GENETIC DIVERSITY AND RELATIONSHIPS AMONG SOME BARLEY GENOTYPES FOR NET BLOTCH DISEASE RESISTANCE USING RAPD, SCOT AND SSR MARKERS

S. A. DORA[1], M. MANSOUR[2], AZIZA A. ABOULILA[1] AND E. ABDELWAHAB[2]

1. Genetics Dept., Faculty of Agriculture, Kafr El-Sheikh University, 33516 Kafr El-Sheikh, Egypt

2. Barley Dept., Field Crops Res. Institute, ARC, Egypt

Barley (*Hordeum vulgare* L.) is the fifth important cereal crop species in crop production world-wide after maize, wheat, rice, and soybean. It is also a model species for genetic studies while it is an annual and diploid self-pollinating species and has a relatively short life cycle. Net blotch of barley, caused by the phytopathogen *Pyrenophora teres* constitutes one of the most serious problems on barley production world-wide (Shipton *et al*., 1973). Net blotch disease cause significant yield loss and affect the grain quality negatively. Losses due to net blotch could reach 50% of yield with possible complete loss depending on cultivar susceptibility and environmental conditions (Steffenson *et al*., 1996). Detection of resistance sources to net blotch and understanding their genetic background are very important in developing new resistant varieties to such disease. Net blotch resistance is controlled by several genes and dependent on the source of resistance, the development stage and the pathotype used for testing (Graner *et al*., 1996; Svobodova *et al*., 2011).

Using molecular marker technology in barley offers high efficiency tools for indirect selection and would enhance the efficiency and accuracy of screening for net blotch resistance. Furthermore, quantitative analysis proved to be useful for determining genes controlling complex traits and provides a more accurate estimation of gene location because of its lower sensitivity to even modest numbers of phenotypic mis-scores (Wright, 1998), barley germplasm identification and classification (Struss and Plieske, 1998).

The association between molecular markers and phenotypes is one of the most significant factors in the field of molecular genetics and molecular breeding. It provides substantial landmarks for elucidation of genetic variability and detection of genomic regions that are responsible for the trait, which plays an essential role in the strategic improvement of barely using marker-assisted selection (Adawy *et al*., 2008).

These molecular markers had been used in barley for detecting genetic diversity and genotype identification. Of these techniques, Random Amplified Polymorphic DNA (RAPD) has several advantages, such as simplicity of use, low

Egypt. J. Genet. Cytol., 46: 139-165, January, 2017
Web Site (*www.esg.net.eg*)

cost, and the use of small amount of plant material. RAPDs were proved to be useful as genetic markers in the case of self-pollinating species with a relatively low level of intraspecific polymorphism, such as cultivated barley (Tinker *et al.*, 1993).

A new molecular marker system called Start Codon Targeted Polymorphism (SCoT) was described by Collard and Mackill (2009), based on the observation that the short conserved regions of plant genes are flanked by the ATG translation start codon. The technique uses single primers designed to anneal the surrounding regions of the ATG initiation codon on both DNA strands. The generated amplicons are possibly distributed within gene regions which contain genes on both plus and minus DNA strands. The utility of primer pairs in SCoTs was described by (Gorji *et al.*, 2011). SCoT markers are reproducible, and it is suggested that primer length and annealing temperature are not the only factors determining reproducibility. They are dominant markers, however, while a number of co-dominant markers are also generated during amplification, and thus they could be used for genetic diversity analysis (Collard and Mackill, 2009).

Microsatellites are widely used as genetic markers because they are co-dominant, multi-allelic, easily scored and highly polymorphic. However, a major drawback of SSR markers is the time and cost required to characterize them (Fisher *et al.*, 1996). SSRs are tandemly arrayed repetitive sequences that are spread throughout the eukaryotic genomes and shown to be the most variable component of the genome with a high level of molecular evolution **(**Hemleben *et al.*, 2000**)**. Microsatellites are suitable for determining paternity, population genetic studies and recombination mapping. It is also the only molecular marker to provide clues about which alleles are more closely related (Goldstein and Pollock, 1997).

This study aimed to: (1) determine the relationship between natural net blotch disease and yield-related characters in 20 barley genotypes and (2) recognize new resistant barley sources and identification of reliable molecular genetic markers for such disease resistance that can be applied in breeding programs.

MATERIALS AND METHODS

Plant material

This study was carried out at the molecular genetic laboratory, Genetics Department, Faculty of Agriculture, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt and at the Experimental Farm at El-Hosainia plain Agricultural Research Station, Elsharkia Governorate, Egypt.

Twenty barley genotypes consisted of eight exotic lines (ICARDA) and twelve local varieties were used to study their reaction to net blotch disease during the two successive growing seasons, 2014/2015 and 2015/2016. Name, pedigree and origin of all used genotypes are presented in Table (1).

Experimental design

Seeds were hand drilled at the recommended sowing rate of barley in Egypt (50 kg/fed.) in the first week of December. Each plot was sown in (4.2 m^2) six rows of 3.5 m long, with 20 cm between rows. This experiment was laid out in randomized complete blocks design with three replications. All cultural practices were applied at the proper time according to Ministry of Agriculture recommendations.

Natural infection with *Pyrenophora teres* conidia, the causal of barley net blotch, was conducted under natural field conditions. Records of the disease were denoted after disease on set using the (0-9) scale adopted by Leath and Heun (1990). Disease symptoms were measured at heading stage.

Data of days to heading, days to maturity, plant height, spike length (cm), number of grains/spike, number spikes/m^2, 1000-grain weight (g), biological yield (kg/fed.), grain yield (kg/fed.), harvest index (HI) and net blotch infection characters were recorded.

Statistical analysis

The components of the analysis of variance were evaluated for each experiment as described by Kearsey and Pooni (1996). Mean performance for all traits of genotypes and cultivars included in this trial were compared using LSD at 0.05 and 0.01 levels of probability. Simple correlation (r) coefficients among all studied traits were calculated according also to Kearsey and Pooni (1996). All statistical analyses were performed using the computer software Costat Computer Program according to (Snedecor and Cochran, 1969).

Genetic polymorphism assessment

DNA isolation and primer selection

DNA was isolated using Cetyl trimethyl ammonium bromide (CTAB)-based procedure for plants from fresh leaves of the used twenty genotypes of barley (Murray and Thompson, 1980). Three different types of DNA markers (five RAPD, three SCoT and eight SSR primers) as shown in Table (2), were used to screen genetic polymorphism among the 20 barley genotypes and identification of molecular markers associated with net blotch disease resistance. These primers were synthesized by iNtRON Biotechnology, Inc, Korea.

Amplification condition

Amplification reactions were applied using 20 µl reaction mixture containing the following; 1 µl of template DNA (40 ng/µl), 1.0 µl of primer (10 pmol/ µl) in RAPD and SCoT analysis, 1 µl from each primer (forward and reverse in SSR analysis, 10 µl 2X PCR Master mix solution [(i-Taq^{TM}) iNtRON Biotechnology] and 7-8 µl of sterile ddH_2O. The reaction mixtures were overlaid with 20 µl of mineral oil per sample. PCR amplification condition was carried out in thermal cycle (Perkin Elmer Cetus) programed. The reaction was subjected to one cycle at

94°C for 2 min. (initial denaturation), followed by 35 cycles of 20 sec. at 94°C, 30 sec.,1 min. and 1 min. at 30, 50, 55°C (for RAPD, SCoT and SSR, respectively) and 30 sec. at 72°C, final extention for 5 min at 72°C (one cycle) then at 4°C for keeping.

Amplification products were separated by horizontal gel electrophoresis unit using 1.5% agarose gel. Electrophoresis was carried out fewer than 70 volts for 15 min., then 90 volts for 90 min. Bands were detected on Benchtop UV-transilliminator and photographed using photo Doc-It^{TM} imaging system. The molecular size of the amplified products was determined against 1 Kb DNA ladder with stain (SibEnzyme) and 1 Kb plus DNA ladder (TIANGEN, cat.no. MD113).

Data analysis

DNA banding patterns generated from RAPD and SSR techniques were analyzed by GelAnalyzer 3 program. Amplification with some arbitrary RAPD primer was repeated three times, and consistent bands for each primer were selected for data generation. Only consistent and reproducible bands were used to run the corresponding statistical analysis. DNA polymorphic bands were registered as discreet variables considering "1" for presence and "0" for absence to construct a binary data matrix. From this matrix, the genetic similarity (GS) was estimated using Nei & Li coefficient's (Nei and Li, 1979) by computational package MVSP 3.1. Also, depending on this matrix, cluster analysis was applied using the same program. The resulting matrix was analyzed on the basis of the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

The informational certainly of primers to differences between genotypes was analyzed by means of the estimation of their Polymorphic Information Content (PIC) and Resolving Power (RP). PIC was calculated using the formula reported by Roldan-Ruiz *et al*. (2000) as follow: $PICi = 2fi(1-fi)$, where *PICi* is the polymorphic information content of the locus i, *fi* is the frequency of the present bands, and (1-*fi*) represents the frequency of the absent bands. The PIC of each primer was calculated using the average PIC value from all loci of each primer. Resolving Power was calculated according to Prevost and Wilkinson (1999) using the formula ($Rp = \sum I_b$), where I_b represents the informative bands, which was calculated with: $I_b = 1-[2 \times (0.5 - p)]$ where *p* is the proportion of genotypes containing the bands.

