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# Molecular genetic analysis of some North African barley germplasms

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**ABSTRACT**

Isozyme and RAPD markers were used to characterize 29 barley accessions, which were collected from North Africa. In addition, resistance gene sequences were employed to develop molecular markers using RT-PCR approach. High level of polymorphism was found with both RAPD and isozyme markers, where RAPD showed that 60 % of amplified bands were polymorphic. Peroxidase showed three polymorphic loci (7 allelic bands). Isozymes cluster analysis successfully separated the barley accessions into three geographically distinct groups. RAPD investigation demonstrated that Egyptian accessions were grouped into two obvious groups. Moreover, the Tunisian accessions showed no distinct clustering, while high dissimilarities were revealed by the Algerian accessions. In the RT-PCR, from six primer pairs selected, primer pair AF092524P1P2 successfully amplified two specific amplicons of approximately (340 & 220 bp) and (360 & 270 bp), respectively in two Egyptian barley genotypes (El-Awamah and Awlad-Ali). One primer pair DN988165P1P2 gave only one specific amplicon in both barley genotypes of 250 and 270 bp, respectively. The markers developed could be used in improving barley crop by assisting in breeding selection of resistance genotypes.

**Key words:** RT-PCR; resistance genes; barley; genetic diversity; RAPD

**IZVLEČEK**

**MOLEKULARNA GENETSKA ANALIZA NEKATERIH SEVERNOAFRIŠKIH GENSKIH VIROV JEČMENA**

Z izoencimskimi in RAPD markerji je bilo ovrednoteno 29 akcesij ječmena, nabranih v Severni Afriki. Za razvoj molekularnih markerjev na osnovi RT-PCR so bila uporabljena nukleotidna zaporedja genov za odpornost. Ugotovljen je bil velik polimorfizem RAPD in izoencimskih markerjev, kjer je bilo 60 % namnoženih RAPD markerjev polimorfnih. Peroksidaza je pokazala tri polimorfne lokuse (7 alelelov). Z analizo izoencimskih skupin so bile akcesije ječmena uspešno razdeljene v tri različne geografske skupine. Raziskava RAPD je pokazala, da se egiptovske akcesije različno povežejo v dve skupini. Tunizijske akcesije niso pokazale različnega povezovanja, pri alžirskih pa so bile ugotovljene velike razlike. V RT-PCR analizi, je od šestih izbranih začetnih oligonukeltodiov par AF092524P1P2 uspešno namnožil dva specifična amplikona s približno 340 in 220 in 360 in 270 baznih parov pri dveh egiptovskih genotipih ječmena (El-Awamah in Awlad-Ali). Par začetnih oligonukleotidov DN988165P1P2 je pomnožil le en specifični fragment pri obeh genotipih ječmena z 250 in 270 baznimi pari. Razviti markerji se bodo lahko uporabili pri izboru genotipov za izboljšanje ječmena v žlahtnjenju na odpornost.

**Ključne besede:** RT-PCR; geni za odpornost; ječmen; genska raznolikost; RAPD markerji

## 1 INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most pivotal cereal crops in the world. It is cultivated in the temperate zones. The haploid genome of barley is about 5.1 Gbp (Mayer et al., 2012). Due to compatibility and inter-fertility of the cultivated and wild species (share a common genome, n =7), wild species of barley and primitive landraces provide precious sources of genetic variability in a number of beneficial traits (Nevo, 1992; Ceccarelli et al., 1995). Consequently, search for genetic variation that might be useful for plant breeding programs is very essential as well as collection and conservation of wild relatives of the cultivated species and the endemic varieties (Brown et al., 1990).

PCR-based molecular markers (e.g. RAPD, SSR, STS, and ISSR) have been used in barley to uncover genetic variation, genotype identification and mapping of genes (Sánchez et al., 1996; Fernández et al., 2002; Matus and Hayes, 2002; Tanyolac, 2003). Particularly, RAPD markers are found to be valuable in case of self-

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pollinating species, which show a relatively low level of intraspecific polymorphism, as in hexaploid wheat (Devos and Gale, 1992; Joshi and Nguyen, 1993) and in cultivated barley (Barua et al., 1993; Chalmers et al., 1993).

