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Laboratory Evaluation of Some Barley Genotypes under Drought and Salinity Stresses

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ABSTRACT

Laboratory evaluation of crop seeds is considered a technique which would be suitable for screening large populations to improve tolerance to adverse conditions such as drought and salinity prior to yield testing. Therefore, this study was conducted on six barley genotypes ('Giza 123', 'Giza 124', 'Giza 126', 'Giza 129', 'Giza 130' and 'Giza 2000') for general and specific evaluation under drought and salinity stresses. Drought stress was induced using polyethylene glycol (PEG) at three levels (5, 7.5 and 10 mg L^{-1}), while salinity stress was induced using mannitol at three levels (5, 7.5 and 10 mg L^{-1}). No germination of any genotype was observed with 10 mg L^{-1} PEG. Results indicate that there were clear and significant differences among genotypes in shoot length, root length, germination percentage, dry weight and seedling vigor index under the treatments of drought and salinity. There was a strong linear relationship between proline content and drought and salinity tolerance. As proline content increased tolerance to drought and salinity stresses increased. Results showed significant differences among genotypes for chemical composition, electrical conductivity (EC), accelerated ageing (AA) and 1000-kernel weight. Data of a phenol test indicated that barley genotypes can be divided into three categories; category 1 includes 'Giza 126', 'Giza 130' and 'Giza 2000', category 2 includes 'Giza 123', and category 3 includes 'Giza 124' and 'Giza 129'. The results of SDS-PAGE showed changes in the protein-banding pattern and band density under different treatments of drought and salinity. 'Giza 126' surpassed other genotypes in terms of drought and salinity tolerance, and hence it can be used in a barley breeding program for drought and salinity tolerance.

Keywords: abiotic stresses, *Hordeum vulgare* L., mannitol, polyethylene glycol, proline

INTRODUCTION

Drought is a worldwide problem, constraining global crop production seriously and global climate change has made this situation more serious (Pan *et al*. 2002; Bayoumi *et al*. 2008). Drought is a complex physio-chemical process, in which many biological macro- and micromolecules are involved, such as nucleic acids, proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements. Currently, study on drought has been one of the main directions in global plant biology and biological breeding.

Salinity is one of the major abiotic stresses which adversely affect crop growth and yield. High concentrations of salt resulting from natural processes or disarrangement in irrigated agriculture result in inhibition of plant growth and yield (Demiral and Turkan 2006). Salinity also induces water deficit even in well-watered soils by decreasing the osmotic potential of soil solutes, thus making it difficult for roots to extract water from their surrounding media (Sairam and Srivastava 2002).

Barley (*Hordeum vulgare* L.) is the world's fourth most important crop in terms of cultivated area (Sondeep *et al*. 2009) and it is considered as one of the most suitable crops, which can be grown over a wide range of environmental conditions. In Egypt, barley cultivated area is mostly under rainfed conditions and newly reclaimed lands.

The present investigation aimed to: (1) find a rapid and easy technique for screening barley genotypes for drought and salinity tolerance, (2) employ gel electrophoresis of protein in the leaves of barley genotypes to evaluate genetic variability under drought and salinity stress conditions, (3) find out the extent of changes in germination behavior stresses, and (4) find out the extent of changes in proline (Pro) content under stress conditions.

MATERIALS AND METHODS

The present study was carried out at the Department of Seed Technology Research, Field Crops Research Institute, ARC during 2009. Barley genotypes included six local cultivars 'Giza 126', 'Giza 2000', 'Giza 124', 'Giza 123', 'Giza 129' and 'Giza 130' obtained from Barley Research Department. The experiments were carried out in a randomized complete block design (RCBD) with four replications.

Salt stress was induced by mannitol (ADWIC, Egypt) treatment. Three salt stresses with osmotic potentials of 5, 7.5 and 10 bars were arranged as described by Elemery *et al*. (1995). Distilled water served as the control. Drought stress was induced using polyethylene glycol (PEG 6000) (BDH Laboratory supply, UK) treatment. Three drought stresses with concentrations of the same osmotic potentials of 5, 7.5 and 10 bars were arranged as described by Michel and Kaufmann (1973).

