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BARLEY SEED STORABILITY AS AFFECTED BY WATER DEFICIT AND FERTILIZING DURING GRAIN DEVELOPMENT

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ABSTRACT: Sustainable agriculture especially organic agriculture is a low input system that implies the efficient use of biological resources. To study the effect of various combinations of organic and chemical fertilizers and water deficit on barley seed storability, seeds which produced in two successive growing seasons of 2007 and 2008 under combinations of different water deficit during grain filling and fertilizing systems were subjected to controlled deterioration and electrical conductivity tests. Results showed that water stress induced after flowering to maturity in the field enhanced seed storability. The seeds stored for 2 and 3 weeks at high temperature (40°C) had greater germination and lower mean germination time than nondeteriorated and 1 week stored seed. There was inconsistency in storability of seeds produced with inorganic fertilizer under water stress conditions. Application of organic fertilizers alone or along with inorganic fertilizers under water stress conditions, enhanced seed storability. Water stress increased seed EC over fertilizing systems in both years; however, in severe water stress (SS) condition, seeds fertilized with biofertilizers had lower EC. Generally, despite water stress during grain development increases EC of offspring seed, but it could stimulate its tolerance to high temperature during longer storage period.

Key Words: Storability; Organic fertilizer; Seed vigor; Drought stress; EC

INTRODUCTION

Seeds as reproductive units are expected to produce new plants in the field. The standard germination test fails to provide accurate information concerning a seed lot's field performance. The latter is called seed vigor. Seed vigor is defined as "those seed properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions and retention of performance after storage" (Black and Bewely, 2000). Some seeds are stored before sowing in conditions that may have negative effect on seed quality. Studies of seed longevity under conventional or optimal storage conditions would take years to complete, so accelerated ageing or controlled deterioration (CD) conditions are utilized to speed the loss of viability. The CD test has been used to assess the vigor of seed lots and to predict their relative longevity by ageing seeds rapidly at elevated temperature and relative humidity (RH) (Powell and Matthews, 2005). High moisture and temperature during storage affect seed properties and reduce seed germination (Abdalla and Roberts, 1969; Panobianco and Viera, 2007). AA and CD tests and electrical conductivity (EC) are able to predict seed storability. There are evidences that seed size, seed composition, variety, physiological damage to seed and environmental conditions during grain filling (parental effect) have significant effects on AA, CD and EC tests (Viera *et al*., 2002; Hrstkova *et al.,* 2006; de Geus *et al*., 2008). Small seeds of sunflower had less germination and emergence percentage after AA test compared to big ones (Kaya and Day, 2008). Drought stress during flowering and grain development in pea increased EC in stressed seed (Fougerex *et al*., 1997), however, drought stress in rape seed (Hashem *et al.,* 1997) and peanut (Ramamoorthy and Basu, 1996) during flowering and grain filling reduced EC. deGeus *et al* (2008) in their experiment on corn found that the quality and germination percentage of seeds produced under low input cropping system, after AA test, was less than seed

produced under the conventional system, while their EC was more.

It is assumed that the seeds produced in areas with limited production resources, specially water and nutrients, face problems with quality and storability. Because of the importance of seed to produce a uniform emergence and good establishment stand after storage, especially in organic and low input systems this study was conducted to compare storability of seeds grown at conventional, organic and low input fertilizing systems under water deficit conditions.

MATERIALS AND METHODS

Plant material

Seed of spring cultivated barley variety. Turkman used in this experiment was provided by the Seed and Plant Breeding Research Institute, Karaj, Iran.

Seed production conditions

Field studies were conducted at the Experimental Farm of the College of Agriculture, University of Tehran, Karaj, Iran (35^o 56' N and 50^o 58' E with an altitude of 1312 m) during the 2006-2007 and the 2007-2008 cropping seasons. The soil was a clay loam with a pH of 8.4 and EC equal to 1.02. Karaj has a long term average annual precipitation of 400 mm (including snow) which it was about 450 mm for first year (2007) of the experiment and 680 mm for the second year (2008).