Recently, cDNA sequences are being used to develop markers, which are very useful since they are gene-based markers (Parchman et al., 2010). Moreover, the progress in sequencing and documentation in public database has facilitated sequence data mining and development of DNA markers without any difficulty (Bhattachryya et al., 2014).

Resistance gene analogs (RGAs) were frequently found to be in close genetic distance to known resistance loci, hence suggesting their possible role in disease resistance responses in plants (Fourmann et al., 2001). The gene sequences, which encode proteins containing a nucleotide-binding site (NBS) and C-terminal leucine-rich repeats (LRRs) constitute the largest class of R genes in flowering plants (Dangl and Jones, 2001).

Wild barley has been known to carry valuable sources of useful genes for barley breeding. For example, genes that are associated with resistance to diseases (Fetch et al., 2003), tolerance to abiotic stress (Ellis et al., 2000), other key agronomic traits (Vanhala and Stam, 2006), and quality traits (Shen et al., 2011).

The development of new barley lines, tolerant to abiotic and biotic stresses is an essential part of the continued improvement of the crop. Moreover, it can assist in the amelioration of other relative crops. Wild barley would be a valuable source of novel genetic variation for environmental stress tolerance. However, it depends on identifying of suitable genetic variation and the development of marker-assisted selection, which allows effective cultivar development (Ellis et al., 2000).

The aims of this study are to uncover the genetic variability in a barley germplasm collected from North Africa, compare peroxidase isozymes and RAPD diversity in the studied materials, and amplify disease resistance sequences from some Egyptian landraces, which could be used as molecular markers in assisted marker selection of the resistance lines of barley.

## 2 MATERIALS AND METHODS

### 2.1 Plant materials

Twenty–nine cultivars, genotypes and landraces of barley were obtained from National Institute of Agricultural Research Tunisia (INRAT), National Institute of Agricultural Research Algeria (INRAA), National Research Centre, Egypt (NRC), and Agricultural Genetic Engineering Research Institute, Egypt (AGERI). For resistance genes amplification, two Egyptian barley genotypes El Awamah & Awlad Ali were selected. The barley cultivar names, places and country of origin are listed in Table 1.

### 2.2 Isozyme analysis

To electrophoretically examine peroxidase (PER, E.C.1.11.1.7) isozymes, crude extraction of the twenty-nine genotypes from Algeria, Tunisia & Egypt was done using 0.1M Tris-HCl buffer in 4°C for two hours (Gottlieb, 1981). Then, the homogenates were centrifuged at 14.000 rpm for 20 minutes at 4°C using Centrion K3 centrifuge (Centrion, UK). Electrophoretic separation of the extracts was carried out in 10 % native PAGE (Laemmli, 1970). The peroxidase enzyme was stained as described by Soltis et al. (1983) as follows: gels were incubated in 100 ml staining solution containing 0.05 M acetate buffer (pH = 5.0) and 65 mg benzidine. Two ml of 0.1 M $CaCl_2$ were added as a catalyst. Finally, two ml of $H_2O_2$ were added as the substrate and the gels were kept in refrigerator until dark brown bands appeared. All isozyme bands were assessed according to their relative distances.

