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BARLEY DROUGHT TOLERANCE IN RELATION TO BIOCHEMICAL GENETIC MARKERS (ISOZYMES)

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ABSTRACT

Seven genotypes of six rowed type barley (five newly bred lines and two released varieties) were used to study the response of these genetic material under drought conditions with polyethylene glycol solutions having water potential of 0, -4, -8, and -12 bars, The objective was to select tolerant barley genotypes to water deficit stress during germination in relation to isozyme variations.

The variables, abnormal seedling percentage (ASP%), dry weight of roots (DWR), dry weight of shoots (DWS) and dry weight of shoots +roots (DWSR) showed a variation coefficient of variability larger than 15%, which reflect a relative high random effect of the water stress on these traits. In meantime the other traits showed smaller variation coefficients, between 5.52 and 12.03%.

The highest physiological grain quality was observed on the barley genotypes H10, H7 and L3 since they showed great germination percentage (GP%) and (DWSR) under the highest water deficit stress (-12 bar). G131 showed the lowest GP%, NSP%, SL (cm), RL (cm), DWS, DWR and DWSR under the same severe stress conditions. The large root length allowed a better soil exploration, however is not a guarantee of improved water absorption. GP% under -4 bar reached two times higher than under -12 bar, such performance well accepted by dry land farmers.

The reduction of water deficit stress from -12 bar to -4 bar caused about 42.5%, 90.6%, 82.7%, 87.8%, 87.8% and 90.3% increasing in GP%, NSP%, SL, RL, DWS, DWR, respectively. Hence, any barley genotypes could be sown in newly reclaimed areas where limited water supply reached -4 bar water potentiality. Meanwhile, under more than -4 bar water deficit conditions, the hulles barley line H10 is recommended for cultivation.

Electrophoretic patterns of the three isozymes tested showed nine monomorphic bands under the four water potentials tested. The band (No.3) was present in all genotypes except genotypes H7, H10 and G131 under water deficit levels. However, in regard to acid phosphatase densitometric analysis, the second band was presented in five genotypes (L3, L26, G126, H6 and G131) and missed for H7 under all treatments. While, it presented only under control treatment of the drought tolerant genotype (H10). The first band fluctuated in its appearance in all genotypes tested under each of water deficit treatments and absent as a result of severe stress. Patterns of the three isozymes tested differentiate all barley genotypes under investigation and failed to give clear cut markers for drought tolerance.

Key words: barley, abiotic stress, drought stress, isozymes, electrophoresis, polyethylene glycol

INTRODUCTION

Barley, (*Hordeum vulgare* L.), is one of the principal cereal crops in the world and is cultivated in all temperate areas (Von Bothmer *et al.*, 1995). Water deficit is a major constraint on plant productivity with an evident effect on plant growth (Rampino *et al*., 2006). This deficit has an evident effect on plant growth that depends on both severity and duration of the stress (Araus *et al*. 2002; Bartels and Souer 2004).

Screening techniques should be fast, easy to apply, inexpensive, and capable to evaluate plant populations. Great advances have been made in recent years in the techniques used to identify markers linked to useful traits. While isozyme electrophoresis techniques have been the basis for most work in crop plants, useful markers have been generated using enzyme electrophoresis methods. The value of markers in analyzing the inheritance of traits in crop plants and

understanding genome structure and organization is now well established (Korzun, 2002).

In vitro selection using polyethylene glycol has been frequently reported in several plant species (Gonzalez-Murua *et al.,* 1985 and Mercado *et al*., 2000). Dolgikh *et al.* (1994) obtained *in vitro* selected drought resistant maize plants using variable levels of polyethylene glycol (PEG 6000). Efficient screening of genetic stocks for drought tolerance is possible if drought conditions are simulated in the laboratory by using osmotic agents which affects germination (Sullivan, 1971). Since the osmotic agent PEG 6000 is non toxic and does not penetrates into the seed it is recommended for several researchers (Willenborg *et al.,* 2005). Simulated drought conditions in the laboratory by using osmotic agents have demonstrated that all traits related with plant development are affected, where the most susceptible traits are seedling and root length, germination, and vigor (Dhanda *et al.,* 2004 and Perez *et al*., 2007).