Seed vigor and seedling characteristics

1. Standard germination

50 pure seeds of each genotype with three replications were germinated in distilled water, mannitol solution and PEG 6000 at -5, -7.5 and -10 atm osmotic pressures. The seeds were placed on two layers of Whatman No. 2 filter paper in glass Petri dishes 150 mm in diameter, and 15 ml distilled water or mannitol solution or PEG 6000 solution were added to each dish. The dishes were placed in a germinator. Seeds were germinated for 7 days at 20°C. Germination counts were made after 4 days and daily till the end of the test. Normal seedlings were counted according to ISTA (1993). Germination percentage was calculated using the following formula outlined by Krishnasamy and Seshu (1990):

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$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Number of tested seed}} \times 100$$

Seed vigor index was calculated using the following formula (Copeland 1976):

$$\text{Seed vigor index} = \frac{\text{Number of seed germinated (1}^{st}\text{ count)}}{\text{Number of days to first count}} + \frac{\text{Number of seeds germinated (last count)}}{\text{Number of days to last count}}$$

2. Accelerated ageing test

The seeds were kept in an ageing chamber at 45°C and 100% relative humidity for 3 days. After ageing, the seeds were sun dried. Seed survival percentage was determined using the standard germination test at 20°C and the mean normal seedling percentage was calculated (AOSA 1983).

3. Electrical conductivity test

The electrical conductivity (EC) of the leachate was determined according to the procedures described by AOSA (1983). Four subsamples of 50 seeds of each cultivar were weighed and placed into plastic cups with 250 ml of distilled water, and held at 25°C. After 24 hr, the electrical conductivity of the leachates was determined using an EC meter (ORION Cat. No. 012210, Thermo Electron Co., USA). The mean values were expressed in μS cm^{-1} g^{-1} seed weight.

4. Seedling characteristics

Normal seedlings obtained from the standard germination test were used for seedling evaluation according to AOSA (1983). Seedling shoot and root length were measured after 8 days of germination test. 25 seedlings from each Petri dish were randomly selected and shoot and root lengths of individual seedling were recorded. The shoots and roots were also dried at 70°C for 72 h.

Seedling vigor index was calculated using data recorded on germination percentage and seedling growth according to ISTA (1985) by the formula:

Seedling vigor index (1) = seedling length (cm) × germination percentage

Seedling vigor index (2) = seedling dry weight (g) × germination percentage

5. Phenol test

Four replications of fifty seeds from each genotype were taken at random and placed over two layers of filter paper (Whatman, 15 cm diameter) previously soaked in 5 ml of distilled water in Petri dishes. The dishes were covered, allowing the seeds with the ventral crease downwards, to soak for 18 h at 22-23°C. The seeds were removed from the distilled water and deposited on two new layers of filter paper in Petri dishes and 5 ml of 0.1, 0.3, 0.5 and 1% (w/v) freshly made phenol solution at pH 4.8 was added. The dishes were covered and incubated at 22-23°C for 1, 2, 3 and 4 hr after which the seeds were classified into five color groups (- = negative, + = light brown, ++ = brown, +++ = dark and ++++ = very dark, as outlined by Saavedra and Laverack 1996).

6. Chemical composition

Samples of about 50 g of air-dried seeds of each genotype were randomly chosen from two replications for estimating seeds chemical composition. Crude protein, total carbohydrates and oil percentage were determined according to the methods of AOAC (2000).

7. Proline determination

Pro was determined in fully expanded leaves according to Pesci and Beffagna (1984).

Data analysis

All data were statistically analyzed by the analysis of variance method according to Snedcor and Cochran (1989). Differences among means were tested by L.S.D at $P = 0.05$. The resulted protein-banding patterns were analyzed in comparison to the protein marker using the computer program (Bio-1D).

