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Invigoration of Low Vigor Sunflower Hybrids by Seed Priming

M. KAUSAR, T. MAHMOOD[1], S.M.A. BASRA† AND M. ARSHAD‡
Department of Agronomy, University of Arid Agriculture, Rawalpindi, Pakistan
†Department of Crop Physiology, University of Agriculture Faisalabad, Pakistan
‡Department of Botany, University of Arid Agriculture, Rawalpindi, Pakistan
[1]Corresponding author's e-mail: qiratm@yahoo.com

ABSTRACT

Sunflower seeds lose their vigor during storage at high temperature and high relative humidity. Present study was conducted to invigorate the performance of low-vigor seeds (exposed to 40±2°C & 100% RH) by seed priming with KH_2PO_4 (Ψ=-1.25 MPa) in four hybrids (Hysun 33, Hysun 38, Hysun 44 & F-330). Priming was effective in reducing the time for 50% germination and mean germination time (MGT) and increase in germination percentage in low-vigor seeds of all hybrids, while EG (energy of germination) and final germination percentage (FGP) in normal seeds displayed a minor increase. Priming of normal/low-vigor seeds improved the vigor of seedling in term of radicle length, plumule length and their root/shoot fresh weight. After cold stress low-vigor-primed and normal-primed exhibited significant change in all emergence parameters but normal and low-vigor seed of Hysun-44 had no significant effect in almost all parameters, except E_{50} of normal and low-vigor seeds and MET of low-vigor seeds of this hybrid. Some proteins were completely lost as a result of AA, which showed reappearance after priming. The proteins with 26, 48, 49, 69, 90, 118, 121,150 and 199 kDa were affected by AA. Priming showed little improvement in the banding pattern and intensity of protein in normal seeds, while low-vigor seeds had significant improvement in the banding pattern and intensity of protein.

Key Words: Accelerated aging; Seed priming; Vigor

INTRODUCTION

Crop production depends heavily on planting of high quality seeds. Rapid and uniform emergence is utmost important, because it is the foundation on which stand establishment is based and potential yield is determined. Absolute longevity depends on initial seed quality, which is controlled by genetic factors such as seed structure and composition, maturity, dormancy, purity, initial viability and vigor (Justice & Bass, 1978) and post-harvest treatments such as forced drying, cleaning and proper storage.

Oil seeds are very sensitive to the harsh environmental conditions. It is hypothesized that their oil content readily oxidize, which deteriorate the seed health in storage (Wilson & McDonald, 1986). These oxidative reactions occur through non-enzymatic auto-oxidation (Vertucci & Leopold, 1986). These deteriorative changes lead to declined germinability (McDonald, 1976) and vigor (Copeland & McDonald, 1995).

Low vigor (aged) seeds could be obtained though accelerated aging (AA) technique (TeKrony, 1995; McDonald, 1995). AA is recognized as an accurate indicator of seed vigor and storability; it correlates with field emergence (Egli & TeKrony, 1996). The seeds that deteriorate rapidly under AA generally show marked reduction in their germinability (McDonald, 1999). The deleterious effects of AA on germination processes are associated with the damage to membrane, nucleic acid and protein level (Fujikura & Karssen, 1995). AA also results in increase in lipid peroxidation, decreased level and activities of antioxidants and several enzymes involved in scavenging free radicals and peroxide (Hsu & Sung, 1997; Bailly *et al*., 1998). This is true for rice (Ray *et al*., 1990), soybean (Trawatha *et al*., 1995), sunflower (Halder *et al*., 1983) and lettuce (Hannan & Hill, 1991). AA leads to same biochemical events as occur in natural aging (Liklatchev *et al*., 1984) the only difference is the rate at which they occur.

Many seed priming treatments have been used to reduce the damage of aging and invigorate their performance in many crops (Basra *et al*., 2003; Farooq *et al*., 2009). Priming includes hydropriming, matripriming, osmopriming, halopriming with or without the use of plant growth regulators (Wahid *et al*., 2008; Farooq *et al*., 2009). Priming is responsible to repair the age related cellular and sub-cellular damage of low vigor seeds that may accumulate during seed development (Bray, 1995). Priming of seed promotes germination by repair of the damaged proteins, RNA and DNA (Koehler *et al.,* 1997). Bailly *et al*. (1998) reported that priming of aged sunflower seeds in PEG progressively restored the initial germinability and resulted in marked decrease in the level of MDA and conjugated dienes, indicating a fall in lipid peroxidation processes. Kathiresan *et*

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al. (1984) found that priming of sunflower seeds with $CaCl_2$, KH_2PO_4, NaCl, $ZnSO_4$, ascorbic acid and succinic acid improved emergence and seedling growth of seedlings.

Seed priming technique with many organic and inorganic salts has been used for invigorating the performance of normal seed in different crops, but very little wok has been done to reduce the damage of aging and invigorate the performance of oil seeds especially sunflower. The objectives of this study were to investigate the effects of accelerated aging on the viability and vigor and to identify the suitable priming protocol for reversing the strength of low vigor sunflower hybrid seeds.

MATERIALS AND METHODS

Four hybrids of sunflower (*Helianthus annuus* L.) Hysun 33, Hysun 38, Hysun 44 (ICI seed corporation Sahiwal) and F-330 (Ayub Agriculture Research Institute Faisalabad, Pakistan) were used as experimental material. The initial seed moisture was: Hysun-33 = 5.69%; Hysun-38 = 4.89%; Hysun-44 = 5.50% and F-330 = 9.3%.

Production of low vigor seed. The seeds were subjected to AA following the methods of Stewart and Bewley (1980) with some modifications. A sealed glass desiccator containing distilled water was placed in a water bath at 40°C to optimize the accelerated ageing by placing the seeds for different durations (120, 144 & 168 h) at the same temperature and RH. Marked decline in the germination percentage and pattern and, and vigor of aged seeds was observed at 168 h.

