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Isozyme Variations in some Wheat Genotypes at Siwa Oasis and Ashmon Habitats

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THE VARIABILITY of grain storage-proteins of twelve wheat genotypes under stress (Siwa Oasis) and normal (Ashmon) habitats were analyzed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). It was clear that small differences were found in the SDS-PAGE analysis of wheat endosperm proteins among the studied genotypes. The defense isozymes [Peroxidase (Px), Polyphenol oxidase (PPO) and Malate dehydrogenase (Mdh)] were detected and their results concluded a prominent role, either directly or indirectly in stress responses. High polymorphisms were observed in most genotypes for Mdh isozymes at Siwa habitat, meanwhile Px isozymes was decreased. Misr-1 genotype attained higher level of Px isozymes under stress habitat as a trait to tolerance response. The changes of PPO isozymes showed a good correlation with the abiotic stress tolerance of wheat genotypes. Thus, PPO increased in Nesr, S8/17, ACSAD-2, ACSAD-3 and NBL genotypes under stress condition (Siwa habitat).

Keywords: Wheat genotypes, Siwa Oasis and Ashmon habitats, Protein profiling, Peroxidase, Polyphenol oxidase and Malate dehydrogenase isozymes.

Wheat (*Triticum* spp.) is a cereal grain, originally from the Levant region of the Near East but now cultivated worldwide (Belderok *et al.,* 2000). Wheat is considered as one of the most important edible crops in Egypt. It provides approximately one-fifth of the total calorific input of the World's population (FAO, 2010). Currently, about 95% of the wheat grown worldwide is hexaploid bread wheat, with most of the remaining 5% being tetraploid durum wheat. In 2010, world production of wheat was 651 million tons, making it the third most-produced cereal after maize (844 million tons) and rice (672 million tons).

Wheat proteins show high complexity and different interactions with each other, thus making them difficult to be characterized. Usually, they are classified according to their solubility. Following the sequential Osborne extraction procedure, albumins, globulins, gliadins and glutenins are isolated. An alternative classification to that described above has been proposed based on composition and structure rather than solubility (Merlino *et al.,* 2009).

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Water stress remains one of the most important factors limiting wheat full genetic potential for yield (El-Fadly *et al.,* 2007). Molecular markers developed by analysis of proteins, isozymes and randomly amplified polymorphic DNA (RAPD) have shown excellent potential to assist selection of quantitative traits (Studer, 1992). Altinkut and Gozukirmizi (2003) conducted a study to identify microsatellite markers associated with water-stress tolerance in wheat. They obtained a DNA fragment of 185 bp that was present in the tolerant individuals but not in the sensitive ones. In an attempt to study the relation between stress and storage proteins, Jaradat and Miller (2001) observed that polymorphisms, based on HMW-glutenins, decreased with increasing aridity of the collection site.

Some researchers (Zhang *et al.*, 2000 and Sairam *et al*., 2001) reported that water stress increased catalase and peroxidase activities in wheat. Polyphenol oxidases (PPO) catalyze the O_2-dependent oxidation of mono- and *o*-diphenols to *o*-diquinones, highly reactive intermediates; the secondary reactions that are believed to be responsible for the oxidative browning that occurs as a consequence of plant senescence, wounding and pathogen infection (Campos *et al*., 2004). Tyagi *et al.* (2000) showed the roles of PPO in the phenylpropanoid pathway, the Mehler reaction, electron cycling, oxygen regulation, flower petal coloration and plant defense. A defensive role of PPO has frequently been suggested due to the inducibility of PPO in response to various abiotic and biotic injuries or signaling molecules (Maki and Morohashi, 2006). Many of these enzymes have a site for proteolytic processing near the carboxy-terminus (Marusek *et al.,* 2006). The PPO enters into contact with the phenolic compounds in the vacuoles only after the occurrence of some type of damage in the plant (Shimizu *et al.,* 2011).