Protein electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) procedure was carried out according to Laemmli (1970). Protein bands were visualized by staining the gel with 0.25% Coomassie Brilliant Blue R-250. Protein band sizes were determined by comparisons with the high molecular weight protein marker.

RESULTS AND DISCUSSION

Effect of PEG (drought stress) and mannitol (salinity stress) on protein patterns using SDS-PAGE

In an attempt to understand the molecular basis of drought and salinity tolerance, proteomics using SDS-PAGE were analyzed to identify protein patterns involved in drought and salinity stresses response in the six barley genotypes. Detection of proteins whose levels were altered by PEG and mannitol stresses was done by comparing patterns from control and PEG and mannitol-treated plants.

PEG (drought stress)

Proteins were extracted from the seedlings, which were treated with 5 and 7.5% PEG and separated by SDS-PAGE. A set of control plants was grown without adding PEG under the same conditions as the stressed plants. Protein bands detected ranged from 12.5 to 711.5 kDa (**Table 1**). Newly synthesis protein bands are indicated by grey shading in **Table 1**. Consequently, these bands can be considered as molecular markers to characterize drought tolerance and interpreted as adaptive bands to drought stress; these newly synthesized proteins might indicate that PEG induced a stress related to genes that produce these drought-inducible proteins. Water deficit alters plant gene expression and leads to specific genes, producing an increase of their transcripts and thus an increase of corresponding proteins (Ingram and Bartels 1996).

'Giza 124', 'Giza 129 and 'Giza 130' exhibited more intense bands under drought stress; bands were faint in control plants. These faint bands may be intact proteins or degradation products (Close *et al*. 1993) considering that band intensity is directly related to protein concentration in barley seedlings (Farooq *et al.* 2009). Various investigators suggested that the low protein concentration is attributed to the decrease rate of protein synthesis, the increase activities of hydrolyzing enzymes, the decreased availability of amino acids or the denaturizing of the enzymes involved in amino acids and protein synthesis (Dubey and Rani 1990; Dubey 1994). Riccardi *et al*. (1998) reported that water deficit induced the expression of proteins not specifically related to this stress, but rather to reactions against cell damage.

Mannitol (salinity stress)

Proteins were extracted from the seedlings, which were treated with 5 7.5 and 10% mannitol and separated by SDS-PAGE. A set of control plants was grown without adding mannitol under the same conditions as the stressed plants. Protein bands detected ranged from 44.6 to 340.4 kDa (**Table 2**). Newly synthesis protein bands are indicated by grey shading in **Table 2**.

'Giza 123, 124, 129 130 and 2000' genotypes exhibited more intense bands under salinity stress but these were faint for control plants. These faint bands may be intact proteins

Table 1 SDS-PAGE of total proteins extracted from the leaves of six barley genotypes under polyethylene glycol (PEG) treatments.

MW	Giza 124			Giza 129			Giza 130			Giza 126			Giza 123			Giza 2000		
	Control	5.0 mg L^{-1}	7. mg L^{-1}	Control	5.0 mg L^{-1}	7.5 mg L^{-1}	Control	5.0 mg L^{-1}	7.5 mg L^{-1}	Control	5.0 mg L^{-1}	7.5 mg L^{-1}	Control	5.0 mg L^{-1}	7.5 mg L^{-1}	Control	5.0 mg L^{-1}	7.5 mg L^{-1}
711.5	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-
688.7	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-
667.5	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
640.2	-	-	-	+	+	+	-	+	-	+	-	-	+	-	+	+	+	-
635.6	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-
617.4	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
594.7	+	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-
555.3	-	-	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	+
543.1	+	+	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+
518.9	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
509.8	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
496.1	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
482.5	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
440.0	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
228.0	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
210.2	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+	+
203.6	+	+	+	+	-	-	+	+	-	-	-	-	+	+	+	+	-	-
193.8	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	+	-	+
184.0	-	-	+	+	-	-	+	+	-	+	-	+	+	+	+	+	+	+
178.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
164.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
148.9	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+
145.3	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-
138.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
131.7	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-
112.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
106.7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
94.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
85.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
77.2	-	-	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-
74.4	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	-	+	+
64.2	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	+	-	-
45.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26.3	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-
21.8	+	+	-	+	-	-	+	-	-	-	-	-	+	+	+	-	-	-
14.3	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
12.5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	19	13	14	22	19	17	19	20	19	21	17	17	20	20	22	24	21	18

