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Genetic Diversity and Identification of Molecular Markers Associated with Leaf Rust Resistance in Barley Genotypes

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Genetic diversity and relationships among 19 Egyptian barley (*Hordeum vulgare*) genotypes including cultivars and promising lines were evaluated. The 19 genotypes were used to detect marker associated with resistance to leaf rust using simple sequence repeat (SSR). Greenhouse was screening to evaluated the seedling stage of leaf rust reaction for the studied genotypes and the field screening analysis was carried out in the Experimental Research Station of Sakha during two growing seasons 2019/2020 and 2020/2021 to evaluate some morphological traits and adult leaf rust reaction among 19 barley genotypes. The genotypes were highly diverse in leaf rust reaction; Giza 131,133,132,136,137, line 6 and line 7 were resistant. Principal components analysis PCA was performed explaining about 53.34 % of total variance which was highly informative. SSR marker, Bmag692, discriminate the resistance and susceptible barley genotypes which it could be useful for the genetic diversity studies of leaf rust in barley. These results will be useful for barley management in terms of biodiversity protection and design of new crosses for disease resistance to leaf rust breeding program.

Key words: *Hordeum vulgar*, leaf rust, *PCA, PIC,* SSR

INTRODUCTION

Barley (*Hordeum vulgare* L.) is diploid (2n=2x=14) one of the cereal crop that is grown all over the world and is ranked fifth in world crop cereal production. Barley can be grown in numerous different climatic regions due to its flexibility to diverse conditions. These climatic conditions include variable growing seasons, temperatures, and precipitation rates (FAO, 2020).

Cultivated barley is affected by many diseases in the different parts of the world and including Egypt where it is commonly affected by leaf rust, scald and net blotches (Williams, 2003). According to Park *et al*., (2003), fungi causing rust diseases like leaf rust, stem rust and stripe rust have hindered the barley for many years as many cereal rust epidemics occurred in the past. Barley leaf rust (BLR) or brown rust, caused by fungal pathogen *Puccinia hordei*, is one of the most important barley diseases worldwide. In experimental conditions, yield losses as high as 60% can happen in highly susceptible barley cultivars, but losses of about half that level are common in practice (Das *et al*., 2007). It has been controlled primarily by the use of resistance cultivars. Resistance breeding can be the economically and environmentally effective strategy to reduce the yield losses caused by rusts. To date, in barley several seedling genes conferring resistance to leaf rust there are currently 28 designated barley leaf rust resistance genes known as Reaction to *P. hordei* (*Rph*) genes (*Rph1* to *Rph28*) has been recorded and mapped, *Rph1–19*, were known as seedling or all–stage resistance (ASR) genes (Park *et al*. 2015; Rothwell *et al*., 2020; Mehnaz *et al*., 2021a). Characterize and postulate the known *Rph* genes (resistance to *Puccinia hordei*) and identify novel sources of ASR (all–stage resistance) and APR (adult plant resistance) to *P. hordei* (Mehnaz *et al*., 2021b).

Improvement of resistant cultivars is one of the most effective and economical resources of controlling leaf rust in barley. Identification and incorporation of new and effective sources of resistance is a key to the success of barley breeding programs. Molecular markers display an important role and considered as a tool in parallels with conventional breeding for barley improvement. Initially, to design breeding program for useful trait is selecting parental genotypes based on its genetic dissimilarity.

The genome of diploid barley is very large at 5,100 Mbp and consists of 80% repetitive (Mascher *et al*., 2017). Simple Sequence Repeats or microsatellite (SSRs) are PCR–based marker and have proved to be useful in barley research as they offer reproducibility, multi–allelic nature, co–dominant inheritance, genome specificity, relative abundance, and adequate good genome coverage (Varshney *et al*., 2005). The SSR markers have been successfully used in detect the genetic diversity in barley leaf rust (Amgai *et al*., 2016). The use of molecular markers has fast tracked breeding programs by permitting marker assisted selection (Langridge and Barr, 2003). Microsatellites or (SSRs) are the ideal markers in cereals research due to their highly polymorphic and co–domi-

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nant characteristics (Gupta and Varshney, 2000). High density SSR–based linkage maps of barley are available (Khatab and Mariey, 2013; Varshney *et al*., 2007; Helal *et al*., 2018), which have increased the probability of finding polymorphic markers for specific chromosomal locations with linked to specific traits. The objective of the present study was to fingerprint and to determine the relationships among Egyptian barley genotypes based on SSR markers and to assessment of genetic diversity and relationships among barley cultivars in order to use them barley breeding programs for leaf rust resistance in Egypt.

MATERIALS AND METHODS

Barley Materials and Field Experiments

Nineteen barley genotypes including Egyptian cultivars and promising lines were used in this study their names and pedigree are shown in Table 1. They grow in the Experimental Research Station of Sakha during two growing seasons 2019/2020 and 2020/2021 to evaluate some agro–morphological traits and leaf rust reaction. Randomized complete block design with three replications was used. Plot size (experimental unit) was 1.8 m^2 (6 rows×0.2 m×1.5 m). Studied Characteristics determined in this study, were heading date (HD), plant height (PH) cm, number of tillers m^{-2} (NT), number of grain $spike^{-1}$ (NGS), spike length (SL), grain yield (GY) t h^{-1} and leaf rust reaction (LR).