Many types of genetic markers have been applied to diversity studies in barley. Interesting results came from some of early work using biochemical markers which showed more variations, and many alleles are associated with adaptation to specific environments (Nevo, 1992).

Isozymes, along with morphological and other protein markers, were used to build the first genetic maps of crops such as maize (*Zea mays* L.) (Goodman and Stuber 1983), tomato (*Lycopersicum esculentum* L.) (Rick, 1983), wheat (*Triticum aestivum* L.) (Hart, 1983), barley (*Hordeum vulgare* L.) (Brown, 1983) and canola (*Brassica napus* L.) Ahmed and Afiah (2008).

The aims of this study were to obtained biochemical markers for drought tolerance in barley, ascertain whether genotypes which treated with three drought levels (-4, -8, -12 bar) using PEG6000, to examine the effect of drought stress on α and β- esterase activity in barley evokes qualitatively similar effects as those under control (0 bar), as a quick and easy method to study response of barley genotypes under drought stress.

MATERIALS AND METHODS

1- Plant Material:

Seven genotypes of six rowed type barley (five newly bred lines and two released varieties) were used to study the response of these

genetic material under drought conditions with polyethylene glycol solutions having water potential of 0, -4, -8, and -12 bars, Name, type, pedigree and/ or selection history of all varieties or newly bred lines are presented in table (1).

The experiment was established in the plant pathology laboratory at Desert Research Center (DRC). Germination was performed in glass jars with constant volume of polyethylene glycol solutions having water potential of -4, -8, and -12 bars. Distillated water was used as control (0 bars). Glass jars were covered with plastic film to avoid changes in the water potential through evaporation solutions. Grains were placed during 13 days in chambers at constant temperature regimes of 20°C and 85 to 100 % relative humidity for germination according to Perez *et al.* (2007). Data collected from the three replicates of this experiment were arranged and statistically analyzed in a split plot design. Percentages of the three variables (germination, normal seedlings and abnormal seedlings) were analyzed on the transformed arcsin square root of percentage data, where data in table are untransformed.

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

Name	Caryopsis type	Pedigree and/or selection history
Line 3	Hulled	Giza126/(ICB 82-1451-8AP-OAP-9AB-0TR) $F_8$3Sel,Mar.
Line 26	Hulled	Giza126/(Arar//2762/BC-2L-2Y-ICB 83-0687-7AP-0AP-1AP) $F_8$26Sel,Mar.
Giza126	Hulled	Baladi Bahteem"/ "SD 729-Por12762-BC
H6	Hulles	Giza126/(ICNB F8 - 654 Sel, 5AP) F_8 H6 Sel, Mar.
H7	Hulles	Giza126/(ICNB F8 - 654 Sel, 5AP) F_8 H7 Sel, Mar.
H10	Hulles	Giza126/(ICNB F8 - 654 Sel, 5AP) F_8 H10 Sel, Mar.
Giza131	Hulles	CM67-B/CENTENO//CAM-B/3/ROW906. 73/4/GLORIA-EAR/COME-B/5/FALCON-BAR/6/LINO

Evaluated variables:

- Germination percentage: at the end of the assay (13 days), the number of germinated grains was assessed for each treatment and replication.
- Normal seedlings percentage: when all seedlings were complete and healthy, with all their structures well developed they were considered normal.
- Abnormal seedlings percentage: Partial and non healthy seedlings, with at least one none well developed structure were considered abnormal.
- Root and seedling length: to determine these two traits, 10 normal seedlings from each treatment in each replicate were measured.
- Dry weight of roots or shoots and roots and roots+shoots: to determine dry weight, 10 normal roots and shoots of each seedling for each treatment in each replicate were placed in drying oven during 48 h at 80 ºC in paper envelopes.