+ = Band presence; - = Band absence; Grey shading = newly-formed bands

or degradation products (Close *et al*. 1993) considering that the band intensity is directly related to protein concentration in barley seedlings (Farooq *et al.* 2009).

Proline

In view of the fact that the accumulation of Pro is tightly controlled by genes and cDNA encoding osmolyte biosynthesis and only achieved when the rate of synthesis prevails over that degradation, probably because too much Pro is toxic to plant cells (Yokota *et al*. 2006; Bayoum *et al*. 2008).

Proline content and drought tolerance

The data presented in **Table 3** shows significant differences among genotypes and among treatments. Results indicated that Pro content increased in all treatments as compared to control. It might be an adaptation to the purpose of which is to overcome the stress conditions and it could supply energy for growth and survival and thereby help the plant to tolerate stress (Sankar *et al*. 2007). These genotypes, which had high Pro content, might increase ability to synthesize osmotic regulators (Pro) for protection from the damage of soil water deficits. Furthermore, Pro may play a role as an enzyme-stabilizing agent and has the ability to mediate osmotic adjustment, stabilized sub-cellular structure and scavenge free radicals (Hassanein 2004).

However, these genotypes which have over-accumulation Pro clearly demonstrated that selection for Pro could be used as a biochemical marker for increased stress tolerance in conventional crop breeding program and could lead to development of varieties and eventually to plants with heritable stress tolerance. In addition to Shivkumar *et al*. (1998) and Silveira *et al*. (2003) who showed that Pro accumulation was indeed a heritable trait and they concluded that selection for high Pro had been effective and played an important role in rehydration of protoplasm and osmotic adjustment are hypothesize to enhance drought tolerance in plants.

Proline content and salinity tolerance

One of the compatible solute which accumulates under salt stress in plants is Pro. In the present study, an increase in Pro accumulation in all genotypes under salinity was found (**Table 4**). Although the precise role of Pro accumulation is still debated, it is often considered as a compatible solute involved in osmotic adjustment (Azooz *et al*. 2004). The accumulation of Pro may be through an increase in its synthesis constantly with inhibition of its catabolism (Yoshiba *et al*. 1997) and may be a mechanism for stress tolerance. However, its role in imparting stress tolerance under saline conditions is controversial. Anyway, understanding the biosynthesis, degradation, transport and role of Pro during

Table 2 SDS-PAGE of total proteins extracted from the leaves of six barley genotypes under mannitol treatments.

M.W	Giza 124				Giza 129				Giza 130				Giza 126				Giza 123				Giza 2000			
	Control	5.0 mg L-1	7.5 mg L-1	10.0 mg L-1	Control	5.0 mg L-1	7.5 mg L-1	10.0 mg L-1	Control	5.0 mg L-1	7.5 mg L-1	10.0 mg L-1	Control	5.0 mg L-1	7.5 mg L-1	10.0 mg L-1	Control	5.0 mg L-1	7.5 mg L-1	10.0 mg L-1	Control	5.0 mg L-1	7.5 mg L-1	10.0 mg L-1
340.4	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
322.6	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
261.4	-	-	-	-	-	-	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
250.0	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
210.5	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+
166.3	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
128.0	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
118.1	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
100.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+
97.2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
94.2	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-
91.7	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	+
91.0	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-
87.3	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+	-	-	+
86.1	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
84.4	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
83.3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-
77.7	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+
70.9	-	-	+	+	-	+	+	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	+	+
55.3	+	+	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	+	-	+	-	-
51.5	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	+	-	-
49.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
47.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
46.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
44.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total	12	12	13	14	14	14	15	16	13	14	12	11	14	14	12	11	13	13	13	16	13	14	12	15

+ = Band presence; - = Band absence; Grey shading = newly-formed bands

Table 3 Effect of polyethylene glycol treatments on seedling vigor index, S/R ratio and proline content of six barley genotypes.