The experimental design was a split plot arrangement based on a randomized complete block design with four replications. The barley Var. Turkeman was sown in 2m by 5m plots with 1-m alleys between replications on March 17^{th}, 2007 and March 1^{th}, 2008 at a rate of 400 seed m^{-2}, respectively. The treatments consisted of three irrigation regimes (main plots) and six soil fertilizing systems (sub-plots). The irrigation treatments were applied at different phenological stages of barley according to Zadoks scale (1974) and consisted of: non-stressed (NS, full irrigation until the end of physiological maturity), moderate stress (MS, ceased irrigation from the beginning of flowering (Zadoks, 65) to the beginning of the grain filling stage (Zadoks, 70) and severe stress (SS, ceased irrigation from the beginning of flowering stage to the end of physiological maturity). Fertilizing systems consisted of no fertilizer (control) (NF), phosphorous and nitrogen biofertilizers (Biofertilizer is a complex of different free living nitrogen fixing and phosphorus solubilizing bacteria) (NB), 100% chemical fertilizer (NPK) (based on soil chemical analysis) (CF), vermicompost (VC) (applied 5 t/ha), 50% chemical fertilizer (NPK)+50% vermicompost (2.5 t/ha) (CV), and finally 50% chemical fertilizer (NPK)+50% biofertilizer (CB), assigned to the sub plots.

Application of chemical fertilizer was performed based on soil analysis. The amounts of N, P and K applied were 105 kg N/ha^{-1}, 32 kg P_2O_5/ha^{-1}, and 170 kg K_2O/ha^{-1}, respectively. Full irrigation was performed at weekly intervals whenever soil moisture reached 50% available soil water in the root growth zone.

After harvest, the barley ears were threshed by hand and were equilibrated to 7-8 percent moisture content (by fresh weight), 1000-seed weight was determined and seeds were stored at 4°C until the laboratory seed tests were performed.

Seed Quality tests

Controlled Deterioration (CD)

Laboratory seed tests were performed at Plant Research International, Wageningen, Netherlands on harvested seeds from both experimental years (2007 and 2008) according to methods of the International Seed Testing Association (ISTA, 1999). Seed moisture content was 7-8 percent. For CD test barley seed from two harvested years (2007 and 2008) were put at 75% RH for 3 days at 20°C (75% RH was made by saturated NaCl salt). After 3 days the seeds reached to 15 percent moisture content. After that one fifth of the seed put at 32% RH (as control seed (nondeteriorated)) to reach 7-8 percent moisture content and the other ones were sealed and stored at 40^0C (75% RH) for 1 week, 2 weeks, and 3 weeks periods. After each storage period a sample was taken and the seeds were put at 32% RH to dry back to 7-8 percent moisture. Germination test was carried out with three types of seed lot: Control (non treated seed, A (3 days in 75%RH and 1 week in 40^0C), B (3 days in 75%RH and 2 week in 40^0C) and C (3 days in 75%RH and 3 week in 40^0C) . Seeds

were considered germinated when radicles emerged at least 2mm. Seedling quality was evaluated after 7 days in respect to the number of normal and abnormal seedlings (ISTA, 2006). Parameters related to germination, such as maximum germination (G_{max}) and mean germination time (MGT, calculated by integration of the fitted germination curve) were calculated using the software package Seed Calculator V3.0 (Plant Research International, Wageningen, The Netherlands). The final number of germinated seeds after the germination period (7 days) were used in analysis of variance using transformation by function of $y = \arcsin\sqrt{p/100}$, were p is the germination percentage.

Electrical conductivity (EC)

Samples of 50 seeds produced under different irrigation and fertilizing systems in both years were weighted and placed in a beaker with 75 mL of distilled water. Beakers were incubated at 20°C and EC readings of the steep water were recorded with conductivity meter 24 Hours after soaking and expressed as $\mu Scm^{-1}gr^{-1}$ (ISTA, 2008). The cell constant was calibrated before each replication using the control standard solution 0.01 mol/L of KCl.