Isozymes Electrophoresis:

Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted to identify isozymes variation among the studied seven barley genotypes. All samples were collected from lab. experiment under four levels of drought (0, -4, -8 and -12 bar) using PEG 6000. Grains were placed during 13 days in chambers at constant temperature regimes of 20 ^{o}C and 85 to 100% relative humidity for germination. Fresh shoot samples for each genotype under each treatment were used separately for isozymes extraction according to Stegemann *et al.* (1985). Isozymes extraction from barley samples was performed separately by homogenizing 0.5g fresh shoot samples in one ml extraction buffer using a mortar and pestle. The extract was then transferred into clean eppendorf tubes and centrifuged at 10000 rpm for five minutes. The supernatant was transferred to new clean eppendorf tubes and kept at –20^{o}C until use for electrophoretic analysis. A volume of 50 µl extract of each sample was mixed with 25µl of treatment buffer, then a volume of 50 µl from this mixture was applied to each well. After electrophoresis, the gels of α or β-esterase were soaked in 0.5 M borate buffer (pH 4.1) for 90 minutes at 4^{o}C. This procedure lowers the pH of the gel from 8.8 to about 7 at which the reaction proceeds readily. The low temperature minimizes diffusion of the protein within the gel. The gel then was rinsed rapidly

in two changes of double distilled water. The gel was stained for esterase activity by incubation at 37^{o}C in a solution of 100 mg α-naphthyl acetate or β- naphthyl acetate (as a substrate) and 100 mg fast blue RR salt in 200 ml of 0.1 M phosphate buffer pH 6.5 (Scandalios, 1964). For acid phosphatase,

Statistical analyses:

The data collected for all barley genotypes tested from lab. experiment were subjected to the ordinary analysis of variance of split plot design on ten seedlings mean basis in each of the three replicates as described by Gomez and Gomez (1984). Significance of differences among means of treatments (T), genotypes (G) and T×G were verified according Waller and Duncan (1969) New LSD.

Densitometric scanning

All gels resulted from protein and isozyme electrophoreses were scanned using Gel Doc 2001 Bio-Rad system. The densitometric scanning of the bands based on its three directions characters, where each band is recognized by its length, width and intensity. Accordingly, relative amount of each band quantity could be measured and scored.

RESULTS AND DISCUSSION

1- Genetic materials response under Drought stress by using PEG 6000:

From the analysis of variance, the mean of all genotypes tested differed statistically ($\alpha \leq 0.05$) for all evaluated traits. Also, differences among means of the osmotic concentrations for all evaluated traits and for the genotypes × osmotic pressure interactions were found as well. This means that the relative performance of the genotypes through the water stress pressures was not the same (Table, 2). Similar results were found by Blum *et al*. (1980) and Perez *et al*. (2007) in wheat using PEG 6000 where genotypes had a different germination rate under numerous osmotic concentrations.

The variables, abnormal seedling percentage (ASP%), dry weight of roots (DWR), dry weight of shoots (DWS) and dry weight of shoots +roots (DWSR) showed a variation coefficient of variability larger than 15%, which reflect a relative high random effect of the

water stress on these traits. In meantime the other traits showed smaller variation coefficients, between 5.52 and 12.03% (Table, 2).

Table (2): Means of germination and vigor of grains evaluated for seven barley genotypes under four polyethylene glycol water potentials.

