PHYSIOLOGICAL AND MOLECULAR MARKERS FOR SALT TOLERANCE IN FOUR BARLEY CULTIVARS

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Abstract

The present investigation was carried out to detect physiological and molecular markers for salt tolerance in barley. Four cultivars of barley were screened for their tolerance against salt stress (9000 ppm). with respect to the performance of some physiological parameters such as germination percentage and abscisic acid (ABA) content and develop RAPD-PCR markers in barley linked to salt tolerance.

The results of physiological analysis revealed a depression in germination percentages and an accumulation of abscisic acid (ABA) content in the stressed plants than those of the controls. The content of abscisic acid was much greater in the tolerant cultivars than in the sensitive ones.

RAPD analysis was done utilizing six 10-*mer* random primers. The results showed the occurrence of some molecular genetic markers associated with salt tolerance. In conclusion, the physiological and molecular markers would be useful in screening different cultivars for their tolerance against salt stress during breeding programs of barley.

Keywords: Barley, *Hordeum vulgare*, Salt stress, Germination, Abscisic acid, RAPD-PCR markers

Introduction

Barley is the main crop grown on a large scale in coastal regions such as the new reclaimed land and in soils with chemical problems (saline soils). Total area of cultivated barley fluctuates from one year to another due to the rainfall amount and its distribution in Egypt. Cultivated production area in the Nile Valley decreased gradually, on the other hand, barley production area increased in the new reclaimed lands under different irrigation systems.

Walia *et al*., (2006) reported that barley (*Hordeum vulgare* L.) is a salt-tolerant crop species with considerable economic importance in salinity-affected arid and semiarid regions of the world.

Plants respond to salt stress through modifications of their morphological, physiological and metabolic processes. Selection of plant cultivars with considerable resistance to salt stress has been considered as economic and efficient means of utilizing salt-prone areas when combined with appropriate management practices (Blum, 1974; Turner, 1991 and Quisenberry, 1992). Therefore, improved tolerance is one of the major objectives in plant breeding programs for crops grown in arid and semi-arid areas (Anderson and Reinbergs, 1985 and Matin *et al.*, 1989).

The effects of salt on germination and early seedling development can be used for rapid screening. On the other hand, salt effects can be avoided at early stages to improve tolerance at later stages (Nieman and Shannon, 1977). The authors however, preferred to conduct a separate experiment to detect the salt effects on seed germination because they assumed that they were dealing with a different set of genes at the seedling stage versus later stages.

Maiti and Huerta (1990) assessed salt tolerance at the seedling stage of 25 genotypes of Sorghum by germinating them in 0.4 M NaCl and CaCl2. They reported that germination percentage could be used as a reliable indicator of salinity resistance.

Mallek *et al*. (1998) studied the effects of salinity on seed germination of six varieties of barley grown in Tunisia. Six salt concentrations were used (NaCl 0 to 153 mM). The effects of NaCl on germination were evaluated by two criteria: radicle emergence from seed and leaf emergence from the coleoptile's tip. The results classify the different varieties according to their salt tolerance. Germination as well as plantlet emergence can be considered as indicators of salt stress tolerance for cereals at the first stages of development.

ABA levels increased in tissues subjected to osmotic stress by salt. Under these conditions, specific genes are expressed that can also be induced in unstressed tissues by the application of exogenous ABA (Skriver and Mundy, 1990). Some of these genes appeared to be a part of a general response to osmotic stress. Napin and β-conglycinin are proteins that constitute a part of the plant's response to osmotic stress. These genes and other ABA-responsive genes are expressed in various plant organs in response to ABA or osmotic stress. Certain ABA-responsive genes may encode RNA-regulatory proteins capable of altering developmental events in plants (Jin *et al*., 2000).

Using four different metabolic processes (crassulacean acid metabolism 'CAM', amino acids metabolism, osmo-protection, and plant defense mechanisms) as indicators, the relationship between salt stress and plant growth regulators in *Mesembryanthemum crystallinum* plant was studied by Thomas and Bohnert (1993). Sodium chloride was found to be

more effective than ABA in stimulating the accumulation of proline and osmotin-like proteins. Generally salt stress increased ABA accumulation but with different rates in different growth stages. Moon *et al*. (1995) compared the effects of salt and exogenous ABA on the roots of some salt sensitive and salt tolerant rice varieties. Endogenous ABA levels showed a transient increase in roots exposed to a salt shock (150 mM NaCl). In the tolerant varieties, ABA concentration increased by 30-fold, whereas it increased only by 6-fold in the sensitive varieties. The abundance of the ABA-induced proteins was highest in the most tolerant varieties. Three ABA-responsive proteins were present at different levels in roots from tolerant and sensitive varieties. Both the salt-induced increase of endogenous ABA levels and the different molecular responses of the root tissues to ABA were associated with the varietal differences in salt tolerance.

Molecular markers such as sodium dodecyl sulphate (SDS)-protein, isozymes and RAPD (Randomly Amplified Polymorphic DNA) have recently shown excellent potentiality to assist selection of quantitative trait loci (QTLs) associated with these traits (Xue *et al*., 2009). Stuber, 1992 represented valuable and reliable tools for the identification of the desired genotypes independent to environmental variations. In addition, marker-assisted breeding can offer an efficient and rapid mean to identify and incorporate adapted germplasms into Egyptian cultivars.

Using the Polymerase Chain Reaction (PCR), single-copy genomic sequences were readily amplified by a factor of more than 10 million copies with high specificity and DNA segments up to 2,000 base pairs (Saiki *et al.*, 1988).

Williams *et al.* (1990) described DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence. These polymorphisms, simply detected as DNA segments amplified from one parent but not the other, are inherited in a Mendelian fashion and can be used to construct genetic maps in a variety of species. They suggested that these polymorphisms to be called RAPD markers.

Bahieldin and Ahmed (1994) tested six barley cultivars (*Hordeum vulgare* L.) for RAPD marker using agarose as well as DGGE (denaturing gradient gel electrophoresis) methodology with 29 arbitrary 10-*mer* primers. Among a total of 418 bands observed, 39 were polymorphic markers. These markers were sufficient to distinguish between barley cultivars. The cultivar-specific markers represented 62% of the total RAPD markers regardless of gel matrix. Most of these markers were scored for the absence of common bands. Cultivar-specific markers can, subsequently, be used in detecting linkage map that involve any polymorphic gene(s). The barley cultivars were surveyed for relationships based on marker differences. In conclusion,

RAPD markers provided a quick and reliable alternative to identify barley cultivars and also as genetic markers for salt tolerance.

