Amplified Fragment Length Polymorphism (ALFP) based molecular analysis of Egyptian barley lines and landraces differing in their resistance and susceptibility to leaf rust and net blotch diseases

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Abstract

Twenty four Egyptian lines and landraces, representing Egyptian barley germplasm were collected and screened for resistance to net blotch (*Pyrenophora teres*) and leaf rust (*Puccinia hordei*) diseases. Two landraces, collected from Rafah (Sinai), Awlad Ali (Marsa Matrouh) and the two lines AT 5 and DT 1 (Barley Dept., ARC) showed extreme resistance to both net blotch and leaf rust. The line AT 42 is the most susceptible one for net blotch and leaf rust. Genetic diversity among the 24 accessions with various levels of resistance and susceptibility to net blotch and leaf rust was evaluated using Amplified Fragment Length Polymorphism (AFLP). AFLP analysis generated large number of polymorphic bands (markers) and allowed easy identification of the different genotypes at the DNA level. The developed AFLP-based dendrogram divided the barley genotypes into two main clusters in accordance with their origin (native landraces), and resistance and susceptibility to diseases (lines derived from the same crosses). The developed AFLP fingerprints for the newly identified valuable net blotch and leaf rust resistant Egyptian barley accessions reported herein could support the future Egyptian barley germplasm collection, preservation and utilization.

Keywords: ALFP, Hordeum vulgare, genetic diversity, Puccinia hordei, Pyrenophora teres

Zusammenfassung

ALFP basierte Molekularanalyse der Resistenz ägyptischer Gersten-Linien und -Landrassen gegen Blattrost und Netzfleckenkrankheit

Mit Hilfe genetischer Marker wurde die große Variabilität der Resistenz 24 ägyptischer Gersten-Linien und -Landrassen gegenüber Blattrost und Netzfleckenkrankheit untersucht. Insbesondere die Landrassen erwiesen sich als beachtenswerte genetische Ressource für die zukünftige Züchtung widerstandsfähiger Gerstensorten.

Schlüsselwörter: ALFP, Hordeum vulgare, genetische Diversität, Puccinia hordei, Pyrenophora teres

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1 Introduction

Barley (*Hordeum vulgare*) ranks as the world's fourth major cereal crop after maize, wheat and rice. It is also one of the world's ancient cereal crops with archaeological remains suggesting that it was first domesticated in the fertile crescent around 10,000 years age- at about the same time as wheat. Barley is very closely related to wheat and this similarity allows the production of fertile hybrids between the two species (Zohary and Hopf, 1988). Improvements in its yield have been due to a large number of factors but improved varieties have certainly played a major role. Disease was seen as the key limitation on yield in the early 1980s and was the major focus of breeding programs. Fungal diseases have been considered as one of the main causes of crop losses over since mankind started to cultivate plants (Oerke et al., 1994). Estimates of yield losses in barley due to net blotch disease only range from 15 % in Canada (Martin, 1985), 35 % in the United States (Steffenson et al., 1996) and can reach up to 40 % in other countries (Shipton et al., 1973). Little attention has been given to large scale cultivation of barley in Egypt and consequently Egyptian barley germplasm. In the Northwest costal region of Egypt, the Bedouins grow their own local adapted genotypes continuously in isolated areas therefore one can expect an interesting genetic diversity, which can be introduced in breeding programs to improve barley cultivars.

The recent development in molecular genetic technology particularly in the development of the so called "Molecular Markers", which are used to reveal polymorphism, has greatly improved research in many disciplines such as Taxonomy, Phylogeny, Ecology, Genetics and Evolutionary biology (Tingey and Del tufo,1993). Molecular markers can also facilitate quantification of existing genetic diversity and uncovering duplicate or very similar genotypes, and the identification of unique variants or genotypes for expanding the useful variation. AFLP is a PCR-based molecular marker that had been used for the characterization and evaluation of germplasm and genetic resources (Vos et al, 1995). AFLP was acknowledged as efficient and powerful tool in the prospection of biodiversity in important crop species (Hongtrakul et al., 1997; Milbourne et al., 1997 and Paul et al., 1997). With the aid of molecular markers, genes of interest were tagged, traced and used in breeding programs, and to select disease resistant germplasm (Stam, 1997).

In the case of barley, molecular marker technology was employed successfully for the identification and classification of barley germplasm (Struss and Plieske, 1998), assessment of the genetic relatedness of barley accessions (Yang and Quiros, 1993), development of molecular markers linked to rust resistance gene (Nachtigall et al., 2000), characterization of genetic diversity in malting barley cultivars, biological and molecular evaluation of native barley germplasm (Czembor, 2001 and Saker 2005a and b and Saker et al., 2005) and mapping of resistance genes (Chelkowski et al., 2003). The objectives of this study were to (1) survey the Egyptian barley germplasm for new resources for resistance to net blotch and leaf rust, (2) develop salient AFLP profiles (fingerprints) of the identified valuable genotypes and landraces, and (3) assess the genetic diversity within the selected landraces differing in their resistance and susceptibility to fungal diseases.

