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RAPD - MARKERS FOR YIELD ABILITY AND DISEASE RESISTANCE IN BARLEY UNDER RAINFED CONDITIONS

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Fifteen genotypes comprised one exotic, two local check varieties and twelve newly bred lines produced through the Desert Research Center (DRC) barley breeding program were grown during two successive seasons ended in 2003/2004 at Maryout experimental farm of DRC. The aim of this study is to obtain reliable molecular markers for drought tolerance and disease resistance in such genotypes.

In the second season, grain yield/plant for the hulled grains type ranged from 3.71g for Giza 126 (G126) to 6.18g for line3. Meanwhile, H_{10} was the best yielded (7.08 g) and Giza 131 gave the relatively lowest grain yield/plant (2.69g) among the hulles grains genotypes under tested rainfall conditions. Also the highest grain yielding ability (newly bred hulles grains line, H_{10}) showed highly resistance for both parameters of net blotch disease (D.S and D.I).

In respect to disease occurrence, genotypes P_4 and G126 were moderately resistant to net blotch disease where disease severity recorded 50 % and 43.3 %, respectively. Whereas, lines 26, Mar19A, 8, and H_{10} were resistant.

RAPD banding pattern for the five primers were scored seven negative molecular markers which were exhibited in the relatively sensitive barley variety G126 and ten positive molecular markers which appeared in the tolerant line3.

On the other hand, lines 8 and 25 (the two high yielding hulled newly bred lines) seemed to be a drought tolerant genotypes as it had a positive molecular markers

(820 bp) for operon primer O_4 and 937 bp for operon primer O_{10}. Also, line 25 had a positive molecular marker of operon primer Z_4 (3000 bp) for high yielding ability under tested rainfall conditions

The negative markers can be used as indicators to discard the newly bred line 24 as it designed in the lowest yielding barley group and had a negative marker (1032 bp) for O10 operon primer.

The two Z operon primers differentiate hulles grains barley genotypes by five positive markers for drought tolerance and net blotch disease resistance. Interestingly, many bands were shown to appear in the tolerant hulles barley lines (H_2, H_6, and Mar19A). Also, similar bands were present in Z_4 and Z_7 operon primers. For this reason, results of both positive and negative molecular size (bp) against different primers indicated that RAPD markers are dominant.

Keywords: barley, drought stress, rainfall conditions, molecular markers, similarity, RAPD.

In Egypt, expansion of barley area in newly reclaimed lands faces the problem of drought stress because of the limited moisture from rainfall or the availability of only brackish water at salt affected soils in other areas. Thus, it has become necessary to develop newly bred lines more adapted to such stress conditions. Pedigree selection was performed through segregating generations based on hulled and hulles grains of barley families derived from selected crosses (DRC barley breeding program).

Net blotch, caused by the fungus *Pyrenophora teres*, is a common disease of barley (*Hordeum vulgare* L.; Steffenson, 1997). Resistance genes for net blotch have proven to be difficult to map due to complex genetic interactions affecting resistance. There are at least three (Graner *et al.*, 1996) or four major genes (Kahn and Boyd, 1982; Steffenson, 1997), as well as minor genes (Arabi *et al.*, 1990). Kahn and Boyed (1982) reported strong genotype-based sensitivity to environmental modification of resistance and susceptibility. (Legge and Tekauz, 1993) have shown that isolates of *P. teres* have very complicated genetic interaction that differs from one population to another.

Molecular tags for resistance genes to this disease would be extremely beneficial for breeders because they could use such tags during the preliminary selection process to track resistance in existing populations or to pyramid resistance into new populations. Markers could be used as diagnostic tools in preference to inoculating the plant with the pathogen, and would negate the need for quarantine facilities. Having molecular markers

for the resistance genes also may lead to a better understanding of the disease and its molecular mechanisms, and may eventually facilitate the isolation of the resistance genes.

Nowadays, polymerase chain reaction (PCR) based molecular markers has developed into controllable tools to analyze genetic relationships and genetic diversity using random amplified polymorphic DNA (RAPD) by Williams *et al.*(1990), Tinker *et al.* (1993), Gonzaler and Ferrer(1993) which is one of such tools. So, RAPD analysis has been used for *Brassica* (Demeke *et al.*, 1992), *Oryza* (Yu and Nguyen, 1994; Mackill, 1995), *Triticum* (Vierling and Nguyen, 1992; Chandrashekhar and Nguyen, 1992; Abdel-Tawab *et al.*, 2003) and cotton (El-Kady *et al.*, 2006). Moreover, RAPD analysis has been used earlier for genetic diversity analysis for Egyptian barley cultivars (Abdelsalam *et al.*, 1998). At the same time, technical simplicity and speed of RAPD methodology is a principal advantage (Gepts, 1993).