Klara *et al*. (2007) determined genetic relationships between 38 barley genotypes with the aid of RAPD, STS (sequence tagged site) and SSR(simple sequence repeat) markers and demonstrated that RAPD markers could be employed both for estimating the relationships between varieties and for variety identification.

Giora and Uri (2012) reported that Genotypic information is required in the form of markers for any quantitative trait loci involved (marker-assisted selection) or of direct knowledge of the genes.

The aim of this work to Study the relative salt tolerance of four cultivars of barley with respect to the performance of some physiological parameters such as germination percentage and abscisic acid (ABA) content and develop RAPD markers in barley linked to salt tolerance.

Materials and Methods

1- Materials:

Grains were provided by Barley Research Department, Field Crops Research Institute, Agriculture Research Center (ARC), Giza, Egypt (Table 1).

Table (1): Serial number, names and Pedigree of the investigated four cultivars of barley used in this study.

Serial No.	**Cultivar's name**	**Pedigree**
1	Giza 125	Giza 117/Bahteem 52//Giza 118/FAO86
2	Giza 126	WI 2291/4/11012-2/70-2245/3/Apam/IB65/A16
3	Rihane 03	As46//Avt/Ath8
4	Giza 124	Giza 117/Bahteem52/Giza 118/FAO86, Line 366.16.2

The four cultivars were sown in three replications under two concentrations (0.0 ppm-9000 ppm) in a sand culture experiment, which was conducted according to the technique of Haekel *et al*. (1981). Modified Hogland solution suggested by Johnson *et al*. (1957) was used as nutrient supplement. Salt solution with concentration of 9000 ppm was prepared by adding NaCl and $CaCl_2$ with a ratio of 3:1 g/l, respectively. Plants were taken for molecular analyses at day 40 from planting in the sand culture.

2- Methods:

Germination percentage (%):

Twenty grains were used from each cultivar (ten in the control and ten in 9000 ppm) Grains for each cultivar were put together in a germination dish at 16°C±1 in growth chamber for 11 days. Measurements for germination percentages were recorded at days 7 through 11 days.

Abscisic acid analysis:

Abscisic acid was extracted, methylated and estimated according to the method adopted by Wasfy *et al*. (1975).

DNA isolation:

DNA isolation from plant tissues was carried out using DNeasy plant Mini Kit (Qiagen Inc. Dneasy plant mini handbook).

RAPD-PCR conditions:

RAPD-PCR reactions were conducted using 6 arbitrary random 10-*mer* primers with the following sequences indicated in Table (2).

Table (2): Random primer names and their sequences for RAPD-PCR analysis.

Primer's name	**Sequence**
OP-A03	5′ AGT CAG CCA C 3′
OP-A10	5′ GTG ATC GCA G 3′
OP-B08	5′ GTC CAC AGG G 3′
OP-B14	5′ TCC GCT CTG G 3′
OP-D20	5′ ACC CGG TCA C 3′
OP-Z11	5′ CTC AGT CGC A 3′

The reaction conditions were optimized and mixtures (50 µl total volume) consisted of dNTPs, $MgCl_2$, 10X buffer, Primer and Template DNA.

The reaction mixtures were overlain with a drop of light mineral oil per sample. Amplification was carried out in a Perkin Elmer 2400 thermocycler programmed for 42 cycles as follows: 94°C/4 min (1 cycle); 94°C/1 min, 37°C/1 min. 72°C/2 min. (40 cycles); 72°C/10 min. (1 cycle) and 4°C (infinitive).

Gel electrophoresis:

Agarose (1.2%) was used for resolving the PCR products. Two standard DNA markers were used {1} a one kbs plus ladder (GIBCOBRL, Cat. No. 10787-026) its molecular sizes (MS) in bp of the 12 marker bands are: 12000, 5000, 2000, 1650, 1000, 850, 650, 500, 400, 300, 200 and 100 {2} a Kb DNA ladder (Stratagene) its molecular sizes in bp of the 8 marker bands are: 12000, 3000, 2000, 1500, 1000, 750, 500 and 250. according to the method of Maniatis *et al.* (1982).

The run was performed for one hour at 100 V in a Bio-Rad™ submarine (8 cm x 12 cm). Bands were detected on UV-transilluminator and photographed by Gel Doc 2000 Bio-Rad™ and analyzed by diversity database V.2.1.1.

Statistical analysis:

The data for germination percentage (Table 3) were statistically analyzed using the method of Gomez and Gomez (1984).

Results

The results of germination rate and germination percentage are presented in Table (3).

Measurements for germination percentages were recorded at days 7 through 11 days.

The statistical analysis for cultivars at day 7 showed no significant differences in germination percentage among cultivars throughout control and salt treatments, and also across treatments. The means for cultivar 4 was higher than those for other cultivars (100.00%) under salt stress condition.

The results from day 8 to day 11 showed significant differences among cultivars throughout treatments and also across treatments. At day 11, cultivar 2 showed the lowest mean of germination percentage (90.55%), while cultivar 4 recorded the highest mean of germination percentage (100.00%) under salt stress condition.

The results of germination rate at day 11, in the sensitive cultivars (1 and 2) showed that the germination rate of control was higher than the germination rate of treated grains.

On the other hand, the results of in the tolerant cultivars (3 and 4) revealed that the germination rate of grains under salt stress condition was higher than germination rate under control except for cv. 3 in which the germination rate of control was slightly higher than that of the salt stress.

The results of abscisic acid contents in the four cultivars of barley are shown in Table (4). Abscisic acid content was generally increased in the four cultivars under salt stress as compared to its content in plants grown under control condition. The increasing folds in abscisic acid content under salt treatment varied among the cultivars. It was about 174.68 and 165.89 folds in the tolerant cultivars (cv. 3 and cv. 4), 57.73 and 133.14 folds in the sensitive cultivars (cv. 1 and cv. 2) respectively.

These results indicated that abscisic acid content increased in the salt tolerant cultivars with higher levels than those of the salt-sensitive cultivars.

Application of RAPD analysis for the identification of barley cultivars and the detection of molecular markers linked to salt tolerance.