**Table 1.** Places, names and country of origin of the barley cultivars and landraces

| Serial | Place of origin or cultivar name | Country of origin |
|---|---|---|
| 1 | Sidimehdi | Algeria |
| 2 | Temasine | Algeria |
| 3 | Kasrmegarine | Algeria |
| 4 | RasEllouche | Algeria |
| 5 | Saida | Algeria |
| 6 | Tichedielt | Algeria |
| 7 | Nailia | Algeria |
| 8 | Rihone-03 | Algeria |
| 9 | Azrir | Algeria |
| 10 | Tozeurt | Tunisia |
| 11 | Tozeur2 | Tunisia |
| 12 | Manel | Tunisia |
| 13 | SidiBeozid | Tunisia |
| 14 | Kibilliz | Tunisia |
| 15 | Tomban | Tunisia |
| 16 | Gabes | Tunisia |
| 17 | KairooaA | Tunisia |
| 18 | Rihan | Tunisia |
| 19 | Jerba | Tunisia |
| 20 | Arish (Sinai) | Egypt |
| 21 | Giza123 | Egypt |
| 22 | Giza 126 | Egypt |
| 23 | Giza 129 | Egypt |
| 24 | Giza 125 | Egypt |
| 25 | Giza 2000 | Egypt |
| 26 | Giza 127 | Egypt |
| 27 | Matrooh (Awama) | Egypt |
| 28 | Giza 131 | Egypt |
| 29 | Giza 130 | Egypt |

### 2.3 DNA extraction and RAPD analysis

DNA isolation of the different barley cultivars, genotypes and landraces was performed using the CTAB method of Doyle and Doyle (1990). For RAPD-PCR analysis, 2 random 10-mer primers OPA3 and OPG3 were used. The random primers used in our study were ordered from SNEF medical, Germany. PCR reactions were conducted in a total volume of 20 µl reaction mix containing 2 µl of 10× reaction buffer, containing 2 mM $MgCl_2$, 2 µl of 0.2 mM dNTP, 0.1µl (0.5 U) of Taq DNA polymerase (Sigma, USA), 40 ng of genomic template DNA, and 10 pmol primer in a preheated thermocycler (Biometra, Germany) under the following conditions: 3 min at 95°C, followed by 44 cycles of 2 min at 92°C, 1 min at 37°C, and 2 min at 72°C. The reaction was finally incubated at 72°C for 10 min. For selecting the optimal conditions of the RAPD PCR, different optimization experiments were performed.

The PCR products were separated by electrophoresis on a 1 % agarose gels using 1× TAE buffer. Then, gels were stained with ethidium bromide (10 mg/ml) and visualized under UV light. A 100 bp DNA ladder (Axygen, USA ) was used as a molecular DNA standard.

### 2.4 Resistance sequences and primers selection

Forty-six sequences showed resistance to different pathogens were retrieved from NCBI gene bank. These sequences were coded for different proteins included definsin (maize and wheat), superoxide dismutase (maize and wheat), catalase (barley and *Cynodon*). Six primer pairs were designed using Primer3 software. After primer selection with the Primer3 program, the complementarity of the primer pairs (primer dimmer and internal complementarity) was checked and the expected annealing temperatures were manually calculated. Table 2 contains the primer sequences, primers length and the expected PCR products in bp for the six primer pairs that were selected from resistance sequences, which were obtained from the NCBI gene bank.

**Table 2:** Oligonucleotide primers used for RT-PCR amplification

| Primer name | Sequence | Primer length | Sequence/plant | Expected PCR fragments |
|---|---|---|---|---|
| AB089942P1 | GGTGTGAAGCGAGCAAGC | 18 bp | Definsin/wheat | 522 bp |
| AB089942P2 | CAGTGGCATCGTTATTACATCA | 22 bp | | |
| AF092524P1 | CTACGTCGCCCACTACAACAAG | 22 bp | SOD/wheat | 565 bp |
| AF092524P2 | CCAACAGCGGGAAACTCAAG | 20 bp | | |
| AJ849917P1 | GGCCACAACGCTAGTACAATCTT | 23 bp | Definsin/*Zea* | 433 bp |
| AJ849917P2 | CATGCGTATCACTCAATCTGCC | 22 bp | | |
| CV064086P1 | CGGCCATGGATCCCTACAAG | 20 bp | CatIso1/barley cDNA | 509 bp |
| CV064086P2 | CTCCTGCATGTTGGTCTTCGG | 21 bp | | |
| DN988165P1 | GTATCTTCATGTCATTGCTCGC | 22 bp | CatIso3/*Cynodon* | |
| DN988165P2 | CTCCGGCTGGTTCCTTTC | 18 bp | | 153 bp |
| X17564P1 | AGGGCACCATCTTTTTCACC | 20 bp | SOD/*Zea* | |
| X17564P2 | GCGACGCTCTTATTTCACGA | 20 bp | | 516 bp |

