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Research Article

Effect of Priming on Various Seeds Quality Parameter in Accelerated Aged Seed Lots of Barley (*Hordeum valgare* L.)

D. S. Chauhan* and D. P. Deswal

Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar

*Corresponding Author E-mail: chauhan@rediffmail.com

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ABSTRACT

The present investigation was conducted at the Department of Seed Science and Technology, CCS Haryana Agricultural University Hisar. The study was planned to determine the effect of aging and priming on seed storage proteins of barley under natural and artificial aged conditions. An effort was also made to define the stability of improvement in seed vigor due to priming. A study was conducted on six barley genotypes Viz.ALFA-93, BH-393,BH-75,BCU-3,RD-2552 and K-551. All Six varieties was divided in two seed lots subjected to accelerated ageing at 42±1°C for 72 hrs on 100% relative humidity. Both fresh and accelerated aged seeds of genotype BH-75 and BH-393 were primed by soaking in water and in an aqueous solution (27.3 gm/ litre) of polyethylene glycol 6000 (PEG 6000) providing an osmotic potential of -1Mpa (Michel & Kaufnann, 1973) for varible time period (12,18,24 &30 hrs) and different observation related to seed vigour i.e. standard germination, vigor index, rate of germination and dehydrogenase activity was recorded. The present study revealed that priming with water and aqueous solution of PEG 6000 significantly increased the standard germination, rate of germination, dehdrogenase activity and vigor index in both the varieties by boosting the repair mechanism of seed. Among all the priming treatments seed soaking of 12 hrs was found most effective.

Keywords: *Barley, Crops, Arid, Semiarid Regions, Environmental Factors*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a common crop grown in the semiarid Mediterranean area, and due to its drought resistance, is one of the most widely grown crops in arid and semiarid regions of the world (Ghazi et al., 2007). Seed deterioration can be defined as the loss of quality, viability and vigour either due to aging or effect of adverse environmental factors. Seed characteristics decrease under long storage condition due to aging. It is the reason of declining in germination, emergence and seedling growth (Soltani et al., 2008).

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Deterioration of seed is serious problem in tropical and subtropical countries like India where high temperature and humidity accelerate the seed ageing phenomenon and seed priming is a unique technique which can allows some of the metabolic processes necessary for germination to occur without germination take place. In priming, seeds are soaked in different solutions with high osmotic potential. This prevents the seeds from absorbing in enough water for radicle protrusion, thus suspending the seeds in the lag phase (Taylor et al., 1998). Seed priming has been commonly used to reduce the time between seed sowing and seedling emergence and to synchronize emergence (Parera & Cantliffe, 1994). In seed priming, the osmotic pressure and the period for which the seeds are maintained in contact with the membrane are sufficient to allow pre-germinative metabolic processes to take place within the seeds up to a level limited to that immediately preceding radicle emergence. Seed priming (Pre-sowing hydration treatments of seeds) is a widely used technique to enhance seed performance, notably with respect to rate and uniformity of germination thereby enabling better crop establishment (Heydecker, 1973; Heydecker, 1978; Bradford, 1986; Parera & Cantliffe 1994; Taylor et al., 1998). Numerous studies have demonstrated that priming is associated with an increase in protein synthesis (Bray et al., 1989; Dell'Aquilla & Bewley, 1989; Davison & Bray 1991; Dell'Aquilla & Sapda 1992).

Osmotica that have showed good potential to enhance germination emergence; growth and grain yield of wheat include solution of potassium hydrophosphate (Das & Choudhury 1996), Polyethylene glycol (Dell'Aquilla & Taranto, 1986) and Potassium chloride (Misra & Dwibedi 1980). On farm-priming of wheat seeds has been found as a mean of promoting rapid germination emergence and to increase seedling vigor and yield (Harris et al., 2001). Seed hydrated for 1-3 h gave better results than those hydrated for shorter or longer duration. The root length and dry matter of seedlings were significantly more for seeds hydrated for 1-3 h. The effectiveness of hydration by moisture equilibrium for 24-48 h followed by dehydration, in controlling the deterioration of cotton seeds, it would eliminate leaching of toxic products from the seed as a possible reason of the beneficial effect. The quenching effect of hydration of the propagation of free radicals and improved membrane integrity have been reported by Davison et al., (1991), Dell' Aquila & Tritto (1991), Garcia et al., 1995 and Chang & Sung (1998). Many studies have related the priming induced germination enhancement with the improvement in membrane integrity as well as the increase in protein and nucleic acid synthesis (Dell' Aquila & Taranto, 1986; Fu et al., 1988; Smith & Cobb, 1991). Disruption of membranes due to maturation and drying is known to be repaired and returned to their normal configuration during priming (Rao et al., 1987; Fu et al., 1988).Therefore the present study was conducted to access the stability of improvement in various quality parameters in marginal seed lots of two barley varieties by using osmo and hydropriming techniques with PEG (6000).