Genotypes	Control				5 mg L-1				7.5 mg L-1				10 mg L-1			
	Seedling vigor index (1)	Seedling vigor index (2)	Shoot / root ratio S/R ratio	Proline	Seedling vigor index (1)	Seedling vigor index (2)	Shoot / root ratio S/R ratio	Proline	Seedling vigor index (1)	Seedling vigor index (2)	Shoot / root ratio S/R ratio	Proline	Seedling vigor index (seedling length)	Seedling vigor index (seedling dry weight)	Shoot / root ratio S/R ratio	Proline
Giza 126	1741	18.16	1.185	1.6	602.3	13.30	1.414	2.2	254.8	5.68	1.277	3.0	0.00	0.00	0.00	0.00
Giza 123	1704	18.47	1.162	1.4	514.7	11.57	1.426	1.7	211.5	4.15	1.125	2.1	0.00	0.00	0.00	0.00
Giza 124	1543	17.90	1.275	1.3	473.6	10.45	1.765	1.6	161.5	3.39	1.094	1.8	0.00	0.00	0.00	0.00
Giza 2000	1521	16.76	1.482	1.3	433.5	9.03	1.687	1.6	135.9	3.05	1.045	2.4	0.00	0.00	0.00	0.00
Giza 129	1344	15.47	1.451	1.4	365.8	7.83	1.754	1.7	99.82	2.12	1.078	2.2	0.00	0.00	0.00	0.00
Giza 130	1489	15.34	1.464	1.2	320.3	6.69	1.604	1.4	57.53	1.15	1.201	2.3	0.00	0.00	0.00	0.00

Where:	L.S.D	C.V.
Seedling vigor index (I)	38.12	4.29 %
Seedling vigor index (2)	0.299	2.40 %
Shoot/root ratio	0.201	11.95 %
Proline	0.052	2.75 %

stress and the signaling events that regulate stress-induced accumulation is vital in developing plants for stress tolerance (Kavikishore *et al*. 2005).

Comparison of drought tolerance among barley genotypes

As screening technique, the survival ability of the six barley genotypes to tolerate chemical desiccation by PEG during the growth of seedling is exhibited in **Table 5**. Seedling development under laboratory conditions have been accepted as suitable growth stages for testing the drought tolerance in barley. It could be speculated that the presence of increased concentrations of PEG during the growth of seedling inhibits the developmental traits and survival of barley seedling (**Table 5**). The results show that there was no germination for all genotypes under 10% PEG treatment. Shoot length, root length, germination percentage and dry weight were always decreased by exposure to all the stress levels tested. It was clear that as the stress level increases, the seedling vigor index decrease (**Table 5**). A similar observation was reported by Radhouane (2007) for pearl millet. The results show that 'Giza 126' was better than the other genotypes under the stress levels tested. The tested genotypes varied significantly in their reaction to PEG. However, the reduction in shoot and root length may be due to an impediment of cell division and elongation leading to kind of tuberization. This tuberization and lignification of the root system allows the plant to enter a slowed-down state, while waiting for the conditions to become favorable again (Fraser *et al*. 1990). This technique would appear to be suitable for screening large populations to improve drought toler-

Table 4 Effect of mannitol treatments on seedling vigor index, S/R ratio and proline content of six barley genotypes.