Malate dehydrogenase (Mdh) functions to maintain malate and pyruvate pools, which are required for operation of the Hatch-Slack cycle and are actively used for neutralization of salt treatment (Eprintsev and Fedorina, 2007). The increase in activity of Mdh was found to be related to salt-induced synthesis of the additional isoforms of Mdh in mesophyll cells (Lea *et al*., 2007). The accumulating ammonia destroyed the cell membranes, which led to damaging of the plant cells. As established, Malate dehydrogenase glutamate (Mdh-GOT) play key role in catabolism of glutamate (Hong *et al.,* 2004).

Therefore, the objective of this work was to detect biochemical (SDS protein and some isozymes) markers associated with stress tolerance or susceptibility gene (s) in wheat genotypes under Siwa Oasis habitat.

Material and Methods

Plant materials

Six genotypes of bread wheat (M1, M2, SH1, L1, L2 and L3), as well as six genotypes of durum wheat (B4, B5, B6, D1, D2 and D3), were used in this study (Table 1). Names, origin, pedigree and/or selection history of the genotypes tested are described by Afiah *et al.* (2014 and 2015).

TABLE 1. Names, origin, pedigree and/ or selection history of the twelve wheat genotypes and lines used in the present study of bread (M1, M2, SH1, L1, L2 and L3) and durum (B4, B5, B6, D1, D2 and D3).

Symbol.	Genotype	Origin	Pedigree and/or selection history
(M1)	Misr-1	Egypt	OASIS/SKAUZ//4*BCN/3/2*PASTOR. CMSSOOYO 1881T-050M-030Y-030M-030WGY-33M-0Y-0S.
(M2)	Misr-2	Egypt	SKAUZ/BAV 92. CMSS96M03611 S-1M-010SY-010M-010SY-8M-0Y-0S.
(SH1)	Shandaweel-1	Egypt	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC. CMSS93B00567S-72Y-010M-010Y-010M-0HTY-0SH.
(L1)	Nesr	CIMMYT/ ICARDA#	ICW85-0024-06AP-300AP-300L-1AP-0AP
(L2)	S8 / 17*	Egypt	R8 tissue culture regenerated double haploid plant
(L3)	Line – 606*	Egypt	Atlas 66/Nap Hall//(NE 70117) SkoresPelka 35/2*RCB - 61 Su 606 – 13 Su -2 Su – 5 Su – 0 Su
(B4)	Baniseuf-4	Egypt	AUSL/5/CANDO/4/BY*2/TACE//II 27655/3/TME//ZB/W*2. ICD88-1120-ABL-0TR-1BR-0TR-6AP-0AP-0SD.
(B5)	Baniseuf-5	Egypt	DIPPERZ/BUSHEN3. CDSS 92B128-1M-0Y-0M-0Y-3B-0Y-0SD.
(B6)	Baniseuf-6	Egypt	BOOMER-21/BUSCA-3. CDSS 95Y001185-8Y-0M-0Y-0B-1Y-0B0SD.
(D1)	ACSAD-2	ACSAD	Gediz" S"- Bar," S"/ Ege // Ruf ,"S" – FG"S".
(D2)	ACSAD-3	ACSAD	MZA" S" × 21563- AA"S"/ PG"S"- FG"S"×GTA"S" ×21563- AA"S"/ 2
(D3)	NBL**	Egypt	**(Mexi 'S'/ Mgha/ 51792// D. 6 – CD 9799)** /(ICD 85-1328- ABL) 9Hekma-8Mat -11Mar-6Mar-3Mar- OMar

CIMMYT: Centro International de Mejoramiento de Maize Y Trigo (Mexico) = International maize and wheat improvement center.

ICARDA: International Center for Agricultural Research in the Dry Areas.

* Newly bred lines released through Desert Research Center wheat breeding program.

Methods

Grains were sown under Siwa Oasis and Ashmon conditions on Nov., 2012. Grains were sown in three replications at an adjusted rate 300 viable grain/m^2. Normal agronomic practices were performed; more details of soil and irrigation water chemical analysis are mentioned by Afiah *et al.* (2014). Metrological parameters were obtained from the observatory at each research station and daily minimum and maximum temperature and rainfall were recorded (Afiah *et al.*, 2014). The molecular and biochemical studies were conducted at Botany Dept., Faculty of Science, Zagazig Univ and Microbial Genetics Lab., Genetics Dept., Fac. of Agriculture, Kafr El-Sheikh.