Hybrid	Initial Germination (%)	Germination (%) of L-V seeds
Hysun -33	93.8	84.5
Hysun -38	97.7	62.2
Hysun-44	99.8	72.2
F-330	91.1	37.8

Priming. Normal/low-vigor seeds of all four sunflower hybrids were primed in the aerated solution (Ψ=-1.25 MPa) of KH_2PO_4 at 25°C for 24 h followed by thorough washing and re-drying near to their original weights.

Seeds vigor evaluation. Effects of AA and priming on seed vigor and viability were assessed by applying tetrazolium chloride (TTC) cold and standard germination tests. For standard germination test, 15 seeds were put in a Petri dish and germination was recorded daily according to the Association of Official Seed Analysts (1990) up to four days. The T_{50} of seedlings was calculated according to the following formula of Farooq *et al*. (2006). Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981). Germination Index (GI) was calculated as described in the Association of Official Seed Analysts (1983). Energy of germination was calculated as the percentage of germinating seeds on 4th day after planting relative to total number of seed tested (Ruan *et al*., 2002).

In the paper towel test, 15 seeds were placed between two moistened sheets of paper towel. The sheets were incubated at 20±2°C in growth chamber replicated thrice. After four days the data regarding normal/abnormal seedling, root/shoot length and root/shoot fresh weight were taken. For TTC test, soaked seeds were peeled carefully with out damaging the embryo and stained in 1% TTC solution for 5 h. Ten seeds were used in each treatment with four replications. The data was recorded for the intensity of color.

Fifty seeds of each treatment soaked in distilled water for 18 h at 25°C after weighing. The leachate was used to determine the electrical conductivity of solutes (Twin Cond. Conductivity Meter, B-173, Horiba Ltd., Japan) and expressed as μS cm^{-1} g^{-1}. Cold test was performed in triplicates according to ISTA (1985).

Protein analysis. For extraction of protein, seed samples were ground to fine powder with mortar and pestle. To extract protein in 1 g of seed flour, 400 μL of the protein extraction buffer (0.05 M Tris-HCl, 0.2% SDS, 5 M Urea & 1% ß-mercaptoethanol) was added to the tube and mixed well by vortexed, centrifuged at 15,000 rpm for 5 min. The extracted crude proteins were recovered as clear supernatant, heated at 90°C for 4 min and stored at -20°C until further analysis. SDS-PAGE was performed by the method described by Laemmli (1970). Proteins were analyzed on 1.5 mm thick and 25 cm long gels run in a dual vertical slab unit (C.B.S. Scientific Co. Del Mar, California 92014 Model: DSG-200-02). From each sample, 15 μL of extract was loaded on a polyacrylamide gel. The resolving gel (10%) and staking gel (4%) were prepared from acrylamide monomer solution (Merck). Electrophoresis was carried out at a constant current of 35-45 mA at room temperature for 8 h. The gels were stained by Coomassie brilliant blue staining for about 4 h and destained overnight. Protein profile of seeds was identified according to differences in the banding pattern and staining intensities.

Statistical analysis. The experiment design for all above tests was completely randomized design in triplicate with two factors for accelerated aging and three factors for priming. The data was analyzed by using a statistical package MSTATC. Data from AA was subjected to the LSD and from priming to DMR for separating the means at $P < 0.05$ according to Steel *et al*. (1997). Correlation analysis was performed where required.

RESULTS

Accelerated aging. Hysun-33 showed significant increase in T_{50} and decrease in GI due to the accelerated aging, however MGT, EG and FGP exhibited non-significant change with respect to their control, while Hysun-38 had pronounced affects of aging with reference to all parameters. Hysun-44 showed some stability and exhibited non-significant results with reference to the T_{50}, MGT, EE and FGP, however, GI decreased significantly. The AA had more drastic change in variety F-330 in all parameters, which all showed statistically significant results (Table I).The affects of AA remained non significant in Hysun-33 and Hysun-38 in almost all attributes of emergence after

Table I. Influence of accelerated ageing on the germination of sunflower seeds

Hybrids	Treatment	T_{50} (days)	Mean germination time (days)	Germination Index	Energy of germination (%)	Final Germination (%)
Hysun-33	Control	1.29 1b	1.55 d	10.75 a	6.52 a	93.8 a
	Low-vigor	2.45 a	2.07 abcd	6.3 b	5.63 a	84.5 a
Hysun-38	Control	1.22 b	1.59 cd	10.58 a	6.52 a	97.7 a
	Low-vigor	2.5 a	2.19 ab	4.03 bc	4.15 b	62.2 b
Hysun-44	Control	1.78 ab	1.77 bcd	9.33 a	6.52 a	99.8 a
	Low-vigor	2.84 a	2.11 abc	5.94 b	5.48 b	72.2 ab
F-330	Control	1.31b	1.58 cd	10.33a	6.52 a	91.1a
	Low-vigor	2.92 a	2.53 a	2.47 c	2.52 c	37.8 c
LSD= 0.05		0.746	0.532	2.298	1.380	20.68

Means sharing the same alphabets differ non- significantly (P>0.05) by LSD test

Table II. Effect of pre-sowing cold stress (7°C for even days) on the daily emergence of normal/L-Vigor seeds of sunflower Hybrids

Hybrids	Treatment	E_{50} (days)	MET (days)	E. Index	FEP (%)
Hysun-33	Normal	0.11 bc	1.64 ab	19.16abc	100a
	L-Vigor	1.03 b	1.89 a	15.86cde	89.30ab
Hysun-38	Normal l	0.15 bc	1.47 ab	19.33ab	96.00a
	L-Vigor	2.24 b	1.79 a	12.66e	74.64 b
Hysun-44	Normal	1..37 c	1.32 b	21.70a	100a
	L-Vigor	1.85 b	1.83 a	15.69de	92.00a
F-330	Normal	1.00 b	1.64 ab	17.83bcd	97.3a
	L-Vigor	5.15 a	1.83 a	8.22f	49.3c
LSD = 0.05		2.674	0.4495	3.393	14.98

Mean sharing the same alphabets differ non-significantly (P > 0.05) by LSD test; E_{50} = Time for 50% emergence, MET = Mean emergence time, EI = emergence index, FEP = Final emergence percentage

cold stress, while Hysun-44 had significant effect of the treatment in E_{50}, MET and EI, however FEP had non-significant change.F-330 showed significant effect in E_{50}, EI and FEP, but insignificant effect was observed in MET of this hybrid cold stress (Table II).