RESULTS AND DISCUSSION

Mean squares of all traits of the studied genotypes in two seasons are presented in Table (3). Results pointed out those mean squares of genotypes were highly significant for all traits in both seasons**.**

Mean performances of the twenty genotypes for eleven different characters under study are presented in Table (4). Overall mean values for days to heading and days to maturity showed that the most

desirable mean values towards the earliness were exhibited by the Giza 133 in both seasons with average values of (84.12 and 84.79 days) for days to heading and (124.03 and 121.40 days) for days to maturity in first and second seasons, respectively. On the other hand, Line 81 possessed the latest genotypes while they recorded (98.67 and 94.87 days) for days to heading and (134.06 and 128.74 days) for days to maturity in first and second seasons, respectively.

Concerning plant height, data in Table (4) showed highly significant differences among the 20 barley genotypes in both seasons , Giza 126, Giza 136 and Line 81 had the highest mean values in both seasons (99.44, 98.57 and 94.90 cm in first season and 88.40, 90.50 and 87.20 cm in second season, respectively). On the other hand, Giza123 and Line 46 had the lowest mean values (84.38 and 85.13 in first season and 81.33 and 81.94 in second season. With respect to spike length Giza131 and Line 9 had the highest mean values (9.76 and 9.55 cm in both seasons). For Number grains/spike, results of mean performance as shown in Table (4) revealed that Giza131 gave the highest mean values for this trait in both seasons (70.10 and 66.69 in the first and second seasons, respectively) followed by Giza 126 and Giza 129 which gave 60.05 and 68.08 and 62.06 and 64.07 in the first and second seasons, respectively.

With respect to number of spikes/m^2, line 9 and Line 15 had the highest mean values in both seasons (700.72 and 680 cm and 698.68 and 663.33 in the first and second seasons, respectively). On the other hand, Giza126, Giza 2000 and Line 46 had the lowest mean values in first season (418.69, 410.04 and 416.06, respectively) and Giza117 and Giza 123 in second season (328.01 and 326.03, respectively). The results of number spikes/m^2 showed wide range between the highest and lowest values, these results are due to barley Line 9 and line 15 are two rowed barley genotypes which have high tillerring capacity compared with six rowed barley. Line 15 showed the highest mean values for 1000-grain weight (60.17 and 59.53 g) in the first and second seasons, respectively. On the other hand, Giza135 (39.77 and 36.90 g) and Line 38 (39.47 and 40.07 g) possessed the lowest mean values for 1000-grain weight in both seasons. Regarding biological yield, line 91 (6945.91 and 6155.77 kg/fed) and Giza134 (6787.12 and 6384.23 kg/fed) possessed the highest mean values in both seasons.

For grain yield, the highest grain yield mean values were obtained by the genotypes Giza133 (2224.71 and 1980 kg/fed), Line 77 (2120.78 and 1858.15 kg/fed) and Line 91 (2085.44 and 1992.69 kg/fed) in both seasons. Whereas the lowest mean values were obtained by, Giza117 (1522.67 and 1266.79 kg/fed) and Giza129 (1491.70 and 1396.15 kg/fed) in both seasons. Moreover, Giza 133 and line 91 showed superiority mean values for grain yield over all the tested barley genotypes. These findings are in agreement with Hartleb *et al.* (1990) and Zaki and

Al-Masry (2008). With respect to harvest index, Giza126 had the highest mean values in both seasons (35.44 and 34.72 in the first and second seasons, respectively).

All traits recorded over the two seasons were significant. Such results indicated that the tested genotypes varied from each other and ranked differently from season to other. These findings are in harmony with reports of El-Gayar *et al*. (1984), Afiah and Abdel-Hakim (1999), Afiah *et al*. (1999) and Zaki and Al-Masry (2008).

For net blotch infection, Line 81 (1.67 and 1.33) possessed the lowest mean values in both seasons. On the other hand, Giza117 (6.67 and 6.67) and Giza 2000 (7.67 and 8.00) had the highest mean values in both seasons. Giza 123, Giza 126 and Giza 136 were moderately susceptible, while other genotypes ranged between resistant to moderately resistant.

Varied response by barley lines confirms that they were genetically diverse and that their response to disease may be under the control of several resistantce genes (Liu *et al*., 2011; Owino *et al*., 2014) which may have conditioned their response to the disease.

Low temperatures coupled with higher relative humidity at El-Hosainia plain Agricultural Research Station may have favored spore production and multiple infections of genotypes (Agrios, 2005; Kosiada, 2008). Maximum spore production has been reported to occur at 25°C and at a high relative humidity (Kosiada, 2008). These conditions may have contributed to the observed significant variations in disease response in the two seasons. Higher amounts of rainfall observed in both seasons at early growth stages may caused a rise in moisture levels in the host plants thus causing increased infection by the pathogens (Agrios, 2005).

Pyrenophora teres has the ability to undergo sexual reproduction and this may cause an increase in frequency of pathotypes that have the ability to adapt to the changes in the genetic makeup of the host population (Statkevičiūtė *et al*., 2010). Such pathotypes can also be increased in frequency due to the influence of selection pressure from growing resistant varieties (McDonald and Linde, 2002).

Correlation coefficient

Correlation coefficient is important in plant breeding where it measures the degree of association between two or more characters. The correlation coefficients among the studied characters of barley genotype are shown in Table (5). Significant positive correlation was observed between days to heading and days to maturity, also significant positive correlation was observed between days to maturity and each of biological yield and grain yield. Significant and positive correlation was detected between plant height and each of spike length, number of grains/spike, number of spikes/m^2, 1000-

grain weight, biological yield and grain yield.

Spike length showed positive correlation with each of number of grains/spike, number of spikes/m^2, 1000-grain weight, biological yield, grain yield and harvest index. Number of grains/spike also showed positive correlation and each of number of spikes/m^2, 1000-grain weight, biological yield, grain yield and harvest index. Significant positive correlations were observed between number of spike/m^2 and each of 1000-grain weight, biological yield, grain yield and harvest index.

Also, 1000-grain weight showed positive and significant correlation with each of biological yield, grain yield and harvest index and negative and significant correlation with net blotch disease. Biological yield showed positive and significant correlation with each of grain yield and harvest index and negative and significant correlation with net blotch disease. Also grain yield showed positive correlation with harvest index. While harvest index showed negative and significant correlation with net blotch disease. From the previous results, it could be concluded that, it is logically presence of positive correlation between grain yield and one or more traits of its components and this happened in this study. Also, negative correlation between net blotch and each of 1000-grain weight, biological yield, grain yield and harvest index was due to the negative effect of net blotch on plant leaf area. Kashif and Khaliq (2004), Saleem *et al*. (2006) and Muhammad *et al*. (2010) found positive correlation between grain yield and most of its components. Riggs *et al.* (1981) reported that a high meaningful and positive correlation was existed between harvest index and grain yield in barley. Kiflu (2009) also reported significant and positive correlation between days to heading and days to maturity.

Molecular diversity assessment

Polymorphism as detected by RAPD analysis

Five RAPD primers were used to study the genetic diversity and relationships among the 20 barley genotypes. These primers produced multiple band profiles (Fig. 1) with different amplified DNA bands (Table 6). The molecular size of the amplified DNA bands ranged from 161 bp to 1656 bp. A total of 48 amplified fragments (loci) were obtained, out of them 34 (70.83%) were polymorphic. The total number of polymorphic DNA fragments ranged from high that was scored by the primer OPH-03 (12), while the lowest number was recorded by primer OPH-01 (3). The polymorphism percentage ranged from 37.5% (OPH-01) to 87.5% (OPH-04). These variations in the number of bands amplified by different primers are influenced by variable factors such as primer structure and number of annealing sites in the genome (Kernodle *et al*., 1993). Results showed also that five fragments out of the 34 polymorphic ones were genotype-specific markers. The re-

solving power (RP) ranged from (7.7 to 15.7). The average polymorphic information content (PIC) was 0.23, ranging from 0.04 (OPH-01) to 0.33 (OPH-04). The percentage of polymorphic bands (70.83) expressed by random primers is in the range of other reports on other RAPD studies of barley which were 74% (Karim *et al*., 2010) and 88% (Ciulca *et al*., 2010). These results agree with those reported by Sosinski *et al*. (2000), Saker (2005) and Zaki and Al-Masry (2008).

The RAPD based-dendrogram (Fig. 4) was divided into two clusters at the genetic similarity percentage 76.2% and each cluster was divided into two subclusters. The first cluster was separated in 81.7% genetic similarity percentage, the first subcluster included the most resistant genotypes and located together such as (Line 77, Line 38, Line 81, Line 26 and Line 15), while the second subcluster consisted of the genotypes (Giza 136, Giza 135 and Line 91). On the other hand, the second cluster which was separated in 79.1% of genetic similarity percentage included most of the susceptible genotypes according to morphological data (Giza 117 and Giza 2000) but they were found in two different subclusters. The other genotypes ranged from moderately resistance or moderately susceptible (Giza 123, Giza 124, Giza 126, Giza 131, Giza 132, Giza 133, Giza 134, Giza 129, Line 9 and Line 46) also found in this cluster. These results agreed with those of Peltonen *et al.* (1996) and Zaki and Al-Masry (2008).