Protein analysis

Total proteins were extracted from all genotype leaves under stress (Siwa habitat) and normal (Ashmon habitat) conditions as in El-Fadly *et al*. (2007). The extracted proteins were electrophoresed using SDS-PAGE and stained according to Laemmli (1970). The banding patterns and the molecular weight were determined against standard M.Wt. markers [Pharmacia Biotech Cat. No.17-0446 (10-225 kDa)].

Isozymes Electrophoresis

Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted to identify isozyme variations among the studied genotypes using three isozyme systems according to Stagemann *et al*. (1985). Fresh and young leave samples for each genotype and location were used separately for isozymes extraction. The studied isozymes are those of peroxidase (Px), polyphenol oxidase (PPO) and malate dehydrogenase (Mdh). Separation of isoenzymes of Px, PPO and Mdh were performed by Native PAGE (30 % polyacrylamide) by loading 50 µg of protein. The gels were run in an electrode buffer composed of 0.025 M Tris and glycine 0.192 M (pH 8.8) for 3 h at 40^{o}C at a constant current of 30 mA.

Isozyms staining and detection (Scialabba et al., 2002)

After electrophoresis, the gels were stained according to their enzyme systems with the appropriate substrate and chemical solutions then incubated at room temperature in dark for complete staining. In most cases, incubation for about 1 to 2 hr was enough.

Peroxidase (Px)

Benzidine di HCl (0.125 g), glacial acetic acid (2 ml) and D.W (up to 50 ml). Gel was placed into this solution and 5 drops of hydrogen peroxide were added. The gel was incubated at room temperature until bands appearance (Brown, 1978).

Polyphenol Oxidase (PPO)

After electrophoresis, the gel was soaked in 0.1 M sodium mono di Phosphate buffer (pH 6.8) solved in 100 mg sulfanilic acid, and then mixed with 30mg athecol solved in 1ml acetone. The gel was incubated at 37^{o}C until bands appeared (Gauillard *et al.,* 1993).

Malate dehydrogenase (Mdh)

0.1M Tris-pH (7.5), 100 ml NAD (30 mg), MTT (20 mg), PMS (5 mg) and maleic acid (1.2g) were mixed. The gel was placed into this solution and incubated at 30 ºC for 30 min until bands appeared (Tesfaye *et al.,* 2001).

Results and Discussion

A. SDS-PAGE analysis of protein

Figure 1.a & b. shows the electrophoretic banding pattern of proteins in the tested wheat genotypes under stress (Siwa habitat) as well as under normal habitat (Ashmon habitat). A total of 10 bands with different molecular weights were scored. These bands were not necessarily appearing in all genotypes. All genotypes

under the two conditions exhibited 5 common bands *i.e.* No. 1, 2, 4, 5 and 6 with molecular weight 10, 25, 50, 75 and 100 kDa, respectively. These bands may be considered as biochemical marker for wheat genotypes under normal and stress conditions. SDS-PAGE shows no new proteins synthesized under Siwa stress condition, moreover protein of 35kDa disappeared in the genotypes of Siwa habitat. The reduction in protein content due to salinity or water stress might be due to increase of proteolysis or inhibition of protein synthesis (Dey and Kar, 1995). Wyedert and Cullen (2010) revealed that protein synthesis was reduced by stress, but a greater effect of stress was shown as protein degradation. Therefore, protein profiles (SDS-PAGE) could be useful markers in genotypes adaptation, on improving the efficiency of wheat breeding programs. Nemati *et al.* (2012) concluded that protein profiles could be useful markers for cultivar identification, registration of new varieties, pedigree analysis and studies of genetic diversity and classification of adapted cultivars. The variation found in protein profiles within the present study might be a response to the extreme conditions of Siwa habitat. This conclusion agrees with that of Marcos-Filho (2005).