		L3	L26	G126	H6	H7	H10	G131	Mean
GP%	Cont.	97.33	98.67	92.1	98.73	96	98.67	98.67	97.17
	-4 bar	85.07	91.43	81.97	90..67	83.9	91.6	83.3	86.21
	-8 bar	67.33	75.67	64	74	64	76	52.67	67.67
	-12 bar	52	44.67	42	50.67	54.33	62	41.33	49.57
	Mean	75.43	77.61	70.02	74.47	74.56	82.07	68.99	75.16
	LSD 0.05		T= 1.66		G=3.41		T×G=6.82		CV=5.52
NSP%	Cont.	97.33	97.33	90.83	98.73	94.77	97.33	94.67	95.86
	-4 bar	83.77	86.3	80.67	89.33	78.57	86.5	80.77	83.70
	-8 bar	46	62.33	54.67	60.67	56	48	39.33	52.43
	-12 bar	9.33	7.33	11.33	10.67	7.67	6.67	2.03	7.86
	Mean	59.11	63.32	59.38	64.85	59.25	59.63	54.20	59.96
	LSD 0.05		T=2.56		G=4.10		T×G=8.2		CV=8.332
ASP%	Cont.	0.1	2.57	1.33	0.1	1.33	1.33	3.83	1.51
	-4 bar	1.33	5.17	1.33	1.33	5.17	5.1	2.57	3.14
	-8 bar	21.33	13.33	9.33	13.33	8	28	13.33	15.24
	-12 bar	42.67	37.33	30.67	40	46.67	55.33	39.33	41.71
	Mean	16.36	14.60	10.67	13.69	15.29	22.44	14.77	15.4
	LSD 0.05		T=2.58		G=4.4		T×G=8.81		CV=34.83
SL(cm)	Cont.	27.16	26.6	26.74	27.96	26.69	27.43	25.74	26.90
	-4 bar	22.9	22.97	22.9	21.56	23.37	20.61	20.77	22.15
	-8 bar	9.53	13.38	11.63	12.46	14.07	10.82	7.63	11.36
	-12 bar	3.38	3.62	3.89	5.18	4.07	3.35	3.31	3.83
	Mean	15.74	16.64	16.29	16.79	17.05	15.55	14.36	16.06
	LSD 0.05		T=1.48		G=1.2		T×G=		CV=9.076
RL(cm)	Cont.	16.73	16.04	15.43	17.10	17.86	16.11	15.13	16.34
	-4 bar	13.21	13.26	12.23	16.34	15.65	13.55	10.55	13.54
	-8 bar	3.41	4.24	4.33	4.61	4.49	3.62	2.94	3.95
	-12 bar	1.25	1.84	2.12	2.73	1.2	1.39	1	1.65
	Mean	8.65	8.85	8.53	10.20	9.80	8.67	7.41	6.37
	LSD 0.05		T=0.87		G=0.57		T×G=1.13		CV=10.82
DWS (mg)	Cont.	621.6	564.6	523	545.8	528.3	451.1	356.4	512.9
	-4 bar	488.5	317.8	334.2	428.7	412.5	334.8	312.7	375.6
	-8 bar	126.9	132.8	136.6	195.2	239.1	124.8	71.54	146.7
	-12 bar	63.96	32.98	47.25	61.68	72.42	17.67	24.48	45.78
	Mean	325.2	262	260.3	307.9	313.1	232.1	191.3	270.25
	LSD 0.05		T=34.05		G=34.42		T×G=68.84		CV=15.52
DWR(mg)	Cont.	224.3	209.1	180.7	183.8	162.7	190.5	149.7	185.8
	-4 bar	174.1	146.1	149.2	172.3	130.9	119.3	94.22	140.9
	-8 bar	44.67	37.42	50.73	70.38	40.93	41.52	26.45	44.59
	-12 bar	13.18	9.78	14.87	22.16	19.69	9.3	6.65	13.66
	Mean	114.1	100.6	98.89	112.2	88.57	90.14	69.25	96.24
	LSD 0.05		T=13.11		G=12.01		T×G=24.02		CV=15.20
DWSR(mg)	Cont.	845.9	773.7	703.7	729.6	691	641.6	506.1	698.7
	-4 bar	662.6	463.9	483.4	601	543.4	454.1	406.92	516.5
	-8 bar	171.57	170.22	187.33	265.58	280.03	166.32	97.99	191.3
	-12 bar	77.14	42.76	62.12	83.84	92.11	26.97	31.13	59.44
	Mean	439.3	362.6	359.19	420.1	401.67	322.24	260.55	366.5
	LSD 0.05		T=39.01		G=36.2		T×G=72.39		CV=12.03

GP: germination percentage; NSP: normal seedling percentage; ASP: abnormal seedling percentage; RL: root length; DWR: dry weight of root; SL: seedling length; DWS: dry weight of seedling; LSD: least significant difference; T: water deficit treatments; G: the seven barley genotypes tested; CV: Coefficient of variability.

- Comparison of genotypes:

Genotypic differences for tolerance to osmotic stress during germination were observed using both criteria; germination test and dry weight of shoots + roots as a scale of vigor. The highest physiological grain quality was observed on the barley genotypes H10, H7 and L3 since they showed great germination percentage (GP%) and (DWSR) under the highest water deficit stress (-12 bar). G131 showed the lowest GP%, NSP%, SL(cm), RL(cm), DWS, DWR and DWSR under the same severe stress conditions. The large root length allowed a better soil exploration, however is not a guarantee of improved water absorption (Dhanda *et al.,* 2004). It could be argued that observed responses were more specific to the species rather than to the genotypes (Blum *et al.,* 1980).