Genotypes	Control				5 mg L^{-1}				7.5 mg L^{-1}				10 mg L^{-1}			
	Seedling vigor index (1)	Seedling vigor index (2)	Shoot / root ratio S/R ratio	Proline	Seedling vigor index (1)	Seedling vigor index (2)	Shoot / root ratio S/R ratio	Proline	Seedling vigor index (1)	Seedling vigor index (2)	Shoot / root ratio S/R ratio	Proline	Seedling vigor index (seedling length)	Seedling vigor index (seedling dry weight)	Shoot / root ratio S/R ratio	Proline
Giza 126	1741	18.16	1.19	1.6	1110	15.59	1.01	2.6	665.7	10.71	1.14	3.7	298.6	5.90	1.24	6.1
Giza 123	1704	18.47	1.16	1.4	961.1	14.62	1.10	1.7	618.8	10.15	1.14	2.1	239.7	4.81	1.32	7.3
Giza 124	1543	17.90	1.28	1.3	868	14.19	1.37	3.4	552.1	9.94	1.28	4.5	219.4	4.22	1.45	7.2
Giza 2000	1521	16.76	1.48	1.3	742.1	12.91	1.26	1.9	497.2	9.16	1.29	4.0	189.5	3.77	1.35	10.3
Giza 129	1344	15.47	1.45	1.4	699.8	12.15	1.19	4.0	455.9	8.52	1.38	4.9	151.4	2.79	1.35	8.6
Giza 130	1489	15.34	1.47	1.2	618.3	11.63	1.10	2.5	392.1	7.70	1.45	3.6	124.7	2.09	1.33	7.6

Where:	L.S.D	C.V.
Seedling vigor index (I)	45.42	3.54 %
Seedling vigor index (2)	0.371	2.07 %
Shoot/root ratio	0.195	9.07 %
Proline	0.187	2.85 %

Table 5 Effect of polyethylene glycol treatments on germination (%), root length, shoot length, dry weight and seed vigor index of six barley genotypes.

Genotypes	Control					5 mg L^{-1}					7.5 mg L^{-1}					10 mg L^{-1}				
	Germination (%)	Root length (cm)	shoot length (cm)	Dry weight (mg)	Seed vigor index	Germination (%)	Root length (cm)	shoot length (cm)	Dry weight (mg)	Seed vigor index	Germination (%)	Root length (cm)	shoot length (cm)	Dry weight (mg)	Seed vigor index	Germination (%)	Root length (cm)	shoot length (cm)	Dry weight (mg)	Seed vigor index
Giza 126	95.0	8.40	9.93	191.1	25.40	75.0	3.33	4.70	177.3	20.46	56.0	2.00	2.55	101.4	15.00	0.00	0.00	0.00	0.00	0.00
Giza 123	95.0	8.30	9.63	194.4	24.65	71.0	3.00	4.25	162.9	18.89	49.7	2.10	2.25	83.60	14.10	0.00	0.00	0.00	0.00	0.00
Giza 124	94.7	7.17	9.13	189.1	24.69	69.0	2.49	4.37	151.4	18.77	44.3	1.74	1.90	76.5	12.25	0.00	0.00	0.00	0.00	0.00
Giza 2000	93.3	6.57	9.73	179.6	24.17	66.0	2.47	4.10	136.8	17.35	39.0	1.70	1.78	78.30	10.90	0.00	0.00	0.00	0.00	0.00
Giza 129	89.0	6.17	8.93	173.8	22.21	61.3	2.17	3.80	127.6	15.86	33.0	1.46	1.57	64.13	9.05	0.00	0.00	0.00	0.00	0.00
Giza 130	89.3	6.77	9.90	171.7	23.01	58.0	2.12	3.40	115.2	14.79	20.7	1.27	1.51	55.60	5.95	0.00	0.00	0.00	0.00	0.00

where:	L.S.D	C.V.
Germination %	1.87	2.27 %
Root length	0.312	6.55 %
Shoot length	0.368	5.76 %
Dry weight	3.107	1.87 %
Seed vigor index	0.716	3.30 %

ance prior to yield testing.