In this study, DNAs were isolated from two sensitive (1 and 2) and two salt tolerant (5 and 6) barley (*Hordeum vulgare* L.) cultivars.

For molecular analysis, DNAs of these cultivars were subjected to PCR against six different random 10 *mer* primers. The number of bands for each primer varied from 1 to 11 bands. The sizes of amplified fragments ranged from 160 to 5200 bp (Plate 1 and Tables 5 and 6).

The results of primer A-03 indicated the occurrence of four monomorphic bands and three polymorphic bands one of them was negative molecular markers for salt tolerance and the remaining were two cultivar specific bands related to cultivar 1 with molecular size 790 bp and 370 bp. (Plate 1 and Tables 5, 6, 7 and 8).

The results of RAPD against primer A-10 indicated the production of one positive marker with molecular size of 970 bp where it was present in the tolerant cultivars 3 and 4. And two negative bands related to sensitive cultivars 1 and 2).wit molecular size 950 and 900 bp respectively. and five monomorphic bands (Plate 1 and Tables 5, 6, 7 and 8).

The results of primer B-08 indicated the possible presence of one positive marker (500 bp) present in cultivars 3 and 4,and one negative marker (630 bp) present in cultivars 1 and 2 and one cultivar specific band related to cv. 2 with molecular size 410 bp (Plate 1 and Tables 5, 6, 7 and 8).

A posistive marker of 420 bp was shown against primer B-14, where it was present in salt tolerant cultivars 3 and 4, two monomorphic bands and one a cultivar specific band for cv. 3 with molecular size of 300 bp were found (Plate 1 and Tables 5, 6, 7 and 8).

Primer D-20 resulted in the production of one negative marker (2730 bp), which, it frequently presented in all the sensitive cultivars (1, and 2). Also the results showed the appearance of one positive marker (2100 bp) that it was presented in the tolerant cultivars 3 and 4. There are six monomorphic bands and two cultivar specific band for cv. 1 (1600 bp) and the other is specific for cultivar 4 (1080bp). (Plate 1 and Tables 5, 6, 7 and 8).

The results of primer Z-11 revealed the possible presence of one positive marker (640 bp) found in tolerant cultivars 3 and 4 only, and also the occurrence of (Plate 1 and Tables 5, 6, 7 and 8), four monomorphic bands and six polymorphic bands one of them was cultivar specific band related to cv. 2 with molecular size 450 bp.

Discussion

Through the last few years great efforts were devoted by researchers working in several areas to identify and select grain crops such as barley plants that exhibit an effective degree of salt-tolerance which consequently enable such plants not only to survive under conditions of their growth in salty soil and newly reclaimed desert lands but also to exhibit a marked exaggerated productivity and yield at harvest. Solving of such problem by selection of highly salt tolerant barley cultivars will assist in plant breeding programs via identification of molecular, physiological and genetical markers that determine salt tolerance capacity (Flowers and Hajibagheri, 2001; Mikiko *et al*., 2001; Rao *et al*., 2002; Witcombe *et al*., 2008 and Munns and Tester, 2008).

As for germination percentage of grains of barley cultivars in response to salt stress, data obtained revealed that salt stress caused a depression in germination. The induced depression showed an obvious variation in its magnitude among each one of barley cultivars. Concerning this, the magnitude of reduction in germination rate and germination percentage was shown to be substantially lower in tolerant cultivars than that recorded for the sensitive cultivars in response to the imposed salinization treatment .these result concomitant with Emre *et al.* (2011) These results might indicate that salt stress might induce the functioning of certain genes related to germination responses through its effect on the content and/or the activity level of certain intermediate organic compound within the target cells. And hence, one can suggest that germination rate and germination percentage can be used as markers that determine the tolerance capacity of barley plants to salt stress during germination and early seedling growth. This might reflect the tolerance capacity of the adult plants towards salinity stress. Such findings are in agreement with those obtained by Alka *et al.* (1981), Kabar and Baltepe (1987), Malki and Waisel (1987), Ramagopal (1988a), Hurkman *et al.* (1989), Salim (1991), Hurkman and Tanaka (1996), Mallek *et al*. (1998), Yamaguchi and Shinozaki (2006), Cutler *et al*. (2010) and Hirayama and Shinozaki (2007, 2010).

There is an overwhelming consensus in the literature that the intermediate organic compound which acts as inducer for functioning of certain genes related to salt-tolerance following exposure of plants to salinity is abscisic acid (Li *et al*., 2010).

The data obtained in the present investigation regarding the changes in abscisic acid (ABA) content of barley cultivars in response to their growth in saline soil revealed that the endogenous abscisic acid content was found to be increased in plants of the investigated cultivars subjected to salt stress when being compared to the same content of the corresponding controls. (Veselov *et al*., 2008) The magnitude of the induced increase in such content varied among cultivars It was about 174.68 and 165.89 folds in the tolerant cultivars (cv. 3 and cv. 4), 57.73 and 133.14 folds in the sensitive cultivars (cv. 1 and cv. 2) respectively.

The above mentioned data pinpoint to the substantial increase in abscisic acid level within the tissues of salt-tolerant cultivars when being compared with the level of abscisic acid within the tissues of salt-sensitive cultivars. (Verslues and Bray, 2006). This manner of response might indicate that salt stress induced an alteration in biosynthesis and activity levels of the endogenous phytohormones in favour of abscisic acid versus other growth hormones (Guoóth *et al*., 2009).

The resulted elevated content and/or activity level of abscisic acid appeared to act as the predominant stimulus for initiating a sequence of

events at transcriptional and translational levels of DNA-RNA-protein machinery performance. In addition, the elevated content and/or activity level of abscisic acid might exert a profound effect on regulation of solute accumulation via alteration of hydraulic conductivity of cellular membranes coupled by an alteration of elasticity of cellular walls facilitating the maintenance of turgidity of salt-stressed cells faster than the cause of what happened in the cells containing a low level of ABA. This suggestion is supported by the findings of Lerner (1985), Stewart and Voetberg (1985), Larosa *et al*. (1987), Thomas *et al*. (1992), Moon *et al*. (1995), Popova *et al.* (1995), Chen Ching-Nen *et al*. (2002) and Lee *et al*. (2003). Ren *et al*., 2007, Merlot *et al*. 2007, Van den Wijngaard *et al*. (2005) and Flowers *et al*. (2010)

There are chemical signals coming from roots in dry or saline soil that reduce leaf growth. These are commonly referred to as 'root signals'. Abscisic acid (ABA) is the obvious candidate for this signal, as it is found in xylem sap, and increases after drought and salinity stress , The hormonal regulation of source–sink relations during the osmotic phase of salinity, the phase when growth rate and development is reduced and before ions build up to toxic levels in leaves, affects whole plant energy balance, and is critical to delay the accumulation of ions to toxic levels (Pe´rez-Alfocea *et al*., 2010).