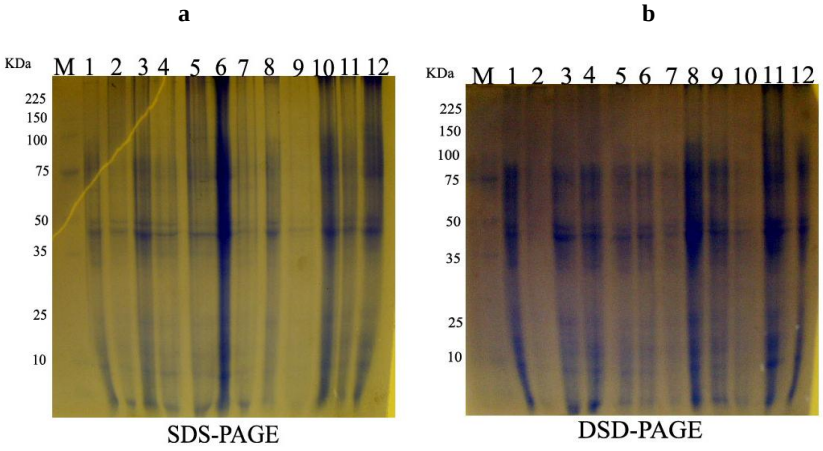

Fig. 1. Protein profile of bread (M1, M2, SH1, L1, L2 and L3) and durum (B4, B5, B6, D1, D2 and D3) wheat genotypes.
a. under stress (Siwa habitat) b. under normal condition (Ashmon habitat).

B. Isozyme electrophoresis

B.1. Polymorphisms of peroxidase isozymes:

Figure 2:a,b and Table 2:a,b show the peroxidase banding patterns of the different wheat genotypes under study exhibited from four to three bands at normal and stress conditions, respectively. The first three bands of peroxidase isozymes were detected for all genotypes under stress (Siwa habitat) and normal

(Ashmon habitat) conditions. Three bands with high density were found in genotype M1 at Siwa habitat, whereas at Ashmon habitat, M2 had three and B5 had four high density bands. The first band (Px1, RF=0.1) was decreased in genotypes at Ashmon habitat, compared to genotypes at Siwa habitat. The fourth band (Px4, RF=0.5) disappeared completely from genotypes at Siwa habitat and some genotypes at Ashmon habitat. Dasgupta *et al.* (2010) reported that four peroxidase isoforms were observed under control and salinity treatment in some mangrove species. Deef *et al*. (2014) showed that M1 and M2 are tolerant genotypes of bread wheat's and may be recommended to be cultivate under Siwa Oasis habitat. Meanwhile, the reverse was true at durum genotypes i.e. most yield and tolerant genotypes D3 and D1 (under Siwa Oasis habitat) showed least peroxidase intensity bands. Cluster and principal component analysis, based on eleven of stress tolerance indices, revealed that D3 and D1 were the most stress tolerant genotypes of durum wheat (under Siwa Oasis habitat) (Afiah *et al.*, 2015).