Maker- assisted selection (MAS) has been becoming the method of choice in facilitating tagging of the desirable traits in many crops (Abdel-Tawab *et al.*, 1997). It was evident that drought tolerance is a complex trait which is greatly affected by the environmental factors. However, Marker-assisted selection could enhance the identification of barley genotypes tolerant to drought stress. This approach would enable the molecular plant breeders to grasp the promising genotypes with more confidence in their merits as selection will be based on genetic rather than phenotypic basis with the elimination of the confounding effects of environmental factors. Moreover, this process is fast, reliable and cost effective which can reduce the required time for barley breeding program to half.

This investigation based on the study of genotypic performance of the tested varieties / lines under stress conditions and comparing them with genetic distance estimated from RAPD markers. The aim of this study is extended to find the relationships between some yield-related traits and drought tolerance in fifteen barley genotypes. Also to obtain reliable molecular markers for drought tolerance and disease resistance that can be used in breeding programs.

MATERIALS AND METHODS

Plant Materials

Fifteen genotypes comprised one exotic, two local check varieties and twelve newly bred lines produced through the Desert Research Center (DRC) barley breeding program were grown at Maryout experimental farm of DRC. These genetic materials descended from high yielded and good combiners of local and exotic parents i.e. G126 and $ICNBF_8$ 654Sel, 5AP, respectively (Afiah and Zaki, 2001). Name, origin, pedigree and/ or selection

history of all varieties or newly bred lines are presented in table (1). Soil of the experimental site characterized as loamy clay, slightly saline (ECe 3.3 dSm^{-1}), calcareous (33.4%$CaCO_3$) and 0.6% organic matter. For not late sowing date, supplementary irrigation by agricultural drainage water (EC about 4.6 dSm^{-1}), is given at sowing date (12 Nov. and 16 Nov., in the 1st and 2nd seasons, respectively). The meteorological data of total seasonal rainfall and its distribution during growth stages for the two growing seasons at Maryout Research Station are presented in table (2).

TABLE (1). Pedigree and classification of barley varieties/ lines under investigation.

Genotype	Origin	Caryopsis type	Pedigree and/or selection history
Line 3	Egypt	Hulled	G126/(ICB 82-1451-8AP-OAP-9AB-0TR) $F_6$3Sel,Mar.
Line 8	Egypt	Hulled	G126/(ICB 82-1451-8AP-OAP-9AB-0TR) $F_6$8Sel,Mar.
Line 10	Egypt	Hulled	G126/(Arar//2762/BC-2L-2Y-ICB 83-0687-7AP-0AP) $F_6$10Sel,Mar.
Line 17	Egypt	Hulled	G126/(Arar//2762/BC-2L-2Y ICB 83-0687-7AP-0AP) $F_6$17Sel,Mar.
Line 24	Egypt	Hulled	G126/(Arar//2762/BC-2L-2Y ICB 83-0687-7AP-0AP) $F_6$24Sel,Mar.
Line 25	Egypt	Hulled	G126/(Arar//2762/BC-2L-2Y ICB 83-0687-7AP-0AP) $F_6$25Sel,Mar.
Line 26	Egypt	Hulled	G126/(Arar//2762/BC-2L-2Y-ICB 83-0687-7AP-0AP-1AP) $F_6$26Sel,Mar.
G126	Egypt	Hulled	Baladi Bahteem"/ "SD 729-Por12762-BC
H_2	Egypt	Hulles	G126/(ICNB F_8 - 654 Sel, 5AP) F_6 H_2 Sel, Mar
H_6	Egypt	Hulles	G126/(ICNB F_8 - 654 Sel, 5AP) F_6 H_6 Sel, Mar.
H_7	Egypt	Hulles	G126/(ICNB F_8 - 654 Sel, 5AP) F_6 H_7 Sel, Mar.
H_{10}	Egypt	Hulles	G126/(ICNB F_8 - 654 Sel, 5AP) F_6 H_{10} Sel, Mar.
MAR 19 A	Egypt	Hulles	(ICNBF_8-596Sel,3AP)/Assala#
P_4	ICARDA*	Hulles	ICNB F_8 - 654 Sel, 5AP
Giza131	Egypt	Hulles	CM67-B/CENTENO//CAM-B/3/ROW906 73/4/GLORIA-EAR/COME-B/5/FALCON-BAR/6/LINO

* ICARDA: International Center for Agricultural Research in the Dry Areas.
\# Assala = Harma-03/Beecher

Each experiment was laid out in randomized complete block design with three replicates. Plot size was 4x5 m. barley grains were broadcasted at the rate of 40 kg/fed. The experiments were bordered by more susceptible varieties to net blotch disease. All agricultural practices were followed as recommended for rainfed areas. During harvest, data were recorded for a random samples of ten guarded plants in each plot for nine traits i.e., Plant height (cm), No. of spikes/plant, spike length(cm) , No. of spikelets, No. of grains/spike, 1000- grain weight (g), Grain yield/plant (g), disease severity(%) and disease incidence. Severity of net blotch infection was

recorded at booting stage on plot bases, disease incidence (severity of infection) was estimated as percentage from 0 to 100 where: ≤ 30 resistant (R), 30.1-50 moderately resistant (MR), 50.1-70 moderately susceptible (MS) and ≥ 70 highly susceptible (S) as described by Tekauz(1985).