Priming. The analysis of resulting data indicate that priming of low–vigor and normal seeds of all hybrids were effective in reducing the T_{50}, MGT, while enhancing GI, EG and FGP with respect to their controls (Table III). Normal and low-vigor seeds of Hysun-33 showed significant reduction in T_{50} and MGT, and increase in GI, EG and FGP but FGP remained un-changed in normal seeds after priming. Pronounced reduction in T_{50} and MGT and tangible improvement in EI, EG and FGP was observed of normal and low-vigor seeds Hysun-38 and Hysun-44, but priming had no significant effects in EG and FGP of normal seeds of these two hybrids with respect to their controls. Low-vigor/P and N/P seeds of F-330 exhibited significant decrease in T_{50} and MGT and increase in GI, EG and FGP after treatment, except EG and FGP of normal seeds which exhibited non-significant effect of the priming strategy.

Mean germination time in all hybrids had strong positive correlation coefficients with 50% germination time of treated seeds and strong negative correlation in germination Index (GI). T_{50} in Hysun -33 had weak negative correlation coefficient with EG and FGP, while F-330 showed strong negative correlation coefficient between T_{50} and GI, EG and FGP. Other two hybrid (Hysun-38 & 44) had strong negative correlation (-0.9186) and other two parameters also showed negative correlation coefficients with T_{50}. The correlation comparison also showed the strong correlation among all germination parameters (Table IV).

Priming had non-significant effect in normal seeds of Hysun-33, they exhibited significant decrease in E_{50} and MET, but no noticeable effect was observed on EI, EE and FEP with respect to their control, however, low-vigor seeds of Hysun-33 had significant invigoration effect in all parameters of emergence after cold stress, (Table V). The treatment showed pronounced decrease in the duration of E_{50} and MET and increase in EI EE and FEP in low-vigor seeds of Hysun-38, but no tangible change was observed in the $E_{50,}$ and MET and EI, however EE and FEP normal-primed seeds of this hybrid had significant effect. Normal seed of Hysun-44 had no significant effect in almost all parameters of emergence, except $E_{50,}$ which had significant decrease in the duration of 50 % emergence of their seedlings. While low-vigor seeds showed significant decrease in E_{50} and MET but no statistically significant effect on the other parameters with respect to their control. Normal seed of F-330 exhibited significant influence of the treatment in E_{50}, MET and EI but non-significant effect was observed in EE and FEP, while low-vigor seeds had prominent change in almost all emergence attributes except EI, which had non-significant effect with reference to their control, while comparison between Low-vigor/P and N/P seeds of all tested hybrids showed significant effect of the treatment after cold stress.

Seed vigor reduction is conformed by the significant reduction in the length of root and shoot in aged seeds, while the rate of reduction in root/shoot length in all hybrids (Fig. 1a & b) might be the clear index of lowering the vigor of seed during aging by increase in the leakiness of membranes (Fig. 2). The data of teterazolium chloride test record showed that viability of the seeds reduced with aging significantly. Weak and low vigor seeds could not perform well in the field conditions that may be the reason of the significant decline in the vigor percentage seedling in aged seed after cold stress (Fig. 3).

Vigor of the emerged seedling reduced drastically after cold stress. This implied that germinated seeds couldn't continue their growth after emergence. All hybrids showed acute loss of high vigor seedling (G-I) in first category (Low-vigor/Control) except Hysun 44. Priming had non-significant increase in highly vigor seedling in all hybrids in second category seeds (Low-vigor/P). In normal seeds only Hysun- 44 had significant improvement after

Table III. Effect of seed priming on germination of Normal/L-Vigor seeds

Hybrids	Treatments	T_{50} (days)	MGT (days)	Germination Index (%)	EG (%)	FGP
Hysun-33	N/ Control	1.12 f	1.37 f	11.8 d	6.35 ab	95.54 ab
	N/Primed	0.73 i	1.19 h	14.45 a	6.66 a	100 a
	L-v/Control	1.70 b	2.56 b	6.70 h	5.98 c	90.43 c
	L-v/Primed	1.56 c	2.11d	9.22 g	6.50 ab	97.63 ab
Hysun-38	N/ Control	1.01 g	1.67 e	9.68 g	6.37 ab	95.57 ab
	N/Primed	0.84 h	1.27 gh	14.13 a	6.51 ab	97.70 ab
	L-v/Control	1.71 b	2.54 b	4.02 jk	4.15 f	62.30 f
	L-v/Primed	1.45 d	2.09 d	5.60 i	4.67 e	73.17 e
Hysun-44	N/ Control	1.15 f	1.62 e	10.45 f	6.45 ab	98343 ab
	N/Primed	0.69 i	1.24 gh	13.49 b	6.66 a	100.0 a
	L-v/Control	1.53 cb	2.79 a	5.53 i	5.33 d	80.00 d
	L-v/Primed	1.29 e	2.15 d	7.19 h	6.22 bc	93.30 bc
F-330	N/ Control	1.23 e	1.32 g	11.15 e	5.93 c	88.90 c
	N/Primed	0.91h	1.21 h	12.50 c	6.22 bc	93.30 bc
	L-v/Control	1.89 a	2.48 b	3.59 k	3.71 g	55.63 g
	L-v/Primed	1.64 b	2.35 c	4.28 j	4.59 e	68.90 e
LSD (0.05)		0.0744	0.0911	0.5904	0.3021	4.5601

Means sharing the same alphabets differ non- significantly (P>0.05) by DMR test. N and L-v stand for unaged (normal) and aged treatments
T_{50} = Time for 50% germination, MGT = Mean germination time, GI = germination index, EG = Energy of germination, FGP = Final germination percentage

Table IV. Correlation comparison among different parameters of germination of control/L-Vigor seedlings after seed priming (a) Hysun-33 (b) Hysun-38 (c) Hysun-44 (d) F-330