Leaf rust assessment experiments

Seedling stage evaluation of leaf rust resistant in greenhouse

Seedlings of 19 tested barley genotypes were grown in rectangular trays in a greenhouse under controlled temperature, humidity and illumination conditions. Seven to 10 day old seedlings at the one and a half leaf growth stage were inoculated in the greenhouse; control seedling was sprayed with distilled water. The seedlings were transferred to an enclosed growth chamber and urediniospores were sprayed over seedlings using an aerosol hydrocarbon propellant pressure pack. The growth chamber door was kept closed for 5 min to allow urediniospores to infect leaves completely. Leaf rust–inoculated seedlings were incubated for 24 hours at room temperature in a dark chamber where continuous mist was created by an ultrasonic humidifier. Three replicates were used for each plant extract used and for the control treatment. Infection type responses were scored 10–12 days after inoculation according to the 0–4 scale used by Park and Karakousis (2002), which 0, 1 and 2 were considered as resistance response. Infection types 3 and 4 considered as the susceptible ones in all treatment of the study. Number of pustules/leaf (Receptivity) was determined by counting the number of uredinia or pustules per leaf area (receptivity) on the upper leaf surface of 19 barley genotypes under study, 14 days after inoculation.

Adult stage evaluation of leaf rust resistant in the field

Nineteen barley genotypes were screened for leaf rust reaction at heading stage at Sakha Experimental Research Station open field under natural infection conditions during normal barley growing season. Disease scoring was conducted according to the modified Cobb's scale (Peterson *et al*., 1948) as extreme resistance (R),

Table 1. Name and pedigree of 19 barley genotypes used in the field experimental and greenhouse

No.	Name	Pedigree
1	Giza 123	Giza 117/FAO 86
2	Giza 130	Comp.cross"229//Bco.Mr./DZ02391/3/Deir Alla 106
3	Giza 131	CM67B/CENTENO//CAMB/3/ROW906.73/4/GLORIABAR/ COME-B/5/FALCON BAR/6/LINO
4	Giza 132	Rihane-05//AS 46/Aths*2Athe/ Lignee 686
5	Giza 133	ICB91-0343-0AP-0AP-0AP-281AP-0AP
6	Giza 134	ICB91-0343-0AP-0AP-0AP-289AP-0AP
7	Giza 135	ZARZA/BERMEJO/4/DS4931//GLORIABAR/COPAL/3/SEN/5/AYAROS
8	Giza 136	Plaisant/7/Cln-B/Ligee640/3/S.P-B//Gloriaar/ Come B/5/Falconbar/6/Linocln-B/A/S.P/Lignee640/3/S.P-B// Gloria-Bar/Come B/5/Falconbar/6/Lino
9	Giza 137	Giza 118 /4/Rhn-03/3/Mr25-//Att//Mari/Aths*3-02
10	Giza 138	Acsad1164/3/Mari/Aths*2//M-Att-73-337-1/5/Aths/ lignee686 /3/Deir Alla 106//Sv.Asa/ Attiki /4/Cen/Bglo."S")
11	Giza 2000	C .C 89/3/Alanda/Hamra//Alanda-01
12	Line 1	Giza 117/3/Alanda/Hamra//Alanda-01
13	Line 2	Giza 117/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/ Cel/WI2269//Ore/4/ Hamra-01
14	Line 3	Giza 2000/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/ Cel/WI2269//Ore/4/ Hamra-01
15	Line 4	Giza 118/3/Alanda/Hamra//Alanda-01
16	Line 5	Giza 118/6/Lignee527/Chn-01//Alanda/5/Arizona5908/Aths// Avt/Attiki/3/S.T.Barley/4/Aths/Lignee686
17	Line 6	BLLU/PETUNIA1//CABUYA/3/Alanda// Lignee527 / Arar
18	Line 7	Rihane03/7/Bda/5/Cr.115/Pro/Bc/3/Api/CM67/4/Giza120/6/Dd/4/Rihane-03
19	Line 8	Giza 124/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/ Cel/WI2269//Ore/4/ Hamra-01

moderate resistance (MR), moderated susceptible (MS) and susceptible (S).

Molecular Analysis

DNA Extraction and SSR -PCR Amplification

Genomic DNA of the 19 barley genotypes was extracted from young leaves using CTAB method according Doyle and Doyle (1990). Polymerase chain reaction (PCR) amplification for both SSR was prepared in volume of 25 μl using 40 ng of genomic DNA, 2 μmol dNTP., 25 mM of $MgCl_2$, 10 pmol of each primer (forward and reverse), a 0.5 μl of 5U of *Taq* polymerase and 12 μl of 10X PCR buffer. PCR cycling for SSR was carried out as the following program; one cycle at 95ºC for 5 min., then 35 cycles was performed as follow: 1 min. at 95ºC for denaturation, 45 sec. 45–55ºC for annealing based on primer and 30 sec. at 72ºC for extension, and then incubated at 72ºC for 7 min. Ten SSR primes were selected based on their linkage with particular leaf rust resistance gene. Amplified products were separated using agarose gel electrophoresis (2%) in 0.5 x TBE buffer against 100 bp DNA Ladder as a size marker.

Data Analysis

Data collected from field experiment were statistically analyzed as a randomized complete block design (RCBD) using analysis of variance (ANOVA) as a combined analysis (Steel *et al*., 1997). Pearson correlation were used to study the relationship between each two studied traits were done using SPSS–16.0 statistical software package (SPSS Inc. Chicago, IL, USA). To study the differences and interrelations between genotypes with respect to measured phenotypic traits and genotypic data a multivariate heatmaps visualizing clustering was constructed using ClustVis program.

The amplified bands from SSR were scored as a binary data. The data were used to estimate the genetic similarity on the basis of number of shared amplification products (Nei and Li, 1979). Polymorphism information content (PIC) values, Resolving power (Rp), marker index (MI) and effective multiplex ratio (EMR) values were done according to (Anderson *et al*., 1993). Phylogenetic trees were constructed based on Jaccard similarity matrix using PAST program (PAleontological Statistics Version 1.94b) was performed to produce a dendrogram using un–weighted pair–group method with arithmetical average (UPGMA) Hammer *et al*., (2001).