Polymorphism as detected by SCoT analysis

Three SCoT primers were used to study the genetic differences and relationships among the 20 barley genotypes as shown in Fig. (2) and Table (6). The molecular sizes of the amplified bands ranged from 169 bp to 2277 bp. A total of 31 major SCoT amplified fragments were obtained, out of them 24 (77.42%) were polymorphic and the polymorphism percentage ranged from 55.56% (SCoT-8) to 91.67% (SCoT-9). The total number of polymorphic DNA fragments ranged from high scored by the primer SCoT-9 (11), to low scored by the primer SCoT-8 (5). These variations in the number of bands amplified by different primers are influenced by variable factors such as primer structure and number of annealing sites in the genome (Kernodle *et al*., 1993).

Results showed that two fragments out of the 24 polymorphic ones were unique (genotype-specific markers). The resolving power (RP) ranged from 9.6 to 12.7 for SCoT-7 and SCoT-8, respectively. The polymorphic information content (PIC) ranged from 0.18 to 0.33 for SCoT-8 and SCoT-9, respectively. The percentages of polymorphic bands expressed by SCoT primers were compared to earlier reports of other SCoT studies on barley. These results agree with those of Amirmoradi *et al.* (2012) who detected a total of 112 bands among 38 accessions belonging to eight annual *Cicer* species using nine SCoT markers, of which 109 were polymorphic. The number of bands

ranged from 7 to 17 with an average of 12.4 per primer. The overall size of amplified products ranged from 220 to 2250 bp. Polymorphism percentage ranged from 86.6% to 100% with average polymorphism of 97% across all accessions.

The dendrogram constructed based on SCoT markers (Fig. 4) was separated at 69.8% similarity percentage into two clusters. The first cluster was divided into two subclusters at 75.3% similarity percentage. The first subcluster contained the most resistant ICARDA genotypes (Line 77, Line 81 and Line 91), while the second subcluster contained the Egyptian genotypes in two groups, Giza 135 in the first group, while Giza 126 and Giza 124 was found in the second group. On the other hand, the second cluster was separated at 75.7% similarity percentage into two subclusters, the first included the genotypes (Giza 136, Line 38, Line 15, Line 26 and Line 46), while the second subclusters included most of susceptible genotypes (Giza117 and Giza 2000) and other genotypes ranged from moderately resistance to moderately susceptible (Giza123, Giza131, Giza132, Giza133, Giza134, Giza129 and Line 9). These results were agreed with those of Karim *et al*. (2010), Adawy *et al*. (2013) and Diab *et al*. (2013).

Polymorphism as detected by SSR analysis

Data in Table (7) were obtained from eight microsatellite primer pairs which were screened against 20 barley genotypes to detect polymorphic markers. The eight SSR primers selected in this study generated a total of 40 major SSR alleles and the number of polymorphic alleles was 29, representing a level of polymorphism of 72.5% as presented in Fig. (3) and Table (7). The number of alleles per primer ranged from 2 in (Bmag0344a) to 8 in (GBM1215 and Bmag0496). The number of polymorphic alleles generated by individual primer pairs ranged from 1 in (Bmac0040 and Bmag0344a) to 8 in (Bmag0496). The average of the total alleles per primer was 5, while the average of polymorphic alleles per primer was 3.63.

The resolving power (RP) of the eight SSR primers ranged from 3.6 to 8.7. Similarly, polymorphic information content (PIC) values ranged from 0.05 to 0.40 demonstrating uniform polymorphism rate among all the eight SSR primers. Polymorphic information content (PIC) refers to the values of a marker for detecting polymorphism within a population or set of genotypes by taking into account not only the number of alleles that are expressed but also the relative frequencies of alleles per locus. As evident, SSR marker Bmag0496 showed the highest level of polymorphism with PIC value of 0.40, whereas the PIC values for the rest of SSR markers were in the range of 0.05-0.32 (Table 7). In this regard, Sipahi (2011) differentiated and identified 34 Turkish barley genotypes using barley SSR markers. Amplification of SSR loci was generated using 17 SSR primers. These SSR primers totally produced 67 alleles ranging from two to six alleles per locus with a

mean value of 3.94 alleles per locus. Also, Khodayari *et al*. (2012) evaluated the genetic diversity of 32 individuals of two-rowed and six-rowed Iranian landraces barley using 17 microsatellite markers. A high level of polymorphism information content (PIC; average = 0.651) and an average of 8.1 allele per locus were observed.

Regarding the SSR-based dendrogram according to (Nei & Li's Coefficient) the dendrogram showed two clusters at similarity percentage of 71.3% as shown in Fig. (4). In the first cluster, Line 38 genotype was separated in a single subcluster from all the other barley genotypes in this cluster. However, the second subcluster has 14 genotypes and was divided into two groups. The first group was separated into two subgroups, the first containing six Lines of ICARDA genotypes and the other containing four Egyptian genotypes. Also, the second group contained four of Egyptian barley genotypes. Meanwhile the second cluster which was divided into two subclusters at similarity percentage of 73.5% containing the genotypes Giza117, Giza124, Giza135, Giza136 and Line 9. Most of the resistant genotypes are located together such as (Line 91 and Line81) and (Line 26 and Line15). On the other hand; susceptible genotypes and moderately resistant or moderately susceptible such as (Giza 117, Giza124, Giza135, Giza136 and Line 9) also were located together. These results confirmed the conclusion mentioned in the performance of the genotypes tested and are in accordance with those reported by Abu Qamar *et al.* (2008) and Svobodova (2011).

Good results could be obtained if we crossed these twenty genotypes because there is a wide diversity among them. It is noteworthy that cluster analysis is a valuable tool for subdividing genotypes into groups including similar and dissimilar lines and has a great value from the breeder's point of view for initiating barley hybrid program. These findings are in line with those obtained earlier by Svobodova *et al.* (2011) and Maniruzzaman (2014).

Comparison of RAPD, SCoT and SSR data

From results presented in Tables (6 and 7), 20 barley genotypes were characterized by nine genotype-specific markers (four positive and five negative) as shown in Table (8). These marker loci were classified as five genotype-specific markers (two positive and three negative) by RAPD primers, two genotype-specific markers (one positive and one negative) by SCoT and SSR primers. Among the 20 genotypes of barley, six showed genotype-specific markers; Line 77 had the highest number of negative markers (three negative markers) using all types, while Line 9 had the highest number of positive markers (two positive markers). These results indicated that all types applied in this study succeeded in showing different molecular marker patterns which can be relied upon in distinguishing among the studied barley genotypes. Although, SCoT marker type had the highest percentage of

polymorphism (77.42%), while RAPD primers were the best in terms of the average of resolving power RP (11.78) and the average number of genotype-specific markers / primer (1.0) as shown in Table (9). These findings were in harmony with that illustrated previously by Fernández *et al.* (2002) in barley.

Phylogenic relationship among 20 barley genotypes as detected by genetic similarity (GS) and cluster analysis using RAPD, SCoT and SSR combined data

The similarity matrix resulting from the combined DNA markers RAPD, SCoT and SSR data were performed to generate correct relationships based on large and different genome regions as shown in Table (10). The highest percentage of genetic similarity (90.7%) was detected between Line 81 and Line 91, indicating that these two barley lines are closely related to each other's, this result agree with SCoT similarity, followed by (87.7%) between Line 15 and Line 26. On the other hand the lowest genetic similarity value (65.2%) was obtained between Giza 124 and Line 46 indicating the wide genetic diversity among them. These results confirmed the result obtained by SSR analysis published by Abu Qamar *et al.* (2008), indicating the wide genetic diversity among them.

Based on combined data, the dendrogram built on the basis of combined data from RAPD, SCoT and SSR analyses as shown in Fig. (4), represents the genetic similarity among the twenty barley genotypes. The dendrogram includes two clusters at genetic similarity percentage of 74.2%, the first cluster contained Giza 135, Line 77, Line 81 and Line 91, while the second cluster was separated into two subclusters at 75.1% similarity percentage. The first subcluster was divided at 78.1% of similarity into two groups, the first containing five of the ICARDA genotypes in addition to Giza136, while the second group containing six of Egyptian barley genotypes. The second subcluster contained the reset Egyptian barley genotypes (Giza 117, Giza 124, Giza 123 and Giza 126).