The primers were ordered from Metabion International AG

### 2.5 RNA isolation

For RNA isolation from the two barley genotypes, leaves of the 7 day old seedlings of the barley genotypes were used. To avoid any contamination with RNase, all solutions were treated overnight with DEPC at a final concentration of 0.1 %. All non-disposable equipment, like glass and porcelain, was cleaned with 0.5 % SDS/0.5 M NaOH and rinsed with DEPC treated water and wiped with 70 % ethanol.

Total RNA was isolated using TRIzol reagent of the TriFast (PeqGOLD) extraction kit. In this method, 0.2 - 0.5 g of fresh leaves was ground to fine powder in liquid nitrogen. The powder was added directly to an Eppendorf tube containing 0.5 ml TRIzol reagent. The homogenate was incubated at room temperature (RT) for 5 min. After adding 0.2 ml chloroform, the tube was repeatedly inverted by hand for 15 sec. The mixture was incubated for 15 - 20 min at RT. Then, the upper aqueous phase was separated by centrifugation at 12000 rpm for 15 min at 4°C. The RNA was precipitated using 1 vol. isopropanol for 10 min at RT and afterwards by centrifugation at 12000 rpm for 10 min at 4°C. The pellet was washed with 70 % ethanol and resuspended in DEPC treated water (0.943 g/ml, BioBasic INC). To check the quality and quantity of the isolated RNA, RNA agarose gel electrophoresis was performed. Electrophoresis was carried out on 1.5 % agarose gel for 1h at 70 V.

### 2.6 RT-PCR analysis

RT-PCR was carried out using Ready-To-Go RT-PCR Beads kit (Amersham Biosciences). Each bead is optimized to allow the first-strand cDNA synthesis and PCR reactions to proceed sequentially (One-step Protocol for RT-PCR). In the One-step protocol,

primers for the first-strand cDNA synthesis and PCR were added along with the template to an RT-PCR Bead.

First, for each reaction tube, 39 µl DEPC-treated water was added. The tube was taped to mix the water with the bead. To dissolve the bead, the tube was incubated on ice for 5 min. and the tube contents were gently pipetted up and down. After that, to each bead (contains 2 units of *Taq* DNA polymerase, 10 mM Tris-HCl, 60 mM KCl, 1.5 mM $MgCl_2$ 200 µM dNTPs, MuLV reverse transcriptase, RNAguard™ Ribonuclease Inhibitor and stabilizer when brought to a final volume of 50 µl) 1 µl of the first-strand primer (0.5 µg), 5 µl of 10 pmol PCR primer pairs (resistance sequences based primers) and 5 µl of the template RNA were added.

For positive control reaction, 50 µl of DEPC-treated water were added to control mix bead and then the entire contents of the control tube were transferred into a tube containing a RT-PCR Bead. The reaction tubes were incubated at 42°C for 15-30 min in heat block or thermal cycler. Then, the reaction tubes were incubated at 95°C for 5 min in order to inactivate the reverse transcriptase. The PCR analysis was carried out in MiniCycler™ (MJ Research) thermocycler for 30 cycles with a 1 min denaturation step at 94°C, 1 min annealing at 58°C and 1 min extension at 72°C. After RT-PCR amplification, the PCR products were resolved on 2 % agarose gel electrophoresis and the amplification profiles of the primer pairs were analyzed.