TABLE (2). Monthly mean rainfall (mm) at Maryout Research Station.

Month	2002/2003	2003/2004
October	4.31	0.25
November	2.03	4.52
December	69.60	41.91
January	38.10	70.86
February	83.80	56.64
March	40.64	2.03
April	0.25	0.25
Total	238.73	176.46

Source: Meteorological Desert Research Center Lab.

Statistical analysis was performed as outlined by Gomez and Gomez (1984). Comparison between means of all traits studied among genotypes was made using new LSD (Duncan, 1955).

DNA extraction

From the field experimental site, young leaves of each genotype were collected from ten plants randomly and then one gram of ten leaves sample was treated with liquid nitrogen and transferred to El-Sheikh Zewayed Biotechnology Lab. of DRC for DNA extraction according to the method of Welsh and Mcleland (1990).

DNA amplification

Protocol for PCR - RAPD reaction was concluded using (10- base) oligonucleotide primers (Operon Technologies Inc., U.S.A) according to Williams *et al.* (1990) in the following sequences:

The PCR amplification conditions were as follows:

Initial extended step of denaturation at 94 °C / 3 min (1 cycle) followed by 94°C/1min. (40 cycle) , primer annealing at 36 °C / 1min and elongation at 72 °C / 1 min (1 cycle) .The 40 cycle was followed by an extended primer extension step at 72 °C / 4 min. Then all completed reactions were held at 4 °C until electrophoresis.

Amplified products were analyzed by electrophoresis in 1.5 % agarose gels with 0.5 x TBE buffer and detected by staining with ethidium bromide solution for 45 min. Gels were photographed under UV light and analyzed by gel documentation system. Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0, respectively.

TABLE (3). List of operon primers and their nucleotide sequence.

Description	Primer sequence 5\ ——► 3\
OP O_2	5\-ACGTAGCGTC-3\
OP O_4	5\-AAGTCCGCTC-3\
OP O_{10}	5\-TCAGAGCGCC-3\
OP Z_4	5\-AGGCTGTGCT-3\
OP Z_7	5\-CCAGGAGGAC-3\

Each reaction mixture (25 µl total volumes) for PCR amplification consisted of:

** 10x reaction buffer with 15mM $MgCl_2$ 2.5 µl
** dNTPs (8 mM) 1.0 µl
** Template DNA (10-50 ng/µl) 0.2 µl
** Taq DNA polymerase (5 U/µl) 0.2µl
** Primer (10 mM) 1.0 µl
** H_2O (dd) 19.3 µl

RESULTS AND DISCUSSION

This study could be divided into two experiments. The first one as field experiment to evaluate 15 barley genotypes for net blotch disease resistance under limited rainfall conditions compared to the recommended variety for rainfed conditions (G126) as well as the newly released cultivar, Giza131 and the ICARDA's hulles parent $ICNBF_8$654Sel,5AP. The second experiment was the molecular study to detect some molecular markers associated with drought stress tolerance.

Field Experiment

The fifteen barley genotypes were planted and irrigated with drainage water (2950 ppm approximately) at Maryout Experimental Research Station of Desert Research Center. The layout was performed in a randomized complete blocks design to detect the most tolerant and sensitive genotypes under drought conditions. Observations were taken (after 21 days from sowing) for disease resistance.

Comparisons between the means of the two types of barley genotypes (hulled and hulles grains) regarding each trait indicated marked differences. Tables (4-a and b) represent mean performance of all traits recorded for hulled and hulles barley genotypes. In the 2nd season grain yield/plant for the hulled grains type ranged from 3.71(g) for G 126 to 6.18 (g) for line3 (Table 4-b). Meanwhile, line H_{10} was the best yielded (7.08 g) and Giza 131 gave the lowest grain yield/plant (2.69 g) among the hulles grains genotypes under Maryout rainfed conditions.

In respect to disease severity data presented in table (4-b) clearly indicate that genotypes P_4 and G126 in the 2nd season were moderately resistant to net blotch disease, it recorded 50 % and 43.3 %, respectively. Whereas, lines 26, Mar19A, 8 and H_{10} were resistant.

Diseases severity was not correlated to the incidence of disease, where most genotypes which recorded different degree of susceptibility to the disease didn't have the same degree of resistance except the genotypes, line 26 and variety G 126.