From our results of SSR, SCoT and RAPD-based phylogenetic relationship study of the selected barley genotypes, it is evident that Egyptian barley genotypes are genetically very close and originated from closely related genotypes. This molecular evidence is confirmed based on data extracted from the historical genetic background of these genotypes. The majority of these genotypes have a common ancestor, at least for one of the parents. For instance, as previously reported by Afiah and Abdel-Hakim (1999) and Saker *et al*. (2005) the ancestors of Giza 124 are Bahteem and Giza 117, the ancestors of Giza 126 are Bahteem and SD 729 and the ancestors of Giza 123 are Giza 117 and FAO 86. It is also evident that RAPD analysis misplaced some of the ICARDA genotypes as well as revealed conflicting and unexpected genetic similarities among other genotypes. Similar observations were reported by Virk *et al*. (2000). In this context, Lombard (2001) concluded that

molecular markers can be used as a tool and a convenient method for application of combine information from a large number of markers. Herein, we could conclude that it is possible to tag the breeding history and the origin of different barley genotypes using a combination of different molecular systems. Previously published data on barley by Russell (1997) and Sakar (2005) indicated that correlations between the relationships revealed by different polymorphism assays can vary widely both within and between species.

Results of this study are considered as the starting point needed to identify the valuable Egyptian barley net blotch resistance germplasm at both the phenotype and genotype levels and draw the attention of breeders and banks of natural plant genetic resources towards this valuable yet neglected germplasm. This is especially significant since molecular analysis combined with biological evaluation has proved to be a promising strategy in the selection of disease resistant germplasm (Haley, 1993).

On the basis of observed responses, it can be concluded that screened barley genotypes and groups contain a number of genes conferring resistance to *P. teres*. These genotypes could be incorporated into breeding program. The expression of such gene(s) is usually dependent on environment and barley genotypes containing such gene(s) are likely to vary in their response to net blotch under different environments. There is a need to establish molecular basis of the observed responses under field conditions. Multiple location studies using the same genotypes are also required to confirm the responses of the genotypes in other environments since environment was found to play a major role in the reaction in some number of screened genotypes.

SUMMARY

To evaluate the resistance of some barley genotypes for net blotch disease and grain yield and its related traits, twenty genotypes (12 local varieties and 8 exotic lines) of barley were used. Expression of severity to foliar infection varied between the evaluated genotypes, Giza 117 and Giza 2000 appeared the highest infection response, Giza 123, Giza 124, Giza 126 and Giza 131 were moderately susceptible, while the other genotypes ranged between resistant to moderately resistant. Line 81 and Line 91 proved to be most resistant genotypes for net blotch. Moreover, Giza 133 and line 91 showed superiority in grain yield values over all the tested barley genotypes and high resistance reaction for net blotch disease. Genetic variability and relationships among the used barley genotypes were evaluated by using five RAPD primers, three SCoT primers and eight SSR primer pairs. A high degree of polymorphism was detected with the three types of DNA markers which recorded 70.83, 77.42 and 72.5%, respectively. Alleles number ranged from 8 to 15, 9 to 12 and 2 to 8 per primer, with averages of 9.6, 10.33 and 5 per RAPD, SCoT and SSR primers, respectively. The

highest percentage of genetic similarity as revealed by combined RAPD, SCoT and SSR data was found between line 81 and line 91 (90.7%), while the lowest similarity percentage was detected between Giza 124 and line 46 (65.2%). Giza 134 and Line 9 genotypes were resistant for net blotch disease while they gave positive genotype-specific markers with RAPD and SCoT analyses. Only Giza 123 genotype gave a positive genotype-specific marker using SSR analysis. Therefore, these genotype-specific markers could be considered as a molecular marker for net blotch disease response under similar conditions.

REFERENCES

Abu Qamar, M., Z. H. Liu, J. D. Faris, S. Chao, M. C. Edwards, Z. Lai, J. D. Franckowiak and T. L. Friesen (2008). A region of barley chromosome 6H harbors multiple major genes associated with net type net blotch resistance. Theor. Appl. Genet., 117: 1261-1270.

Adawy, S., M. Saker, W. Hagag and H. El Itriby (2008). AFLP-based molecular analysis of Egyptian barley lines and landraces differing in their resistance and susceptibility to leaf rust and net blotch diseases. J. of Landbauforschung, 58: 125-134.

Adawy, S. S., A. A. Diab, A. I. Sayed, S. D. Ibrahim, S. I. El-Morsy and M. M. Saker (2013). Construction of genetic linkage map and QTL analysis of net blotch resistance in barley. IJABR, 4: 348-363.

Afiah, S. A. N. and A. M. Abdel-Hakim (1999). Heterosis, combining ability, correlations and path coefficient analysis in barley (*Hordeum vulgare* L.) under desert conditions of Egypt. Proc. 1st Pl. Breed. Conf., Cairo Univ., Special Issue of Egypt. J. Pl. Breed., 3: 53-66.

Afiah, S. A. N., H. A. Sallam and S. A. H. Khattab (1999). Evaluation of divergent barley (*Hordeum vulgare* L.) genotypes under certain environments. Ann. Agric. Sci., Moshtohor, 37: 973-988.

Agrios, G. N. (2005). Plant pathology. 5th Edition. Elsevier Academic Press, UK.

Amirmoradi, B., R. Talebi and E. Karami (2012). Comparison of genetic variation and differentiation among annual *Cicer* species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. Plant Syst. Evol., 298: 1679-1688.

Ciulca, A., S. Ciulca, E. Madosă, S. Mihacea and C. Petolescu (2010). RAPD analysis of genetic variation among some winter barley cultivar. Romanian Biotechnol. Lett., 15: 19-24.

Collard, B. C. Y. and D. J. Mackilla (2009). Start codon targeted (SCoT) polymorphism: simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol. Biol. Rep., 27: 86-93.

Diab, A. A., M. A. Atia, E. H. Hussein, H. A. Hussein and S. S. Adawy (2013). A multidisciplinary approach for dissecting qtl controlling high-yield and drought tolerance-related traits in durum wheat. Int. J. of Agrci. Sci. and Res., 3: 99-116.

El-Gayar, M. A., A. M. Esmail, A. M. Hegazi and M. T. Hegab (1984). Estimates of genetic and environmental variability in barley (*Hordeum vulgare* L.) under Nile valley and Sinai conditions. Proc. 9th Int. Cong. for Statistics, Computer Science, Social and Demographic Research, p: 485-497.

Fernández, M. E., A. M. Figueiras and C. Benito (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor. Appl. Genet., 104: 845-851.

Fisher, P. J., R. C. Gardner and T. E. Richardson (1996). Single locus microsatellites isolated using 5' anchored PCR. Nuc. Acids Res., 24: 4369-4371.

Goldstein, D. B. and D. D. Pollock (1997). Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. J. Hered., 88: 335-342.

Gorji, A. M., P. Poczai, Z. Polgar and J. Taller (2011). Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. Am. J. Pot. Res., 88: 226-237.

Graner, A., B. Foroughi and A. Tekauz (1996). RFLP mapping in barley of a dominant gene conferring resistance to scald (*Rhynchosporium secalis*). Theor. Appl. Genet., 93: 421-425.

Haley, S., P. Miklas, J. Stavely and D. Kelly (1993). Identification of RAPD markers linked to a major rust resistance gene block in common bean. Theor. Appl. Genet., 86: 505-512.

Hartleb, H., U. Meyer and C. O. Lehmann (1990). Resistance behaviour of common barley to different isolates of *Drechslera teres* (Sacc.) Shoem. Archiv für Phytopathologie und Pflanzenschutz, 26: 257-264.

Hemleben, V., T. Schmidt, R. A. Torres-Ruiz and U. Zentgraf (2000). Molecular cell biology: role of repetitive DNA in nuclear architecture and chromosome structure. Progress in Botany, 61: 91-117.

Karim, K., B. Chokr, S. Amel, H. Wafa, H. Richid and D. Nourdine (2010). Genetic diversity of Tunisian date palm germplasm using ISSR marker. Int. J. of Botany, 6: 182-186.

Kashif, M. and I. Khaliq (2004). Heritability, correlation and path coefficient analysis for some metric traits in wheat. Int. J. Agri. Bio., 6: 138-142.

Kearsey, M. and H. Ponni (1996). The Genetical Analysis of Quantitative Traits. Chapman and Hall. London. U.K.

Kernodle, S. P., R. E. Cannon and J. G. Scandalios (1993). Concentration of primer and template qualitatively affects product in RAPD-PCR. Biotechniques, 1: 362-364.

Khodayari, H., H. Saeidi, A. A. Roofigar, M. R. Rahiminejad, M. Pourkheirandish and T. Komatsuda (2012). Genetic diversity of cultivated barley landraces in Iran measured using microsatellites. Int. J. of Biosci., Biochemistry and Bioinformatics, 2: 287-290.

Kiflu, A. (2009). Agronomic Evaluation of Ethiopian Barley (*Hordeum vulgare* L.) Landrace Populations under Drought Stress Conditions in Low Rainfall Areas of Ethiopia. An MSC Thesis Submitted to Swedish Biodiversity Center.

Kosiada, T. (2008). Influence of temperature and daylight length on barley infection by *Pyrenophora teres*. Plant Protection Res., 48: 9-15.

Leath, S. and M. Heun (1990). Identification of powdery mildew resistance genes in cultivars of soft red winter wheat. Plant Dis., 74: 747-7582.