2.3.3 Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA) using MSTATC (Michigan State Univ., East Lansing, MS, USA) and SAS (SAS Inst., 1990) programs. Homogeneity of error variances was tested using Bartlett's Chi-square. Since the χ^2 was not significant, a combined analysis of the data was performed for two years and used for showing the results.

RESULTS

Field experiment

In both years the 1000-seed weight was reduced by water stress and this effect was greater in the relative drier production year of 2008 (Table 1). Although the germination percentage was not significant ($P<0.05$) for the irrigation and fertilization treatments, but the germination time was significant for the mentioned treatments ($P>0.05$) (Table 1). Drought stress decreased germination percentage although it was not significant. Seeds produced under severe stress during development geminated faster, expressed as a shorter mean germination time (MGT), than seeds produced under full irrigation. Seeds produced under mild water stress showed an intermediate rate of germination. Combined analysis over the two production years showed, it became apparent that seeds produced with nitrogen fixing and phosphorus solubilizing bacteria (NB) were heavier than those produced with 100% chemical fertilizer (CF). Seeds produced with 50% chemical fertilizer plus 50% biofertiliser (CB) fertilization germinated significantly faster, while absence of fertilizer (NF) or fertilization with vermicompost (VC) resulted in significantly slower seed germination (Table 1).

Seed quality tests

Controlled deterioration

Mean Germination Time (*MGT*)

There were significant interaction effect of drought stress and deterioration and fertilizing and deterioration on MGT ($P<0.01$). Drought stress in the field decreased MGT (Figure 1).The seeds which deteriorated for 2 and 3 weeks started germination faster than control (not deteriorated) and 1 week deteriorated seeds, however, seeds produced under sever stress in field and deteriorated for 3 weeks germinated later than 2 weeks deteriorated seeds. Results showed that seed storing for one week in high temperature and relative humidity increased MGT (Figure 1).

Table 1. Effects of irrigation and fertilizing treatments on 1000 seed weight of barley seeds (var. Turkaman) produced over two years, 2007 and 2008

		1000 seed weight (gr)	MGT (day)	Germination (%)
Year	2007	42.10 a	1.89 a	84.00 a
	2008	36.65 b	1.75 b	82.38 a
Irrigation system	NS	46.67 a	1.97 a	85.61 a
	MS	37.41 b	1.82 b	83.67 a
	SS	34.03 c	1.69 c	80.30 a
Fertilizing system	NF	39.32 ab	1.91 a	81.34 a
	NB	41.22 a	1.83 ab	84.08 a
	VC	39.70 ab	1.92 a	83.17 a
	CV	38.60 ab	1.77 bc	79.88 a
	CB	39.40 ab	1.72 c	84.68 a
	CF	38.00 b	1.79 bc	86.00 a

* Means with the same letter(s) are not significantly different at $P < 0.05$ level.
NS= normal irrigation until the end of the plant physiological maturity
MS= ceased irrigation from the beginning of flowering (Zadoks, 65) to the initiation of seed filling stage (Zadoks, 70).
SS= ceased irrigation from the initiation of flowering stage.
NF= no fertilizing, NB= phosphatic and nitrogenous biofertilizer, VC=vermicompost,CV=50% chemical fertilizer including NPK+50% vermicompost, CB= 50% chemical fertilizer including NPK + 50% biofertilizer and CF= 100% chemical fertilizer

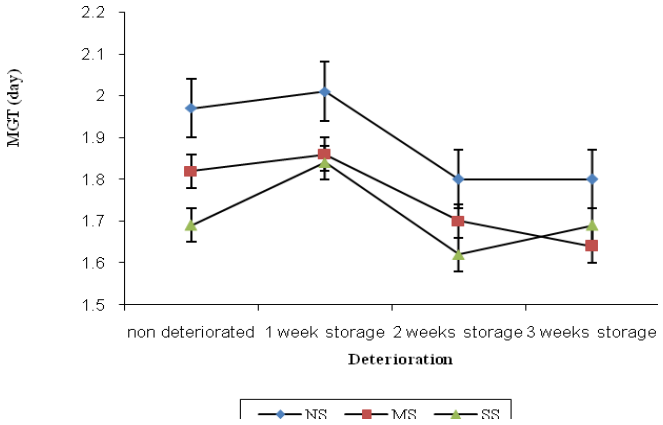

Figure 1. Averaged mean germination time (over two years, 2007 and 2008) of deteriorated barley seeds harvested from plants subjected to drought stress
NS= normal irrigation until the end of the plant physiological maturity
MS= ceased irrigation from the beginning of flowering (Zadoks, 65) to the initiation of seed filling stage (Zadoks, 70).
SS= ceased irrigation from the initiation of flowering stage (Zadoks, 65) to the end of the physiological maturity.
Bars indicate ± SD of means