### 2.7 Data analysis

Peroxidase loci were labeled sequentially with those migrating closest to the anodal end designated as number 1, while alleles at each locus were labeled alphabetically from the most anodal band. A tree illustrating the genetic diversity among barley landraces and varieties, using unweighted pair-group method with arithmetic average (UPGMA), was generated based on peroxidase isozymes data using NTSYS-pc version 2.1 (Rohlf, 2000). The RAPD amplicons (bands) were recorded as 0 (absence) or 1 (presence) and were also analyzed by cluster analysis using the UPGMA method using the NTSYS-pc version 2.1. The goodness of fit of the cluster analysis, for both of isozymes and RAPD data, was determined by calculating the cophenetic value matrix from the tree matrix in order to carry out the Mantel test (Mantel, 1967) to determine the agreement between the two matrices.

## 3 RESULTS

### 3.1 PER isozyme analysis

Only three loci (PER 1, PER 2 and PER 3) were obtained from peroxidase enzyme analysis in the 29 accessions and varieties of barley. Out of these three loci, two loci (PER 2 and PER 3) were polymorphic in all accessions obtained from Algeria and Tunisia. All the three loci (PER 1 “one band”, PER 2 “2 bands” and PER 3 “one band”) were monomorphic in Egyptian varieties and landraces, where three accessions (Giza 129, Matrouh-Awama and Giza 131) showed one band with slower electrophoretic mobility. PER 1 and PER 3 were monomorphic with only one band shown in the Egyptian accessions and varieties. PER 3 displayed three polymorphic isozymes (bands) in the Algerian and Tunisian landraces. However, in case of PER 1 and PER 2, two polymorphic bands were observed in Algerian accessions. Nevertheless, in the Tunisian accessions, PER 1 (one band) was monomorphic and PER 2 (one band) was also monomorphic. Three Tunisian accessions (Tomban, Gabes and KairooaA) showed one unique allele in locus PER 2.

Based on the profiles of peroxidase isozymes, cluster analysis grouped all Egyptian barley varieties into one main cluster group at a distance of 1.5 (Fig. 1). However, only one landrace (Matrouh) was contained into a separate subgroup with two accessions (Manel and Tozeurt) from Tunisia and one accession (Sidimehdi) from Algeria. Similarly, Algerian and Tunisia accessions were grouped into one main at the same distance (1.5). Two accessions (Azrir: Algeria and Gabes: Tunisia) were found in one of the subclusters with two Egyptian barley varieties. A unique main cluster contained only one Algerian accession (Temasine), see Fig. 1. Cophenetic correlation based Mantel t-test was r = 0.800, which indicated a good fit to the dendrogram obtained with the cluster analysis.

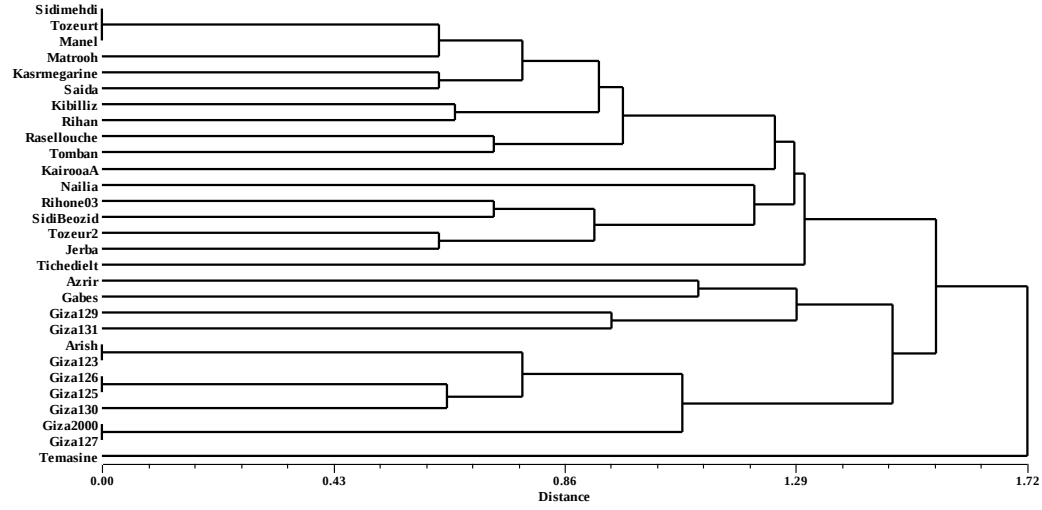


**Figure 1:** Cluster analysis of the isozymes-derived data of 29 barley accessions collected from three North African countries