2 Materials and methods

2.1 Plant Material

Seed samples of twenty four barley landraces and lines representing Egyptian barley germplasm and differing in resistance and susceptibility to leaf rust and net blotch were used in this study. Cultivated genotypes (lines) were kindly provided by the Barley Department, Field Crops Research Institute, Agricultural Research Center (ARC), Ministry of Agriculture, Egypt. Meanwhile, native barley landraces, grown by Bedouins were collected from different locations of Egypt. i.e., Awlad Ali, El-Kasr and El-Awama villages (Northwest Coast, Marsa Matrouh), Rafah, El-Sheikh Zowaid and Al-Arish (Northeast coast, Sinai). The name and pedigree of these lines are shown in Table 1.

2.2 Genomic DNA isolation

Plants were grown in a greenhouse. Five plants were sampled from each genotype and total cellular DNA of the pooled sample was prepared as described by DNeasy Plant Mini Kit (cat no. 69104). Agarose gel electrophoresis confirmed that the DNA was of high molecular weight with no degradation or contaminating RNA.

2.3 AFLP analysis

The AFLP procedure was performed with minor modifications according to the protocol of Vos et al. (1995) that is supplied with the AFLP Analysis System I kit (Gibco, BRL). Approximately 500 ng DNA was digested simultaneously with *EcoRI* and *MseI* at 37 °C for 2 hr. The digested samples were incubated at 70 °C for 15 min to inactivate the restriction endonucleases. *EcoRI* and *MseI* adapters were ligated to the digested samples at 20 °C for 4 hr. This was done to generate template DNA for amplification. Preamplification was carried out with + 1 - primers each carrying one selective nucleotide (*EcoRI* + A and *MseI* + C) in a thermocycler for 20 cycles (94 °C/30s, 56 °C/60s and 72 °C/60s). The amplification products were diluted 50

Table 1:
The name, pedigree and location lines and landraces of barley

Code	Lines and Landraces	Name	Pedigree	Locations
1	El-Arish	-----	------	
2	El-Sheikh-Zowaid	-----	------	Sinai
3	**Rafah**	-----	------	
4	**Awlad- Ali**	-----	------	
5	El-Kasr	-----	------	Marsa - Matrouh
6	El-Awama	-----	------	
7	AT 1	Linaze-Bar/Robust/Qunina	CMB93.910-A-1Y-1Y-1N-OY	
8	AT 58	Lignee 527/Chn01//Gustoe/4/ Rhn-08/3/DeirAlla 106//DL71 /Strain205	Sk.Sel98/99 no.134-2-1F3SP	
9	AT 40	PI2325/Maf102//Cossak/3/Trump/4/Aths/Rihano1	------	
10	AT 42	Mari/Aths*2//Arizona5908/At-hs*Arar/Lignee 527	Egypt, 99/00.Scr,1/4	
11	AT 10	Aths/Lignee686//Orge905/Cr. 289-53-2	Egypt, 00/01.Scr1 no. 34	
12	AT 11	Lignee527/NK1272//ULB70-63	Egypt, 00/01.Scr1 no. 33	
13	AT 25	Rhn/Lignee527	ICB 82-0897-4AP-OAP	
14	AT 26	Lignee527/NK1272//Alanda	ICB 91-0517-7APP-OAP	
15	AT 4	Lignee527/NK1272//UL76252/Jaidor	ICB 91-1019-21AP-OAP	Barley Dept., ARC
16	AT 6	QB813.2	BCB99-00 no.46	
17	AT 3	Aths/Rihane-01// Sawsan/ Lignee640	Egypt, 99/00.Sci,1 no. 9	
18	AT 22	Lignee527 / NK1272	------	
19	**AT 5**	26216/6/CI 010214/CM67/U. Sask 1800//Pro/CM67/ 3/ DL70/5/Nacha 2	ICB 94-0561-OAP	
20	AT 29	Lignee527// Chn-01 //Allanda	ICB 93-0372-0AP-8AP-OAP	
21	AT 15	Giza 123	------	
22	AT 20	Rod586/Nopal*s/3/PmB//Aths//Bc/4/F2CC33 MS/CI 07555	ICB 93-434-0AP-9AP-OAP	
23	**DT 1**	------	Arona5908/AdHis"Lignee	
24	DT 2	------	Dhm.Unk.	