Storage for 1 week considerably increased MGT. However, longer storage for 2 and 3 weeks significantly decreased MGT in all fertilizing systems also there was not any significant difference between fertilizing systems. Nondeteriorated seeds germinated as early as 2 and 3 weeks deteriorated seeds when were grown in field under integrated and conventional systems (CF, CV and CB) which received chemicals. Unlike, its MGT was significantly high either received no fertilizer or organic fertilizer including biofertilizer as well as vermicompost (Figure 2).

In all fertilizing systems across all irrigation systems, the one week deteriorated seeds had higher MGT (Figure 2).

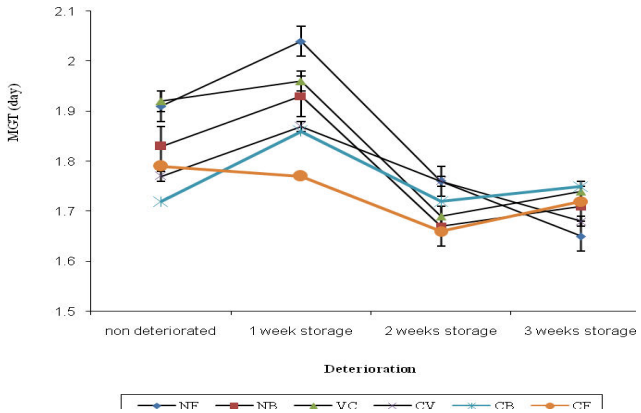

Figure 2. Effects of fertilizing systems and storage across all irrigation systems on mean germination time of barley seeds. NF= no fertilizing, NB= phosphatic and nitrogenous biofertilizer, VC= vermicompost,CV=50% chemical fertilizer including NPK+50% vermicompost, CB= 50% chemical fertilizer including NPK + 50% biofertilizer and CF= 100% chemical fertilizer.

Bars indicate ± SD of means

Germination

($P<0.05$). Water stress affected on seed storability as the germination of nondeteriorated seed decreased with increase in water stress however it was not seen in deteriorated seeds. When seeds produced under full irrigation condition in field, seed storage decreased their germination particularly in 2 and 3 weeks storage which their germination decreases significantly (Figure 3). Also significant decrease was seen in germination after storage

Interaction effect of drought and deterioration was significant on germination percentage in seeds produced under MS water stress condition although germination of 2 and 3 week stored seed didn't change in compare with corresponding seeds which produced under full irrigation. However, when seeds produced under SS water condition, not only longer storage (2 and 3 weeks) decreased germination but also increased it significantly rather non deteriorated and one week deteriorated seeds (Figure 3).

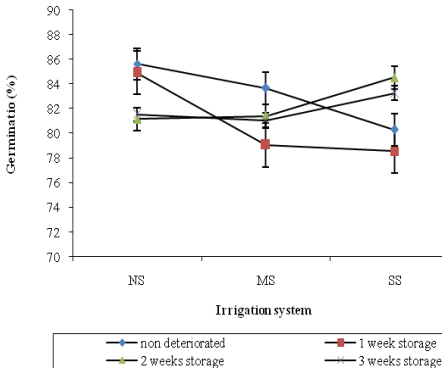

Figure 3. Effect of irrigation regimes and storage on germination percentage of barley seeds over two years.
NS= normal irrigation until the end of the plant physiological maturity
MS= ceased irrigation from the beginning of flowering (Zadoks, 65) to the initiation of seed filling stage (Zadoks, 70).
SS= ceased irrigation from the initiation of flowering stage (Zadoks, 65) to the end of the physiological maturity.
Bars indicate ± SD of means