It is worthy to note that the highest grain yielding ability (newly bred hulles barley line H_{10} showed high resistance for both parameters of net blotch disease, D.S and D.I (Tables 4-a and b). These results partially agreed with those of Afiah and Moselhy (2001). Therefore, the selected lines of low and high yield were used as assisted genotypes to obtain molecular markers associated with drought tolerance by using PCR- RAPD technique.

TABLE (4-a). Mean performance of all traits recorded during the first season (2002/2003).

Genotype	Plant height (cm)	No. of spikes/plant	Spike length(cm)	No. of spiklets	No. of grains/spike	1000-grain weight (g)	Grain yield/plant (g)	D.S%	D.I%
Line 3	54g	5.33ab	6.5 ef	23.67 a-c	31.3 d	48 37 c	6.77 cd	28.33 e	46.67 b
Line 8	54.5g	4 67a-c	7 5 b-e	21 67 c	34.3 bc	42.83 d	6 63 de	10 i	58.33 a
Line 10	73c	5.67 a	6.83 d-f	25 a-c	30.3 d	41.6 d	7 17 cd	20 g	11 67 h
Line 17	58 67f	4.33 b-d	7 c-f	23.67 a-c	32 c-e	41.2 d	5 1 fg	8 i	11.67 h
Line 24	81.33a	4 33 b-d	8.17 a-e	23 bc	22.7 f	43.33 d	5 67 ef	30 e	23.33 e-g
Line 25	58 67f	4 33 b-d	8 67 a-c	23.67 a-c	45.33 a	43.27 d	6.9 cd	60 a	23 33 e-g
Line 26	62.5e	4.67 a-c	9.33 a	25.67 ab	33 cd	51.63 a	7.3 b-d	25 f	21.67 f-g
G 126	51.5g	3.67cd	8 17 a-e	21 67 c	29.7 e	30.97 f	4.13 gh	50 b	26 67 d-f
H_2	76.33b	5.67 a	8.5 a-d	23 bc	34.7 bc	34.87 e	5.33 f	30 e	21.67 fg
H_6	62.17e	4 33 b-d	8.83 ab	25.67 ab	31 d	51.13 bc	7.33 ab	20g	20 g
H_7	69.67d	4.67 a-c	8.83 ab	22.33 bc	39 b	48.13 c	7 cd	60 a	31.67 d
H_{10}	62.5e	4.67 a-c	9.17 ab	27 a	36 33 b	47 84 c	8.37 a	15 h	11.67 h
MAR 19 A	59 67e	5 ab	7.5 b-c	21.67 c	37.5 b	43.97 d	7.8 a-c	25 f	28.33 de
P4	70cd	4.23 b-d	4.67 gh	16 d	31 d	30 7 f	4.14 h	43.33 c	40 c
Giza 131	72cd	3.33 d	4 h	16.33 d	24.9 e	35.47 e	3.4 h	36 67 d	40 c
Grand mean	64 43	4.59	7.58	22.67	30.87	42.35	6 2	30.76	27.78

Means within columns followed by the same letter(s) are not significantly different by Duncan's Multiple Range test ($P \leq 0.05$)

D.S: Diseases Severity %

D.I: Disease incidence %

TABLE (4-b). Mean performance of all traits recorded during the second season (2003/2004).

Genotype	Plant height (cm)	No. of spikes/plant	Spike length(cm)	No. of spiklets	No. of grains/spike	1000-grain weight (g)	Grain yield/plant (g)	D.S%	D.I%
Line 3	51.33g	4.39a-c	6cd	22fg	29 e	38.22 e	6.18 bc	30 e	28.33 d
Line 8	52.23g	4.07 b-d	7bc	21.33g	34.33 ab	36.34 f	5.89 cd	10 g	50 a
Line 10	70.67d	5 01 a	8.83 a	30.67 a	32.33 c	37.37 ef	5.53 d	20 f	11.67 f
Line 17	48.5h	3.76 cd	6.67bc	21g	25.3 f	38.49 e	4.36 e	50 c	11.67 f
Line 24	78b	4.07 b-d	9 a	23.67 d-f	25.3 f	49.57 a	4.68 e	25 ef	21.67 e
Line 25	61f	4.7 ab	9.07 a	25 b-d	21.7 g	40.92 d	5.81 cd	70 a	28.33 d
Line 26	63.67e	4.39 a-c	9 a	25.67bc	31.7 cd	37.04 ef	6.06 cd	30 e	28 33 d
G 126	50.5g	3.45 d	7.5 b	23.33d-f	31.7 cd	36.34 f	3.71 f	43.33 c	11.67 f
H_2	71d	4.7 ab	8.83 a	24.33 c-e	34.33 ab	30.51 h	4.56 e	30 e	21.67 e
H_6	60.33e	4.39 a-c	8.77 a	23 ef	32.67 bc	46.5 b	6.12 bc	40 d	20 e
H_7	70d	4.7 ab	8 77 a	26.33 b	34.3 ab	41.31 d	6 8 ab	60 b	35 c
H_{10}	63 33e	4.7 ab	9 a	24.33 c-e	35.33 a	41.5 d	7.08 a	10 g	11 67 f
MAR 19 A	60 f	4.39 a-c	7.33 b	21 g	36.64 a	40.43 d	6.21 bc	25 ef	25 de
P4	70.67d	4.07 b-d	4.33 f	17 h	31.33 cd	28.3 i	3.4 fg	50 c	45 b
Giza 131	73.67c	3.76 cd	4.67 ef	17 h	22 g	32.91 g	2.69 g	40 d	38.33 c
Grand mean	62.99	4.31	7.65	23.04	30.53	38.38	5.27	35.56	25.89