- Effects of water stress:

As the osmotic pressure was stronger, the manifestation of all traits was significantly ($\alpha \leq 0.05$) poorer, except for abnormal seedling percentage (ASP) (Table,2). The traits NSP%, SL and RL were highly vulnerable to water stress, since they showed smallest values under -8 and -12 bars, which means that the starch could not be used by the embryo to produce in normal manner (Dhanda *et al.,* 2004). It is important to set up that under -4 bars it was possible to reach a GP% and two times higher than -12 bar, performance well accepted by farmers from dry lands (Thill *et al.*, 1979). Similar results have been found on wheat (*Tritricum aestivum*), barley (*Hordeum vulgare*), and rice (*Oryza sativa*) using PEG solutions (Dighe and Rajurkar, 1984).

Ingeneral, the reduction of water deficit stress from -12 bar to -4 bar caused about 42.5%, 90.6%, 82.7%, 87.8%, 87.8% and 90.3% increasing in GP%, NSP%, SL, RL, DWS, DWR, respectively (not shown in tables). Hence, any barley genotypes could be sown in newly reclaimed areas where limited water supply reached -4 bar water potentiality. Meanwhile, under more than -4 bar water deficit conditions, the hulles barley line H10 is recommended for cultivation. Tawfik *et al*. (2007) reported similar results in faba bean crop. Also, Afiah *et al.* (2010) reported similar trend of genotypic variation under salt stress in the same barley genotypes tested on the bases of molecular genetic markers (ISSR-PCR).

Biochemical genetic markers "Isozymes":

Electrophoretic analysis of isozymes is known to be a good technique to qualitative methods for detection of genetic differences among individual genotypes. For this reason, three isozymes including esterase (Est.) and acid phosphatase (Acp.) were used to evaluate the effect of the environmental stress (water deficit) on the studied seven barley genotypes.

Esterase is a gene family controlling enzymes that hydrolyze ester bond in lipids to produce plant energy for biochemical reactions. The data included in the present work were obtained by using two different substrates; α-naphthyl acetate and β- naphthyl acetate.

***α*-esterase:**

Electrophoretic patterns of α-esterase isozyme for all genotypes are illustrated in figure (1-A) and densitometrically analyzed in table (3). and detected, nine bands, all of them were monomorphic under the four water potentials tested. The previously findings of Abdel-Tawab *et al*. (1989) were in line with our results.

***β*- esterase**

β-esterase electrophoretic patterns for the studied barley genotypes are visualized in Figure (1-B) and denistometrically analyzed as shown in table (3). The obtained result revealed that six bands were present. The band (No.3) was present in all genotypes except genotypes H7, H10 and G131. under all water deficit levels. These results agreed with those found by Abdelsalam *et al.* (2005), who reported a negatively correlated marker with drought tolerance in cotton genotypes.

Acid phosphatase

Acid phosphatase electrophoretic patterns are shown in figure (1-C) and densitometric analysis are summarized in table (3). Two bands were found and exhibited variation in their densities and intensities. However, the second band was presented in five genotypes (L3, L26, G126, H6 and G131) and missed for H7 under all treatments. While, it presented only under control treatment of the drought tolerant genotype (H10). The first band fluctuated in its appearance in all genotypes tested under each of water deficit treatments however, it absent as a result of severe stress for all barley genotypes under investigation. These results are in harmony with

those earlier reported by Abdelsalam *et al*. (1998) and El-Saied and Afiah (2004).

These results lead to the assumption that acid phosphatase isozyme patterns did not give clear-cut markers for the discrimination between drought tolerance and drought sensitivity of the genotypes under study, except the first band which could be considered biochemical genetic marker as it absent for all genotypes tested under severe stress (-12 bar).