The preceding suggestion might explain the pivotal possible role of ABA in inducing tolerance for some barley cultivars exposed to salt stress (Etehadnia *et al*., 2008). And hence, one can infer that the potentiality of barley cultivars to tolerate salinity stress appeared to be determined through a modulation in ABA level within the tissues of salt-stressed plants. Supporting this view, Sauter *et al*. (2002) The mechanisms by which ABA is rerouted from cell-to-cell is not known with precision, but it might circulate as an inactive glucose ester conjugate. The chemical properties of ABA glucose ester are well suited for its long distance translocation in the xylem as it has low biomembrane permeability (Jang and Hartung, 2007). The ABA conjugate is stored in vacuoles or apoplastic space (Dietz *et al*., 2000), which is then released into the active form by apoplastic and endoplasmic reticulum b-glucosidases (Lee *et al*., 2006) in response to salinity.

The efficiency of using RAPD analysis to detect molecular markers for economically important traits, a logical step for efficient gene mapping and genotyping of individuals, useful tools for the rapid development of genetic information in plants are important to distinguish between different cultivars by comparing polymorphism in genomic fingerprints using RAPD (Skolnick and Wallace, 1988; Welsh and McClelland, 1990; Williams *et al.* 1990; Echt *et al.*, 1992; Vierling *et al.*, 1994.; Bahieldin *et al*., 1994 and Thudi *et al*., 2010).DNA markers are innumerable, highly polymorphic and

at the same time reliable, not influenced by the environment, lack pleiotropic or epistatic effects.(Grover *et al*., 2012)

From the obtained results the presence of positive marker band for salt tolerance in a salt-sensitive cultivar can be explained on the basis that bands with similar molecular sizes might represent amplified DNA fragments with different sequences. This explanation is inconsistence with the findings of Rieseberg (1996) and Abdel-Tawab *et al.*, 1998. Moreover, the occurrence of molecular and/or specific bands in salt-tolerant cultivars and their absence in salt sensitive cultivars is in agreement with the results of Abdel-Tawab *et al.*, 1997.

A common set of genes or their products which play active roles in the mechanism of salt tolerance have been cloned in many plant species under salinity stress e.g., barley (Hurkman *et al*., 1989, Narita *et.al*.2004.) and tomato (Chen and Plant, 1999).

The observed alteration in the DNA fragments of barley cultivars exposed to salinity stress may be attributed to the activation of the defense responsive genes whose transcripts and expression are controlled under salinity stress. Supporting this view, Muramoto *et al.* (1999). who isolated a cDNA clone, Bnuc1, encoding a nuclease I from leaves of salt stressed barley, noticed that the transcript of Bnuc1 gene increased dramatically in barley leaves under salt stress. They reported that the salt-inducible nuclease activity possibly corresponds to this gene.

In addition, transcriptional regulation is assumed to be responsible for developmental changes in gene expression whereas both transcriptional and post-transcriptional controls are important during stress (De Rocher and Bohnert, 1993 and Fukuda and Tanaka 2006).

From the obtained results, it is suggested that salinity stress elicited a concurrent sequence of events within the tissues of barley plants. Such events appeared to be triggered at cellular and sub-cellular levels through the whole stages of growth and development of salt stressed plants with difference in magnitude among the investigated barley cultivars. The apparent differential responses of such cultivars might be related to the impact of salinity stress on the endogenous content of abscisic acid, which acts as an inducer for alteration in DNA structure implicating RNA transcription and gene expression. Supporting this view, El-Hashami, (2007). who demonstrated that abscisic acid is a regulator involved in the control of changes in specific genes expression e.g., rab genes, which occur in response to water deficit. Also Kantar *et al* (2010) showed a positive correlation between levels of mRNA expression and suppression of their target mRNA transcripts in dehydration-stress-treated barley.

Such manner of change could be reflected in a modulation in DNA, fingerprints of salt-stressed barley plants which ultimately determine their capacity to tolerate their growth under salt stress conditions.

Table (3): Means of germination percentages at 7 through 11 days after putting grains of the four barley (*Hordeum vulgare* L.) cultivars to germinate under control and 9000 ppm salt stress conditions.

Treatment	Cultivars	Days				
		7	**8**	**9**	**10**	**11**
Control	**1**	73.00 AB	93.62 AB	100.00 A	100.00 A	100.00 A
	2	81.30 AB	100.00 A	100.00 A	100.00 A	100.00 A
	3	56.34 AB	100.00 A	100.00 A	100.00 A	100.00 A
	4	77.20 AB	89.50 AB	89.53 ABC	89.53 ABC	89.53 ABC
Salt stress	**1**	80.33 AB	85.67 AB	92.83 AB	92.83 AB	92.83 AB
	2	81.17 AB	90.55 AB	90.55 AB	90.55 AB	90.55 AB
	3	85.33 AB	94.67 AB	94.67 AB	96.86AB	96.86 AB
	4	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A

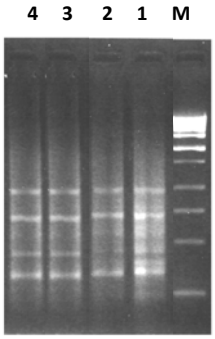
Table (4): Abscisic acid concentrations (mg/100 g fresh weight) in leaves of the four barley (*Hordeum vulgare* L.) cultivars under control and 9000 ppm salt stress conditions.