Means within columns followed by the same letter(s) are not significantly different by Duncan's Multiple Range test ($P \leq 0.05$)
D.S: Diseases Severity %
D.I: Disease incidence %

Molecular Markers for Drought Tolerance

RAPD molecular markers

In this regard, RAPD markers were developed for drought tolerance in the tested genotypes. DNA isolated from each of the two contrasting genotypes; G126 and line 3 (hulled), Giza 131 and H_2 (hulles) was compared to all other local and exotic lines. These genotypes were tested against 12 operon primers; five 10-mer random primers i.e.; (O_2, O_4, O_{10}, Z_4 and Z_7) of them were informative as shown in tables (5-a and b), and figure (1). The total numbers of bands exhibited by each of the five PCR reactions were 6, 7,6,4,6 and 5,11,11,7,6 bands, for hulled and hulles grains, respectively. Banding pattern for the five primers were scored as present (1) or absent (0).

Table (6) illustrates unique bands generated by the five operon primers used which identified 13 out of the 15 barley genotypes tested. Op, Z_4 differentiate G126 by the unique band of 3917 bp molecular size.

Table (7a) shows seven negative molecular markers which were exhibited in the relatively sensitive barley variety (G126) and ten positive molecular markers which appeared in the tolerant line3. It is note-worthy that, No.2 and No.6 (the two high yielding hulled newly bred lines, 8 and 25) seemed to be

a drought tolerant genotypes as it had a positive molecular markers (820 bp) for operon primer O_4 and 937 bp for operon primer O_{10}. Also, line 25 had a positive molecular marker of operon primer Z_4 (3000 bp) for high yielding ability under Maryout rainfed conditions (Tables 5-a and b).

TABLE (5-a). Molecular size in bp of the amplified polymorphic (non-unique) DNA bands generated by 5 DNA random primers (O_2, O_4, O_{10}, Z_4 and Z_7) used for identifying the 8 hulled barley genotypes.

Genotype	O_2	O_4	O_{10}	Z_4	Z_7
Line 3	2000-1873-1307	1951-1076-820	1483-1170-937	3000-1242-529	3000-1859-1230-1000-703
Line 8	1873-1791-1000	1880-1076-820	1170-937	2885-1242-529	3000-1859-1230-1000-703
Line 10	1933-1873-1000	1951-1129-820	1170-937	1242-529	3000-1859-1230-1000-703
Line 17	1933-1307-1000	1951-1129-820	1483-1170-937	2885-1242-529	3000-1859-1230-1000-703
Line 24	1873-1000	2028-1129-820	2020-1483-1170-1032-937	3000-1242-529	3000-1859-1230-1000-703
Line 25	2000-1873-1000	2028-1129-820	2020-1170-937	3000-1242	3000-1859-1230-1000-703
Line 26	1873-1791-1000	2144-1129	2020-1483-1170-985	1242	3287-1859-1230-1000-703
G 126	2000-1873-1000	2144-1129	1483-1170-1032-985	1242	3000-1859-1230-1000-703
No. of polymorphic bands	6	7	6	4	6

TABLE (5-b). Molecular size in bp of the amplified polymorphic (non-unique) DNA bands generated by 5 DNA random primers (O_2, O_4, O_{10}, Z_4 and Z_7) used for identifying the 7 hulles barley genotypes.