RESULTS

Field experiments data analysis

Morphological traits

The results of analysis of variance of all studied traits a cross the two growing seasons exhibited significant differences among the genotypes for all studied characters based on the least significant difference

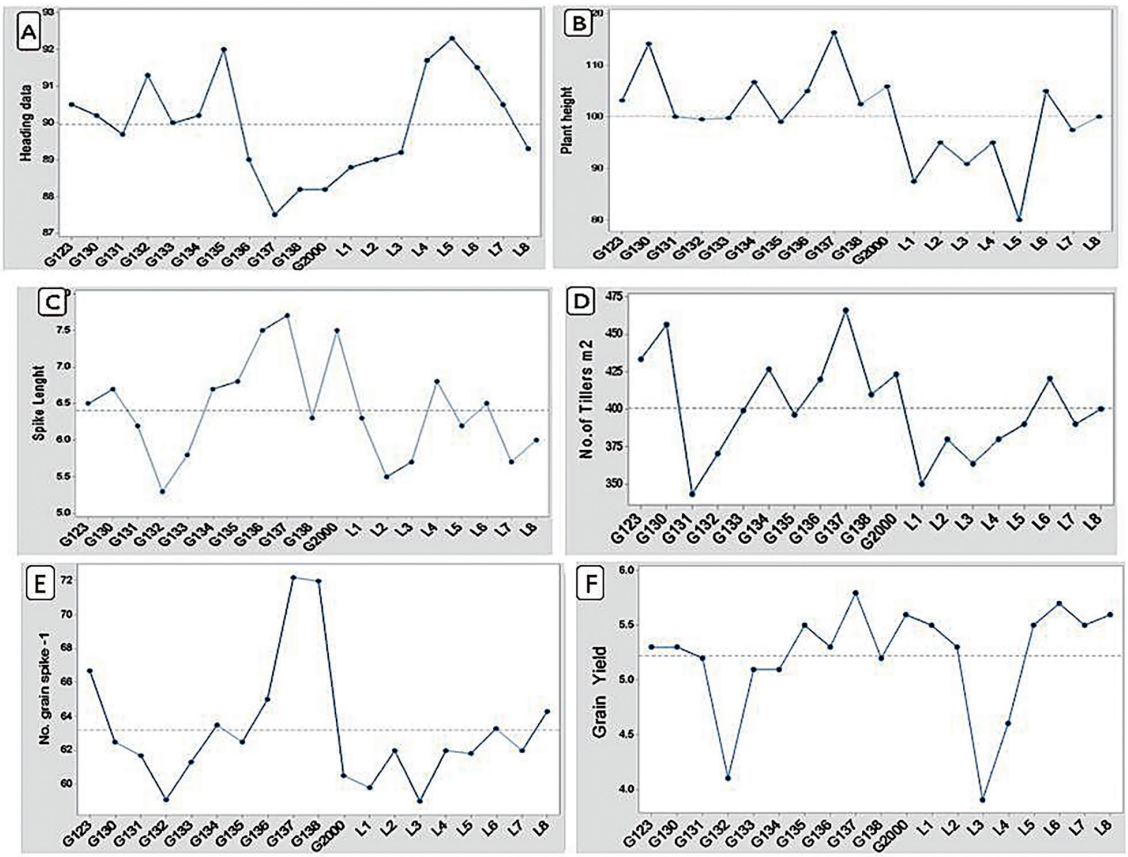

Fig. 1. The main effects plot showed the averages values for **A:** heading data, **B:** plant height, **C:** spike length, **D:** number of tillers m^2, **E:** number of grain spike and **F:** grain yield among the 19 Egyptians barley genotypes grown in two seasons2019/2020 and 2020/2021.

(LSD). The significant difference in values between the different genotypes for HD,PH,SL,NGS,NT and GY were (LSD=1.72,3.33, 1.12, 1.64,4.49, 0.29) respectively. This provides an evidence for the possibility to carry out a sufficient selection program on the basis of these traits using the studied genotypes. The main effect plot of the average values was shown in Figure 1A, the. results varied significantly in heading data, Giza 137 was the earliest genotypes with average value of (87.5 days) while the latest genotypes was Line 5 with values of (92.3) days. Combined means performance of plant height clearly indicated that the cultivar Giza 137 was the tallest cultivar (116.4 cm^2). However Line 5 was the shortest cultivar (80.0 cm) as shown in Figure 1B. Regarding the main effect plot of spike length Figure 1C, the results indicated that cultivar Giza 137 had the highest spike length (7.7 cm), however Giza 132 had the lowest spike length (5.3 cm). The mean performance of number of grains $spike^{-1}$ indicted that Giza 137 gave the highest no. of grains $spike^{-1}$ was (72.2 grains $spike^{-1}$), however, the lowest no. of grains $spike^{-1}$ was produced by Line 3 which gave 59.0 grains $spike^{-1}$ were showed in Figure 1E. Concerning number of tillers m^{-2}, the average values of the genotypes as displayed in Figure 1D revealed that the cultivar Giza 137 gave the highest number of tillers m^{-2} (466.3 tillers m^{-2}), while, the lowest number of tillers m^{-2} was obtained by cultivar Giza 132 with value of 343.2 tillers m^{-2}. Concerning the results of the main effect plot of grain yield results showed in Figure 1F indicated that cultivar Giza 137 had maximum value (5.8 t h^{-1}). However, line 3 had minimum value of grain yield (3.9 t h^{-1}).

Inoculation and leaf rust disease reaction on barley cultivars

Disease assessment was done on 19 tested barley genotypes to leaf rust at two growing stage (seedling stag at greenhouse and heading stage at Sakha open field under natural infection conditions).

Greenhouse experiments

The results of leaf rust reaction of seedling 19 barley genotypes showed a significant difference in values between the different genotypes was (LSD=3.02). The main effects plot of LR reaction among all 19 barley genotypes were presented in Fig. 2 which results indicated that Giza 131, Giza 132, Giza 136, Giza 137, Line 6 and Line 7 scored one under green house at seedling stage as resistance genotypes, while Giza123, Giza 2000 and Line 3 scored four as susceptible genotypes. However for moderated resistance MR Giza 130, Giza 133, Giza 138, Line 1 and line 2 scored two, but Giza 134, Giza 135, line 3, line 4 and line 7 scored three as a moderated susceptible.