Cultivars	**Control**	**Salt stress**	**Relative ABA content* (X-Folds)**
1	50.58	57.73	1.15
2	30.89	133.14	4.31
3	4.90	174.68	35.65
4	3.60	165.89	46.08

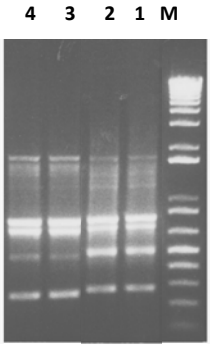
* Relative ABA content = $\frac{\text{Treatment}}{\text{Control}}$

Plate (1): DNA polymorphism of the four barley (*Hordeum vulgare* L.) cultivars generated by the six random 10 *mer* primers. M refers to DNA standards with MS shown in the Materials and Methods.

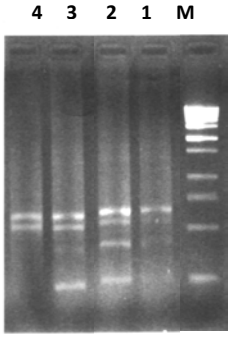
OP-A03



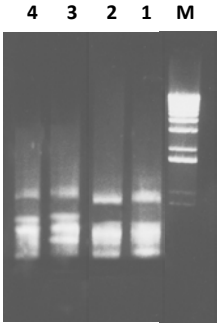
OP-A10



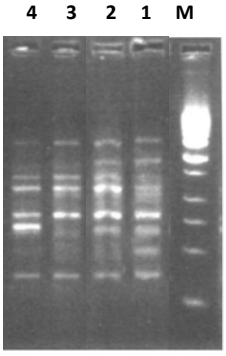
OP-B08



OP-B14



OP-D20



OP-Z11

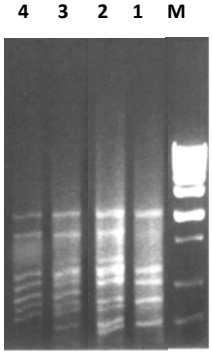


Table (5): DNA polymorphism using randomly amplified polymorphic DNA (RAPD) markers by six random 10 *mer* primers for the sensitive (cvs. 1-2) and the tolerant (cvs. 3-4) cultivars of barley (*Hordeum vulgare* L.).

Primer's name	Molecular size (bp)	intensities (%)			
		cv. 1	cv. 2	cv. 3	cv. 4
OP-A03	950	6.42	5.91	5.16	5.70
	790	4.11	-	-	-
	690	7.70	5.98	5.87	5.99
	560	8.69	5.02	-	-
	430	5.24	3.15	4.68	4.75
	370	4.33	-	-	-
	330	8.36	7.50	7.17	7.30
OP-A10	1110	3.11	3.01	4.96	4.95
	970	-	-	6.33	5.69
	950	4.14	4.71	-	-
	900	4.20	5.08	-	-
	580	13.30	13.80	15.50	14.10
	520	14.40	13.80	14.9	15.20
	390	14.10	14.60	4.59	4.92
	160	5.18	5.16	7.54	6.64
OP-B08	630	6.17	7.40	-	-
	560	-	4.67	6.90	7.02
	500	-	-	6.07	6.59
	410	-	5.69	-	-
	250	10.00	8.76	10.50	-
OP-B14	470	17.50	17.70	14.00	10.30
	420	-	-	9.98	10.30
	340	32.70	36.10	17.30	31.50
	300	-	-	15.00	-
	250	11.40	9.78	-	7.38
OP-D20	5200	3.50	4.05	3.79	3.66
	2730	4.20	3.50	-	-
	2100	-	-	3.74	1.96
	1840	2.69	5.75	3.73	2.95
	1720	4.12	8.15	7.44	5.59
	1600	3.73	-	-	-
	1120	2.07	2.61	-	2.45
	1230	7.31	7.06	6.77	5.27
	1080	-	-	-	8.96
	850	7.82	6.26	5.04	-
	590	6.36	6.60	4.87	4.02
	490	1.71	1.48	2.29	-
	380	5.64	5.32	5.01	4.35
OP-Z11	1580	8.27	8.42	6.77	4.43
	1120	-	12.50	7.08	3.95
	870	-	4.51	3.00	-
	820	6.62	7.22	8.88	4.33

	760	6.01	6.10	2.34	4.17
	640	-	-	4.27	5.76
	590	7.76	3.53	3.30	3.05
	280	-	2.81	2.30	1.67
	490	3.45	5.07	2.42	-
	450	-	1.45	-	-

Table (6): DNA monomorphic and polymorphic bands using randomly amplified polymorphic DNA (RAPD markers) by the six random 10 *mer* primers among the four barley (*Hordeum vulgare* L.) cultivars.

Primer's name	**Monomorphic bands**	Polymorphic bands			**Toltal No. of bands**	**Polymorphism %**
		DNA markers	**Cultivars specific bands**	**Non DNA markers**		
Op-A03	**4**	**1**	**2**	**0**	**7**	**42.86**
Op-A10	**5**	**3**	**0**	**0**	**8**	**37.50**
Op-B08	**0**	**2**	**1**	**2**	**5**	**100.00**
Op-B14	**2**	**1**	**1**	**1**	**5**	**60.00**
Op-D20	**6**	**2**	**2**	**3**	**13**	**53.85**
Op-Z11	**4**	**1**	**1**	**4**	**10**	**60.00**

Table (7): Molecular markers of the DNA by using randomly amplified polymorphic DNA (RAPD markers) by the six random 10 *mer* primers for the sensitive (cvs. 1-2) and the tolerant (cvs. 3-4) cultivars of barley (*Hordeum vulgare* L.).(+) means present, (-) means absent.

Primer's name	**Molecular weight (bp)**	**Cultivars**			
		cv. 1	cv. 2	cv. 3	cv. 4
Op-A03	560	+	+	-	-
Op-A10	970	-	-	+	+
	950	+	+	-	-
Op-B08	630	+	+	-	-
	500	-	-	+	+
Op-B14	420	-	-	+	+
Op-D20	2730	+	+	-	-
	2100	-	-	+	+
OP-Z11	640	-	-	+	+

Table (8): Molecular markers of the DNA (cultivar specific band) by using randomly amplified polymorphic DNA (RAPD markers) by the six random 10 *mer* primers for the sensitive (cvs. 1-2) and the tolerant (cvs. 3-4) cultivars of barley (*Hordeum vulgare* L).(+) means present, (-) means absent.