### 3.2 RAPD analysis

RAPD analysis based on two random primers (OPA3 and OPG3) showed that about 60 % of amplified bands were polymorphic. The number of bands (alleles) ranged from 3 to 12 per primer, with an average of 6 per primer. Cluster analysis based on RAPD data revealed that the two accessions (Sidimehdi and Temasine) from Algeria were clearly separated at a distance of 2.47 as two identical genomes from all other barley accessions. The Egyptian accessions were grouped into two obvious groups; one contained 4 accessions (Giza126, Giza2000, Giza127 and Giza125) and the second contained two accessions (Giza123 and Giza129). However, only one accession (Giza130) was out of these two groups. Moreover, it was obvious that the accessions of each group were genetic invariable (showed high similarity). The Tunisian accessions showed no distinct clustering. The most high dissimilarities were revealed by the Algerian accessions (Fig. 2). Cophenetic correlation based on Mantel t-test, used to measure the goodness of fit of RAPD cluster analysis, was r = 0.977, which showed a very good fit to the generated dendrogram.

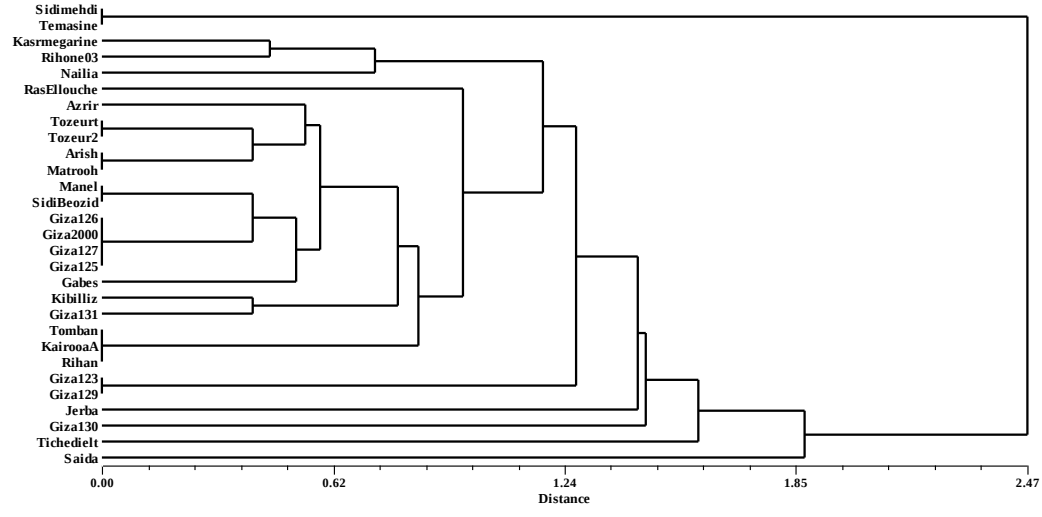


**Figure 2:** Cluster analysis of the RAPD-derived data of 29 barley accessions collected from three North African countries

### 3.3 RT-PCR and cDNA analysis

The total RNA was isolated with TriFast method, which ensured high quality total RNA in sufficient amount. RNA was checked for quality and quantity using agarose gel electrophoresis. To amplify the selected resistance gene-sequences from the Egyptian barley genotypes, which may represent candidate sequences

for the resistance gene analogs of barley; comparative RT-PCR amplifications with each primer pair (Table 2) were performed. The total RNA isolated from two Egyptian barley genotypes (El-Awamah and Awlad-Ali) was used as template for RT-PCR analysis. The genomic DNA of the barley genotypes was also used to check and compare the amplification profiles when using RNA and DNA as templates. In case of DNA template, ordinary PCR analysis was performed.