The sources of names and pedigrees was provided from Barley Research Department/ARC

fold in TE buffer and stored at -20 °C. Selective amplification was carried out with *EcoRI* + 3 - primers and *MseI* + 3 - primers and 5µl of the diluted PCR products from the preamplification. Eighteen primer pair combinations were employed in this study. The PCR amplification was performed as follows: one cycle at 94 °C for 30 s, 65 °C for 30 s and 72 °C for 60 s, followed by 13 cycles of touch-down PCR in which the annealing temperature was lowered by 0.7 °C every cycle. This was followed by 23 cycles at 94 °C for 30 s, 56 °C for 30 s and 72 °C for 60 s.

2.4 Gel analysis

The reaction products were mixed with equal volumes of formamide loading buffer (98 % formamide,10mm EDTA, bromophenol blue and xylene cyanol), denaturated by incubating at 90 °C for 3 min and quickly cooled on ice. The products were analyzed on 8 % denaturing polyacrylamide gels. The gel was run at constant power (50 - 55 W) until the dye was about 2/3 down the length of the gel. The gel was silver stained using a DNA silver staining kit (Promega), according to the manufacturer instructions.

2.5 Analysis of AFLP data

AFLP polymorphic bands were scored as present (1) or absent (0). Estimates of similarity among all genotypes were calculated according to Dice coefficient (Sneath and Sokal, 1973) definition of similarity: siJ = 2a / (2a + b + c), where siJ is the similarity between two individuals i and J, a is the number of bands present in both individuals, b is the number of bands present in i and absent in J, and c is the number of bands present in J and absent in i, the matrices of similarity were analyzed by the Un weighted Pair-Group Method Analysis (UPGMA) and the dendrograms were obtained.

2.6 Pathogens

For artificial infestation of different accessions, local isolates (Egyptian biotypes) of *Puccinia hordei* and *Pyrenophora teres* were isolated from the barley plants grown

in Experimental Farm of National Research Center at El-Kanater El- Khairea Egypt. The isolates were purified by single spore, maintained and propagated. Strains of conidiosores (*P. hordei*) were produced on leaves of their original barley lines in the greenhouse. For inoculation, pieces of infected leaves with abundant sporulating colonies or pustules (10 mm in diameter) were washed in 100 ml sterilized distilled water containing 0.01 % Tween-80. The spore suspension adjusted to 10^5 spores /ml was used. Frequent virulence checks assured their purity throughout the experiment. Single conidia of each *P. teres* isolates were increased on V-8 juice agar at 20 °C (Miller, 1955). After seven days in culture, the cultures were washed from the agar surface, filtered through a 330µm strainer and made up into an aqueous suspension containing 12,500 conidia/ml and 0.01 % Tween-80.

2.7 Field evaluation

Field screening using naturally occurring inoculum was used to identify resistance against net blotch and leaf rust diseases in different accessions. Barley seeds were sown at rows spacing of 15 cm with width measuring 3.0 m of each accession at the NRC experimental farm at El- Kanater El- Khairea during 2004 and 2005 seasons. Planting were made on October,15th using a randomized complete block design with three replicates for each line. Each replicate consisted of 20 plants. Irrigation was carried out as recommended. Diseases incidence of leaves was assessed 20 days intervals as the percentage of necrotic flag area or covered by lesion colonies and the lesions numbers per leaf (the fifth leaf from the top of the plant). Disease incidence of heads is calculated as the proportion of barley heads with symptoms out of a predetermined number of heads. Mean number of spores/cm^2 were counted during growth periods using hemocytometer. Data were recorded per 10 plants, 20 days intervals on randomly selected one lines per replicate.

2.8 Evaluation under greenhouse conditions

Resistance and susceptibility of the 24 barley accessions to net blotch and leaf rust was determined, following artificial infection of plants with spore suspensions of *Pyrenophora teres* and *Puccinia hordei* under greenhouse conditions. Pots (20 cm diameter) containing sandy loam soil were sowed with five seeds of each accessions and grown in the greenhouse at 20 ± 2 °C. Pots were arranged in a complete randomized design with three replicates for each line. Plants were sprayed with spores suspension of each fungus at concentration of 10^5 spores/ml with 0.01 % surfactant, using a hand held atomizer onto expanded leaves (about 0.5 ml per leaf). Each treatment was replicated three times. Plants were grown for 18 - 24 h and 98 % relative humidity at greenhouse temperature in a dark room and then returned to the glasshouse for disease development for 15 day. Diseases severity were measured using a 0 - 9 scale (0 resistant, 9 susceptible) according to Finckh et al., 1999). Data were subjected to analysis of variance and treatment means were compared by an approximate Student's t-test ($p < 0.05$).