Primer's name	**Molecular weight (bp)**	Cultivars			
		cv. 1	cv. 2	cv. 3	cv. 4
OP-A03	790	+	-	-	-
	370	+	-	-	-
OP-B08	410	-	+	-	-
OP-B14	300	-	-	+	-
OP-D20	1600	+	-	-	-
	1080	-	-	-	+
OP-Z11	450	-	+	-	-

References:

Abdel-Tawab, F. M.; Dhindsa R. S.; Rashid, M. A.; Bahieldin, A. and Abo-Doma, A. (1998): Marker-assisted selection for salt tolerant genotypes in Sorghum (*Sorghum bicolor* L.) Proc. 3rd Arab Conf. Modern Biotech. & Areas of Application in the Arab World, 14-17 December, Cairo, Egypt. P. 661-672.

Abdel-Tawab, F. M.; Eman, M. Fahmy; Bahieldin, A. and Hala, F. E. (1997): Molecular markers for salt tolerance in some inbreeds in maize (*Zea mays* L.). Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, Egypt, 5: 389-417.

Anderson, M. K. and Reinbergs, E. (1985): Barley breeding. American Society of Agronomy, Madison, Wisconsin, USA. P. 232-268.

Alka, P. K.; Kumar, A.; Masih, S. N. and Shamshery, A. P. (1981): Tolerance of some barley *Hordeum vulagre* varieties to salt stress at seedling stage. Indian Journal of Plant Physiology. Vol. 24 (4): 304-311.

Bahieldin, A. and Ahmed, I. A. (1994): Use of Agarose-RAPD molecular markers for the identification of some barley cultivars. Egyptian Journal of Genetics and Cytology. Vol. 23: 81-94.

Bahieldin, A.; Eman, M. Fahmy.; Gad El-Karim, G. A.; El-Domyati, F. M.; Hassan, H. Z. and Salam, T. Z. (1994): Detection of molecular genetic markers for salt tolerance in wild wheats. Egyptian Journal of Genetics and Cytology. Vol. 23: 95-105.

Blum, A. (1974): Ecotypic response in sorghum to drought stress. II. Leaf tissue water relations. Crop Science. Vol. 14: 691-692.

Chen, C. and Plant, A. (1999): Salt-induced protein synthesis in tomato roots: The role of ABA. Journal of Experimental Botany. Vol. 50: 677-687.

Chen Ching-Nen; Chu, C. C.; Zentella, R.; Pan, S. M.; Ho and T. H. David (2002): AtHVA22 gene family in *Arabidopsis*: Phylogenetic relationship,

ABA and stress regulation, and tissue-specific expression. Plant Molecular Biology. Vol. 49 (6): 633-644.
Cutler, S. R., Rodriguez, P. L. and Finkelstein, R. R. (2010): Abscisic acid: Emergence of a core signaling network. Annual Review of Plant Biology. Vol. 61: 651-679.
De Rocher, E. and Bohnert, H. (1993): Development and environmental stress employ different mechanisms in the expression in the plant gene family. Plant Cell. Vol. 5: 1611-1625.
Dietz, K. J., Sauter, A., Wichert, K., Messdaghi, D. and Hartung, W. (2000): Extracellular b-glucosidase activity in barley involved in the hydrolysis of ABA glucose conjugates in leaves. Journal of Experimental Botany. Vol. 51: 937-944.
Echt, C. S.; Erdahl, L. A. and McCoy, T. J. (1992): Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. Genome. Vol. 35: 84-87.
El-Hashami, N. (2007): Effects of Abscisic Acid On Stomatal Development in Wheat Seedlings. Journal of Science and its Applications. Vol. 1: (1): 1-5.
Emre, Y.; Feyza, T.; Cuneyt, U. and Filiz, G. (2011): Physiological responses of elite barley (*Hordeum vulgare* L.) cultivars to salt stress at germination stage. Current Opinion in Biotechnology. Vol. 22: 150-152.
Etehadnia, M.; Doug, R.; Waterer, K. and Tanino, K., (2008): The method of ABA application affects salt stress responses in resistant and sensitive potato lines. Journal of Plant Growth Regulation. Vol. 27: 331-341.
Flowers, T. J. and Hajibagheri, M. A. (2001): Salinity tolerance in *Hordeum vulgare*: Ion concentrations in root cells of cultivars differing in salt tolerance. Plant and Soil. Vol. 231: 1-9.
Flowers, T. J., Galal, H. K. and Bromham, L. (2010): Evolution of halophytes: Multiple origins of salt tolerance in land plants. Functional Plant Biology. Vol. 37: 604-612.
Fukuda, A. and Tanaka, T. (2006): Effects of ABA, auxin, and gibberellin on the expression of genes for vacuolar H^+-inorganic pyrophosphatase, H^+-ATPase subunit A, and Na^+/H^+ antiporter in barley. Plant Physiology and Biochemistry, 44: 351-358.
Giora, B. A. and Uri, L. (2012): Marker-assisted selection in plant breeding. Plant Biotechnology and Agriculture. Pp. 163-184.
Gomez, K. A. and Gomez, A. A. (1984): Statistical Procedures for Agricultural Research. John Wiley and Sons Inc., USA. Pp. 303.
Grover, S.; Singh, Y.; Pareek, N. and Malik, C. P. (2012): DNA fingerprinting: approaches and applications with emphasis on random amplified polymorphic DNA (RAPD). The Journal of Plant Science Research. Vol. 28 (1): 1-14.

Guoóth, A.; Tari, I.; Gallé, A.; Csiszar, J.; Pécsvaradi, A.; Cseuz, A. and Erdei, L. (2009): Comparison of the drought stress responses of tolerant and sensitive wheat cultivars during grain filling: changes in flag leaf photosynthetic activity, ABA levels and grain yield. Journal of Plant Growth Regulation. Vol. 28: 167-176.

Haekel, M. S.; El-Abasiri, A.; Abo El-Enin, R. A. and Gomaa, A.S. (1981): Studies on salt tolerance in barley and wheat: 1. Screening technique. Fourth International Barley Genetics Symposium. Edinburgh. Pp. 974.

Hirayama, T. and Shinozaki, K. (2007): Perception and transduction of abscisic acid signals: Keys to the function of the versatile plant hormone ABA. Trends in Plant Science. Vol. 12: 343-351.

Hirayama, T. and Shinozaki, K. (2010): Research on plant abiotic stress responses in the post-genome era: Past, present and future. The Plant Journal. Vol. 61: 1041-1052.