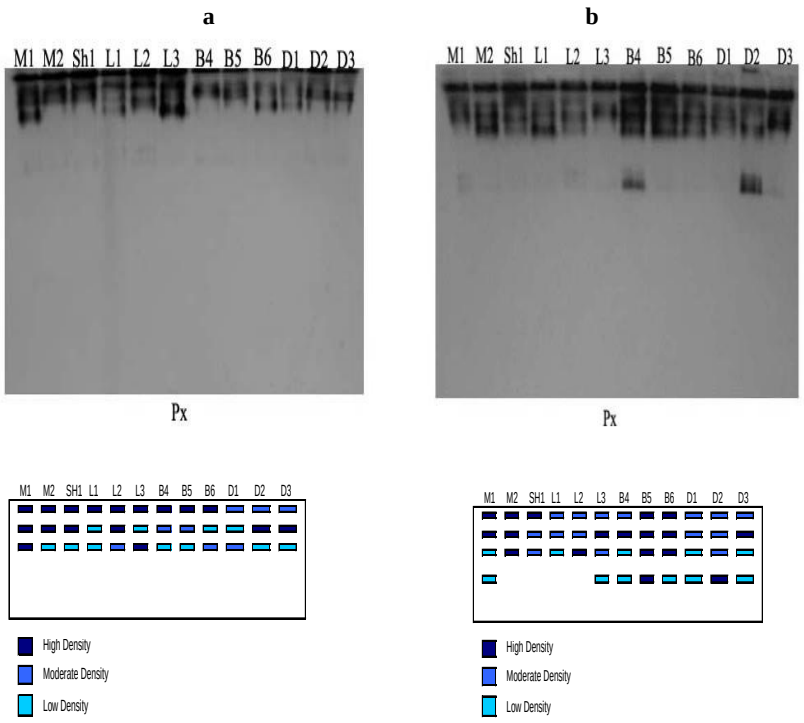

Fig. 2. Photograph of electrophoretic peroxidase isozyme patterns of bread (M1, M2, SH1, L1, L2 and L3) and durum (B4, B5, B6, D1, D2 and D3) wheat genotypes.
a. under stress (Siwa habitat) b. under normal condition (Ashmon habitat).

TABLE 2. Peroxidase isozymes intensity of bread (M1, M2, SH1, L1, L2 and L3) and durum (B4, B5, B6, D1, D2 and D3) wheat genotypes.
a. under stress (Siwa habitat) b. under normal condition (Ashmon habitat).

a

Peroxidase isozymes	RF	bread						durum					
		M1	M2	SH1	L1	L2	L3	B4	B5	B6	D1	D2	D3
Px1	0.1	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{+}	1^{+}	1^{+}
Px2	0.2	1^{++}	1^{++}	1^{++}	1^{-}	1^{++}	1^{-}	1^{+}	1^{+}	1^{-}	1^{-}	1^{++}	1^{++}
Px3	0.3	1^{++}	1^{-}	1^{-}	1^{-}	1^{+}	1^{++}	1^{-}	1^{-}	1^{+}	1^{+}	1^{-}	1^{-}

b

Peroxidase isozymes	RF	bread						durum					
		M1	M2	SH1	L1	L2	L3	B4	B5	B6	D1	D2	D3
Px1	0.1	1^{++}	1^{++}	1^{++}	1^{+}	1^{+}	1^{+}	1^{+}	1^{++}	1^{++}	1^{+}	1^{+}	1^{+}
Px2	0.2	1^{++}	1^{++}	1^{+}	1^{+}	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}
Px3	0.3	1^{-}	1^{++}	1^{+}	1^{-}	1^{++}	1^{+}	1^{-}	1^{++}	1^{++}	1^{-}	1^{+}	1^{-}
Px4	0.5	1^{-}	0	0	0	0	1^{-}	1^{-}	1^{++}	1^{-}	1^{-}	1^{++}	1^{-}

1^{++} = High Density, 1^{+} = Moderate Density, 1^{-}= Low Density, 0 = Absent

B. 2. Polymorphisms of polyphenol oxidase isozymes

The obtained results in Fig. 3: a,b and Table 3:a,b showed that two polyphenol oxidase(PPO) isozymes were detected in the wheat genotypes under the two conditions under study. Generally, stress (Siwa habitat) showed higher density isozyme polymorphisms of polyphenol oxidase in most genotypes. It is clearly noticeable that no clear relationship is evident between polyphenol oxidase isozymes and stress tolerance or susceptibility in wheat with exception of few bands. PPO are synthesized in the cytoplasm in the form of preproteins (60–70 kDa) and have a signal peptide in the amino terminus, which internalizes them in the chloroplast, where they are processed by stromal peptidases into functional proteins (54–62 kDa) and then imported into the thylakoid lumen (Marusek *et al.*, 2006).

