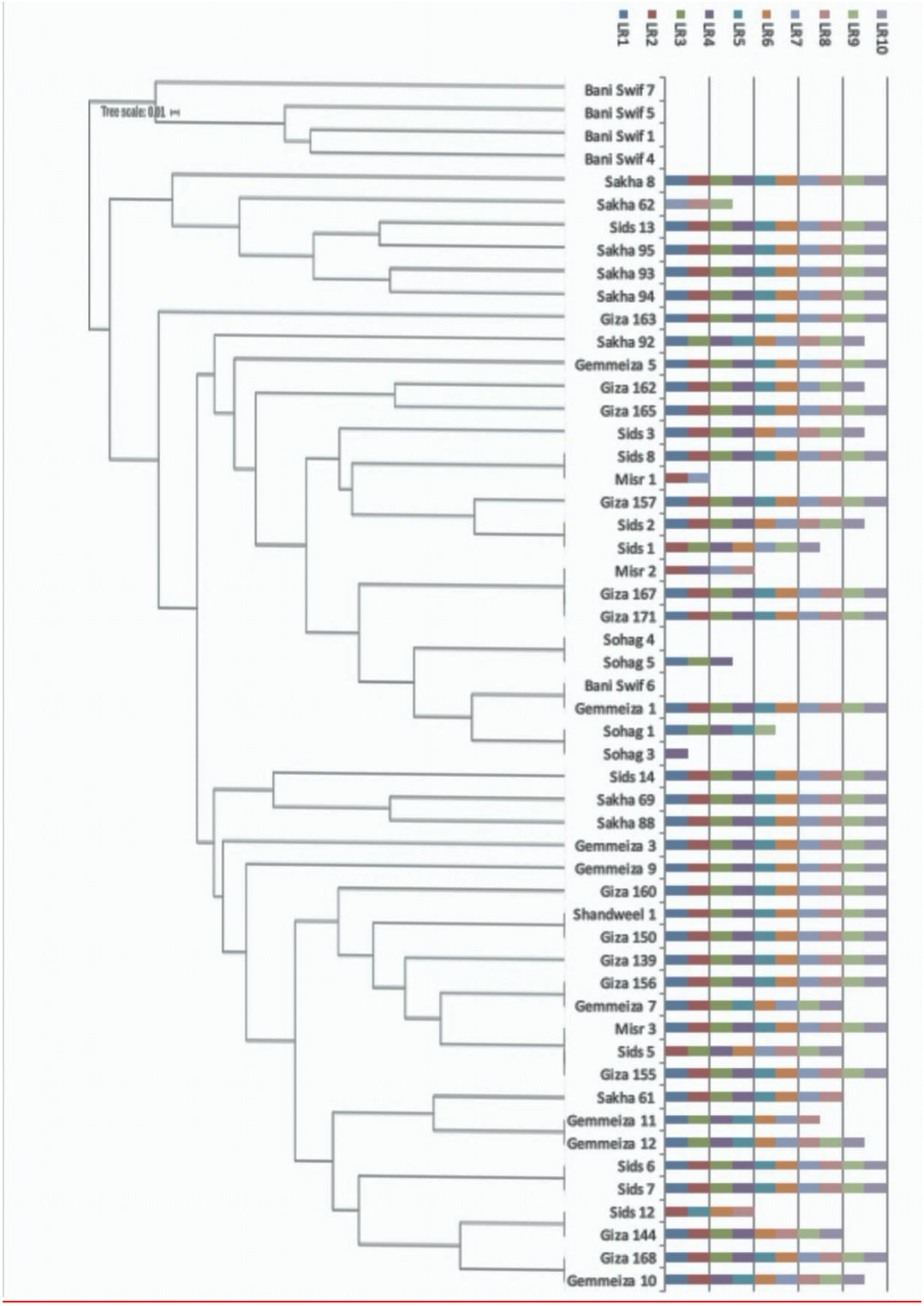

Fig. 2. Comparison between the reaction of the Egyptian wheat varieties under study against 15 different leaf rust races (on the right side) and the cluster analysis which was done by the genotype data of the SSR markers used (on the left side) [Each colour in the histogram represents the sensitivity against a leaf rust race as shown in the figure, the varieties that did not show any reaction (tolerant) had no colours. It was deduced that there are 4 main clusters, A, B, C and D, each represent a similar group according to the genotype data, also they had similar reaction against the leaf rust rust races]