Open filed experiments condition

At heading stage the adult plant of 19 barley genotypes were scored and the results were shown in (Fig. 3)

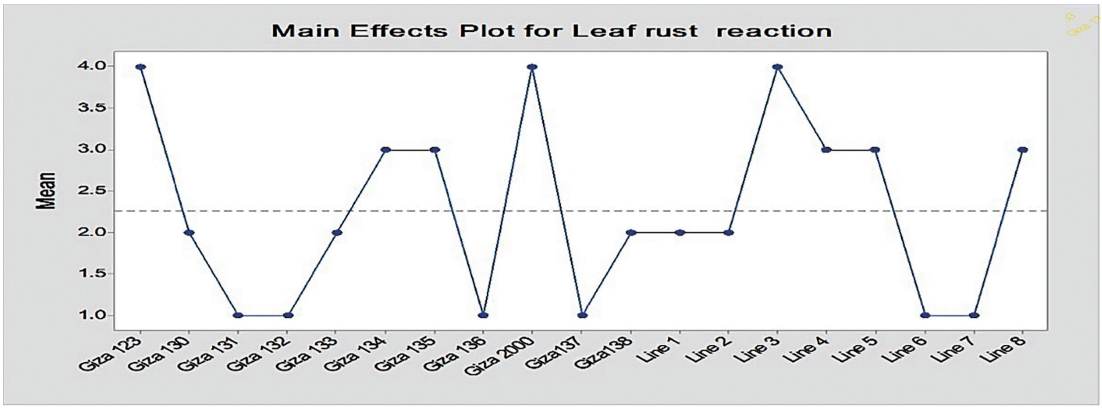

Fig. 2. Main effects plot for leaf rust reaction of 19 seedling barley genotypes under greenhouse.

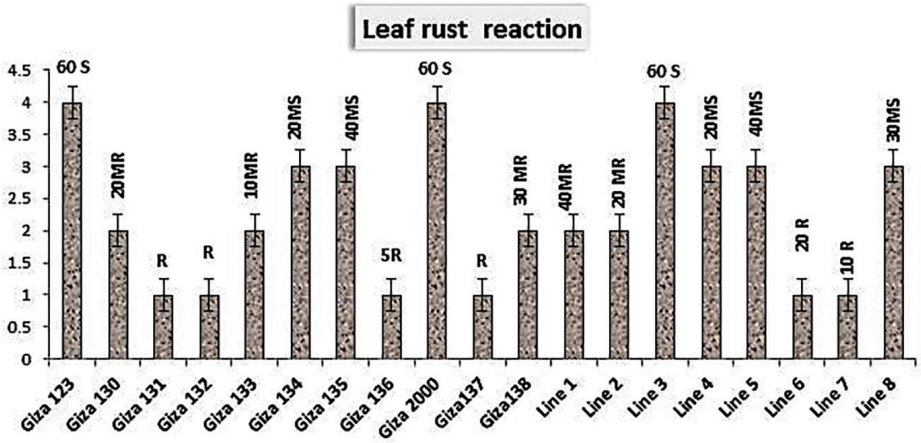

Fig. 3. Leaf rust reaction for the 19 barley genotypes in open field experiments.

indicted that Giza 131, Giza132, Giza 136, Giza 137, Line 6 and Line 7 rated to R type which recorded (R, R, 5 R, R, 20 R and 10 R) respectively. However, Giza123, Giza 2000 and Line 3 rated to S type they recorded (60S) where could consider them as susceptible genotypes. While Giza 130, Giza 133, Giza 138, Line 1 and line 2 rated to moderated resistance MR type they recorded (20, 10, 30, 40, 20 MR) respectively, on other hand, Giza 134, Giza 135, line 4, line 5 and line 8 rated to moderated susceptible MS type they recorded (20, 40, 20, 40 and 30 MS) respectively.

Relationships among phenotypic studied traits and leaf rust reaction

Pearson correlation coefficient and the principal component analysis (PCA) analysis were applied to understand the relationships among analysis phenotypic studied traits and leaf rust reaction.

Pearson correlation coefficient

The heatmap results of Pearson correlation coefficient among leaf rust reaction and the six agro–morphological studied traits showed a significant positive correlation coefficient among leaf rust reaction and grain yield (0.19), spike length (0.06) and heading data (0.05). Whereas, negative significant correlation was obtained among leaf rust reaction and number of grains spik^{-1} (–0.09), number of tillers m^{-2} (–0.40) and plant height (– 0.49) as shown in Figure 4.

Principal component analysis (PCA)

The loading plot analysis of PCA were performed using Nei's (1973) distance matrix presented in the horizontal axis as showed in Fig. 5 indicated the direction of association among all studied traits. The first two components PCA had Eigen value more than one and contributed 66.31 of the total variation. The first PCA1 axis, showed 43.13% of the total variation due to plant height, spike length, no. grains spike^{-1}, number of tillers m^{2} and grain yield were loaded in right side of the horizontal axis according to their positive correlations with most other traits. The second PCA2 axis represents 23.01% of the total variability due to heading data leaf rust reaction were located in the left side (negative) of the horizontal axis according to their negative correlations with most other traits.