(a)

	T_{50} (days)	MGT(days)	G. Index	EG (%)	FGP (%age)
T_{50} (days)	1				
MGT(days)	0.942886	1			
G.Index	-0.95383	-0.98862	1		
EG (%)	-0.24896	-0.0842	0.232548	1	
FGP (%age)	-0.24848	-0.08349	0.231846	0.9999996	1

(b)

T_{50} (days)	**1**				
MGT (days)	0.91192	1			
G.Index	-0.9186	-0.99804	1		
EG (%)	-0.6636	-0.91152	0.898497	1	
F G EP (%age)	0.66189	-0.91045	0.897113	0.999986	1

(c)

T_{50} (days)	**1**				
MGT (days)	0.91192	1			
G.Index	-0.9186	-0.99804	1		
EG (%)	-0.6636	-0.91152	0.898497	1	
F G P (%age)	-0.66189	-0.91045	0.897113	0.999986	1

(d)

T50 (days)	**1**				
MGT (days)	0.983498	1			
G.Index	-0.99098	-0.9988	1		
EG (%)	-0.95915	-0.99319	0.987984	1	
F G P (%age)	-0.95907	-0.99321	0.987964	0.999999	1

T_{50} = Time for 50% germination, MGT = Mean germination time, GI = germination index, EG = Energy of germination, FGP = Final germination

treatment, while Hysun-33 showed no noticeable change in results, but Hysun-38 had some improvement in normal seed with respect to its control but this improvement was statistically non-significant, However F-330 showed adverse effect of the treatment on the vigor of seedling after cold stress. Hysun-44 showed significant difference in normal seeds and control after cold stress (Fig. 4 & 5).

SDS-page. Fig. 6 shows that some proteins were completely lost as a result of AA, which showed reappearance after priming. Marker range was 10–200 kDa. It was observed that the proteins with approximately 26, 48, 49, 69, 90, 118, 121,150 and 199 kDa showed effect of the AA. Priming showed little improvement in the banding pattern and intensity of protein in normal seeds, while low-vigor seeds had significant improvement in the banding pattern and intensity of protein. The highlighted band showed reversal with reference to their presence and intensity.

DISCUSSION

Accelerated aging treatment caused a decline in both vigor and germinability of sunflower hybrid seeds. Present data of germination revealed that all hybrids showed over all similar behaviour except small differences, because they all have different genetic makeup (Table I). T_{50}, MGT and GI affected by the AA but Hysun-33 and 44 showed non-significant MGT, which are in contradiction with some previous research (Wahid *et al*., 2008). They reported that high temperature with low RH cause faster field emergence in cotton seed. Seeds of *Stryphnodendron polyphyllum* exhibited resistance against the exposure of high temperature (70°C) for 24 h without imbibition the rate and percentage of emergence was not affected significantly (Tambalini & Perez, 1999). The pattern of seed aging is in general, described in terms of its water content during storage. Under AA conditions oil seed absorbed more water than other seeds. This may be due to the denaturation of seed protein at high temperature and moisture levels (Krishnan *et al*., 2003). Krishnan *et al*. (2004) reported that soybean seed absorbed more moisture content than wheat seeds at 45°C than 35°C temperature.

Table V. Effect of seed priming on the emergence of seedling after cold stress (7°C for 7 days)

Hybrids	Treatments	E_{50} (days)	MET(days)	E. Index	EE (%age)	FEP(%age)
Hysun-33	N/ Control	1.17 cd	1.77 ef	17.75 b	3.73 bcde	93.33 abcde
	N/Primed	1.03 e	1.57 gh	18.07 b	3.89 abc	97.33 abc
	L-v/Control	1.49 b	2.02 bc	12.20 fg	3.25 fg	81.33 f g
	L-v/Primed	1.22 c	1.92 d	15.28 d	3.68 bcd	92.07 bcde
Hysun-38	N/ Control	1.11 cde	1.45 hi	16.54 c	3.14 g	78.67 g
	N/Primed	0.99 e	1.39 i	16.83 c	3.52 ef	88.00 ef
	L-v/Control	1.65 a	2.26 a	11.35 g	2.61 h	65.33 h
	L-v/Primed	1.50 b	2.10 b	12.39 f	3.15 g	77.66 g
Hysun-44	N/ Control	1.02 e	1.41 i	18.27 a	3..95 ab	98.67 ab
	N/Primed	0.84 f	1.32 i	19.07 a	4.00 a	100.0 a
	L-v/Control	1.19 c	2.12 b	12.82 ef	3.57 de	89.33 d
	L-v/Primed	1.03 e	1.87 de	13.53 e	3.84 abcd	96.00 abcd
F-330	N/ Control	1.19 c	1.86 de	15.28 d	3.63 cde	90.67 cde
	N/Primed	1.06 de	1.65 fh	16.21 c	3.73 abcd	93.33 bcde
	L-v/Control	1.41 b	2.28 a	5.150 h	1.71 j	42.67 j
	L-v/Primed	0.84 f	2.08 b	5.910 h	2.20 i	54.67 i
LSD		0.1176	0.1391	0.9079	0.2733	6.860

Means sharing the same alphabets differ non- significantly (P>0.05) by DMR test. N and L-v stand for unaged (normal) and aged treatments
E_{50} = Time for 50% emergence, MET = Mean emergence time, GI = Emergence index, EE = Energy of Emergence, FEP = Final Emergence percentage

Fig. 1. Effect of accelerated ageing on the (a) Redicle length (b) Plumule length (cm) of emerged seedlings of Sunflower seeds, Vertical bars are ± se