Comparison of salinity tolerance among barley genotypes

The ability of the six barley genotypes to tolerate chemical salinity by mannitol during the growth of seedlings is

Table 6 Effect of mannitol treatments on germination (%), root length, shoot length, dry weight and seed vigor index of six barley genotypes.

Genotypes	Control					5 mg L^{-1}					7.5 mg L^{-1}					10 mg L^{-1}				
	Germination (%)	Root length (cm)	shoot length (cm)	Dry weight (mg)	Seed vigor index	Germination (%)	Root length (cm)	shoot length (cm)	Dry weight (mg)	Seed vigor index	Germination (%)	Root length (cm)	shoot length (cm)	Dry weight (mg)	Seed vigor index	Germination (%)	Root length (cm)	shoot length (cm)	Dry weight (mg)	Seed vigor index
Giza 126	95.0	8.40	9.93	191.1	25.40	86.3	6.38	6.47	180.5	22.08	68.0	4.58	5.20	157.6	17.88	49.0	2.73	3.37	120.4	12.75
Giza 123	95.0	8.30	9.63	194.4	24.65	81.0	5.60	6.17	179.1	20.42	65.0	4.45	5.07	156.2	16.87	44.0	2.35	3.10	109.2	11.79
Giza 124	94.7	7.17	9.13	189.1	24.69	81.0	4.52	6.20	175.2	20.49	64.3	3.77	4.82	154.4	15.94	41.0	2.23	3.12	102.9	1061
Giza 2000	93.3	6.57	9.73	179.6	24.17	78.7	4.18	5.25	164.2	19.15	61.7	3.53	4.53	148.6	14.89	38.0	2.13	2.85	99.30	9.93
Giza 129	89.0	6.17	8.93	173.8	21.96	75.0	4.27	5.07	162.0	17.96	58.3	3.31	4.50	146.0	14.25	31.0	2.08	2.80	90.03	9.43
Giza 130	89.3	6.77	9.90	171.7	23.01	71.3	4.13	4.53	163.0	16.69	55.7	2.88	4.17	138.4	13.45	24.7	2.17	2.88	84.67	8.02

where:	L.S.D	C.V.
Germination %	1.20	1.72 %
Root length	0.403	5.43 %
Shoot length	0.299	3.17 %
Dry weight	4.691	1.89 %
Seed vigor index	0.776	2.72 %

shown in **Table 6**. Seedling development under laboratory conditions is suitable for testing the salinity tolerance in barley. The presence of increased concentrations of mannitol during the growth of seedlings appeared to inhibit the developmental traits and survival of barley seedlings (**Table 6**). The tested genotypes varied under the stress levels tested. A trend of decreasing germination percentage, root length, shoot length, dry weight and seedling vigor index with increasing mannitol concentrations was found. At 10 mg L^{-1} mannitol, germination was highly inhibited. Inhibition was greatest in 'Giza 130' among all genotypes. Inhibition of germination due to salinity has been reported in greegram (Misra and Dwivedi 2004). Decreasing germination due to increasing salinity can be correlated to the nature of salinity to reduce imbibition of water due to lowered osmotic potentials of the medium that cause changes in metabolic activity (Yupsanis *et al*. 1994). Moreover, salinity perturbs the plant hormone balance (Khan and Rizvi 1994) and reduces the utilization of seed reserves (Ahmad and Bano 1992). Seedling vigor was estimated by means of seedling shoot and root length. Shoot and root growth were reduced by salinity stress (**Table 6**). Salt stress inhibits the efficiency of the translocation and assimilation of photosynthetic products (Xiong and Zhu 2002) and might have caused the reduction in shoot growth. Reduction in plant growth has also been attributed to reduced water absorption due to an osmotic effect, nutritional deficiency on account of an ionic imbalance and a decrease in many metabolic activities (Kumar *et al*. 2005). 'Giza 126' surpassed other genotypes in salinity tolerance. This technique would appear to be suitable for screening large populations to improve salinity tolerance prior to yield testing.