Figure 3 showed that the primer combinations AB089942P1P2 and CV064086P1P2 amplified 3 weak amplicons in both barley genotypes (El-Awamah and Awlad-Ali). The primer pair AF092524P1P2 successfully amplified two PCR amplicons of approximately (340 & 220 bp) and (360 & 270 bp), respectively in both barley genotypes (Fig. 3). However, primer pair DN988165P1P2 gave only one amplicon in both barley genotypes (El-Awamah and Awlad-Ali) of 250 and 270 bp, respectively. The primer pairs AJ849917P1P2 and X17564P1P2 failed to amplify any fragments from both barley genotypes.

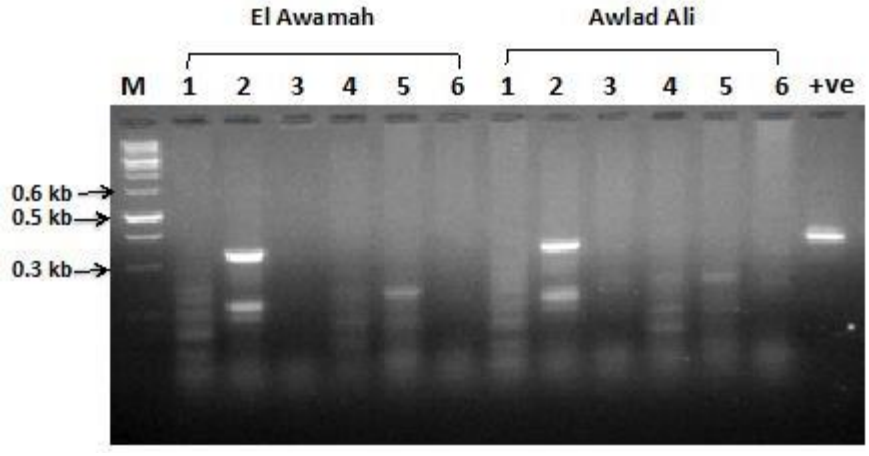


**Figure 3:** PCR amplification of specific DNA markers based on primers derived from resistance gene ortholog sequences, cDNA was used as template. +ve: positive control

Although, the primer combinations AB089942P1P2 and CV064086P1P2 have amplified three faint bands in the One-Step RT-PCR, they were successful to amplify only one specific PCR fragments when genomic barley DNA (El-Awamah) was used as template. The two PCR fragments were of approximately 230 bp and 200 bp, respectively (Fig. 4). Similarly, primer pair DN988165P1P2 amplified only one specific fragment of 700 bp. The X17564P1P2 primer pair failed to amplify any PCR product, while primer pairs AF092524P1P2 and AJ849917P1P2 amplified very faint fragment of 450 bp.

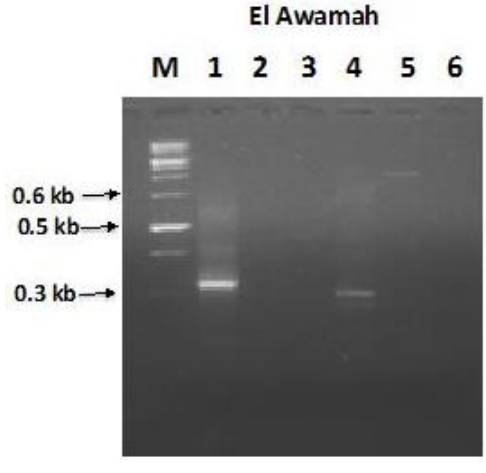


**Figure 4:** PCR amplification of specific DNA markers based on primers derived from resistance gene ortholog sequences, genomic DNA extracted from El-Awamah landrace was used as template

## 4 DISCUSSION

In this study, high genetic variability was observed among the barley landrace accessions and varieties collected from the three North African countries (Algeria, Egypt and Tunisia). However, relatively low genetic diversity was shown among the landrace accessions and varieties from the same region. In addition, development of molecular markers based on pathogen-resistance gene sequences was successful and two gene-based primer pairs amplified specific DNA fragments, which could be used in barley crop improvement.