Liu, Z., R. E. Simon, P. O. Richard and L. F. Timothy (2011). *Pyrenophora teres*: Profile of an increasingly damaging barley pathogen. Mol. plant pathology, 12: 1-19.

Lombard, V., P. Dubreuil, C. Dillmann and C. Baril (2001). Genetic distance estimators based on molecular data for plant registration and protection. Review Acta Hort., 546: 55-63.

Maniruzzaman, M. (2014). Polymorphism study in barley (*Hordeum vulgare*) genotypes using microsatellite (SSR) markers. Bangladesh J. Agril. Res., 39: 33-45.

McDonald, B. A. and C. Linde (2002). The population genetics of plant pathogen and breeding strategies for durable resistance. Euphytica., 124: 163-180.

Muhammad, I. Kh., H. Makhdoom, M. Zulkiffal, A. Nadeem and S. Waseem (2010). Correlation and path analysis for yield and yield contributing characters in wheat (*Triticum aestivum* L.). African J. of Plant Sci., 4: 464-466.

Murray, M. G. and W. F. Thompson (1980). Rapid isolation of high molecular weight plant DNA. Nuc. Acids Res., 8: 4321-4325.

Nei, M. and W. H. Li (1979). Mathematical model for studing genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America, 76: 5269-5273.

Owino, A. A., J. O. Ochuodho, J. O. Were and N. Rop (2014). Response of spring and winter barley to *Pyrenophora teres* under high and medium altitude zones of Kenya. Int. J. of Res. in Agri. and Food Sci., 2: 2311-2476.

Peltonen, S., M. Jalli, K. Kammiovirta and R. Karijalainen (1996). Genetic variation in *Drechslera teres* population as indicated by RAPD markers. Annals of Appl. Biol., 128: 465-477.

Prevost, A. and M. J. Wilkinson (1999). A new system for comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appl. Genet., 98: 107-112.

Riggs, T. J., P. R. Hanson, N. D. Start, D. M. Miles, C. L. Morgan and M. A. Ford (1981). Comparsion of spring barley varieties in England and Wales between 1880 and 1980. J. of Agril. Sci., 97: 599-610.

Roldan-Ruiz, I. J. D., E. Van Bockstaele, A. Depicker and M. De Loose (2000). AFLP markers reveal high polymorphic rates in Ryegrasses (*Lolium* spp.) Mol. Breed., 6: 125-134.

Russell, R., D. Fuller, M. Macaulay, G. Hatz, A. Jahoor, W. Powell and R. Waugh (1997). Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs, and RAPDs. Theor. Appl. Genet., 86: 975-984.

Sakar, M. M. (2005). A biological and molecular characterization of some Egyptian barley genotypes which are resistant to net blotch disease. Cellular & Mol. Biol. Letters, 10: 265-280.

Saker, M. M., M. Nachtigall and T. A. Kuehne (2005). Comparative assessment of DNA fingerprinting by RAPD, SSR and AFLP in genetic analysis of some barley genotypes. Egypt. J. Genet. Cytol., 97: 81-97.

Saleem, U., I. Khaliq, T. Mahmood and M. Rafique (2006). Phenotypic and genotypic correlation coefficients between yield and yield components in wheat. J. Agric. Res., 44: 1-6.

Shipton, A., N. Kahn and R. L. Bayd (1973). Net blotch of barley. Rev. Plant Pathol., 52:269-290.

Sipahi, H. (2011). Genetic screening of Turkish barley genotypes using simple sequence repeat markers. J. of Cell and Mol. Biology, 9: 19-26.

Snedecor, C. W. and W. G. Cochran (1969). Statistical Methods 6th ed. Iowa State Univ. Press, Ames, Iowa, USA.

Sosinski, B., M. Gannavarapu, L. D. Hager, L. E. Beck, G. J. King and C. D. Ryder (2000). Characterization of microsatellite markers in peach (*Prunus persica* (L.) Batsch). Theor. Appl. Genet., 101: 421-428.

Statkevičiūtė, G., G. Brazauskas, R. Semaškienė, A. Leistrumaitė and Z. Dabkevičius (2010). *Pyrenophora teres* genetic diversity as detected by ISSR analysis. Agriculture, 97: 91-98.

Steffenson, B. J., P. M. Hayes and A. Kleinhofs (1996). Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley. Theor. Appl. Genet., 92: 552-558.

Struss, D. and J. Pliescke (1998). The use of microsatellite markers for detection of genetic diversity in barley populations. Theor. Appl. Genet., 97: 308-315.

Svobodova, L. L., L. Stemberková, M. Hanusová and L. Kučera (2011). Evaluation of barley genotypes for resistance to *Pyrenophora teres* using molecular markers. J. of Life Sci., 5: 497-502.

Tinker, N. A., M. G. Fortin and D. E. Mather (1993). Random amplified polymorphic DNA and pedigree relationships in spring barley. Theor. Appl. Genet., 85: 976-984.

Virk, S., J. Zhu, H. Newbury, G. Bryan, M. Jackson and B. Ford-Loyd (2000). Effectiveness of different classes of molecular markers for classifying and revealing variations in rice (*Oryza sativa*) germplasm. Euphytica, 112: 275-284.

Wright, W. (1998). Evolution of non-associative learning: behavioral analysis of a phylogenetic lesion. Neurobiol Learn Mem., 69: 326-337.

Zaki, K. I. and A. I. S. Al-Masry (2008). Detection of biochemical genetic markers for net blotch disease resistance and barley grain yield. Egypt. J. Phytopathol., 36: 1-17.

Table (1): Name, pedigree and origin of the twenty barley genotypes used in the present study.

No.	Genotype	Pedigree	Origin
1	G 117	Baladi 16/Palestine 10	Egypt
2	G 123	Giza 117//FAO86	Egypt
3	G 124	Giza 117/Bahteem 52// Giza 118/FAO 86	Egypt
4	G 126	BaladiBahteem/SD729-por12762-Bc	Egypt
5	G 132	Rihane-05//As46/Aths*2" Aths/ Lignee686	Egypt
6	G 133	Carbo/Gustoe	Egypt
7	G 134	Alanda-01/4/WI 2291/3/Api/CM67//L2966-69	Egypt
8	G 2000	Cr366-13-1/Giza121	Egypt
9	G 129	Deir Alla 106/Cel//As46/Aths*2	Egypt
10	G 131	CM67-B/CENTENO//CAM-B/3/ROW906.73/4/GLORIA-BAR/ COME-B/5/ FALCON – BAR /6/ LINO	Egypt
11	G 135	ZARZA/BERMEJO/4/DS4931//GLORIA-BAR/COPAL/3/SEN/5/AYAROS	Egypt
12	G 136	PLAISANT/7/CLN-B/LIGEE640/3/S.P-B//GLORIA-AR/COME-B/5/FALCON-BAR/6/LINOCLN-B/A/S.P-/LIGNEE640/3/S.P-B//GLORIA-BAR/COME-B/5/FALCON-BAR/6/LINO	Egypt
13	Line 9	E.ACACIA/DEFRA//PENCO/CHEVRON-BAR	ICARDA
14	Line 15	CANELA/GOB89DH//CANELA/GOB82DH/4/ARUPO/K8755// MORA/3/ALELI/5/ SCARLETT	ICARDA
15	Line 26	DEFRA/CL 128//PFC 88209	ICARDA
16	Line 38	P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1/6/ M111/7/ LEGACY/3/ SVANHALS-BAR/MSEL//AZAF/GOB24DH	ICARDA
17	Line 46	CANELA/CI 4196	ICARDA
18	Line 77	LAMOLINA96/6/P.STO/3/LBIRAN/UNA80// LIGNEE640/4/BLLU/5/PETUNIA 1	ICARDA
19	Line 81	LA MOLINA96/LEGACY	ICARDA
20	Line 91	P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1/6/BRS180	ICARDA

Table (2): Name and sequence of RAPD, SCoT and SSR primers used in this study.

Primer No.	Primer type	Primer name	Primer sequence (5'→3')
1	RAPD	OPH-01	GGTCGGAGAA
2		OPH-02	TCGGACGTGA
3		OPH-03	AGACGTCCAC
4		OPH-04	GGAAGTCGCC
5		OPH-05	AGTCGTCCCC
6	SCoT	SCoT-7	ACAATGGCTACCACTGAC
7		SCoT-8	ACAATGGCTACCACTGAG
8		SCoT-9	ACAATGGCTACCACTGCC
9	SSR	GBM1215	F ATGACCAGAAAACGCCTGTC R GGATTCTGCACACACGAGAA
10		Bmac0040	F AGCCCGATCAGATTTACG R TTCTCCCTTTGGTCCTTG
11		GMS006	F TGACCAGTAGGGGCAGTTTC R TTCTTCTCCCTCCCCCAC
12		Bmag0496	F AGTATAACCAACAGCCGTCTA R CTATAGCACGCCTTTGAGA
13		Bmag0344a	F GATCCAACTATATTAACAAAGCC R TGAGGGTATGTACCACTAGCT
14		Bmag0103a	F AAAATATTGGCATGAGCTTAG R ATCAAAGATCACATCCTTCC
15		Bmag0500	F GGGAACTTGCTAATGAAGAG R AATGTAAGGGAGTGTCCATAG
16		Bmag0173	F CATTTTTGTTGGTGACGG R ATAATGGCGGGAGAGACA

Table (3): Estimated mean squares of different agronomic traits for different genotypes in 2014/15 and 2015/16 growing seasons.