Simple sequence repeats analysis (SSR)

Ten microsatellite primer pairs previously mapped the barely chromosomes (Grain Genes database) were used in this study. These primers were screened against 19 barley genotypes in an attempt to detect polymorphic markers. The polymorphism level of theses ten simple

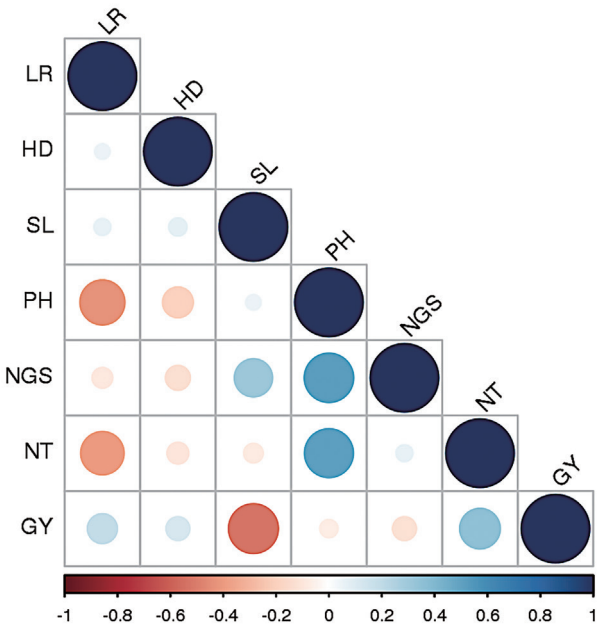

Fig. 4. Heatmap of Pearson correlation coefficient among leaf rust reaction and all studied traits.

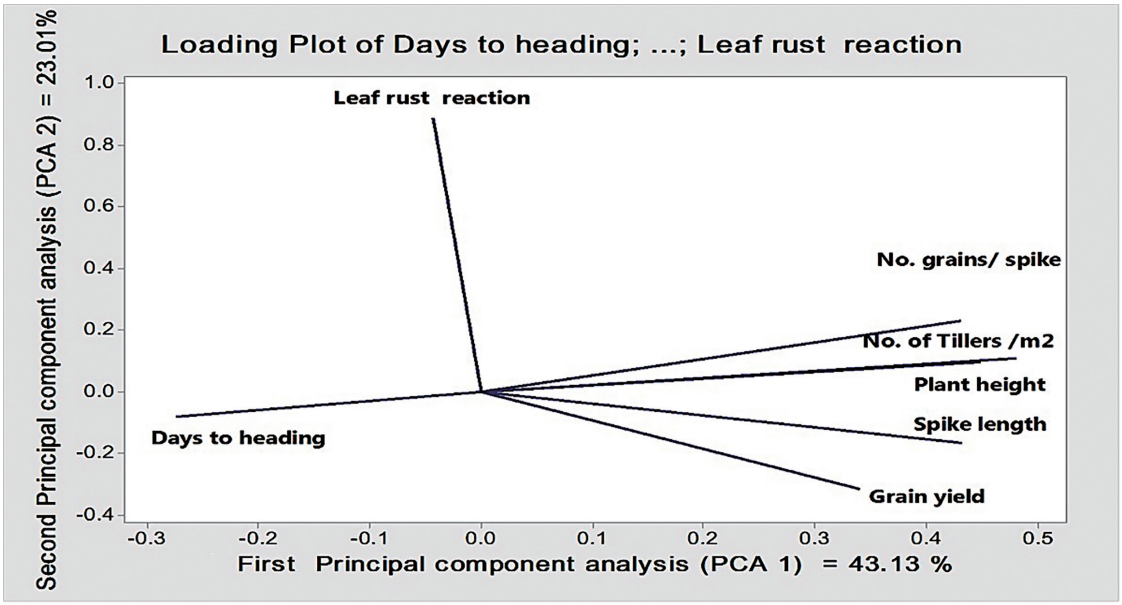

Fig. 5. Loading plot analysis of PCA designated the direction of association among all studied traits.

sequence repeats (SSRs) primers were displayed in Table 2. Eighteen alleles were revealed form ten SSR using studied 19 Barley genotypes with an average of 1.4 alleles per locus. The outstanding six primer pairs (Bmac 0032, Bmac 0036, GMB1402 Bmag 692, Bmac 0125 and Bmag 0011) generated clear fragment patterns with high polymorphism (100%). The polymorphism information content (PIC) value of each SSRs marker ranged from 0.33 for (Bmac0032, 1H) to 0.33 for 0.38 for (Bmag692, 2H). The SSR (GMB1402, 1H) generate high marker efficiency indices such as number of alleles (NA), number of polymorphism bands (NPB), percentage of polymorphism (PP%), polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI), diversity index (DI) and resolving power (RP) values were (3, 3, 100%, 0.37, 1.42, 0.02, 0.81 and 1.68) respectively. As shown in Fig. 6 and Table 2, the resistance genotypes were shared a band with size 180 bp, susceptible genotypes have only one band with 200 bp. However, other genotypes which have moderate reaction to leaf rust have band with around two bands 180 and 200 bp.

Cluster alanysis

Genetic relationships among 19 Barley genotypes based on ten SSR primers data were presented in a UPGMA cluster dendrogram using Jaccard similarity coefficient matrices presented two main clusters (Fig. 7A&B). The first cluster 1 inculed the resistance and modreated resistance genotypes for leaf rust divided in to two sub clusters. The sub cluster1 were include six resistant genotypes R were (Giza 136, Giza 137, Giza 132, Giza 131, Line 6 and Line 7) and the second sub-cluster, included five moderate resistant MR genotypes (Giza 130, Giza 138, Giza 133, Line 1 and Line 2). However second cluster conformed the susceptible and modreated susceptible genotypes for leaf rust recation were divided in two sub cultster. The three susceptible genotypes (Giza 123, Giza 2000 and Line 3) were found in the frsit sub cultster and the second sub comprised the five moderate susceptible genotypes (MS) (Giza 134, Giza 135, Line 4, Line 5 and Line 8) as shown in Figure 7A&B.