Genotype	O_2	O_4	O_{10}	Z_4	Z_7
H_2	2000-1138	1880-1650-763-740-631-500-473	1909-1699-1558-1335-937	3140-1959-1290	3132-2036-1301
H_6	1030	2638-1650-763-740-500-473	1909-1862-1699-1558-1335-937	3140-1959-1290-575	3287-2036-1301
H_7	1933-1873-1030	2307-1650-763-740-500-473	1862-1699-1558-1288-937-785-695-548-500	3140-1879-1290-575	3132-2036-1301-836
H_{10}	1933-1030	2638-763-740-500-473	1862-1558-1335-937-695-548-500	1879-1290-575	3287-2036-1301-836
MAR19A	1030	2307-1650-846-763-740-500-473	1862-1558-1288-937-785-695-548-500	2956-1879-1290-575	3379-2036-1301-836
P4	1873-1030	2144-1650-846-763-740-631-500-473	1699-1335-937-785-695-548-500	2956-1959-1290	3379-2036-1301
Giza 131	1933-1138-1030	2307-1650-846-763-740-631-500-473	1699-1558-1335-937-695-548-500	392-1290	2036-1301
No. of polymorphic bands	5	11	11	6	6

TABLE (6). Molecular size in bp of the amplified polymorphic (unique) DNA bands generated by 5 DNA random primers (O_2, O_4, O_{10}, Z_4 and Z_7) used for identifying the 15 barley genotypes.

Genotype	O_2	O_4	O_{10}	Z_4	Z_7
Line 3	1105	-	-	-	2326-1563
Line 8	-	-	-	-	897
Line 10	-	-	1245	2713	-
Line 17	-	-	-	-	-
Line 24	-	1566	-	-	-
Line 25	-	1679	-	-	2453
Line 26	-	-	872	3813	-
G 126	-	-	-	3917	-
H_2	2159-1511	-	-	-	-
H_6	2089	-	-	-	-
H_7	-	-	-	-	-
H_{10}	-	-	-	3092	2169
MAR 19 A	2228	-	-	-	-
P4	2311	-	-	-	-
Giza 131	-	1186	-	-	3394

The negative markers can be used as indicators to discard the newly bred line 24 as it designed in the lowest yielding barley group and had a negative marker (1032 bp) for Z_{10} operon primer. The two Z operon primers differentiate hulles grains barley genotypes by five positive markers for drought tolerance and net blotch disease resistance as shown in table (7-b).

Interestingly, many bands were shown to appear in the tolerant hulles barley genotypes (Lines H_2, H_6 and MAR19A) Also, fig. (2) indicated the presence of similar bands in Z_4 and Z_7 operon primers. Hence, results of both positive and negative molecular sizes (Table 7-b) against different primers indicated that RAPD markers are dominant.

There is no way to make linkage between these RAPD markers and drought-tolerance or disease resistance genes. In other words, they can be successfully used as RAPD markers for the best genotypes but not for the stress-tolerance gene(s). Many investigators developed markers for different characters, e.g. Terzi (1997), Baum *et al.* (1998), and Strelchenko *et al.* (1999).

The most reliable productive primers from a total five used primers after repeating the PCR two times to insure the data were explained in table (8).

The highest percentages of polymorphic bands among all tested

genotypes were O_4, O_{10} and Z_7 primers. Based on RAPD markers polymorphism, similarity matrix was developed by SPSS version-10 computer package system. Distinct RAPD bands were scored, while weak products were ignored. Similarity values used were calculated between pairs of genotypes based on simple matching coefficient (Table 9).

As showen in the dendrogram (Fig.2), the studied fifteen barley genotypes were divided into two main groups. The first group comprises the eight hulled genotypes while the second one includes all hulles genotypes. The first group was further divided into five subgroups. The closest relationship was scored among line 10 and line17.

Moreover, the dendrogram illustrated in fig. (2) based on RAPD analysis successive in separating the 15 barley genotypes into two main clusters that separated the two types of barley caryopsis (hulled and hulles grains). This cluster further separated into two sub-clusters, within the first sub-cluster of hulles grains type, the two biotic and abiotic stress tolerant newly bred lines H_7 and Mar19A were grouped together. These genotypes share the same genetic background (Table 1).

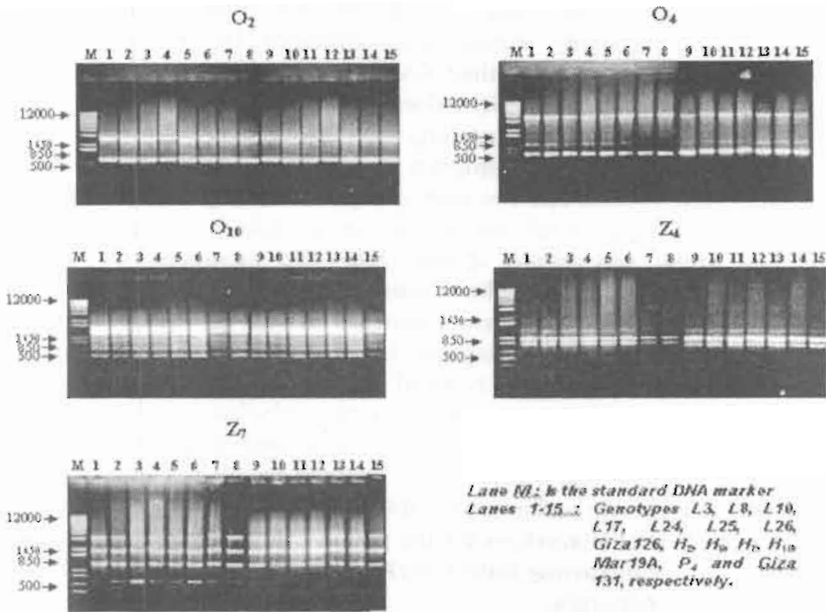
TABLE (7-a). RAPD markers for the polymorphic primers with the two contrasting hulled barley genotypes in regard to drought tolerance.