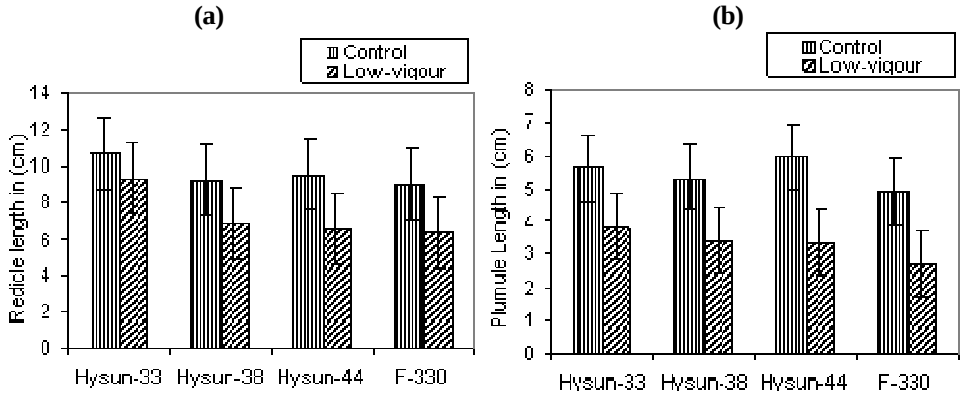
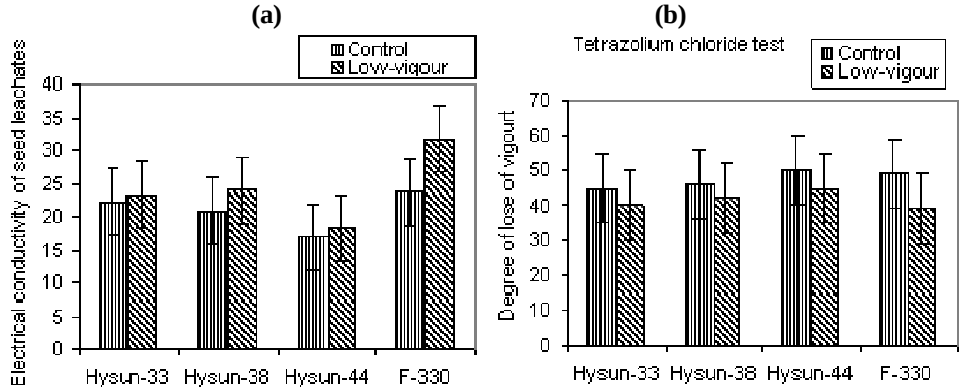


Fig. 2. Influence of accelerated ageing (a) on the membrane integrity evaluated by the electrical conductivity of seed leachates (µs/cm) and (b) on the vigor/viability of seeds by TTC test, Vertical bars are ± se



Seed vigor reduction is conformed by the significant reduction in the length of root and shoot in aged seeds, while the rate of reduction in root/shoot length in all hybrids (Fig. 1a & b) might be the clear index of lowering the vigor of seed during aging, which is supported by the previous results of decline in germination and vigor at all water content level by decrease in the growth of embryonic axis (Abdul-Baki & Anderson, 1973) increase in the leakiness of membranes (Wahid *et al*., 2008) (Fig. 2). It is also suggested that membrane become more susceptible to imbibitional damage

with aging (Van Bilsen *et al*., 1994). The parallel decline in seed vigor and membrane performance might have the causal relationship. Most workers reported that the leakage might be due to the loss of metabolic energy for membrane transport mechanism. Several workers have proposed that lipid within the membrane autoxidized/peroxidized cause aging loss membrane integrity (McDonald, 1976). Thus, the first step in seed deterioration would be the loss of the membrane integrity, leading to an increase in its permeability and causing leakage of cellular solution during seed soaking (Wilson & McDonald, 1986).

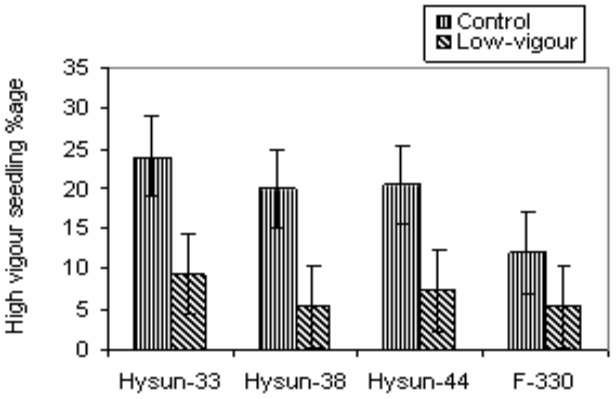
Abdul-Baki and Baker (1973) found that mitochondria of aged seeds are more fragile. The data of teterazolium chloride test record showed that viability of the seeds reduced with aging significantly, these results could be explained on the bases of above discussion. Mitochondrial activity is the sign of viability when it will be damaged then seed would show the loss of viability. Weak and low vigor seeds could not perform well in the field conditions that may be the reason of the significant decline in the vigor percentage seedling in aged seed after cold stress (Fig. 3).

Primed seeds usually exhibit the increased germination rate, reduced mean germination time, greater germination uniformity and some time greater total germination percentage in many plant species (Jett *et al*., 1996; Hardegree & Van Vactor, 2000). While the data (Table III) revealed that priming was effective in reducing the time for 50% emergence and MGT and increase in germination percentage in low-vigor seeds of almost all hybrids except F-330, while FGP both in normal and low-vigor seeds showed minor increase. These results are similar to the wok done by Stofella *et al.* (1992) for pepper seeds and Tarquis and Bradford (1992) for lettuce seeds.

Priming presumably allowed some repairs of damaged to membrane caused by deterioration. In this study the primed seeds in both normal and low-vigor showed better germination pattern and higher vigor level than non-primed. These were consistent with the Ruan *et al.* (2002) findings on rice seedling establishment in flooded soil and Hampton and TeKrony's (1995) view that high vigor seed lot would perform better in field performance under environmentally stressed seed bed conditions than low-vigor seed lots. Primed seeds might have better plasma membrane structure by slow hydration (Jett *et al.,* 1996). Priming also causes to reduce the adherence of seed coat due to imbibition, which may permit to emerge out redicle without resistance as Nascimento and West (1998) reported that the priming minimizes seed coat adherence during emergence of muskmelon seeds.