Hurkman, W. J.; Fornari, C. S. and Tanaka, C. K. (1989): A comparison of the effect of salt on polypeptides and translatable messenger RNA in roots of a salt-tolerant and a salt-sensitive cultivar of barley. Plant Physiology. Vol. 90 (4): 1444-1456.

Hurkman, W. J. and Tanaka, C. K. (1996): Effect of salt stress on germin gene expression in barley roots. Plant Physiology. Vol. 110 (3): 971-977.

Jang, F. and Hartung, W. (2007): Long-distance signalling of abscisic acid (ABA): The factors regulating the intensity of the ABA signal. Journal of Experimental Botany. Vol. 59: 37-43.

Jin, S.; Chen, C. C. S. and Plant, L. (2000): Regulation by ABA of osmotic-stress-induced changes in protein synthesis in tomato roots. Plant Cell Environ. Vol. 23: 51-60.

Johnson, C. M; Stout, P. R.; Broyer, R. C. and Carlton, A. B. (1957): Comparative chlorine requriments of different plant species. Plant and Soil. Vol. 8: 337-353.

Kabar, K. and Baltepe, S. (1987): Alleviation of salinity stress on germination of barley seeds by plant growth regulators. Turkish Journal of Biology. Vol. 11 (3): 108-117.

Kantar, M.; Unver, T. and Budak, H. (2010): Regulation of barley miRNAs upon dehydration stress correlated with target gene expression. Functional and integrative Genomics. Vol. 10 (4): 493-507.

Klara, M.; LLdiko, K.; Csaba, K.; Judit, B.; Laszlo, L. and Zoltan, B. (2007): Efficiency of different marker systems for genotype fingerprinting and for genetic diversity studies in barley (*Hordeum vulgare* L.). South African Journal of Botany. Vol. 73 (1): 43-48.

Larosa, C. P.; Hasegawa, P. M.; Rhodes, D.; Clithero, J. M.; Watad, A. A. and Bressan, R. (1987): Abscisic acid stimulated osmotic adjustment and its

involvement in adaptation of tobacco to NaCl. Plant Physiology. Vol. 85: 174-181.
Lee, J. T.; Prasad, V.; Yang, P. T.; Wu, J. F.; Ho, T. H. David; Charng, Y. Y. and Chan, M. T. (2003): Expression of *Arabidopsis* CBF1 regulated by an ABA/stress inducible promoter in transgenic tomato confers stress tolerance without affecting yield. Plant, Cell and Environment. Vol. 26: 118-119.
Lee, K. H., Piao, H. L., Kim, H. Y., Choi, S. M., Jian, F., Hartung, W., Hwang, I., Kwak, J. M., Lee, I. J. and Hwang, I. (2006): Activation of glucosidase via stress-induced polymerization rapidly increased active pools of abscisic acid. Cell. Vol. 126: 1109-1120.
Lerner, H. R. (1985): Adaptation to salinity at the plant cell level. Plant and Soil. Vol. 89: 3-14.
Li, C.; Lv, J.; Zhao, X.; Ai, X.; Zhu, X.; Wang, M.; Zhao, S. and Xia, G. (2010): TaCHP: a wheat zinc finger protein gene down-regulated by abscisic acid and salinity stress plays a positive role in stress tolerance. Plant Physiology. Vol. 154: 1211-1221.
Maniatis, T.; Fritsch, E. F. and Sambrook, J. (1982): Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Publisher, New York. Pp. 2028.
Matin, M. A.; Brown, J. H. Feguson, H. (1989): Leaf water potential, relative water content and diffusive resistance as screening techniques for drought resistance in barley. Agronomy Journal. Vol. 80: 100-105.
Maiti, R. K. and Huerta, M. L. T. (1990): Genetic variability in seedling resistance to salinity and drought in *Sorghum*. Sorghum Newsletter. Vol. 31: 54.
Malki, E. and Waisel, Y. (1987): Effect of pressure on germination of seeds of wheat (*Triticum aestivum* cv. Barqai) in saline and in non-saline media. Physiologia Plantarum. Vol. 70: 73-77.
Mallek, M. E.; Boulasnem, F. and Salem, M. B. (1998): Effects of salinity on seed germination for cereals grown in Tunisia. Cahiers Agricultures. Vol. 7 (2): 153-156.
Merlot, S.; Leonhardt, N.; Fenzi, F.; Valon, C.; Costa, M. and Piette, L. (2007): Constitutive activation of a plasma membrane H(+)-ATPase prevents abscisic acid-mediated stomatal closure. EMBO Journal. 26: 3216-3226.
Mikiko, L. K.; Levesley, A.; Koebner, R. M. D.; Flowers, T. J. and Yeo, A. R. (2001): Quantitative trait loci for component physiological traits determining salt tolerance in rice. Plant Physiology. Vol. 125: 406-422.
Moon, A. A.; Bouw, G.; Prinsen, E.; Van Montagu, M. and Straeten, D. (1995): Molecular and physiological responses to abscisic acid and salt in roots of salt-sensitive and salt-tolerant Indian Rice varieties. Plant Physiology. Vol. 107: 177-186.