Operon primer	Marker/primer	Positive M.S.(bp)	Negative M.S.(bp)
O_2	3	1307-1105	1000
O_4	5	1951-1076-820	2144-1129
O_{10}	5	1335-937	1032-985-695
Z_4	2	3000	3917
Z_7	2	2326-1563	
Total bands	17	10	7

M.S (bp) = molecular marker

TABLE (7-b). RAPD markers for the polymorphic primers with the two contrasting hulles barley genotypes in regard to drought tolerance and disease resistance.

Operon primer	Marker/op	Positive M.S.(bp)	Negative M.S.(bp)
O_2	1	-	1138
O_4	3	-	1650-846-631
Z_4	2	1879-575	-
Z_7	4	3287-2169-836	3394



Lane M: is the standard DNA marker
Lanes 1-15 : Genotypes L3, L8, L10, L17, L24, L25, L26, Giza126, H_5, H_6, H_7, H_{10}, Mar19A, P_4 and Giza 131, respectively.

Fig. (1). RAPD fingerprints of fifteen barley genotypes using five random primers (O_2, O_4, O_{10}, Z_4 and Z_7).

TABLE (8). Polymorphism percentages in the tested hulled and hulles barley genotypes generated by five operon primers.

primer	Mono -morphic bands	Polymorphic bands		Total bands	Polymorphic %
		unique	Non- unique		
O_2	2	6	9	17	88.24
O_4	0	3	16	19	100
O_{10}	0	2	16	18	100
Z_4	2	3	11	16	87.5
Z_7	0	6	11	17	100
Total	4	20	63	87	95.4

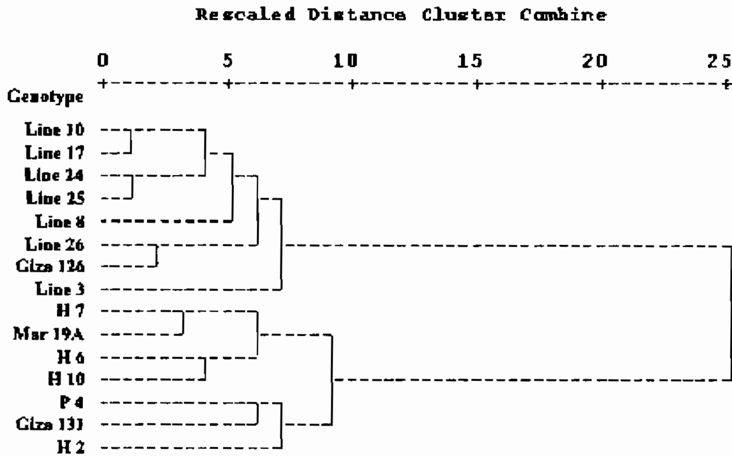


Fig. (2). Distribution of clusters on dendrogram of fifteen barley genotypes using five random primers.

TABLE (9). Similarity matrix among the 15 barley genotypes based on RAPD analysis.

Genotype	Line 3	Line 8	Line 10	Line 17	Line 24	Line 25	Line 26	G 126	H_2	H_6	H_7	H_{10}	MAR19A	P_4
Line 8	806	-	-	-	-	-	-	-	-	-	-	-	-	-
Line 10	.806	.873	-	-	-	-	-	-	-	-	-	-	-	-
Line 17	.851	.873	1.00	-	-	-	-	-	-	-	-	-	-	-
Line 24	.806	.829	.917	.917	-	-	-	-	-	-	-	-	-	-
Line 25	.783	.806	.895	.851	.980	-	-	-	-	-	-	-	-	-
Line 26	.615	.783	.783	.783	.829	.806	-	-	-	-	-	-	-	-
G 126	.737	.806	.851	.851	.895	.873	.938	-	-	-	-	-	-	-
H_2	.132	.226	.164	.164	.100	.195	100	.195	-	-	-	-	-	-
H_6	.034	.132	.132	.132	.067	.100	.132	.100	806	-	-	-	-	-
H_7	067	.164	.286	.226	.164	.195	.164	.195	.737	.851	-	-	-	-
H_{10}	.100	.195	.256	.265	.132	.164	.195	.164	.615	.873	.851	-	-	-
Mar 19 A	.000	.100	.164	.164	.100	.132	.100	.132	.589	.806	.917	.806	-	-
P_4	.195	.286	.286	.226	.226	.256	.286	.316	.783	.760	.737	.665	.783	-
Giza 131	.067	.164	.226	.226	.100	.132	.100	.132	.783	.713	.783	.806	.737	829