2.9 Growth analysis

At harvest stage, plant height and branch number were measured, then, the samples were dried at 60 °C for 4 days before obtaining plant dry weight. Grain dry weight was also measured.

2.10 Statistical analysis

Data were subjected to analysis of variance and treatment means were compared by an approximate Student's t-test ($P < 0.05$).

3 Results

3.1 Survey for resistance to net blotch and leaf rust:

A glance on data of disease severity presented in Table 2 indicates clearly that about half of the tested lines and landraces had high levels of resistance to the net blotch disease under open field conditions. The extremely resistance accessions included Rafah, Awlad Ali, El Awama, AT 1, AT 58, AT 10, AT 11, AT 25, AT 26, AT 4, AT 5, AT 6 and DT 1. Line AT 42 was identified as the most susceptible to *Pyrenophora teres*. The reactions of other landraces ranged from moderate to high resistance. In the case of the extremely resistant landraces, no symptoms were recorded until the harvest time (180 - 200 days). Symptoms were recorded during the first two months of cultivation in susceptible and moderate resistance accessions. In the case of rust, the interactions of the 24 barley accessions with the *Puccinia hordei*, expressed as disease severity (area) revealed a high degree of variation in disease resistance. The extremely resistance landraces to leaf rust included Rafah, Awlad Ali, El Sheik Zowaid, AT 1, AT 58, AT 3, AT 6, AT 5, AT 22, AT 29 and DT 1 (Table 2). The line AT 42 is also the most susceptible one for net leaf rust.

Based on the evaluation of the collected germplasm in open field under natural infection conditions, plants were categorized as extreme resistance (R), moderate resistance (M) and susceptible (S). Seeds were collected and subjected to evaluation under greenhouse conditions. Reactions against different local pathogen races (Egyptian biotypes) of *Pyrenophora teres* and *Puccinia hordei* were recorded

Table 2:

Infection responses (disease severity) of the 24 barley accessions to *Pyrenophora teres* and *Puccinia hordei* pathogens grown under natural infested field conditions). Response of selected barley lines against net blotch and leaf rust after 15 days of inoculation with spore suspension under greenhouse conditions. A 0 - 9 scale was used for rating disease severity (0 = resistant, 9 susceptible).

Accessions	Net blotch severity[x] % under natural field conditions						Net leaf Rust severity[x] % under natural field conditions						Response against Net blotch	Response against Leaf rust
	80	100	120	140	160	180	80	100	120	140	160	180		
El-Arish	0[y]	0	0	0	0	0.23	0[y]	0	0	0	16.5**	25.3**	0.00	3.36
El-Sheikh-Zowaid	0	3.54	11.5	12.3	15.6	15.6	0	0	0	0	0	0	0.87	0.00
Rafah	0	0	0	0	0	0	0	0	0	0	0	0	**0.00**	**0.00**
Awlad- Ali	0	0	0	0	0	0	0	0	0	0	0	0	**0.00**	**0.00**
El-Kasr	0	0.65	1.56	1.65	1.87	1.98	0	0	0	0	3.56	8.54	0.27	0.00
El-Awama	0	0	0	0	0	0	0	0	0	0	1.25	5.65	0.00	0.79
AT 1	0	0	0	0	0	0	0	0	0	0	0	0	0.97	0.00
AT 58	0	0	0	0	0	0	0	0	0	0	0	0	3.65	0.00
AT 40	4.65*	20.6*	58.9*	78.6*	85.6*	91.2*	0	5.32	6.45	0.23	28.6*	49.3*	0.00	4.5
AT 42	5.65*	22.6*	55.6*	75.8*	95.5*	100*	0	0.21	0.98	28.3*	38.0	51.2*	7.23	4.68
AT 10	0	0	0	0	0	0.34	0	0	0.28	30.5*	2.78	49.0	0.00	1.06
AT 11	0	0	0	0	0	0	0	0	0	0.21	28.0	39.0	0.00	2.8
AT 25	0	0	0	0	0	0	0	0	0	0	2.35	3.54	0.00	0.75
AT 26	0	0	0	0	0	0	0	0	0	0	1.56	1.36	0.00	1.00
AT 4	0	0	0	0	0	0	0	0	0	0	19.3**	28.5**	0.00	2.54
AT 6	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
AT 3	4.23	6.54	33.6	60.5*	75.8*	100*	0	0	0	0	0	0	6.23	0.00
AT 22	5.65*	12.3*	45.1*	76.6*	81.6*	100*	0	0	0	0	0	0	5.14	0.00
AT 5	0	0	0	0	0	0	0	0	0	0	0	0	**0.00**	**0.00**
AT 29	0	0	0	0	0.21	0.12	0	0	0	0	0	0	0.42	0.00
AT 15	0	0	0	0.21	0.41	1.21	0	0	0	0	23.6**	31.6**	0.32	4.0
AT 20	2.3	6.78	8.98	10.3	11.5	11.6	0	0	0	0	1.25	6.54	5.6	0.8
DT 1	0	0	0	0	0	0	0	0	0	0	0	0	**0.00**	**0.00**
DT 2	2.35**	5.65**	20.5**	66.5**	74.5**	80.6**	0	0	0	0	0.89	1.32	0.32	0.21