Genetics similarity index

The results of genetics similarity index consuming Jaccard similarity coefficient as showed in Fig. 8 showed that the highest similarity value (0.92%) was observed among both line 2 and Giza 133 and Line 1. Similarly, another high similarity among Line 3 and Giza 123 and Giza 2000 was 0.89 as highly susceptible genotypes. Correspondingly, high genetics similarity were found among all the six resistance barley genotypes ranged

Table 2. List of multiplexing sets of the used ten SSR primers among 19 barley genotypes for leaf rust reaction

No.	Primer name	motifs	Ch. L	NA	NPB	P%	PIC	EMR	MI	DI	RP
1	Bmac 0032	(AC)7(CA)13(AT)19	1H	2	2	100	0.33	1.32	0.02	0.50	1.16
2	GBM10402	(AAC)5	1H	3	3	100	0.37	1.42	0.02	0.81	1.68
3	GMS003	(GT)15	2H	1	0	0	0.00	1.00	0.00	0.00	0.00
4	Bmac 0125	(AG)19	2H	3	3	100	0.34	1.58	0.01	0.73	1.16
5	Bmac 0211	(CT)16	1H	1	0	0	0.00	1.00	0.00	0.00	0.00
6	HVM36	(GA)13	2H	1	0	0	0.00	1.00	0.00	0.00	0.00
7	Bmac 0316	(AC)19	6H	2	2	100	0.35	1.16	0.01	0.67	0.53
8	Bmac 213	(AC)23	1H	1	0	0	0.00	1.00	0.00	0.00	0.00
9	Bmag 0011	(AG) 25	7H	2	2	100	0.34	1.11	0.01	0.70	0.63
10	Bmag692	(CT)19	2H	2	2	100	0.38	1.21	0.02	0.64	0.84
	Total			18	14	600	3.83	11.79	0.09	4.05	6.00

Which Ch L.: chromosome Location, NA: no. of alleles; NPB: number of polymorphic bands, P%: polymorphism (%); PIC: polymorphism information content; EMR: effective multiplex ratio, MI: marker index, DI: diversity index; and RP: resolving power

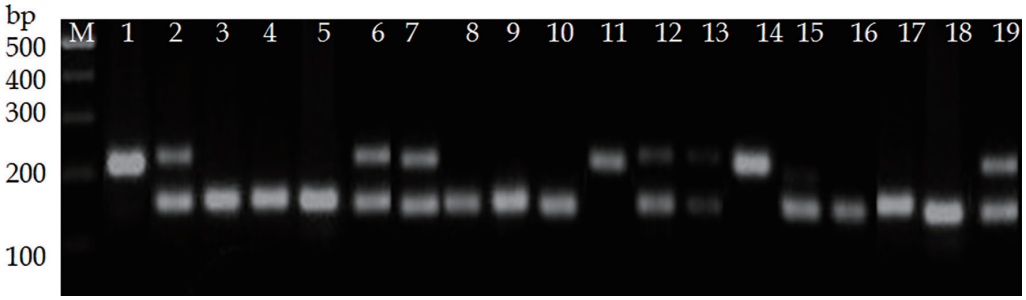

Fig. 6. Banding pattern of Bmag692 marker, the samples are described in Table 1, according to their identification codes.

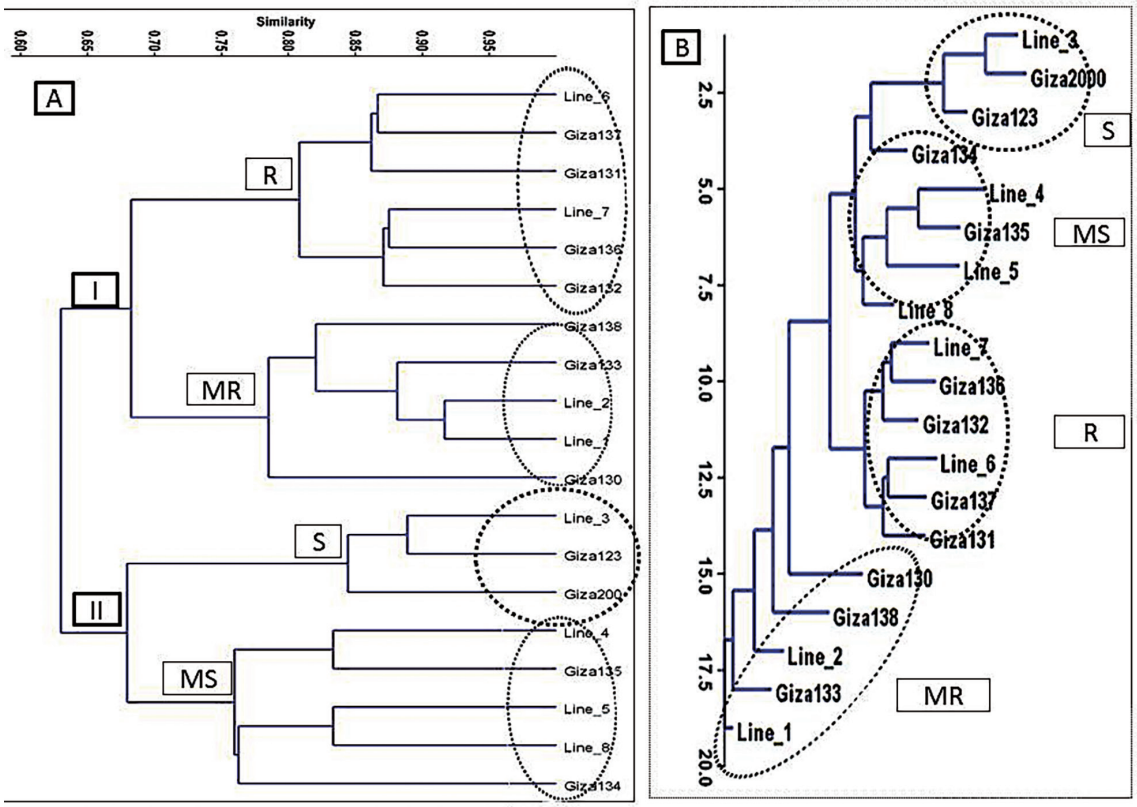

Fig. 7. Dendrogram showing clustering pattern of all the19 Egyptian barley genotypes (A) rooted tree and (B) un–rooted tree using 10 SSR markers.