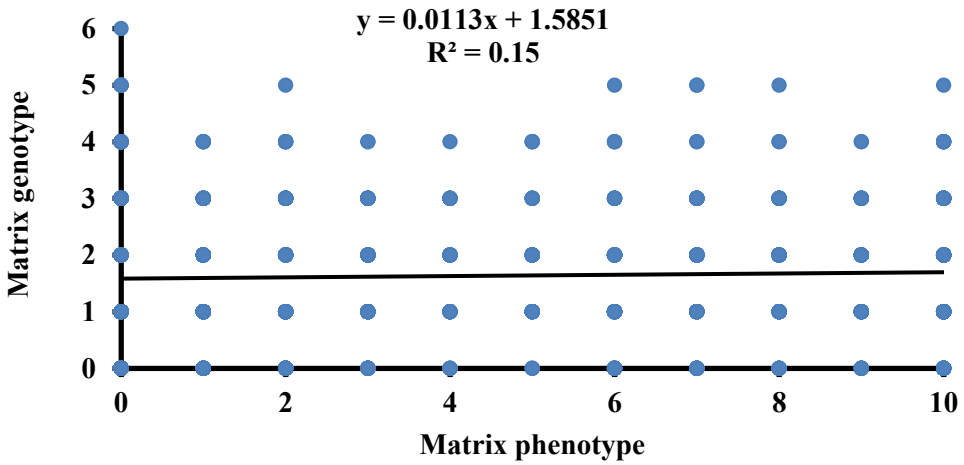

Fig. 3. Correlation between phenotypic and molecular characterization data to detect variations between 53 wheat varieties

TABLE 4. Analysis of molecular variance (AMOVA)

Source	D.f.	S.S.	M.S.	Est. Var.	%
Among Pops	1	3.454	3.454	0.173	7%
Within Pops	51	126.207	2.475	2.475	93%
Total	52	129.660		2.648	100%

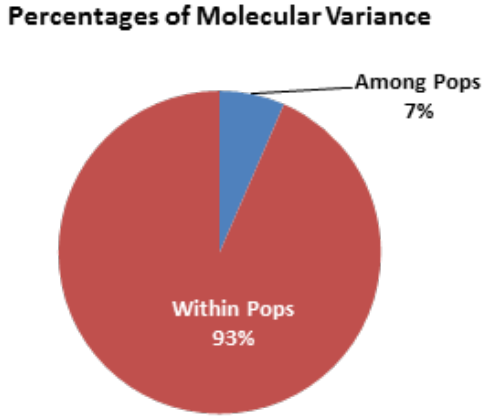

Fig. 4. The variation among populations and within populations

Discussion

Genetic diversity analysis in wheat is very important to understand the genetic relationship of the germplasm and their management and then used in breeding improvement in breeding program. Moreover, maintenance and use of these germplasm, facilitating breeders in devising methods to achieve valuable variation in the breeding programs (Al-Doss et al., 2013). Different studies have been performed on comparisons of evaluation of genetic diversity using phenotypic data and molecular marker analysis in plant protection, plant biology, and breeding program (Eivazi et al., 2008). Genetic diversity using molecular marker is more effective than morphological characters because in molecular marker, a huge amount of data are present within the same species, while in morphological characters, many drawbacks can be avoided by using the molecular characterization (Chinnusamy et al., 2007; Shinozaki et al., 2007).

Molecular markers methods have been used in advanced plant breeding program to develop important characters in plants. Several studies have assessed genetic diversity and phylogenetic relationship between wheat genotypes (Khan et al., 2015; Baloch et al., 2017).