[x] Disease severity was expressed as the percentage of leaves area with disease symptoms
[y] Each value is the mean of three replicates

Disease was different from the mean of pure stands at *P < 0.05 and ** P < 0.01

after 15 days of inoculation (Table 2). The results of evaluation under greenhouse conditions are in general agreement with the results of evaluation in open field under natural infection conditions. In other words, the majority of extremely resistant landraces based on the results of field evaluation behaved in the same fashion under greenhouse conditions (Table 2). Combined data of field evaluation and greenhouse evaluation for net blotch and leaf rust indicated that four landraces, collected from Rafah (Sinai), Awlad Ali (Marsa Matrouh) and the two lines AT 5 and DT 1 (Barley Dept., ARC) showed extreme resistance to both net blotch and leaf rust. While, the line AT 42 was the most susceptible one for both diseases net blotch and leaf rust. The rest of investigated lines and landraces were moderately resistant to one of the two diseases (Table 2).

3.2 AFLP analysis

AFLP fingerprints of the 24 barley accessions were performed using 20 AFLP primer combinations (Table 3). AFLP amplification data revealed a total number of 1203 amplified DNA fragments ranging in length from 40 to 1000 bp, out of them, 588 were polymorphic, i.e. the percentage of polymorphism was 4.8 %. The polymorphic bands were distributed across the genotypes. On average, 48.88 distinguishable bands were observed per primer combi-

nation, and an average of 20.4 of these AFLP bands was polymorphic. The number of amplicons (bands) in the profiles varied, depending on the primer combination and genotype.

Table 3:

AFLP primer combinations, total number of amplicons, polymorphic amplicons, polymorphism percentage and levels of polymorphism detected by different primer combinations for the 24 barley accessions

Primer omb. code	Primer combination	Total number of amplicons	Polymorphic amplicons	Polymorphism %	Fragments size range scored
1/ 5	E-AAC/M-CTA	83	14	16.9	50 - 500
1 / 8	E-AAC/M- CTT	28	21	75	60 - 200
2 / 5	E-AAG/M-CTA	66	22	33.33	70 - 500
2 / 7	E-AAG/M-CTG	88	32	36.36	40 - 520
2 / 8	E-AAG/M-CTT	27	18	66.67	107 - 520
3 / 3	E-ACA/M-CAG	46	20	43.48	94 - 490
3 / 7	E-ACA/M-CTG	63	14	22.22	80 - 500
4 / 2	E-ACC/M-CAC	80	34	42.5	50 - 800
4 / 5	E-ACC/M-CTA	41	29	70.73	65 - 550
4 / 7	E-ACC/M-CTG	65	20	30.77	50 - 600
5 / 4	E-ACG/M-CAT	64	14	21.88	65 - 450
6 / 3	E-ACT/M-CAG	52	48	92.31	60 - 500
6 / 7	E-ACT/M-CTG	59	35	59.32	45 - 520
6 / 8	E-ACT/M-CTT	59	36	61.02	40 - 500
7 / 3	E-AGG/M-CAG	31	27	87.1	60 - 490
7 / 5	E-AGG/M-CTA	85	47	55.29	82 - 1000
7 / 8	E-AGG/M-CTT	65	40	61.54	60 - 375
8 / 2	E-AGG/M-CAC	74	53	71.62	65 - 570
8 / 3	E-AGG/M-CAG	79	40	50.63	68 - 870
8 / 6	E-AGG/M-CTC	48	24	50	50 - 517
Total		**1203**	**588**	**48.88**	

3.3 Unique positive and negative AFLP markers

Data extracted from AFLP analysis using twenty AFLP primer combinations led to the development of unique specific markers to identify the 24 barley accessions. Each of these primer combinations revealed unique markers characterizing one or more genotypes. The total number of unique bands across the 24 barley accessions was 143 unique markers .These markers ranged in size from 60 bp to 460 bp (Table 4). The total number of unique markers per accession ranged from 1 to 11. The highest number was exhibited by DT 1 and DT 2 while the lower number of unique marker was revealed by El-Awama, AT 1, AT 25, AT 6 and AT 3. Figure 1 shows the salient AFLP fingerprints of the investigated lines and landraces using primer combination (5/3).