Munns, R. and Tester, M. (2008): Mechanisms of salinity tolerance. Annual Review of Plant Biology. Vol. 59: 651-681.
Muramoto, Y.; Watanabe, A.; Nakamura, T. and Takabe, T. (1999): Enhanced expression of a nuclease gene in leaves of barley plants under salt stress. Gene (Amsterdam). Vol. 234 (2): 315-321.
Narita, Y.; Hiromu, T.; Toshihide, N.; Akihiro, U.; Weiming, S. and Tetsuko, T. (2004): Characterization of the salt-inducible methionine synthase from barley leaves. Plant Science, 167: 1009–1016.
Pe´rez-Alfocea, F., Albacete, A., Ghanem, M. E. and Dodd, I. C. (2010): Hormonal regulation of source-sink relations to maintain crop productivity under salinity: A case study of root-to-shoot signalling in tomato. Functional Plant Biology. Vol. 37: 592-603.
Nieman, R. H. and Shannon, M. C. (1977): Screening plants for salinity tolerance. P. 359-367. In: M. J. Wright. Plant adaptation to mineral stress in problem soils. Beltsville, Maryland.
Popova, L. P.; Stoinova, Z. G. and Maslenkova, L.T. (1995): Involvement of abscisic acid in photosynthetic process in *Hordeum vulgare* L. during salinity stress. Journal of Plant Growth Regulation. Vol. 14 (4): 211-218.
Quisenberry, J. E. (1992): Breeding for drought resistance and plant water use efficiency. In: Breeding Crops for Less Favorable Environments (M. N. Christiansen & C. F. Lewis, eds.), John Wileyand Sons, New York. P. 193-212.
Ramagopal, S. (1988a): Regulation of protein synthesis in root, shoot and embryonic tissues of germinating barley during salinity stress. Plant, Cell and Environment. Vol. 11 (6): 501-516.
Rao, D. L. N.; Giller, K. E.; Yeo, A. R. and Flowers, T. J. (2002): The effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (*Cicer arietinum*). Annals of Botany. Vol. 89: 563-570.
Ren, H., Gao, Z., Chen, L., Wei, K., Liu, J., Fan, Y., Davies, W. J., Jia, W. S. and Zhang, J. H., (2007): Dynamic analysis of ABA accumulation in relation to the rate of ABA catabolism in maize tissues under water deficit. Journal of Experimental Botany. Vol. 58: 211-219.
Rieseberg, L. H. (1996): Homology among RAPD fragments in interspecific comparisons. Molecular Ecology. Vol. 5: 99-105.
Saiki, R. K.; Gelfand, D. H.; Stoffet, S.; Scharf, S. J.; Higuchi, R.; Mullins, G. T. K. B. and Erlich, H. A. (1988): Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. Vol. 239: 487-494.
Salim, M. (1991): Comparative growth responses and ionic relations of four cereals during salt stress. Journal of Agronomy and Crop Science. Vol. 166 (3): 204-209.

Sauter, A.; Dietz, K. J. and Hartung, W. (2002): A possible stress physiological role of abscisic acid conjugates in root-to-shoot signaling. Plant, Cell and Environment. Vol. 25: 223-228.
Skolnick, M. H. and Wallace, B. R. (1988): Simultaneous analysis of multiple polymorphic loci using amplified sequence polymorphisms (ASPs). Genomics. Vol. 2: 273-279.
Skriver, K. and Mundy, J. (1990): Gene expression in response to abscisic acid and osmotic stress. Plant Cell. Vol. 2: 503-512.
Stewart, G. R. and Voetberg, G. (1985): Relationship between stress-induced ABA and proline accumulations and ABA-induced proline accumulation in excised barley leaves. Plant Physiology. Vol. 79: 24-29.
Stuber, C. W. (1992): Biochemical and molecular markers in plant breeding. Plant Breeding Reviews. Vol. 9: 37-61.
Thomas, J. C.; McElwain, E. F. and Bohnert, H. J. (1992): Convergent induction of osmotic stress-responses. Plant Physiology. Vol. 100: 416-423.
Thomas, J. C. and Bohnert, H. J. (1993): Salt stress perception and plant growth regulators in the halophyte *Mesembryanthemum crystallinum*. Plant Physiology. Vol. 103: 1299-1304.
Thudi, M.; Senthilvel, S.; Bottley, A.; Tom Hash, C.; Reddy, A. R.; Feltus, A. F.; Paterson, A. H.; Hoisington, D. A. and Varshney, R. K. (2010): A comparative assessment of the utility of PCR-based systems in pearl millet. Euphytica. Vol. 174 (2): 253-260.
Turner, L. B. (1991): The effect of water stress on the vegetative growth of white clover (*Trifolium repens* L.): Comparison of long-term water deficit and short-term developing water stress. Journal of Experimental Botany. Vol. 20: 520-525.
Van den Wijngaard, P. W.; Sinnige, M. P.; Roobeek, I.; Reumer, A. and Schoonheim, P. J. (2005): Abscisic acid and 14-3-3 proteins control K channel activity in barley embryonic root. Plant Journal. Vol. 41: 43-55.
Verslues, P. E. and Bray, E. A. (2006): Role of abscisic acid (ABA) and *Arabidopsis thaliana* ABA-insensitive loci in low water potential-induced ABA and proline accumulation. Journal of Experimental Botany. Vol. 57: 201-212.
Veselov, D. S.; Sharipova, G. V.; Veselov, S. U. and Kudoyarova, G. R. (2008): The effects of NaCl treatment on water relations, growth, and ABA content in barley cultivars differing in drought tolerance. Journal of Plant Growth Regulation. Vol. 27: 80-386.
Vierling, R. A.; Xiang, Z.; Joshi, C. P.; Gilbert, M. L. and Nguyen, H. T. (1994): Genetic diversity among elite sorghum lines revealed by restriction fragment length polymorphism and random amplified polymorphic DNAs. Theoretical and Applied Genetics. Vol. 87: 816-820.

Walia, H.; Wilson, C.; Wahid, A.; Condamine, P.; Cui, X. and Close, T. J. (2006): Expression analysis of barley (*Hordeum vulgare* L.) during salinity stress. Functional and integrative Genomics. Vol. 6 (2): 143-156.

Wasfy, W.; Shindy, W. W. and Orrin Smith, E. (1975): Identification of plant hormones from cotton ovules. Plant Physiology. Vol. 55: 550-554.

Welsh, J. and McClelland, M. (1990): Cell and fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Research. Vol. 18 (24): 7213-7218.

Williams, J. G. K.; Kubelik, A. E.; Livak, K. J.; Rafalski, J. A. and Tingey, S. C. (1990): DNA polymorphism amplified by arbitrary primers as useful genetic markers. Nucleic Acids Research. Vol. 18 (22): 6531-6535.

Witcombe, J. R.; Hollington, P. A.; Howarth, C. J.; Reader, S. and Steele, K. A. (2008): Breeding for abiotic stresses for sustainable agriculture. Philosophical Transactions of the Royal Society B: Biological Sciences. Vol. 363: 703-716.

Xue, D.; Huang, Y.; Zhang, X.; Wei, K.; Westcott, S.; Li, C.; Chen, M.; Zhang, G. and Lance, R. (2009): Identification of QTLs associated with salinity tolerance at late growth stage in barley. Euphytica. Vol. 169 (2): 187-196.

Yamaguchi, S. K. and Shinozaki, K. (2006): Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annual Review of Plant Biology. Vol. 57: 781-803.