Seed chemical analysis

Data presented in **Table 7** shows the chemical composition, electrical conductivity (EC), accelerated ageing (AA) and 1000-kernel weight of all six cultivars. Protein percentage varied from 8.3 to 12.8%. The highest protein percentage was observed in 'Giza 123' and 'Giza 130', while 'Giza 129' had the lowest percentage. Carbohydrate percentage ranged from 59.0 to 65.7%. 'Giza 129' and 'Giza 2000' had the highest value; the lowest percentage was in 'Giza 123' and 'Giza 126'. The oil percentage varied from 4.1 to 4.8%.

Data indicated that EC values ranged from 31.3 to 43 µS cm^{-1} g^{-1}, where 'Giza 126' gave the highest vigor grain, while the lowest vigor grain was obtained from genotype 'Giza 130'. It was clear that electrolyte leakage measured from high vigor grain was less than that measured from low vigor grain because the higher vigor grain was able to recognize their membranes more rapidly and repair any damage to a greater extent than the low vigor grain. Also, 1000-kernel weight varied from 35 to 57.9 g. The heaviest 1000-kernel weight was obtained from 'Giza 2000', while the lowest 1000-kernel weight was obtained from 'Giza 129'. Data indicated that AA germination test ranged from 60.7 to 82.7%. 'Giza 124' gave the highest AA value, while the lowest value was obtained from 'Giza 130'. Many investigators used seed chemical analysis to discriminate among genotypes (Abd-Alla *et al*. 2007; Katja *et al*. 2009).

Table 7 Protein percentage, carbohydrate percentage, oil percentage, electrical conductivity, accelerated ageing and 1000-kernel weight of six barley genotypes.

Genotypes	Protein (%)	Carbohydrate (%)	Oil (%)	Electrical conductivity (µScm^{-1}g^{-1})	Accelerated ageing (%)	1000-kernel weight
Giza 126	9.3	59.6	4.1	31.3	70.7	52.7
Giza 123	12.8	59.0	4.2	33.0	68.3	49.7
Giza 124	12.0	60.9	4.7	34.2	82.7	53.4
Giza 2000	9.3	65.4	4.5	37.0	77.3	57.9
Giza 129	8.3	65.7	4.8	40.5	67.7	35.0
Giza 130	12.0	61.8	4.4	43.0	60.7	41.7
L.S.D. 5%	1.17	1.10	0.411	1.61	6.81	1.69
C.V. %	6.06	0.97	5.06	2.42	5.26	1.92

Phenol test

This test is used for discrimination among genotypes of cereal crops such as barley, rice and wheat. Data in **Table 8** show the reactions among the six genotype kernels and different concentrations of phenol. The observations were scored after 1, 2 and 3 hr. Data indicated that genotypes can be divided into three categories; category 1 includes 'Giza 126', 'Giza 130' and 'Giza 2000', category 2 includes 'Giza 123', and category 3 includes 'Giza 124' and 'Giza 129'. This test has been used to discriminate among genotypes of wheat (Selim 2004), barley (El-Sayed *et al*. 2007) and rice (Nethra *et al*. 2007).

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Table 8 Visual assessment of phenol reaction of six barley genotypes grains.

Conc. / Genotypes	0.1 %			0.3 %			0.5 %			1 %			2 %		
	1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.
Giza 126	+	++	++	+	++	++	+	++	++	++	+++	+++	++	++++	++++
Giza 123	-	+	+	-	+	+	+	++	++	+	++	++	++	++	++
Giza 124	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
Giza 2000	+	++	++	+	++	++	+	++	++	++	+++	+++	++	++++	++++
Giza 129	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
Giza 130	-	+	+	-	+	+	+	++	++	+	++	++	++	+++	+++

Where: (-) = negative color; (+) = light brown; (++) = brown; (+++) = dark brown; (++++) = black.

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