Electrical conductivity

Under full irrigation regime (NS) EC was almost the same as in all fertilizing system in both years, but it increased in seeds produced under water stress condition especially in second the year (2008). This was because of pronounced effect of water stress due to drier weather and higher temperature in 2008 (Figure 4). Cordova-Tellez and Burris (2002) showed that high rate of embryo dehydration because of high temperature or drought prevents alignment of lipid bodies in plasma membrane and it tends to cell leakage increment and reduction of seed quality. EC value in SS water regime was less than MS in 2008; however in 2007 it was higher in SS. Results of CD test also showed that in 2008 seeds which were produced under sever stress condition in field had higher germination after storage for 2 and 3 weeks compared to seeds produced under MS stored for corresponding periods.

The seeds produced under water stress and fertilized with chemical fertilizer (CF) showed more EC, however, seed produced with biological fertilizers (NB and CB), especially at higher water stress regime (SS), showed lower EC. There might be some beneficial effect of phosphorous solubilizing bacteria on cell membrane phospholipids.

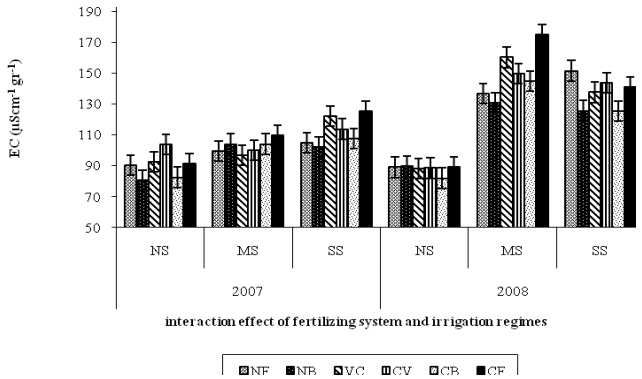

Figure 4. Effect of fertilizing systems and irrigation regimes on EC of barley seeds over two years.
NS= normal irrigation until the end of the plant physiological maturity
MS= ceased irrigation from the beginning of flowering (Zadoks, 65) to the initiation of seed filling stage (Zadoks, 70).
SS= ceased irrigation from the initiation of flowering stage (Zadoks, 65) to the end of the physiological maturity.
NF= no fertilizing, NB= phosphatic and nitrogenous biofertilizer, VC= vermicompost,CV=50% chemical fertilizer including NPK+50% vermicompost, CB= 50% chemical fertilizer including NPK + 50% biofertilizer and CF= 100% chemical fertilizer.

Bars indicate ± SD of means

DISCUSSION

The lower MGT in stressed seed in this experiment can be explained by smaller seed size (Maleki Farahani *et al* (2010) and Cula *et al* (2006). Also Ellis and Marshal (1998) found that in barley grain size affected germination time, as small seeds germinated faster than big ones. The same results also found out by Kaya and Day (2008) with sunflower seeds.

Results showed that seed storing for one week in high temperature and relative humidity increased MGT. This is in agreeing with finding of Matthews *et al.*, (2011).

Lower germination in stressed seed which stored for one week or non deteriorated seeds is consistent with Kaya and Day (2008) who found seed size affects seed susceptibility to AA test. However, in more deteriorated seed there were no significant differences between stressed and unstressed seeds (Figure 3).

Greater germination of severe stressed seed (SS) which stored for longer period (2 and 3 weeks) rather than non stressed seed (NS) may be related to induction of tolerance to deterioration because of water stress in field. Despite of Kaya and Day (2008) the stressed seeds had smaller seed size but showed more germination after longer storage. The seeds which were produced under severe water stress matured earlier than NS and MS. There is evidence that seed maturity has positive effects on storability at severe conditions after harvesting (Lammerts van Bueren *et al.,* 2003).