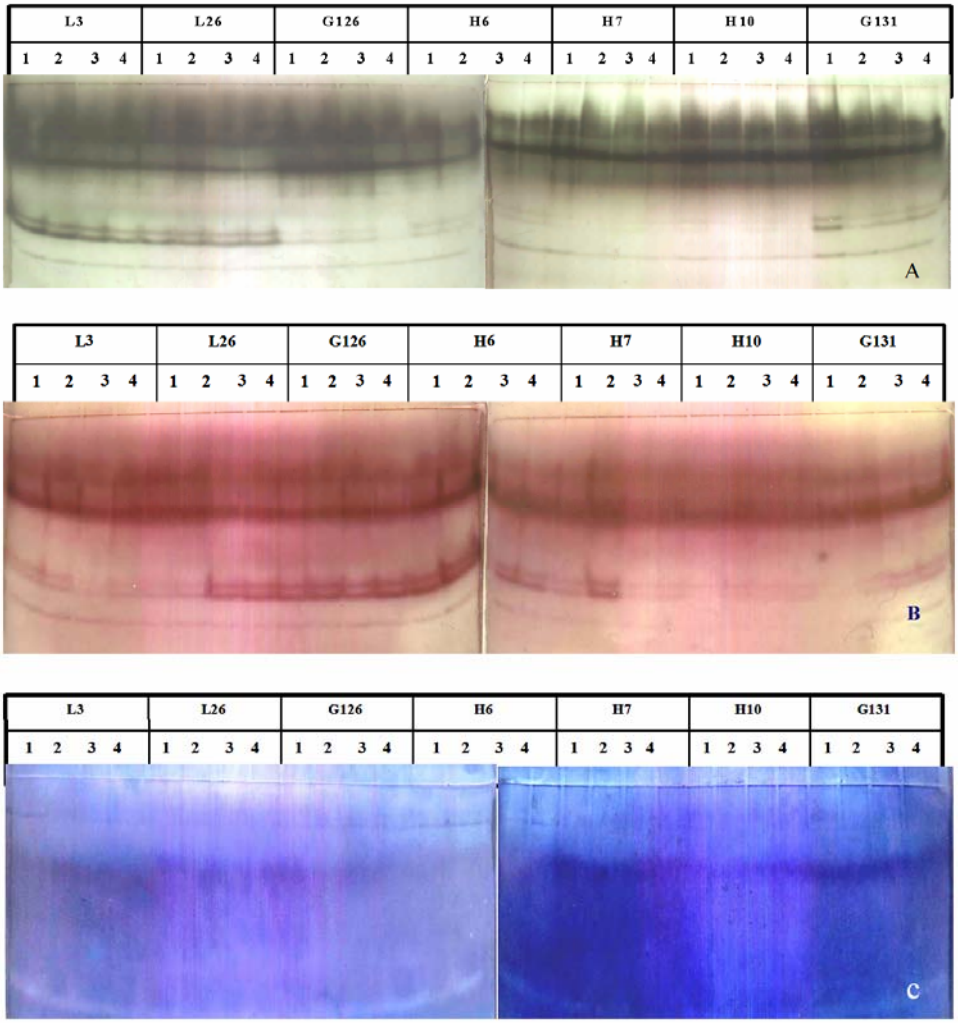

Figure (1): Electrophoresis banding-patterns of acid phosphatase, α- and β-esterases extracted from seven barley genotypes under four drought stress levels. A) α- esterase; B) β- esterase and C) acid phosphatase.

Table (3): Isozymes polymorphism in seven barley genotypes using three isozyme systems (α esterase, β esterase and acid phosphatase).

E.S.	Band No.	L3				L26				G126				H6				H7				H10				G131			
		Cont.	-4 bar	-8 bar	-12 bar	Cont.	-4 bar	-8 bar	-12 bar	Cont.	-4 bar	-8 bar	-12 bar	Cont.	-4 bar	-8 bar	-12 bar	Cont.	-4 bar	-8 bar	-12 bar	Cont.	-4 bar	-8 bar	-12 bar	Cont.	-4 bar	-8 bar	-12 bar
α-esterase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	8	1	1^{++}	1^{++}	1^{++}	1	1^{++}	1^{++}	1^{++}	1	1^{+}	1^{+}	1	1	1^{+}	1	1	1	1	1	1	1	1	1	1	1	1	1	1^{+}
	9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total bands		9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
β-esterase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	1	1^{+}	1	1^{+}	1	1	1^{++}	1^{++}	1	1^{++}	1^{++}	1^{++}	1	1^{++}	1^{+}	1^{+}	1	1^{++}	1	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total bands		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5	5	5	5	5	5	5	5	5	5	5	5
*Aph**	1	0	1	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0
	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	0	1	1	1	1
Total bands		1	2	2	1	1	1	2	1	2	1	1	1	2	2	1	1	0	0	0	0	2	0	0	0	2	2	1	1