Source of variation	d.f.	Days to heading		Days to maturity		Plant height		Spike length	
		2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Replications	2	1.68ns	12.69*	2.98ns	1.01ns	14.96ns	13.62 ns	0.23	0.03
Genotypes	19	39.62**	16.09**	32.23**	7.58**	53.1**	22.21**	3.96**	3.86**
Error	38	2.21	2.79	2.35	1.72	14.06	10.96	0.41	0.48
Source of variation	d.f.	No. grains/spike		No. spikes/m^2		1000 grain weight		Biological yield	
		2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
Replications	2	3.69	3.58	5.39	25.52*	5.39	25.52*	135590	19699
Genotypes	19	375.71**	349.23**	74.87**	109.31**	74.87**	109.31**	1556226**	951996**
Error	38	13.19	15.06	7.88	6.30	7.88	6.30	116202	70676
Source of variation	d.f.	Grain yield		Harvest index		Net blotch			
		2014/15	2015/16	2014/15	2015/16	2014/15		2015/16	
Replications	2	9880	4943	1.29	4.62	1.26		0.20	
Genotypes	19	148285**	146228**	11.701**	11.38**	11.07**		12.12**	
Error	38	12463.04	8001.16	4.76	2.23	1.49		0.67	

* and ** indicate significant mean squares at 0.05 and 0.01 levels, respectively.

Table (4): Mean performance estimates of barley traits for genotypes in 2014/15 and 2015/16 growing seasons.

Genotypes	Days to heading (day)		Days to maturity (day)		Plant height (cm)		Spike length (cm)		No. grains/spike		No. spikes/m^2		1000 grain weight (g)		Biological yield (kg/fed)		Grain yield (kg/fed)		Harvest index		Net blotch infection	
	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2014/15	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
G 117	92.71	87.15	127.20	125.14	89.50	83.94	7.19	7.19	56.03	54.92	426.01	328.01	49.57	48.40	4832.44	4018.85	1522.67	1266.79	31.68	31.53	6.67	6.67
G 123	91.30	89.45	129.70	126.45	84.38	81.33	8.00	8.00	60.82	58.27	438.07	326.03	52.40	51.50	5338.80	5116.05	1697.51	1634.77	31.99	31.99	5.67	6.67
G 124	94.67	89.11	131.67	125.36	87.47	79.45	6.90	6.90	52.13	48.00	450.10	356.71	48.07	47.63	5439.92	5229.23	1575.12	1512.92	29.02	29.10	4.33	4.33
G 126	95.69	89.47	131.11	126.01	99.44	88.40	6.74	6.74	60.05	62.06	418.69	367.47	48.87	40.57	5337.54	5115.00	1892.00	1776.92	35.44	34.72	5.33	7.00
G 132	95.02	91.00	131.33	126.00	94.85	87.53	7.02	7.02	56.13	52.03	482.24	442.73	49.33	53.53	4774.76	4683.46	1521.07	1449.46	31.86	30.95	2.67	4.00
G 133	84.12	84.79	124.03	121.40	87.03	87.67	5.44	5.44	56.03	54.67	492.03	390.67	51.93	53.37	6689.90	5698.85	2224.71	1980.00	33.25	34.81	2.67	2.00
G 134	96.07	89.67	133.33	124.75	95.78	87.99	7.69	7.69	58.43	50.18	454.07	406.68	45.67	42.30	6787.12	6384.23	1978.25	1916.54	29.17	30.03	1.33	2.67
G 2000	93.94	89.72	132.75	125.83	91.00	85.17	7.69	7.69	59.04	52.67	410.04	373.02	52.50	57.20	6259.72	5749.62	1986.51	1903.85	31.73	33.07	7.67	8.00
G 129	86.67	89.69	124.81	125.00	93.92	85.34	9.40	9.40	68.08	64.07	494.00	357.33	47.87	48.20	4733.30	4581.92	1491.70	1396.15	31.32	30.53	2.33	1.67
G 131	92.36	94.42	129.00	125.70	94.36	88.10	9.76	9.76	70.10	66.69	434.41	368.70	49.13	45.90	5420.80	4975.38	1651.71	1473.33	30.44	29.65	2.33	3.67
G 135	88.68	86.90	126.71	124.71	93.20	86.37	7.04	7.04	54.74	50.81	476.68	351.33	39.77	36.90	5170.24	4966.42	1609.08	1546.79	31.11	31.14	3.67	2.67
G 136	91.40	90.64	130.81	125.02	98.57	90.50	7.33	7.33	56.88	51.00	474.00	378.69	51.43	53.07	6089.31	5292.69	1713.29	1641.41	28.11	31.01	5.33	4.67
Line 9	94.00	89.67	130.67	127.87	94.21	86.11	9.55	9.55	28.74	26.72	700.72	698.68	53.33	45.50	6463.70	5419.62	1918.90	1809.92	29.73	33.41	1.33	2.67
Line 15	86.85	86.72	129.83	125.35	95.83	87.00	9.02	9.02	27.37	24.73	680.00	663.33	60.17	59.53	5942.82	5508.46	1691.18	1611.92	28.75	29.27	1.67	1.67
Line 26	89.00	91.33	132.71	127.67	92.00	83.80	9.08	9.08	65.36	64.34	464.04	414.70	46.13	43.23	6799.18	6003.46	1992.69	1873.38	29.28	31.20	1.33	2.33
Line 38	84.69	88.09	124.11	126.13	92.00	84.67	8.69	8.69	64.18	61.05	450.10	390.00	39.47	40.07	5782.74	5076.92	1622.80	1345.38	28.07	26.55	2.00	1.67
Line 46	93.44	91.49	131.47	127.67	85.13	81.94	7.35	7.35	56.02	52.69	416.06	370.10	56.20	47.50	5871.82	5026.15	1925.60	1561.15	32.87	31.06	2.00	3.00
Line 77	94.33	90.73	133.87	126.38	96.73	88.21	7.02	7.02	54.72	52.11	425.52	366.07	49.80	48.83	6429.54	5724.23	2120.78	1858.15	33.14	32.45	3.33	2.67
Line 81	98.67	94.87	134.06	128.74	94.90	87.20	6.54	6.54	50.67	46.71	517.63	500.19	43.20	41.13	6604.62	5584.62	1915.38	1795.96	28.97	32.19	1.67	1.33
Line 91	88.77	90.45	128.20	124.70	94.85	87.58	7.70	7.70	59.10	57.75	454.10	444.71	46.13	42.50	6945.91	6155.77	2085.44	1992.69	30.02	32.37	2.00	2.67
F test	**	**	**	**	**	*	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
LSD 0.05	2.46	2.76	2.54	2.17	6.19	5.47	1.06	1.06	6.01	6.41	51.18	40.18	4.64	4.15	563.45	439.42	184.52	147.85	3.60	2.47	2.02	1.35

* and ** indicate significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table (5): Simple correlation coefficients among all studied traits as average of the two seasons data.

Traits	Days to heading	Days to maturity	Plant height	Spike length	No. of grains/ spike	No. of spikes /m^2	1000 grain weight	Biological yield	Grain yield	Harvest index
Days to maturity	0.78**									
Plant height	0.16	0.10								
Spike length	-0.19	0.02	0.61**							
No. of grains/spike	-0.02	-0.24	0.48**	0.93**						
No. of spikes/m^2	-0.09	0.14	0.26*	0.39*	0.83**					
1000-grain weight	-0.08	0.05	0.59**	0.92**	0.38*	0.28*				
Biological yield	0.13	0.33*	0.38*	0.68**	0.50**	0.28*	0.86**			
Grain yield	0.20	0.28*	0.42*	0.28*	0.58**	0.61**	0.84**	0.89**		
Harvest index	0.20	0.06	0.19	0.39*	0.98**	0.39*	0.44**	0.71**	0.64**	
Net Blotch	0.14	0.08	-0.23	-0.16	0.11	-0.21	0.26*	-0.36*	-0.14	-0.40*

* and ** indicate significant at P $\leq$ 0.05 and P$\leq$ 0.01, respectively.

Table (6): Number and types of the amplified DNA bands as well as the polymorphism percentage generated by the five RAPD and three SCoT primers for 20 barley genotypes.