PPO in the mesophyll chloroplast has been proposed to have a role in the Mehler reaction, photo reduction of molecular oxygen by PSI, and regulation of plastidic oxygen levels (Trebst and Depka, 1995, Badger *et al*., 2000). The Mehler reaction is a potentially important nondestructive sink for excess photosynthetic electrons under conditions of water stress (Siwa habitat) compared with normal condition (Ashmon) may help prevent over-reduction of components of linear electron transport, in agreement with Haupt-Herting and Fock (2002). When excess excitation energy is not dissipated by protective mechanisms, it is used to form cytotoxic ROS (Maki and Morohashi, 2006). Photoinhibition and ultimately photooxidative damage will occur if the combined capacity of ROS scavenging systems is exceeded. D2 and D3 genotypes of elevated PPO are expected to have improved stress tolerance due to contribute to the Mehler reaction.

Our results evaluated the response of tolerant wheat genotypes to stress (Siwa habitat) condition with increased PPO activity. It an adaptive strategy under water stress since it could reduce further water loss and allow limited nutrients to be partitioned to younger tissues (Thipyapong, *et al*., 2004). Induction of PPO activities under water stress has been previously demonstrated in tomatoes (English-Loeb *et al.,* 1997) and in coconut (Shivishankar, 1988). However, further investigation is needed to well-defined responsiveness of PPO to various abiotic and biotic stresses, depending on plant genotype and environmental as well as ecological context, may be an adaptive strategy of plants to cope with a multitude of stresses that often occur simultaneously (Krishnamoorthy *et al.,* 2004 & Raj *et al.,* 2006).

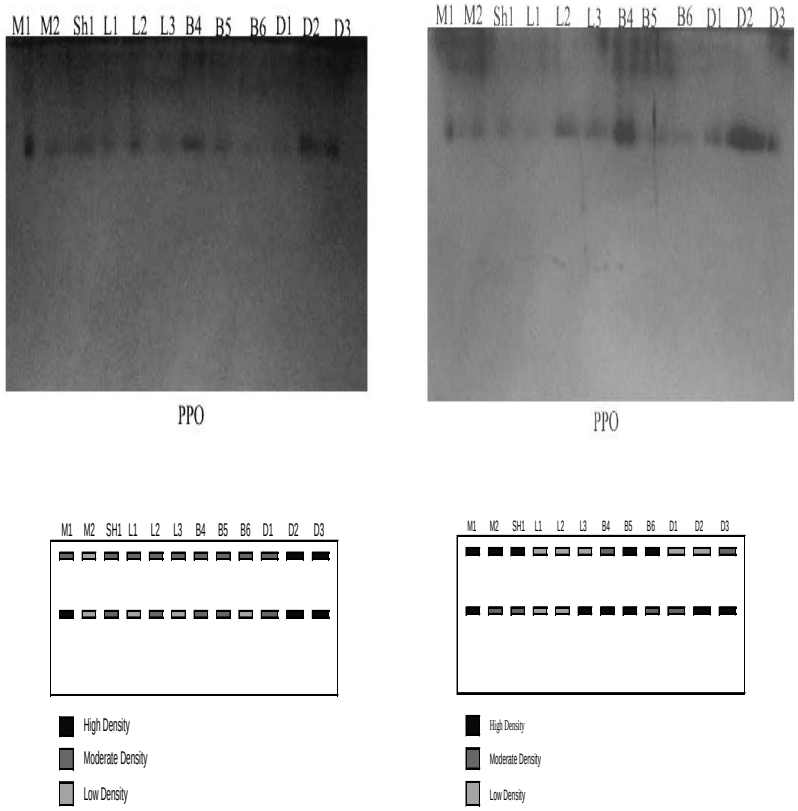

Fig. 3. Photograph of electrophoretic polyphenol oxidase isozyme patterns of bread (M1, M2, SH1, L1, L2 and L3) and durum (B4, B5, B6, D1, D2 and D3) wheat genotypes.
a. under stress (Siwa habitat) b. under normal condition (Ashmon habitat).