A number of cytological and metabolic factors of seed ageing have been identified (Schwember and Bradford, 2010). Lipid peroxidation, resulting in membrane damage as well as the generation of toxic byproducts, is well documented in stored seeds (Mcdonald, 1999; Davies, 2005; Seo *et al*., 2011). Decrease in activities of various peroxide scavenging enzyms (Goel *et al*., 2003) and oxidative damage to DNA and proteins is also likely to be involved in seed ageing (Rao ***et al.,*** 1987; Bailly ***et al.,*** 2008). Formation of isoaspartyl residues may be factors in loss of protein function during deterioration (Oge ***et al.,*** 2008; Rajjou ***et al***., 2008). Also Bernal-lugo and Leopold (1992) found that changes in carbohydrate during storage can affect cell membrane permeability and reduction of physiological quality and germination.

On the other hand, antioxidants, heat shock proteins (HSPs), and enzymes to repair protein damage may be involved in ameliorating the effects of ageing on seed longevity (Kibinza ***et al***., 2006; Prieto-Dapena ***et al.,*** 2006; Oge ***et al.,*** 2008; Almoguera ***et al***., 2009). Our results clearly are consistent with these cellular protective processes that Oge ***et al*** (2008) found in their work.

Skriver and Mundy (1990) described that ABA levels increase in tissues subjected to osmotic stress by desiccation, salt, or cold as well as oxidative stress (Chizhova *et al.,* 2005). Here ABA could be increased in seed under field water stress especially under severe stress (SS) consequently it can increase expression of *PIMT1* in stressed seed (Oge ***et al.***, 2008), this increase in *PIMT1* activity can affect on seed longevity of stressed seed.

As the results show, effects of different fertilizing systems and drought stress in field on storability of seeds are depended up on environmental conditions and agronomic practices in each year. Based on averaged results over years, it can be concluded that under drought stress conditions, integrated and biological fertilizing systems have the ability to produce more reliable seeds with more storability potential compared to conventional system because these seeds were able to have a stable performance over years.

Regarding to EC, as drought stress increased in the field, the seed leakage also increased in both years (2007 and 2008) although it was more in 2008 than 2007. This result is in agreement with Fougerex *et al*., (1997) finding but is in contrary with Hashem *et al.,* (1997) and Ramamoorthy and Basu (1996). Our results support Samarah and Al-Issa (2006) findings so adverse climatic conditions including high temperature and low relative humidity during grain development of barley cause reduction of barley seed vigor through increment of EC so this condition was more available in 2008. deGeus et al (2008) described that before seeds reach physiological maturity some specific metabolic processes such as the migration of lipid bodies to the plasma membrane occurs as a step towards the acquisition of desiccation tolerance. Here high EC could be a result of water stress in field on cell membrane. It is clear that water deficit in barley increases EC although further experiments are needed to study the leakage

composition to find exact effect of water stress in mother plant on off springs.

Fertilizing with chemical fertilizers reduced seed quality through more EC while application of integrated fertilizers (chemical along with biological fertilizer) enhanced seed quality. These results demonstrated the beneficial effect of biological fertilizer in seed production especially in water deficit situation with producing seeds of higher storability and vigor. Since the water stress in field increased EC and germination after longer storage period in CD test, it is assumed that here EC is not a suitable index to evaluate seed vigor in stressed seeds as other scientists (Abdul-Baki, and Anderson, 1970; Cheng *et al.,* 2005; Panobianco and Viera, 2007) showed EC test is not a suitable way to evaluate seeds with high vigor because more leakage like sugars occur in these seeds. Thus as Cheng et al (2005) described it is recommended to measure other parameter like Na^+/Ka^+ ratio.

CONCLUSION

Totally, among different environmental conditions regarding to water and nutrient availability, based on our results it can be concluded that water is more important factor than nutrient as it could affect seed longevity and viability. There was inconsistency in storability of seeds produced with different fertilizers. However, application of organic fertilizers especially biofertilizer containing nitrogen and phosphorus solubilizing bacteria alone or along with inorganic fertilizers under water stress conditions, enhanced seed storability. Generally if barley seeds develop under full irrigation, their storage potential will reduce. However development of seeds under water stress especially after anthesis and during seed maturity enhances seed quality through more germination after storage at high temperature and relative humidity.

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