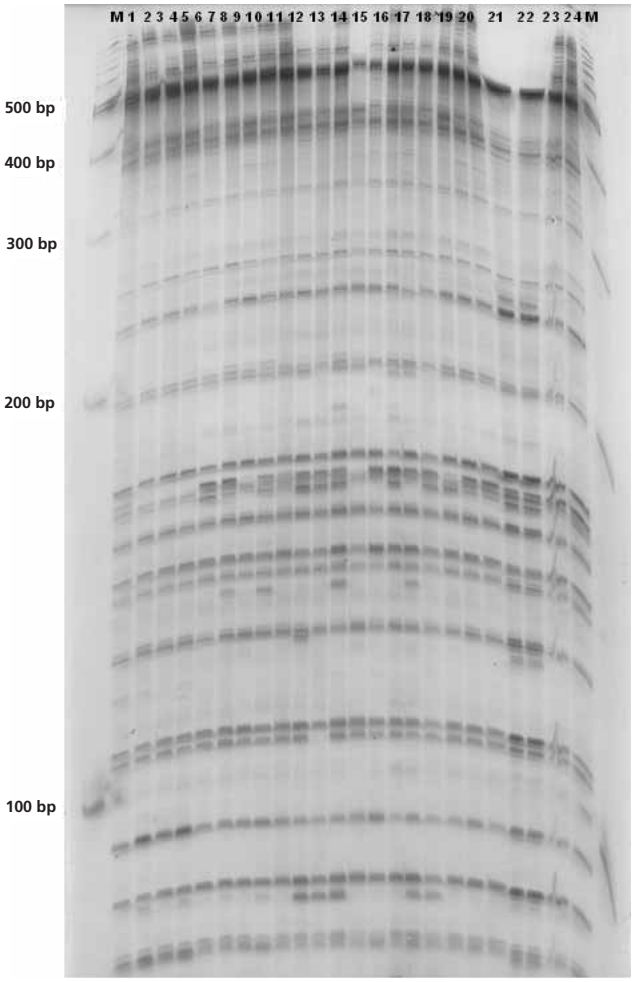

Figure. 1:

AFLP profiles of the twenty four barley landraces and lines, generated using primer combination 5/3

3.4 Genetic relationships among investigated lines and landraces

The developed AFLP-based dendrogram (Figure 2) grouped the 24 barley accessions into two main clusters. The first cluster included all native landraces collected from Sinai and Marsa Matrouh regions, i.e. El-Arish (1), El-Sheikh Zowaid (2), Rafah (3), Awlad Ali(4), El-Kasr (5) and El-Awama (6) in addition to AT 1 (7) and AT 58 (8). The second cluster comprised two subculsters. The first subculster contained 7 lines namely, DT 1 (23), DT 2 (24), AT 20 (22), AT 15 (21), AT 29 (20) and AT 5 (19). The second sub cluster contained the rest of investigated accessions (9 lines). The least average differences (the highest genetic similarity) was scored between El-Sheikh Zowaid and Rafah landraces (0.97), meanwhile, the lowest genetic similarity was detected between DT 2 (24) and Awlad Ali (0.87). The developed AFLP-based dendrogram indicated clearly that all barley landraces collected from Sinai showed highest similarity

Table 4:
Discriminative AFLP markers could distinguish among the 24 Egyptian barley accessions

Marker (bp) Accessions		E-AAC/ M-CTA (1-5)	E-ACC/ M-CAC (4-2)	E-AGG/ M-CAT (5-4)	E-AAG/ M-CTA (2-5)	E-AAC/ M-CTA (3-3)	E-ACA/ M-CAG (3-7)	E-ACT/ M-CAG (6-3)	E-ACT/ M-CTG (6-7)	E-ACT/ M-CTT (6-8)	E-AGG/ M-CAG (7-3)	E-AGG/ M-CTA (7-5)	E-AGG/ M-CAC (8-2)	E-AGG/ M-CAG (8-3)
1	Al-Arish	91							100	245	97, 98, 110, 143, 146, 305, 360			
2	El-Sheikh Zowaid					136, 138						97, 98, 110, 305, 360		
3	Rafah									348	220, 245	110, 184, 305, 360		73, 76, 134
4	Awlad Ali		76								245	110, 143, 184, 305, 360	257	
5	El Kasr									100	220, 245	378		
6	El Awama											82		
7	AT 1									348		82		
8	AT 58								218, 220	177		95, 96	149, 150	
9	AT 40			65, 72, 75					243				161, 163	134
10	AT 42	233		72, 75				139						
11	AT 10													
12	AT 11		380, -400, 460				314			93		82		
13	AT 25			186			314		243					
14	AT 26		-76, 380, 400, 460	- 65, 75			314					96		
15	AT 4				345		405		268, 380					
16	AT 6				345						440		257	
17	AT 3		238		145		405							
18	AT 22			223									100, 103	
19	AT 5	100	60, 91							124		97, 98		
20	AT 29		91				283	139	188				103, 111, 114, 149, 150	193
21	AT 15					136, 138		110		135		82	65	
22	AT 20					108, 110, 136, 138		110, 158, 160		85, 93				
23	DT 1	100, 179				108, 110, 136, 138		110, 158, 160		85, 93				
24	DT 2					108, 110, 136, 138		110, 158, 160		93, 124, 135			65	