The improvement in germination and vigor of normal/low-vigor seed might be due to reserve mobilization of food material, activation and re-synthesis of some enzymes, DNA and RNA synthesis start during osmotic priming. Rapid embryo growth resulted when the obstacle to germination was removed (Khan, 1992). These changes include macromolecular synthesis, several enzyme activities, increase in germinating power and vigor and overcoming of

Fig. 3. Influence of pre-sowing cold stress at (7°C for seven days) on the high vigor seedling (G-I) of normal/low-vigor seeds



dormancy (Khan, 1992; Smith & Coob, 1992). Early studies have shown that osmotic priming in sweet pepper and tomato seeds is associated with DNA synthesis (Lantieri *et al.*, 1994).

This research revealed that the priming improved root/shoot length in normal seed approximately in all hybrids but the low-vigor seed showed no change at all or minute increase in all hybrids. The results of the normal seed in consistent with work done by Stofella *et al.* (1992) for pepper seeds and Tarquis and Bradford (1992) for lettuce seeds. The results of normal seeds in present study was not co-related with Afzal *et al.* (2002), who reported that osmopriming does not enhance the root/shoot length. These were correlated with low-vigor seeds, which showed no improvement. Root fresh weight of the normal treated seeds had visible increase in size in all hybrids except Hysun-38, which showed some adverse results but these were non-significant. Low-vigor seed of all hybrids exhibited no significant change in the root weight of treated seed with respect to their control. Shoot weight of the seedling in normal and low-vigor seed of all hybrids showed increase but it was statistically non significant except F-330, which had prominent effect on the weight of the normal shoot. The results of the normal seed were supported by the result of Lanteri *et al.* (1996) on the pepper seeds (*Capsicum annuum*). They found that the priming of the normal (un-aged) seeds always induced nuclei of embryo root tips to enter the synthetic phase by inducing the DNA synthesis. But same treatment had different effects on the Low-vigor seeds, which might depend on the degree of deterioration during accelerated aging. Present study revealed that the low-vigor seeds had intense damage during AA, which may allow the seeds to germinate but they could not revive their vigor after priming treatment.

Generally, seed storage caused a decrease in the protein content, which may be related to oxidation of the amino acids, due to the increase in the respiratory activity and advance in the deterioration process of the stored seeds. Thus, prolonged seed storage would increase the metabolic activity of the seeds and consequently decrease the reserve

Fig. 4. Influence of seed priming on the vigor of control/low vigor seeds, (a) root fresh weight and (b) shoot fresh weight (g)

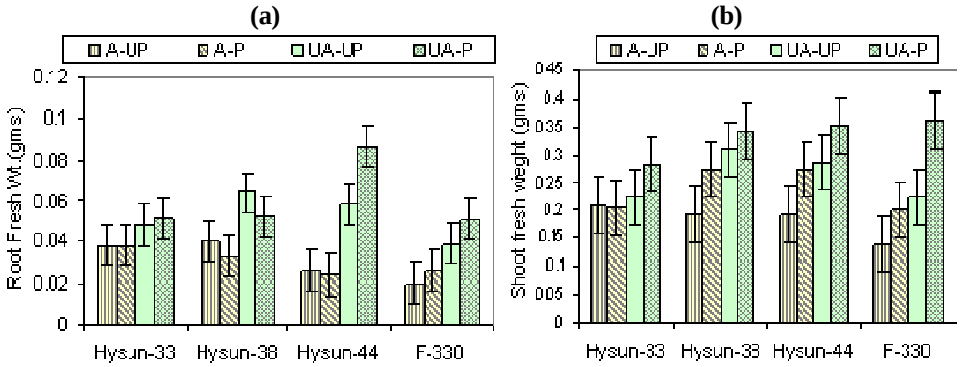
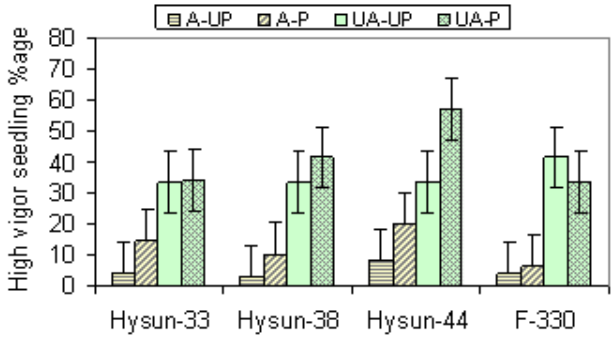


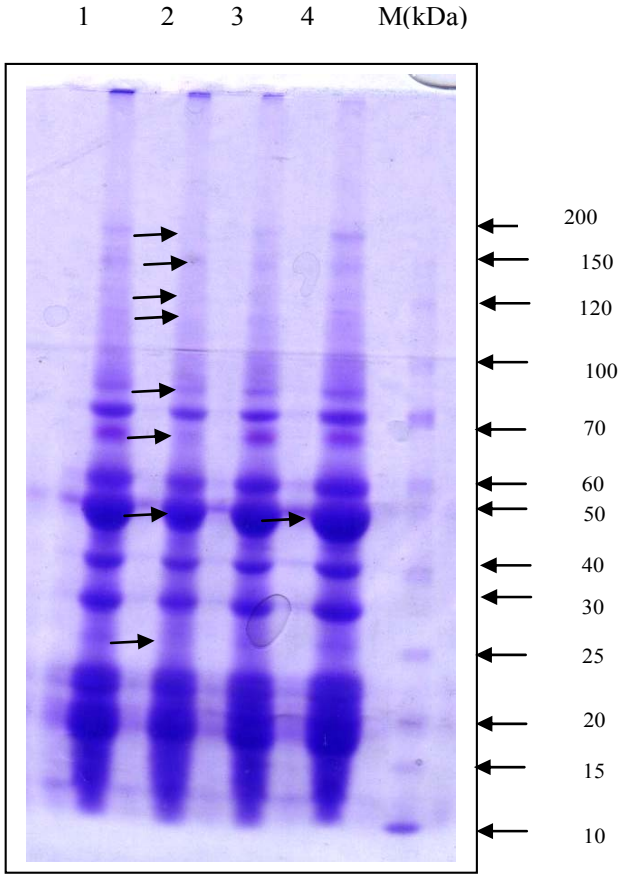
Fig. 5. Influence of seed priming on the vigor of emerged seedling of control/low-vigor seeds of Sunflower hybrids after cold stress (7°C for seven days), P and U-P stand for primed and un-primed treatments