TABLE 3. Polyphenol oxidase isozyme intensity of bread (M1, M2, SH1, L1, L2 and L3) and durum (B4, B5, B6, D1, D2 and D3) wheat genotypes.

a a. under stress (Siwa habitat) b. under normal condition (Ashmon habitat).

Polyphenol oxidase isozymes	RF	bread						durum					
		M1	M2	SH1	L1	L2	L3	B4	B5	B6	D1	D2	D3
PPO1	0.1	1^{+}	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{++}	1^{++}
PPO2	0.4	1^{++}	1^{-}	1^{+}	1^{-}	1^{+}	1^{-}	1^{+}	1^{+}	1^{-}	1^{+}	1^{++}	1^{++}

b

Polyphenol oxidase isozymes	RF	bread						durum					
		M1	M2	SH1	L1	L2	L3	B4	B5	B6	D1	D2	D3
PPO1	0.1	1^{++}	1^{++}	1^{++}	1^{-}	1^{-}	1^{-}	1^{+}	1^{++}	1^{++}	1^{-}	1^{-}	1^{+}
PPO2	0.4	1^{++}	1^{+}	1^{+}	1^{-}	1^{-}	1^{++}	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}	1^{++}

1^{++} = High Density, 1^{+} = Moderate Density, 1^{-}= Low Density, 0 = Absent

B.3. Polymorphisms of malate dehydrogenase isozymes

The results of polyacrylamide gel electrophoresis on malate dehydrogenase (Mdh) isozymes of wheat genotypes under normal (Ashmon habitat) and stress (Siwa habitat) conditions are shown in Fig. 4:a,b and Table 4:a,b. The isozyme profiles showed four bands for malate dehydrogenase. These bands occurred in all genotypes under the two studied conditions. Genotypes cultivated at Siwa (stress) had higher activities of Mdh in all genotypes. The fourth band (Mdh4, RF=0.9) was detected with high density for all genotypes under stress (Siwa habitat) and normal (Ashmon habitat) conditions. Similar results were previously obtained by Sairam *et al*. (2001) and Zhang *et al.* (2000), who observed that water stress, increased some isozyme polymorphisms. Thus, the activity of Mdh is serving as objective enzyme test for determining cereal genotypes with high tolerance to salt stress.

Conclusion

Protein profiles (SDS-PAGE) could be useful markers for genotypes adaptation, by improving the efficiency of wheat breeding programs. The most stress tolerant genotypes D3 and D1 (under Siwa Oasis habitat) showed least peroxidase intensity bands. PPO isozyme intensity was increased in response to stress with highest magnitude of induction in older leaves and corresponding abscission zones. It might facilitate cell death preferentially in these tissues, as an adaptive strategy under stress, since it could reduce further water loss and allow limited nutrients to be partitioned to younger tissues. Wheat genotypes with high Mdh activity should have greater tolerance to stress, whereas low Mdh activity would indicate less tolerance.