amongthem. The same observation was true for landraces collected from Marsa Matrouh and Agricultural Research Center (ARC). The two lines AT 1 (7) and AT 58 (8) belonging to ARC were grouped in cluster 1 with native landraces of Marsa Matrouh. Also, it was observed that some lines such as DT 1 (23) and DT 2 (24), previously known as tolerant and sensitive to salt stress, respectively are grouped together in one subcluster. Similarly the two lines AT 42 (10) and AT 40 (9) (susceptible to rusts) were grouped together.

3.5 Correlation between molecular analysis and biological evaluation

When the results of the molecular analysis are considered with respect to the resistance of different landraces to net blotch and leaf rust, one can notice from data in Table 1 and Figure 2, that Rafah and Awlad Ali (extremely resistant landraces) were grouped together in cluster 1 and the second two extremely resistant lines (DT 1 (23)

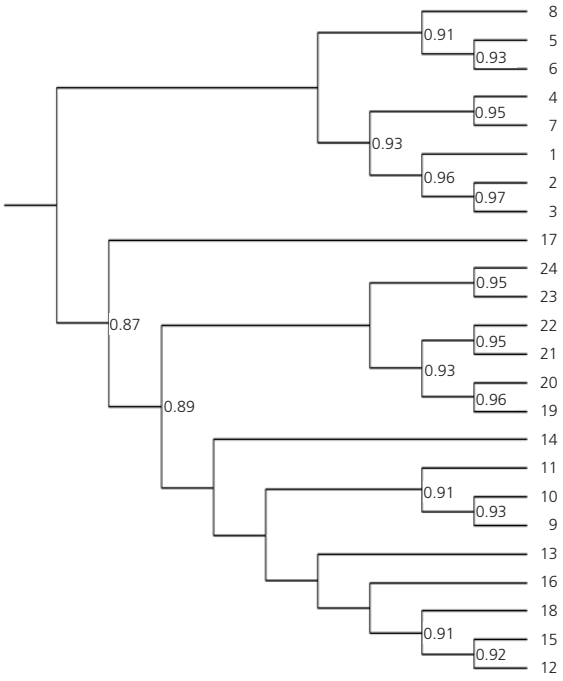

Figure 2:

AFLP -based dendrogram of the twenty four Egyptian barley landraces and lines

Table 5:

Distribution of selected polymorphic AFLP bands between leaf rust resistant and susceptible barley lines

Primer comb. code	Resistant		Susceptible		Size of polymorphic bands
	AT 1	AT 58	AT 40	AT 42	
1 / 5	-	-	+	+	70, 71
2 / 5	-	-	+	+	84, 128 , 132, 165
2 / 7	+	+	-	-	142
3 / 7	-	-	+	+	146
3 / 3	-	-	+	+	128, 129, 130
4 / 2	-	-	+	+	270
4 / 5	+	+	-	-	189
5 / 3	+	+	-	-	125
5 / 7	+	+	-	-	79, 80, 200
	-	-	+	+	440
6 / 7	-	-	+	+	140
6 / 8	+	+	-	-	273
	-	-	+	+	178
6 / 3	-	-	+	+	122, 168, 170
	+	+	-	-	140
7 / 8	+	+	-	-	93
8 / 2	+	+	-	-	119, 120, 155, 156
8 / 3	+	+	-	-	235
8 / 6	-	-	+	+	174
8 / 7	+	+	-	-	273
	-	-	+	+	178
5 / 4	-	-	+	+	72, 75

Table 6:

Distribution of selected polymorphic AFLP bands between net blocth resistant and susceptible barley lines