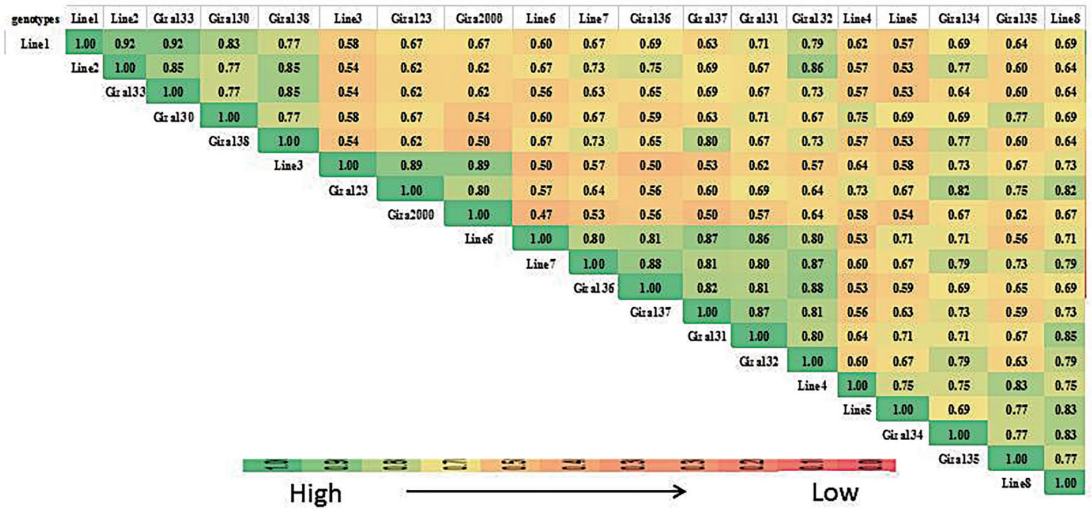

Fig. 8. Genetics Similarity for 19 barley based Jaccard similarity coefficient.

between (0.80%) were observed between both (Line 6 and Line 7) and (Giza 131 and Giza 132) to (0.88%) were observed between Giza 136 and both of (Line 7 and Giza 132) While, the lowest similarity value was (0.47%) were found among resistance (Line 6) and susceptible (Giza 2000) genotypes.

Genetic diversity indices

The genetic diversity indices such as Simpson index (SI), Shannon's diversity index (SDI) and Berger–Parker index (BPI) were important indices in order to estimate the levels of genetic diversity among the 19 Egyptian barley genotypes were presented in Table 3. The achieved highest values of SI and SDI were found in resistance genotypes Giza 132 with values (0.9825 and 4.0435) respectively, while the lowest values of SI and SDI were found in susceptible genotype Line 3 by (0.9756 and 3.714) respectively. While, the lowest BPI value was found resistance genotypes Giza 132 with value 0.0177 and the highest BPI was observed in susceptible genotype Line 3 by 0.0244.

Multivariate clustering heatmap analyses

Multivariate clustering heatmap analyses were used to recognize the differences between the morphological data clusters and the molecular data clusters as well as their interaction in order to comprehend the interrelations among the 19 barley genotypes and their reaction to leaf rust diseases. The cluster heatmap were made using Euclidean distance and ward linkage using through R software as showed in Fig. 9. The nineteen barley genotypes were found in row dendrograme which clustered into two main clusters, first cluster including the six resistant genotypes were (Giza 136, Giza 137, Giza 132, Giza 131, Line 6 and Line 7). Second cluster divided into sub cluster, first sub consisted of three susceptible

Table 3. Genetic diversity among 19 barley genotypes using ten SSR primer combinations

Cultivars	Total polymorphic band	Simpson Index	Shannon's information index	Berger–Parker index
Giza 123	9	0.9773	3.7840	0.0227
Giza 130	9	0.9815	3.9890	0.0185
Giza 131	14	0.9818	4.0070	0.0182
Giza 132	15	0.9825	4.0433	0.0175
Giza 133	12	0.9811	3.9700	0.0189
Giza134	12	0.9804	3.9320	0.0196
Giza135	11	0.9804	3.9320	0.0196
Giza 136	14	0.9818	4.0072	0.0182
Giza 137	14	0.9824	4.0430	0.0175
Giza 138	11	0.9811	3.9700	0.0189
Giza 2000	9	0.9787	3.8500	0.0213
Line 1	11	0.9804	3.9320	0.0196
Line 2	11	0.9804	3.9320	0.0196
Line 3	9	0.9756	3.7140	0.0244
Line 4	10	0.9800	3.9120	0.0200
Line 5	10	0.9792	3.8710	0.0208
Line 6	14	0.9821	4.0250	0.0179
Line 7	13	0.9815	3.9890	0.0185
Line 8	50	0.9804	3.9320	0.0196
Average	51.53	0.9805	3.9386	0.0195