In this study, 12 microsatellite markers were used to detect genetic variation between the 53 tested wheat varieties. In general, the correlation analysis between genetic variation and morphological trait tended to be low (0.15). Low correlation between distances derived from quality traits and SSR markers in wheat genotypes (Eivazi et al., 2008). Javier et al. (2005) also found that, low correlation between genetic similarities estimates depends on the three methods; SSR, RAPD and AFLP.

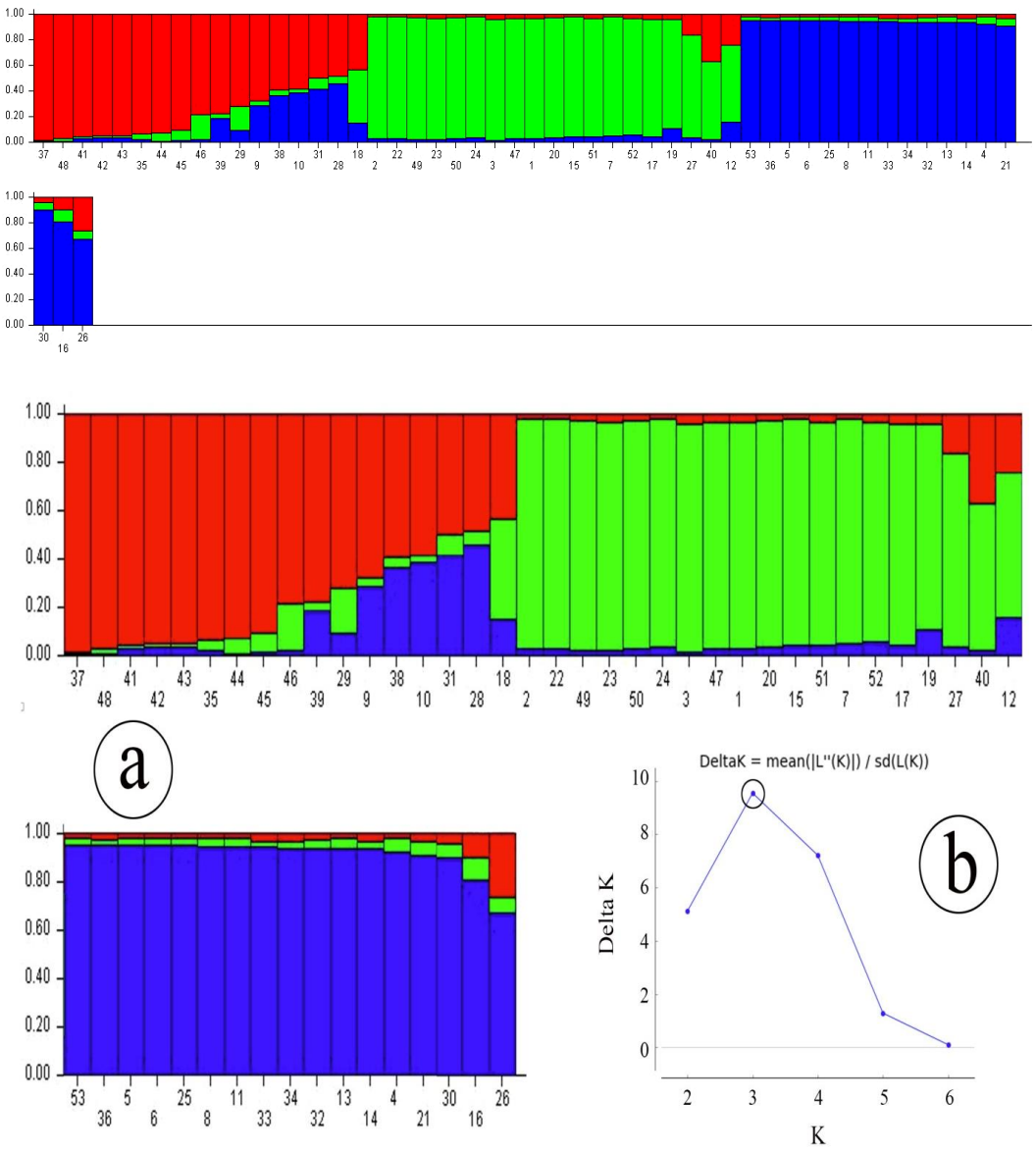

Fig. 5. Population structure analysis of Egyptian wheat varieties, (a) The colored columns represent the varieties distributed in three groups (shown in three different colors); and each line represents a population structure with a hypothetically given K-value; (b) ΔK for population structure with the hypothetical cluster (K) value from 1 to 6

In our study, this low correlation probably due to the few number of used primers in this study, which lead to a few number of amplified loci. Moreover, bread wheat belongs to a hexaploid wheat which genome size is very large (Lagudah et al., 2001); therefore, the used primers were not sufficient enough to cover this a large genome. On the other hand, there are different reasons may be occurred for the observed significant differences between the dendrograms of the tested marker systems. As mentioned before, the wheat genome size is very large; therefore, different markers may detect and amplify different regions of wheat genome. Scoring and detecting of RAPD polymorphism would be more subject to error than detecting and scoring of the other dominant polymorphisms (Kumar, 1999). Scores of RAPD for mapping reasons are used to tests for expected segregation ratio, leading to rejection of many of unreliable markers. This is not possible in the

germplasm analysis case, wherever there is no a prior expectation for the distribution of alleles between wheat genotypes. Increasing the number of polymorphisms would be expected to reduce the impact of scoring errors and unreliable bands (Kumar, 1999).