In summation, the results of this investigation provided some PCR-RAPD based molecular markers associated either positively or negatively with barley genotypes productivity. That could be used to enhance breeding

programs aimed to improve its drought tolerance by pyramiding genes controlling this polygenic character by the aid of marker-assisted selection. It is feasible that more markers can be generated for drought tolerance if more random primers were used. At least, the RAPD marker developed from this study can consequently be used in any further study to identify stress-tolerant genotypes in barley or any other field crop.

This concept has been advocated by several investigators who stated that molecular markers have several advantages over the traditional phenotypic markers that were previously available to plant geneticists. They offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular marker linked to that trait (Abdelsalam *et al.*, 1998 and Abdel-Tawab *et al.*, 2003). In addition, this approach is more reliable, environment independent, reproducible, rapid and cost-effective.

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كاشفات جزيئية لتحسين إنتاجية الشعير وتحمله للأمراض تحت ظروف الزراعة المطرية

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خلال الموسمين الزراعيين ٢٠٠٢/٢٠٠٣و ٢٠٠٣/٢٠٠٤تم تقييم ١٥ من تراكيب الشعير الوراثية المتباينة (صنف مستورد واثنين من الاصناف المحلية واثنى عشر سلالة مرباة حديثا ناتجة من برنامج تربية الشعير بمركز بحوث الصحراء, وذلك فى محطة بحوث مريوط التابعة للمركز. تهدف الدراسة الى بحث العلاقة بين الكفاءة المحصولية وكل من تحمل الجفاف والاصابة بمرض التبقع الشبكى وكذلك للتعرف على كاشفات جزيئية لتحمل الجفاف ومقاومة المرض.

وبالنظر إلى بيانات موسم النمو الثاني (٢٠٠٣/٢٠٠٤) يتضح أن محصول حبوب النبات للتراكيب الوراثية المغطاة تراوح بين ٤,٣٧ جم للصنف جيزة (١٢٦) و ٧,٢٧ جم للسلالة رقم (٣) كما أن السلالة عارية الحبوب H_{10} كانت الأفضل محصولاً ٨,٣٣ جم، مقارنة بالصنف جيزة (١٣١) الذي أعطى ٣,١٧ جم/نبات تحت ظروف التجربة كما أبدت السلالة H_{10} مقاومة عالية لمرض التبقع الشبكي.

وقد أظهرت نتائج شدة الإصابة أن الأب الرابع P_4 والصنف G126 كانت متوسطة التحمل لمرض التبقع الشبكي حيث سجلت ٥٠%،٤٣,٣% على التوالي . بينما التراكيب الوراثية ٢٦ ، MAR19A ، ٨، H_{10} كانت مقاومة للاصابة بالمرض.

من خلال استعراض نتائج التفريد الجزيئى للحامض النووى DNA وجد ان العدد الكلى من الحزم bands التى ظهرت بكل من البادئات العشوائية الخمسة(O_2, O_4, O_{10}, Z_4 and Z_7) بنظام RAPD-PCR كانت ٨،٦،٩،٧،٥ حزمة على التوالى.كما ان البوادىء الخمسة سجلت سبعة كاشفات سالبة وعشرة موجبة مقارنة بالسلالة المرباة حديثاً رقم١ (Line 3).

ومن ناحية أخرى فإن السلالتين عاليتا الإنتاجية مغطاة الحبوب مرباه حديثاً وهما ٨ و ٢٥ تميزتا بكاشفات جزيئية موجبة (820 bp) بالنسبة للبادىء O_4 و 937 bp بالنسبة للبادىء O_{10}. أيضاً السلالة ٢٥ بها معلمات جزيئية موجبة للبادىء Z_4 (3000 bp) للانتاجية العالية تحت الظروف المطرية بمريوط.

المعلمات الجزيئية السالبة يمكن أن تستخدم كدلائل لتمييز السلالة ٢٤ وذلك مع البادىء O_{10} (1032 bp). كما ان البادئين Z_4 وZ_7 يمكن استخدمهما للتفرقة بين السلالات عارية الحبوب بواسطة خمسة معلمات جزيئية موجبة وذلك لمقاومة مرض التبقع الشبكى والتحمل للجفاف.