Molecular marker technique	Primer name	Molecular size range	Number of amplified fragments				Total number of amplified fragments	Polymorphism %	Resolving power (RP)	Polymorphic information content (PIC)
			Monomorphic	Polymorphic						
				Without genotype-specific markers	With (+ or -) genotype-specific markers	Total				
RAPD	OPH-01	309-1455	5	0	3	3	8	37.50	15.7	0.04
	OPH-02	161-1372	2	7	0	7	9	77.78	13.5	0.26
	OPH-03	238-1656	3	11	1	12	15	80.00	12.4	0.29
	OPH-04	387-1565	1	6	1	7	8	87.50	7.7	0.33
	OPH-05	222-1285	3	5	0	5	8	62.50	9.6	0.24
Total			14	29	5	34	48	70.83	58.9	1.16
Average			2.8	5.8	1	6.8	9.6		11.78	0.23
SCoT	SCoT-7	316-2277	2	7	1	8	10	80.00	9.6	0.25
	SCoT-8	256-1109	4	4	1	5	9	55.56	12.7	0.18
	SCoT-9	169-1978	1	11	0	11	12	91.67	11.7	0.33
Total			7	22	2	24	31	77.42	34	0.76
Average			2.33	7.33	0.67	8	10.33		11.33	0.25

Table (7): Number of the amplified DNA bands as well as the polymorphism percentage generated by the eight SSR primers.

Primer name	*Chr. location	No. of alleles		% polymorphism	Mol. size range of allels (bp)	RP	PIC
		Total	Polymorphic				
GBM1215	6H (23.1cM)	8	6	75.00	94 - 740	8.7	0.29
Bmac0040	6H (47.8cM)	4	1	25.00	112 - 378	6.8	0.12
GMS006	6H (37.9cM)	7	6	85.71	90 – 396	5.8	0.32
Bmag0496	6H (44.4cM)	8	8	100.00	93 – 516	7.7	0.40
Bmag0344a	6H (48.9cM)	2	1	50.00	115-182	3.9	0.05
Bmag0103a	6H (84.3cM)	4	3	75.00	103 – 379	4.0	0.31
Bmag0500	6H (0.00cM)	3	2	66.67	104 – 214	3.6	0.27
Bmag0173	6H (34.3cM)	4	2	50.00	89 - 658	6.5	0.22
Total		40	29	72.50		47.0	1.98
Average		5	3.63			5.88	0.25

RP; resolving power, PIC: polymorphic information content
*The information for chromosomes assignments was obtained from www. Graingenes.com

Table (8): Barley genotypes characterized by positive and negative genotype-specific markers and their molecular sizes (bp) and total number of markers for each genotype using RAPD, SCoT and SSR analysis.

Genotype	Type of DNA marker	Positive genotype-specific markers			Negative genotype-specific markers			Total No. of markers
		Primer	Mol. Size (bp)	No.	Primer	Mol. Size (bp)	No.	
Giza 123	SSR	GBM1215	740	1	--	--	--	1
Giza 134	SCoT	SCoT-7	1556	1	--	--	--	1
Line 9	RAPD	OPH-03 OPH-04	1019 1430	2	--	--	--	2
Line 38	SSR	--	--	--	Bmag0344a	182	1	1
Line 46	SCoT	--	--	--	SCoT-8	630	1	1
Line 77	RAPD	--	--	--	OPH-01	1178 748 472	3	3
Total		4			5			9

Table (9): Comparison of DNA marker types in different barley genotypes.

Marker type	No. of primer	Gel polymorphism					Total number of amplified bands	Average number of band / primer	Polymorphism %	Average number of genotype-specific marker / primer	Average value of resolving power (RP)	Average value of polymorphic information content (PIC)
		Polymorphic without genotype-specific marker	Genotype-specific marker			Polymorphic with genotype-specific marker						
			(+)	(-)	Total							
RAPD	5	29	2	3	5	34	48	9.60	70.83	1.00	11.78	0.23
SCoT	3	22	1	1	2	24	31	10.33	77.42	0.67	11.33	0.25
SSR	8	27	1	1	2	29	40	5.00	72.50	0.25	5.88	0.25
Total	16	78	4	5	9	87	119	24.93		1.92	28.99	0.73

Table (10): Genetic similarity (GS) matrix for 20 barley genotypes according to combined data from RAPD, SCOT and SSR analyses.

Genotypes	G 117	G 123	G 124	G 126	G 132	G 133	G 134	G 2000	G 129	G 131	G 135	G 136	Line 9	Line 15	Line 26	Line 38	Line 46	Line 77	Line 81
G 123	0.814																		
G 124	0.861	0.819																	
G 126	0.741	0.827	0.789																
G 132	0.77	0.787	0.721	0.810															
G 133	0.797	0.800	0.759	0.839	0.811														
G 134	0.800	0.775	0.746	0.757	0.814	0.844													
G 2000	0.763	0.795	0.696	0.806	0.832	0.863	0.838												
G 129	0.808	0.784	0.772	0.781	0.872	0.822	0.825	0.830											
G 131	0.785	0.789	0.761	0.729	0.800	0.844	0.848	0.853	0.839										
G 135	0.724	0.701	0.730	0.727	0.657	0.693	0.726	0.703	0.696	0.710									
G 136	0.737	0.714	0.712	0.696	0.713	0.752	0.754	0.806	0.752	0.831	0.803								
Line 9	0.737	0.757	0.727	0.725	0.755	0.812	0.754	0.821	0.794	0.846	0.738	0.813							
Line 15	0.748	0.726	0.725	0.750	0.711	0.791	0.750	0.786	0.803	0.735	0.828	0.791	0.791						
Line 26	0.745	0.789	0.708	0.760	0.787	0.828	0.817	0.795	0.850	0.817	0.761	0.771	0.771	0.877					
Line 38	0.725	0.717	0.701	0.741	0.770	0.725	0.741	0.791	0.795	0.815	0.724	0.812	0.782	0.806	0.841				
Line 46	0.692	0.714	0.652	0.710	0.727	0.752	0.800	0.776	0.766	0.800	0.754	0.781	0.766	0.776	0.843	0.812			
Line 77	0.719	0.740	0.710	0.736	0.725	0.705	0.765	0.771	0.748	0.735	0.750	0.761	0.701	0.757	0.836	0.791	0.776		
Line 81	0.759	0.750	0.722	0.773	0.735	0.759	0.761	0.781	0.771	0.746	0.791	0.757	0.714	0.822	0.868	0.786	0.757	0.849	
Line 91	0.713	0.733	0.676	0.743	0.732	0.699	0.700	0.764	0.715	0.714	0.803	0.725	0.681	0.764	0.787	0.755	0.681	0.819	0.907

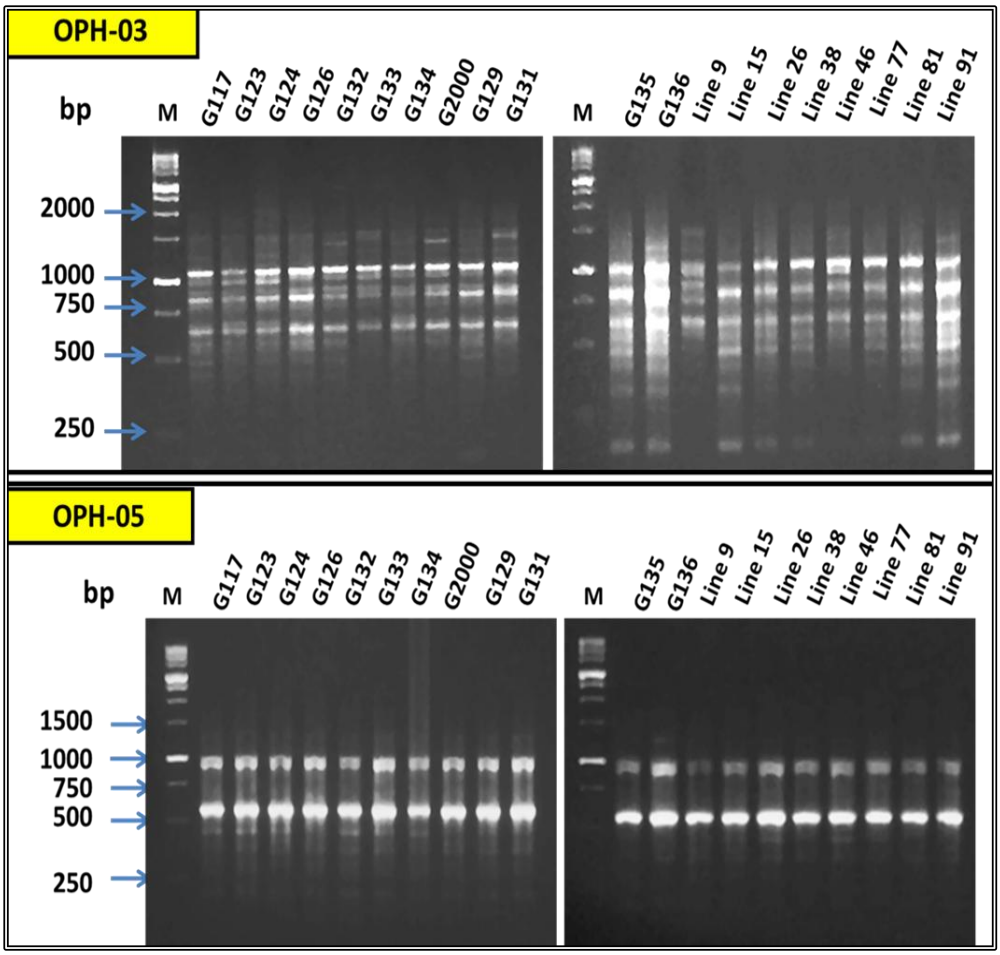

Fig. (1): RAPD patterns of the 20 barley genotypes revealed by primer OPH-03 and OPH-05. M: marker 1 Kb DNA ladder.