Primer comb. code	Resistant		Susceptible		Size of polymorphic bands
	AT 4	AT 6	AT 3	AT 22	
1 / 8	+	+	-	-	60, 75, 107, 108, 110, 127, 128, 129, 131, 189
2 / 5	+	+	-	-	128, 132, 167, 265, 310, 345
2 / 7	+	+	-	-	80
2 / 8	-	-	+	+	315
3 / 3	-	-	+	+	350
3 / 7	-	-	+	+	190
4 / 5	-	-	+	+	174, 175, 185, 215, 310, 330, 340
4 / 7	+	+	-	-	120, 152, 209
5 / 7	-	-	+	+	122
5 / 3	+	+	-	-	79, 375
6 / 3	-	-	+	+	129, 133, 140, 300 340, 430, 180, 182
6 / 7	-	-	+	+	275, 285, 340, 345, 350, 365, 370, 420, 425, 430, 433, 480, 490
6 / 8	-	-	+	+	83, 184, 240
7 / 3	-	-	+	+	145, 155, 172, 175
7 / 5	-	-	+	+	185, 187, 200, 700, 900, 1000
7 / 8	-	-	+	+	325, 375
8 / 2	+	+	-	-	158, 159, 160, 165, 167, 170, 290, 292
8 / 3	-	-	+	+	120, 122, 123, 142, 250, 262
8 / 6	-	-	+	+	490
8 / 7	-	-	+	+	170, 185

and AT 5 (19)) were clustered together in subculster 1 belonging to cluster 2. The two lines grouped together with native landraces in cluster 1 were also resistant to leaf rust. These observations encouraged us to analyze the results of molecular analysis of crosses (lines) developed from the same parents and previously identified as resistant (R) and susceptible (S) to leaf rust and net blotch in the light of resistance and susceptibility, and absence and presence of unique markers (Table 5 and 6). Because each two groups of plants subjected to comparison having the genetic make up except resistance and susceptibility, so somebody has to expect some degree of linkage between markers and resistance or susceptibility and some of the mentioned markers herein can be useful for future mapping of resistance genes.

4 Discussion

Net blotch (*Pyrenophora teres*) and leaf rust (*Puccinia hordei*) are serious diseases of great negative impact on barley and cereals production in Egypt. Therefore, biological and molecular evaluation of any new native barley germplasm is vital to enrich our knowledge on the abilities of existing germplasms and enabling us to predict hybrid performance, select parents for crossing in crop improvement programmes, and clone new natural plant resistance genes (Saker, 2005 a and b Saker et al., 2005). The most interesting and striking output of the biological evaluation part of this study is the resistance of some line and landraces to net blotch and leaf rust. Also, the level of resistance is varied from extreme resistance to high and moderate resistance. This conclusion was reported by other investigators in barley against leaf rust (Mamadov et al., 2003) and net blotch (Weiland et al., 1999 and Raman et al., 2003).

Based on this observation, it can postulated that such lines and landraces have a genetic make up enabling them to resist different types of fungal diseases. It is well-known that invasion of plant cells by one pathogen stimulate the expression of a set of resistance genes involved in resistance. This postulation is quite acceptable because some of susceptible lines are susceptible to net blotch and leaf rust also. The variations in the level of resistance recorded herein confirm the involvement of more than one gene in net blotch and leaf rust resistance (Ho et al., 1996; Steffenson et al., 1996).

Regarding the AFLP analysis, the percentage of polymorphism (4.8 %) detected in the present study is very low compared with previously published AFLP analysis of barley, which ranged from 21 % (Saker, 2005a) to 61.3 % (Maheswaran et al., 1997) and 77 % (Saker et al., 2005). This lowest polymorphism is due to the narrower genetic pools of different genotypes and landraces under investigation, especially 18 lines out of the 24 landraces were developed from common ancestors. The rest of landraces were grown by Bedouins in completely isolated area in Sinai and Marsa Matrouh, so high degree of genetic similarity within the group could be expected. However, the lowest polymorphism may also be attributed to the used primer combinations and/or scoring method followed in this study, only reproducible and distinct bands were scored, meanwhile weak and ambitious bands were excluded (Vos et al., 1995). In this context, Russel et al. (1997) reported high genetic similarity (0.924) within the spring types of barley.

The clustering patterns of AFLP-based dendrograms of the investigated landraces agree with the origin of different groups of lines and landraces depending on its pedigree and resistance traits. Therefore, the use of AFLP analysis in barley is highly recommended to reveal genetic diversity in genetically close genotype and landraces (Saker et al., 2006). In this context, Saker et al. (2005a) indicated that the Egyptian barley genotypes have probably originated from closely related ancestors and possess high degree of genetic similarity. Also they concluded that AFLP can be applied to differentiate closely related genotypes of the same origin. The same conclusion is derived from the RFLP data of Clegg and Brown (1984) who concluded that variability is lower in cultivated barley than in wild barley, possibly as a result of domestication.

It could be concluded that new promising resources for resistance to net blotch and leaf rust were recorded in Egyptian barley germplasm. The outcome of this study indicated that molecular analysis combined with biological evaluation has proved to be a promising strategy in the selection of disease resistant germplasm, as previously reported by Haley et al. (1993). Extensive investigations on pest resistance genes in barley have led to the identification of about 107 resistance genes (Chelkowski et al., 2003).

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