substance content and reduce the dry material weight of the seeds (Bewley & Black, 1994). The SDS PAGE studies provide clear understanding about the mechanism of accelerated aging and priming. The results showed that the proteins with approximately 26, 48, 49, 69, 90, 118, 121, 150 and 199 kDa effected by the AA. Wahid *et al.* (2008) reported variation in the protein expression of sunflower achenes after priming with various chemicals. Priming showed little improvement in the banding pattern and intensity of protein in normal seeds, while low-vigor seeds had significant improvement in the banding pattern and intensity of protein (Fig. 6). Several authors (Bray *et al.,* 1989; Smith & Coob, 1992) have shown that osmotic priming in seeds from some oil species has caused significant increases in the soluble protein content, compared with non-treated seeds from these same species. They further found that the increase in protein synthesis could be related to greater germination capacity and performance under field conditions shown by the osmoprimed seeds. Khan (1992) found that twice amino acids were incorporated in proteins during the first 24 h of imbibitions of sweet pepper seeds in PEG solutions (osmotic priming).

In crux, seed priming overcame the inhibitory effect of low temperatures and increased germination and the stand

Fig. 6. SDS PAGE profile of *sunflower* (1) normal, (2) low vigor unprimed, (3) normal primed, (4) low vigor primed and (M) molecular marker R-250 (10-200 KDa)



establishment. As results indicated, all hybrids showed acute lose of vigor (G-I) in first category (low-vigor/un-primed) except Hysun 44. Priming had no increase in high vigor seedling in all hybrids in second category seeds (low-vigor/primed). With differences among the cultivars, ageing deteriorated the seed quality, which was improved by seed priming, as a result of repair mechanisms. Thus, low-vigor seeds could benefit from priming treatments.

REFERENCES

Abdul-Baki, A.A. and J.D. Anderson, 1973. Vigor determination in soybean seed by multiple criteria. *Crop Sci.*, 13: 630–633

Afzal, I., S.M.A. Basra, N. Ahmad, M.A. Cheema, E.A. Warraich and A. Khaliq, 2002. Effect of priming and growth regulator treatment on emergence and seedling growth of hybrid maize *Zea mays*. *Int. J. Agric. Biol.,* 4: 303–306

Association of official seed Analysts, 1983. *Seed Vigor Testing Handbook*, p: 93. Contribution no. 32 to the Handbook of Seed Testing

Bailly, C., A. Benamar, F. Corbineau and D. Come, 1998. Free radical scavenging as affected by accelerated ageing and subsequent priming in sunflower seeds. *Physiol. Plant,* 104: 646–652

Basra, S.M.A., E. Ullah, E.A. Warriach, M.A. Cheema and I. Afzal, 2003. Effect of storage on growth and yield of primed canola *Brassica napus* seeds. *Int. J. Agric. Biol.*, 5: 117–120

Bewley, J.D. and M. Black, 1994. Seeds: physiology of development and germination. Plenum Press, New York, p. 445

Bray, C.M., 1995. Biochemical processes during osmopriming of seeds. *In*: Kigel, J. and G. Galili (eds.), *Seed Development and Germination*, pp: 767–789. New York, Basel, Hong Kong, Maceldekker

Bray, C.M., P.A. Davison, M. Ashraf and R.M. Taylor, 1989. Biochemical changes during osmotic priming of leek seeds. *Annl. Bot.,* 63: 185–193

Copeland, L.O. and M.B. McDonald, 1995. *Principals of Seeds Science Technology*. Chapman and Hall, New York, USA

Egli, D.B. and D.M. TeKrony, 1996. Seedbed conditions and predctions of field emergence of soybean seed. *J. Prod. Agric.*, 9: 365–370

Ellis, R.A. and E.H. Roberts, 1981. The quantification of aging and survival in orthodox seeds. *Seed Sci. Technol.*, 9: 373–409

Farooq, M., S.M.A. Basra, M.A Cheema and I. Afzal, 2006. Integration of presowing soaking, chilling and heating treatments for vigor enhancement in rice *Oryza sativa* L. *Seed Sci. Technol.*, 34: 189–196

Farooq, M., S.M.A. Basra, A. Wahid, N. Ahmad and B.A. Saleem, 2009. Induction of drought tolerance in rice (*Oryza sativa* L.) by exogenous application of salicylic acid. *J. Agron. Crop Sci.*, 195: in press

Fujikura, Y. and C.M. Karssen, 1995. Molecular studies on osmoprimed seeds of cauliflower: a partial amino acid sequence of a vigor-related protein and osmopriming-enhanced expression of putative aspartic protease. *Seed Sci. Res.*, 5: 177–182.

Halder, S., S. Kole and K. Gupta, 1983. On the mechanism of sunflower seed deterioration under two different types of accelerated aging. *Seed Sci. Technol.,* 11: 331–339

Hannan, R.M. and H.H. Hill, 1991. Difference in lipid profiles from fresh and aged lettuce *Lactuca sativa* L. seed determined by capillary supercritical fluid chromatography and GC/mass Spactrometry. *J. Seed Technol.*, 15: 79–90

Hardegree, S.P. and S.S. Van Vactor, 2000. Germination and emergence of primed grass seeds under field and simulated-field temperature regimes *Annl. Bot.*, 85: 379–390

Hsu, L.J. and J.M. Sung, 1997. Antioxidant role of glutathione associated with accelerated aging and hydration of triploid watermelon seeds. *Physiol. Plantrum*, 100: 967–974

ISTA, 1985. International Rule for Seed Testing. 1985. *Seed Sci. Technol.,* 13: 299–355

Jett, L.W., G.E. Welbau and R.D. Morse, 1996. Effect of matric and osmotic priming treatments on Broccoli seed germination. *J. American Soc. Hort. Sci.*, 121: 423–429