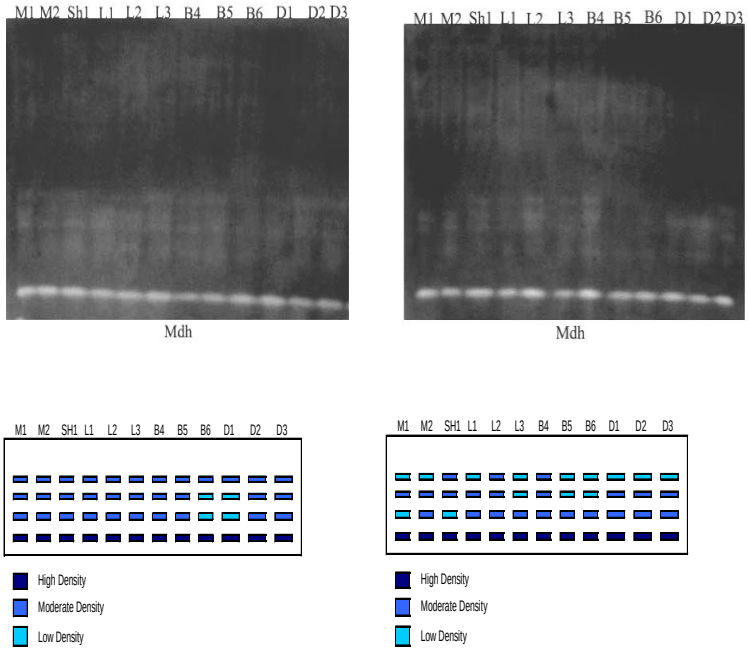

Fig. 4. Photograph of electrophoretic malate dehydrogenase isozyme patterns of bread (M1, M2, SH1, L1, L2 and L3) and durum (B4, B5, B6, D1, D2 and D3) wheat genotypes.
a. under stress (Siwa habitat) b. under normal condition (Ashmon habitat).

TABLE 4. Malate dehydrogenase isozyme intensity of bread (M1, M2, SH1, L1, L2 and L3) and durum (B4, B5, B6, D1, D2 and D3) wheat genotypes.
a a. under stress (Siwa habitat) b. under normal condition (Ashmon habitat).

Malate Dehydrogenase isozymes	RF	bread						durum					
		M1	M2	SH1	L1	L2	L3	B4	B5	B6	D1	D2	D3
Mdh1	0.5	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}
Mdh2	0.6	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{-}	1^{-}	1^{-}	1^{+}	1^{+}
Mdh3	0.7	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{-}	1^{-}	1^{-}	1^{+}	1^{+}
Mdh4	0.9	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}

b

Malate Dehydrogenase isozymes	RF	bread						durum					
		M1	M2	SH1	L1	L2	L3	B4	B5	B6	D1	D2	D3
Mdh1	0.5	1^{-}	1^{-}	1^{+}	1^{-}	1^{+}	1^{-}	1^{+}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}
Mdh2	0.6	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{-}	1^{+}	1^{-}	1^{-}	1^{+}	1^{+}	1^{+}
Mdh3	0.7	1^{-}	1^{+}	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}
Mdh4	0.9	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}

1^{++} = High Density, 1^{+} = Moderate Density, 1^{-}= Low Density, 0 = Absent.

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تباين نظائر الانزيمات لبعض التراكيب الوراثية للقمح تحت ظروف بيئتي واحة سيوة وأشمون

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تم تحليل البروتينات لاثنتى عشرة سلالة وراثية لنبات القمح باستخدام SDS-PAGE تحت تاثير الاجهاد البيئي (بيئة سيوة) مقارنة بالظروف الطبيعية (بيئة أشمون). واظهرت النتائج تباينا كميا وكيفيا محدودا بين السلالات النامية في بيئة سيوة وتلك النامية في الظروف الطبيعية. ولذا يعتبر تحليل البروتين باستخدام PAGE-SDS مفيدا لتقييم التقلبات والمورثات الجينية التي تساعد في تحديد برامج تربية القمح. تمت ايضا دراسة تعدد اشكال المتشابهات الانزيمية لسلالات نبات القمح المختارة تحت نفس ظروف الاجهاد (واحة سيوة) والظروف العادية (أشمون) ولوحظ زيادة تعدد الأشكال لنظائر انزيم المالات دهيدوجنيز في معظم السلالات النامية في واحة سيوة بينما انخفض تعدد الاشكال لنظائر انزيم البيروكسيديزفي هذه البيئة. وأظهرت النتائج أيضا أن تعدد الاشكال لنظير انزيم البولي فينول اوكسيديز يمكن أن يستخدم كمؤشر على مقاومة الاجهاد لمعظم سلالات نبات القمح بالاضافة الى استخداماته الاخرى في فسيولوجيا النبات. وأظهرت الدراسة ايضا ان نظائر الانزيمات قيد الدراسة متمثلة في نظائر البيروكسيديز والبولي فينول أوكسيديز والمالات دهيدوجنيز تلعب دورا هاما سواء بشكل مباشر أو غير مباشر في مقاومة الاجهاد البيئي.