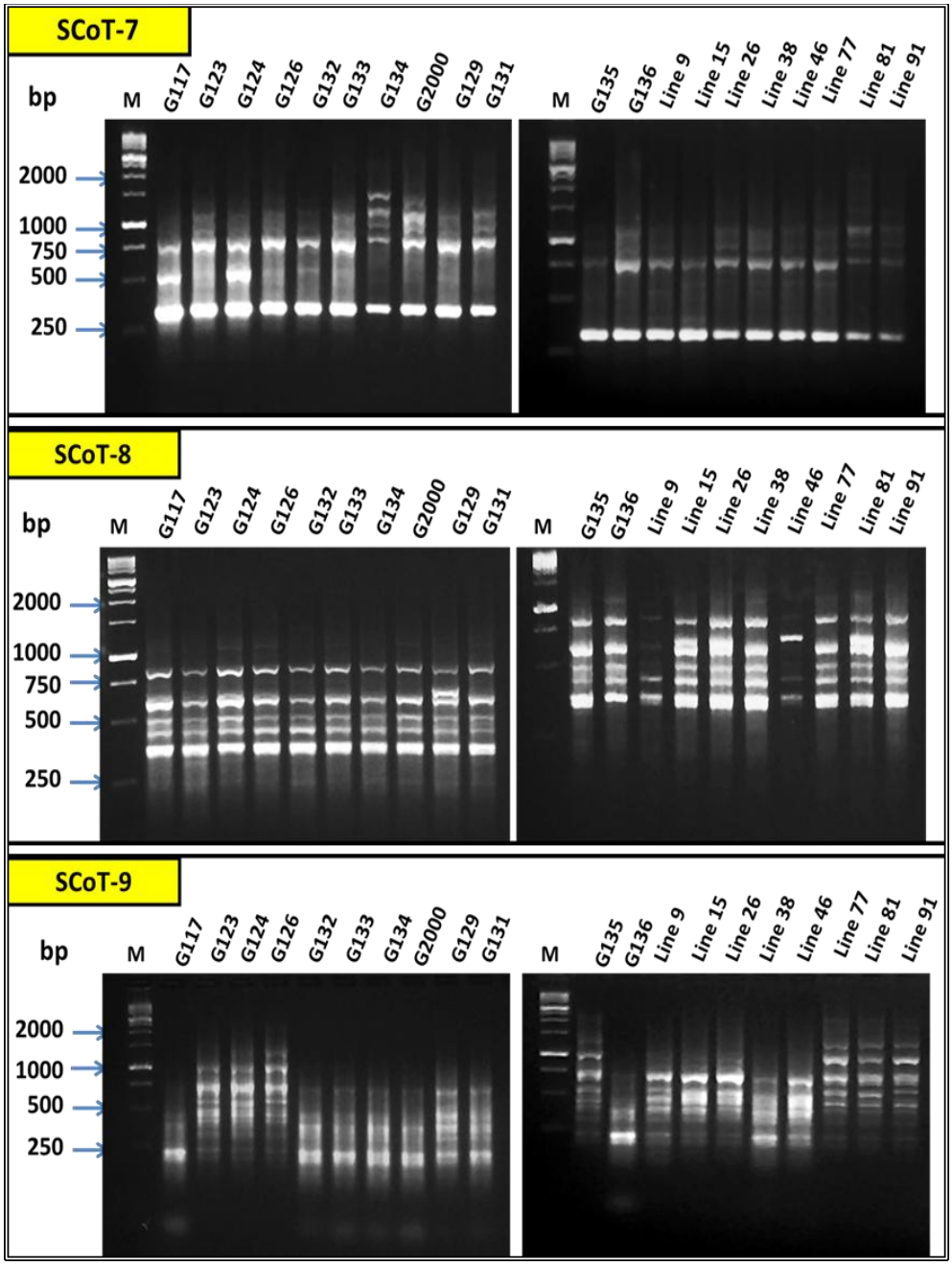

Fig. (2): SCoT patterns of the 20 barley genotypes revealed by primers SCoT-7, SCoT-8 and SCoT-9. M: marker 1 Kb DNA ladder.

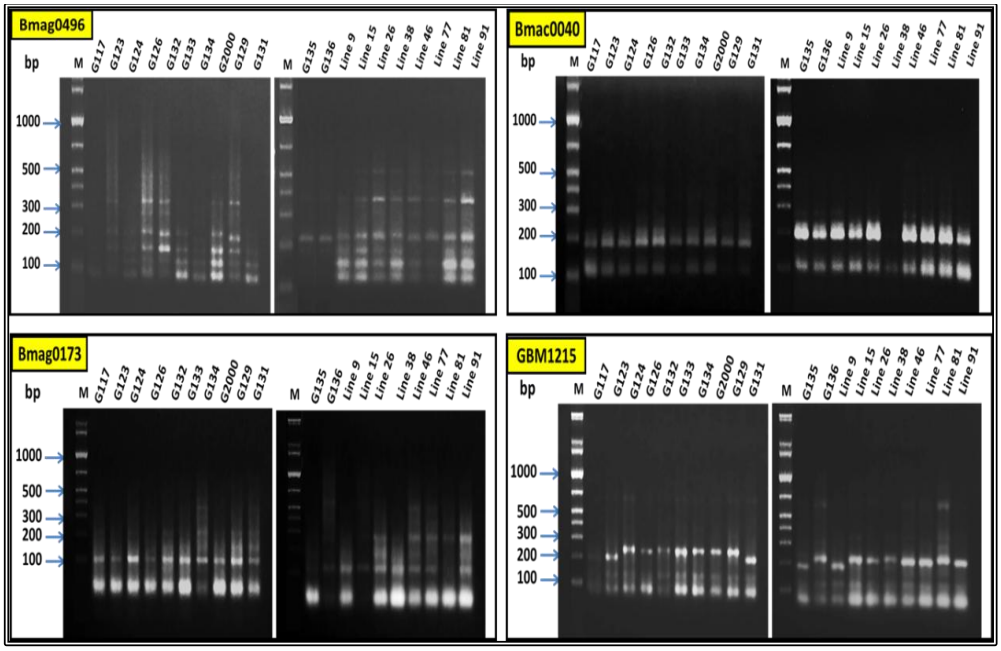

Fig. (3): SSR patterns of the 20 barley genotypes as revealed by primers (Bmag0496, Bmac0040, Bmag0173 and GBM1215). M: marker 1 Kb plus DNA ladder.

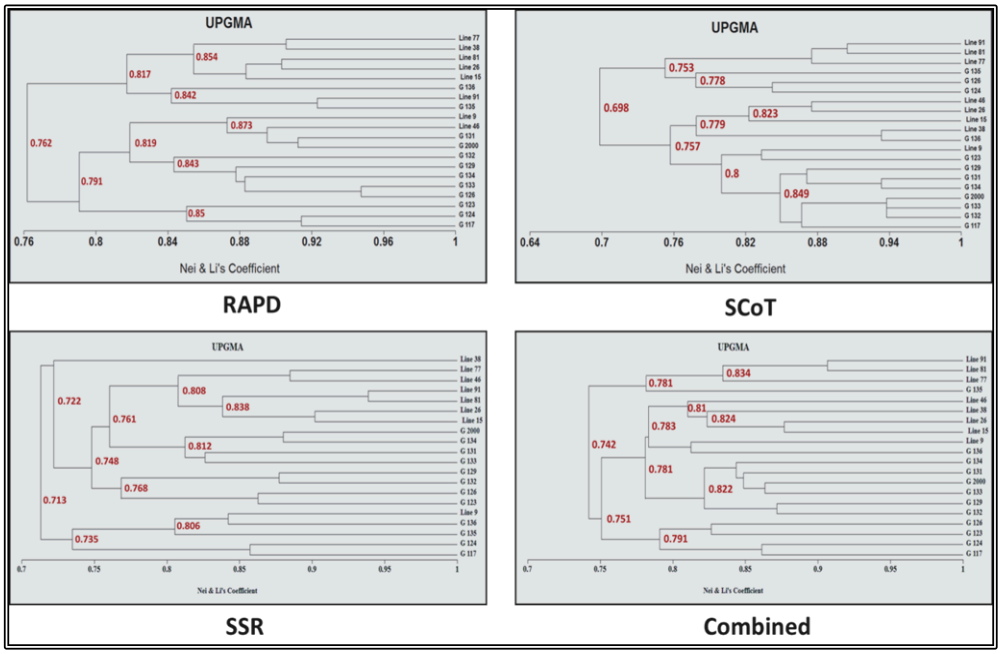

Fig. (4): Dendrogram based on UPGMA cluster analysis showing the genetic similarity percentage between the 20 studied barley genotypes based on RAPD, SCoT, SSR and combined data.