Justice, O.L. and L.N. Bass, 1978. *Principle and Practices of Seed Storage*, p: 289. Hand Book No. 506, USDA, Washington DC

Kathiresan, K., V. Kalyani and J.L. Gnanarethinam, 1984. Effect of seed treatments on field emergence, early growth and some physiological processes of Sunflower *Halianthus annuus* L. *Field Crop Res.*, 9: 215–217

Khan, A.A., 1992. Preplant physiological seed conditioning. *Hort. Rev.,* 14: 131–181

Koehler, K.H., B. Voigt, H. Spittler and M. Schelenz, 1997. Biochemical events after priming and priming of seeds *In*: Ellis, R.H., M. Black, A.J. Murdoch and T.D. Hong (eds.), *Basic and Applied Aspects of seed Biology, Proc. 5th Int. Workshop on Seeds Reading*, pp: 531–536

Krishnan, P., D.K. Joshi, S. Nagarajan and A.V. Moharir, 2004. Characterization of germinating and non-germinating wheat seeds by nuclear magnetic resonance NMR spectroscopy. *European J. Biol. Phys.*, 33: 76–82

Krishnan, P., S. Nagarajan, M. Dadlani and A.V. Moharir, 2003. Characterization of wheat *Triticum aestivum* and soybean *Glycine max* seeds under accelerated ageing conditions by proton nuclear magnetic spectroscopy. *Seed Sci. Technol.*, 31: 541–550

Laemmli, U.K., 1970. Cleavage of structural protein during assembly of the head of bacteriophage T4. *Nature*, 22: 680–685

Lanteri, S., E. Nada, P. Belletti, L. Quagiottil and R.J. Bino, 1996. Effects of controlled deterioration priming on germination and nuclear replication in seeds of pepper *Capsicum annuum* L. *Annl. Bot.*, 77: 591–597

Lantieri, S., F. Saracco, H.L. Kraak and R.J. Bino, 1994. The effects of priming on nuclear replication activity and germination of pepper *Capsicum annuum* and tomato *Lycopersicon esculentum* seeds. *Seed Sci. Res.,* 4: 81–87

Liklatchev, B.S., G.V. Zelensky, G.Y. Kiashko and Z.N. Schevchenko, 1984. Modelling of seed ageing. *Seed Sci. Technol.*, 12: 385–393

McDonald, B.M., 1999. Seed deterioration: physiology, repair and assessment. *Seed Sci. Technol.*, 27: 177–237

McDonald, M.B., 1976. A review and evalution of seed vigor tests. *Proc. Assoc. Official Seed Anal.*, 56: 109–139

McDonald, M.B., 1995. Standardization seed vigor test. *In:* Van De Venter, H.A. (ed.), *ISTA Seed Vigor Testing Seminar*, pp: 88–97. ISTA, Copenhagen, Denmark

Nascimento, W.M. and S.H. West, 1998. Priming and seed orientation affect emergence and seed coat adherence and seedling development of muskmelon transplants. *Hort. Sci.*, 33: 847–848

Ray, M.B., S. Halder and K. Gupta, 1990. Differential responses of early and late cultivation of rice *Oryza stiva* L. seeds under accelerated ageing. *Seed Sci. Technol.*, 18: 823–831

Ruan, S., Q. Xue and K. Tylkowska, 2002. The influence of priming on germination of rice *Oryza sativa* L. seeds and seedling emergence and performance in flooded soil. *Seed Sci. Technol.*, 30: 61–67

Smith, P.T. and B.G. Coob, 1992. Physiological and enzymatic characteristics of primed, redried, and germinated pepper seeds *Capsicum annuum* L. *Seed Sci. Technol.*, 20: 503–513

Steel, R. D.G., J.H. Torrie and D.A. Dickey, 1997. *Principle and Procedures of Statistics: ZA Biomaterical Approach,* 3rd edition. MeCraw Hill, New York

Stewart, R.R.C. and J.D. Bewley, 1980. Lipid peroxidation associated with accelerated aging of soybean axis. *Plant Physiol.*, 65: 245–248

Stofella, P.J., D.P. Lipucci, A. Pardossi and F. Tognoni, 1992. Seedling root morphology and shoot growth after seed priming or pre-gemination of bell pepper. *J. American Soc. Hort. Sci.,* 27: 214–215

Tambalini, M. and S.C.J.G.A. Perez, 1999. Physiological effect of temperature and thermal stress on the seed imbibition of *Stryphnodendron polyphyllum*. *J. Trop. For. Sci.*, 11: 680–689

Tarquis, A.M. and K.J. Bradford, 1992. Prehydration and priming treatments that advance germination also increase the rate of deterioration of lettuce seed. *J. Exp. Bot.,* 43: 307–317

TeKrony, D.M., 1995. Accelerated Ageing. *In*: Van De Venter, H.A. (ed.), *ISTA Vigor Test Seminar*, pp: 53–72. Copenhagen

Trawatha, S.E., D.M. TeKerony and D.F. Hildebrand, 1995. Relationship of soybeans seed quality of fatty acids and C-aldehyde level during storage. *Crop Sci.,* 35: 1415–1422

Van Bilsen, D.G.J.L., F.A. Hoekstra and J.H. Crowe, 1994. Altered phase behavior in membranes of aging dry pollen may cause imbibitional leakage. *Plant Physiol.,* 104: 1193–1099

Vertucci, C.W. and A.C. Leopold, 1986. Physiological activities associated with hydration leveling seeds. *In*: Leopold, A.C. (ed.), *Membranes, Metabolism and Dry Organisms*, pp: 35–49. Cornell University Press, Ithaca, New York

Wahid A, A. Noreen, S.M.A. Basra, S. Gelani and M. Farooq, 2008. Priming-induced metabolic changes in sunflower (*Helianthus annuus*) achenes improve germination and seedling growth. *Bot. Stud.*, 49: 343–350

Wilson, D.O. and. M.B. McDonald, 1986. The lipid peroxidation model of seed ageing. *Seed Sci. Technol.,* 14: 269–300

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