* *Aph*: acid phosphatase; 1: low density (faint); 1^{+}: moderate density and 1^{++}: high density.

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تحمل الشعير للجفاف وعلاقته بالكاشفات الوراثية البيوكيميائية (مشابهات الإنزيمات)

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أجرى هذا البحث للتعرف على استجابة سبعة تراكيب وراثية من الشعير (خمسة سلالات مرباة حديثاً بالإضافة الى صنفين معتمدين للزراعة بمصر) للاجهاد الجفافى باستخدام محلول البولى ايثيلين جليكول حيث يحقق شد رطوبى بمستويات صفر، 4، 8، 12 ضغط جوى. ويعتبر الهدف من الدراسة هو انتخاب تراكيب وراثية من الشعير تتحمل الجفاف خلال مرحلة الانبات وعلاقة ذلك بالاختلافات فى مشابهات الانزيمات.

- كان معامل الاختلاف مرتفعاً نسبياً (أكبر من 15%) لصفات: النسبة المئوية للبادرات الشاذة- الوزن الجاف للجذور- الوزن الجاف للساق والأوراق وكذلك الوزن الجاف للبادرة بالكامل، مما يعكس ارتفاع درجة التأثير العشوائى للإجهاد المائى على هذه الصفات، وكان العكس صحيحاً فى باقى الصفات تحت الدراسة حيث تراوح معامل الاختلاف بين 5,52 و 12,03.

- بدراسة الصفات المرتبطة بالكفاءة الفسيولوجية للحبوب عند الانبات تحت أعلى شد رطوبى (12- bar) حققت السلالات L3, H7, H10 أعلى القيم لصفات النسبة المئوية للإنبات والوزن الجاف للبادرة فى حين أبدى الصنف جيزة 131 أقل القيم لمعظم الصفات قيد البحث. ومن الجدير بالذكر أن زيادة طول الجذر يسمح للنبات باستغلال أمثل لظروف التربة (ماء وعناصر غذائية متاحة) ولكن لا يضمن تحسن درجة الامتصاص.

- بحساب النسبة المئوية للنقص فى مستوى أداء الصفات ما بين شد رطوبى 12- bar ,4- اتضح الارتفاع النسبى حيث وصل 42,5 % لنسبة الانبات ، 90,6% لنسبة البادرات العادية، 82,7% لطول البادرات، 78,8% لطول الجذر، 87,8% للوزن الجاف للسيقان والأوراق، 90,3% للوزن الجاف للجذور مما يدعو للتوصية بزراعة أى تراكيب وراثية من الشعير بمناطق الاستصلاح التى تعانى من محدودية كميات المياه المتاحة تحت شد رطوبى لا يتعدى 4 ضغط جوى، أما إذا زاد الاجهاد الجفافى عن ذلك فيوصى بزراعة السلالة المرباة حديثاً H10.

- التفريد الكهربى للمشابهات الانزيمية لكافة التراكيب الوراثية تحت معاملات الاجهاد المائى المختلفة حدد تسعة حزم كلها متماثلة لإنزيم α-esterase وخمسة متماثلة بالاضافة الى حزمة واحدة متباينة فى حالة انزيم β-estersae، أما إنزيم acid phosphatase فحدد حزمتان متباينتان غابت الأولى فى كافة التراكيب الوراثية تحت مستوى الاجهاد الأعلى (12- bar).

ومن الجدير بالذكر أن مشابهات الانزيمات تحت الدراسة نجحت فى التفريق بين كافة تراكيب الشعير المختبرة بالرغم من أنها لم تعطى حدود فاصلة لكاشفات وراثية بيوكيميائية خاصة بتحمل الإجهاد الجفافى.