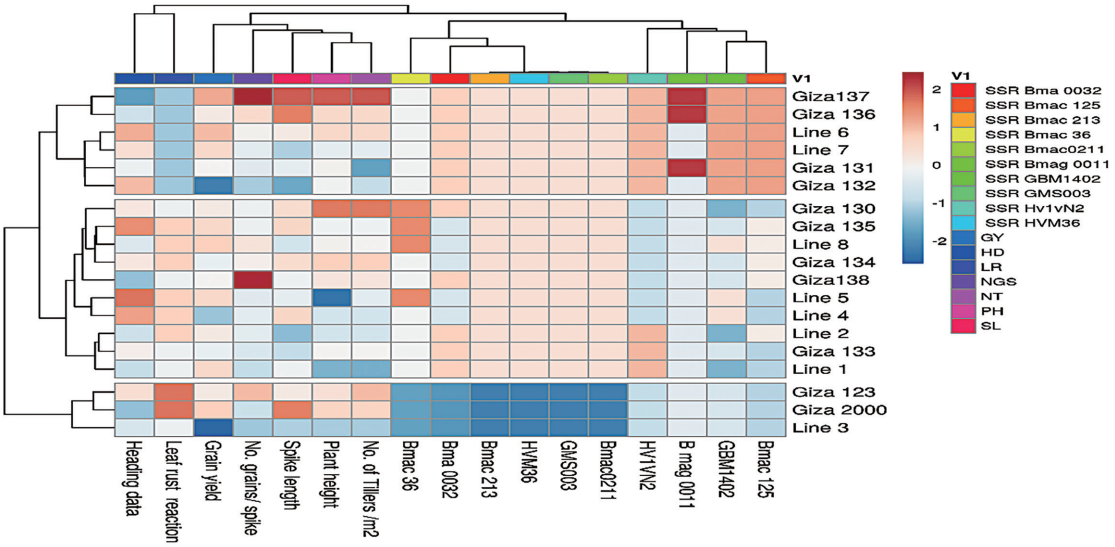

Fig. 9. Multivariate heatmap illustrating the genetic diversity of 19 Egyptian six-rowed barley genotypes, based on the 10 SSR primers and seven morphological traits using the module of a heatmap of ClustVis to study the leaf rust reaction.

genotypes (Giza 123, Giza 2000 and Line 3) and the second sub include moderated susceptible and resistance genotypes. Column dendrogram show the similarity between seven morphological traits and SSR primer.

DISCUSSION

Primarily in plant breeding selected genotypes were chosen for crossing based on their phenotypic traits (Zeven, 1996). Recently, selection resulted in significant genetic erosion in the major crops, including barley. Due to high adaptation potential of varied stress including rust, it is necessary to use more genes of resistance (Dreiseitl, 2003). To pyramid resistance genes successfully, closely linked and breeder friendly markers are necessary to use in assist selection. The present study aimed to characterize new sources of resistance to *P. hordei* to help barley breeders by differentiating the resistance sources currently available to control this disease. The genotypes Giza 131, 132, 133, 136, 137, Lines 6 and 7 were reported to resistance to leaf rust.

Leaf rust is serious disease of great harmful impact on barley production in Egypt. Therefore, a morphological and molecular evaluation of barley germplasm is essential to improve our knowledge on the abilities of accessible germplasms and enabling us to predict new cultivars performance, select parents for crossing in crop improvement programmers, and clone new natural plant resistance genes (Saker, 2005).

Partial resistance to *P. hordei* in barley causes slow rusting in the form of reduced infection frequency, increased latent period and reduced sporulation. Under field conditions it is difficult to select resistance genotypes because all genotypes produce a susceptible reaction type (Golegaonkar *et al*., 2009). In the present study, the filed evaluation resulted in the identification of resistance of some barley genotypes to leaf rust and the level of resistance varied from susceptibility to resistant. It is worthy to note that Giza 131, 132, 136, 137, Line 6 and Line 7 showed resistance response throughout the obtained combined data under greenhouse and field screening. These results were in good harmony with Mamadov *et al*. (2003) and Adawy *et al*. (2008) which reported that the level of resistance is varied from extreme resistance to high and moderate resistance in barley against leaf rust under open field under natural infection conditions. In addition, outstanding fingerprints of the identified valuable cultivars were successfully created using the SSRs. The widespread of *Rph14* in Europe, North America, South America, and Africa, it could be a useful source of resistance especially if it is combined with other seedling resistance gene(s) to raise leaf rust resistance as reported by Fetch *et al*. (1998). *Rph14* located on the short arm of chromosome 2H, SSR marker, Bmag692, linked closely to *Rph14* (Golegaonkar, 2007). In this study SSR marker Bmag692 was amplify two bands one of them with size 180bp was found in most all resistance genotypes it could be as marker for leaf rust in barley. The close linkage and co-dominance of Bmag692 mean that it will be useful in assisting selection for resistance genes like *Rph14* (Golegaonkar, 2007). The efficiency of using this marker in MAS could be improved by either identifying a second marker flanking *Rph14* or by further fine mapping studies. Currently, only few seedling resistance genes are effective, several studies (Golegaonkar *et al*., 2009; Park, 2008) stressed to identify new sources of resistance to leaf rust in barley, including adult plant resistance (APR). Recently, the first gene conferring APR to leaf rust in barley, *Rph20* was mapped on chromosome 5HS (Hickey *et al*., 2011). Two markers linked to *Rph20*, EBmag0833 and bPb–0837, were reported by Liu *et al*. (2010), who proposed the use of bPb–0837 in marker assisted selection for APR against *P. hordei*. The widespread effectiveness of *Rph14* means that it could be useful source of resistance, especially if it is combined with other seedling resistance genes to increase durability (Park *et al*., 2003).

Screening more primers should identify more closely linked markers; the obtained results demonstrated that the marker Bmag692 is the closest marker to the leaf rust resistance locus. The information and the leaf rust–resistant germplasm identified in this study represent a useful resource for breeders to further diversify the genetic basis of leaf rust resistance in barley. The use of more markers could be allowing the further selection of markers that would map closer to the leaf rust. Finding and characterizing such sources of resistance to *P. hordei* in barley could facilitate their utilization in breeding programmers.

AUTHOR CONTRIBUTIONS

The authors contributed to the study conception and design, Material preparation, data collection, by [Samah A. Mariey, Sherin Ph. Mikhail and Mohamed Bosily], data analysis [Samah A. Mariey and Sabah Morsy], molecular work and data analysis were performed by [Ismael A. Khatab and Samah A. Mariey] and review and paper preparation for journal were performed by [Ismael A. Khatab and Toshihiro Kumamaru]. The first draft of the manuscript was written by [Samah A. Mariey] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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