Conclusion

In the study we used marker already linked to genes of resistance to rust. However, the comparison between the biodiversity results obtained from phenotypic and genotypic data indicated low correlation indicating that genetic diversity obtained by molecular marker is not representing the real diversity of the population used in this study. This may belong to new genes in our Egyptian population and interaction between genes affected the phenotype. This study is the first study to link between the MAS applied in wheat and the phenotypic data applied on Egyptian varieties. Therefore, we recommend more study to develop better markers linked to resistant genes present in Egyptian varieties.

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التقييم المظهري والجزيئي للتنوع الوراثي لأصناف القمح المصري

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التنوع الوراثى بين الأنواع النباتية مهم لتحسين صفات النبات. يعتبر تقديره ضروريًا جدًا للمساعدة في إختيار التراكيب الوراثية في برامج التربية. علاوة على ذلك، فهو أساس التحسين الفعال والناجح للمحاصيل ويمكن تقديره بعدة طرق، مثل استخدام الصفات المورفولوجية (مقاومة صدأ الأوراق) والمعلمات الجزيئية. في هذه الدراسة، تم تقدير التنوع الوراثى بين أصناف القمح الـ ٥٣ المختبرة باستخدام ١٥ سلالة من صدأ الأوراق تحت ظروف الصوبة الزجاجية في مرحلة البادرة. بالإضافة إلى ذلك ، تم تقدير التنوع الوراثى باستخدام عشرة معلمات جزيئية (معلمات المايكروساتالايت) مرتبطة بجينات مقاومة للصدأ. أظهر تحليل شجرة القرابة إلى وجود ثلاث مجموعات فرعية بناءً على البيانات المظهرية والجزيئية. عموما، تم الحصول على مستوى منخفض من الإرتباط بين البيانات (r=0.15) المظهريه والبيانات الجزيئيه، بينما التحليل الجزيئي أكثر كفاءة لتقدير التنوع الوراثي. يعد التحليل الجزيئى طريقة فعالة لقياس التنوع الوراثى لأنه لا يتأثر بالبيئة ويمكن تطبيقه بوقت أسرع ودقة ولا يحتاج إلى معلومات نسب سابقة والتي يمكن أن تحسن كفاءة برامج التربية.