The possible explanation of the low genetic diversity among varieties (collected from one country) is that they have a relatively narrow gene pool. Similar finding has been observed in the polish old oat cultivars, where low genetic variation of the old cultivars collection was noticed, which has been related to the entirely limited gene pool of such cultivars (Boczkowska et al., 2014).

Evaluation of the degree of genetic diversity within cultivated barley and its related wild germplasm is necessary for barley crop improvement and for the conservation of barley genetic resources (Boczkowska et al., 2014; Gepts, 2006). Therefore, estimation of genetic diversity of the North African barley landrace accessions and varieties were examined using isozymes and RAPD molecular markers.

RAPD markers provide a powerful tool for studying all aspects of genetic variability and genetic structure of the populations. Genetic data derived from isozymes are more robust this due to isozymes are codominant markers. However, isozyme analysis is restrained because fewer number of loci are generated. RAPDs are dominant markers. Therefore, there is less information per band. However, RAPD analysis has several advantages over other marker types: it has more loci that can be tested with RAPD, it is simple, has low cost, and it needs little amount of plant DNA (Garcia Mas et al., 2000).

It is well known that genetic variability of the wild populations is essential for plants to be adapted to environment (Nevo et al., 1997). Canadian durum wheat cultivars, which were analyzed by AFLP markers, showed that the cultivars that had been bred from landraces were characterized by higher genetic heterogeneity compared to those that were derived from commercial cultivars (Soleimani et al., 2002).

The current results indicated that it is possible to use specific primers based on the resistance gene sequences to amplify PCR products using cDNA or DNA as templates. Also, the amplified PCR products would help in identification of RGAs from barley. Wild barley and Middle Eastern landraces have already proven to be a very beneficial source of genes for modern crop improvement (Ellis et al., 2000). The most obvious example is the development of barley varieties that have *mlo* allele, which showed resistant to powdery mildew (Thomas et al., 1998).

Similar approach was used to develop gene-based markers, which were used to construct a dense linkage map in yellow fever mosquito *Aedes aegypti* (Linnaeus in Hasselquist, 1762). In this approach, cDNA sequences were downloaded from GenBank and primers were set to amplify PCR fragments of about 500 bp (Fulton et al., 2001). The identification of QTL and in special cases, gene cloning, are steps in the process of building a program for the genetic manipulation of abiotic stress tolerance (Ellis et al., 2000) without using transformation.

Fourmann et al. (2001) reported the development and mapping in *Brassica napus* L. of a series of resistance-gene analogs based on existing sequences of nucleotide-binding resistance genes. Some of the sequences could be amplified in *B. oleracea* L. and *B. rapa* L. and were employed as helpful markers, which were linked to disease resistance in the three major cultivated *Brassica* species.

Recently, barley genetic resources were mined for genes and alleles relevant for non-specific resistance (NR) to powdery mildew, which is caused by *Blumeria graminis* (DC.) Speer.f. *hordei* (*Bgh*). In that study, eleven candidate genes were identified, where they showed significant SNP or haplotype associations with the *Bgh*-phenotypes in a worldwide collection of spring barley (Spies et al., 2012).

Marker assisted selection (MAS) signifies that DNA markers, which are tightly-linked to target loci are exploited as a substitute for assist population phenotyping (Collard et al., 2005). In our study, some of the sequences amplified specific DNA fragments (Fig. 4), which could also be used for marker assisted selection in the breeding program of barley crop improvement.

Similarly, Giovanelli et al. (2002) developed gene-based markers, which were associated with cotyledon-stage downy mildew (*Hyaloperonospora parasitica* (Pers.) Constant.) resistance in broccoli. They proposed that such markers could be used for marker-assisted selection to generate downy mildew resistant varieties.

In conclusion, genetic diversity analysis revealed that relatively low genetic variability was found among the landraces and varieties from the same region. Using RT-PCR-based approach, it was successful to amplify gene-based DNA marker bands, which could be employed in the breeding of disease-resistant barley genotypes.

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