MATERIALS AND METHODS

Seed material comprised of six varieties of Barley *Viz*. ALFA-93, BH-393,BH-75,BCU-3,RD-2552 and K-551 having germination above minimum seed certification standard (MSCS) i.e.85% was collected at the time of sowing and stored in ambient conditions and present research work was carried out in the laboratory of Department of Seed Science and Technology CCS Haryana Agricultural University, Hisar from 2004 to 2005. For defining the variables for artificial ageing, seed of all six varieties were artificially aged on two temperature and time variables (42±1ºC/45±1ºC /48 hrs, 42±1ºC/45±1ºC /72 hrs.) and observations were recorded after ageing. Aged as well as non aged seed lots were evaluated for following tests.

Conditioning of seeds

The freshly harvested seeds of all the genotypes were subdivided into two lots. The first lot was kept as such and designated as fresh seed lot for further

tests and observations. While other lot of all the genotypes was exposed to accelerated ageing conditions and designated as aged seed lot.

Accelerated ageing

To observe the optimum time for accelerated ageing test, i.e., period to 50% reduction of seed germination, sufficient number of seed in a single layer from each seed lot of the six genotypes was taken on wire mesh tray fitted in plastic boxes having 40 ml of distilled water. The boxes were placed in ageing chamber after closing their lids. The seeds were aged at 40±1°C at different time intervals and tested for standard germination in three replications of 100 seeds each. The number of normal seedlings was counted on 7th day and expressed in percentage.

Seed from both seed lot was used to conduct following vigor and viability tests as detailed below. Each test was conducted in a completely randomized design with three replications.

Priming of seeds

Fresh and accelerated aged seeds of genotype BH-75 and BH-393 were primed by soaking in water and in an aqueous solution (27.3 gm/ litre) of polyethylene glycol 6000 (PEG 6000) providing an osmotic potential of -1Mpa (Michel & Kaufnann, 1973) for variable time period, and following seed lots were prepared.

1. Fresh seed non-primed
2. Fresh seed water primed (12,18, 24 & 30 hrs)
3. Fresh seed PEG primed (12,18, 24 & 30 hrs)
4. Accelerated aged seed non primed.
5. Aged seed water primed (12,18, 24 & 30 hrs)
6. Aged seed PEG primed (12,18, 24 & 30 hrs)

The following observations were recorded on all the seed lots as per ISTA, 1999

1. Standard germination test (%)
2. Electrical conductivity (µs/cm/seed)
3. Rate of germination.
4. Dehydrogenase activity test (optical Density)
5. Seed vigor index- 1.
6. Seed vigor index-11.

Standard Germination Test (%)

One hundred seeds of each genotype replicated thrice were placed on moistened paper (B.P.) and placed in seed germinator at 20±1°C temperature with 90-95 per cent relative humidity. The final count of germinated seeds was taken on 7th day and normal seedlings were expressed as percent germination of total seed (ISTA, 1999).

Rate of germination (%)

The number of seedlings emerged were counted on each day up to final count of seedling and rate of germination was calculated as described by Maguire (1962).

$$\frac{\text{No. of seedlings emerged}}{\text{first day of count}} + \text{-------} \frac{\text{No of seedlings emerged}}{\text{final day of count}}$$

Electrical conductivity test (EC) (□s/cm/seed)

Twenty normal and uninjured seeds were soaked in separate beakers each containing 50 ml of distilled water. Seeds were immersed completely in water and beakers were covered with foil. Thereafter, these samples were placed in germinator at 20°C for 24 hours in dark. The electrical conductivity of seed leachates was measured by conductivity bridge meter (ISTA, 1999).

Dehydrogenase activity (DHA) test

The method was suggested by Kittock & Law (1968). Reduction of 2,3,5-triphenyl tetrazolium chloride to red formazan by

dehydrogenase enzymes in seed embryos is the basic principle for topographical tetrazolium test for seed viability but the method described here is a quantitative method, which may be used to determine varying dehydrogenase activity between seed lots of similar viability and therefore, it is a measure of seed Vigor. To conduct DHA test, the representative seed samples of each lot, replicated thrice were grounded to pass through 20-mesh screen. The 200 mg flour was soaked in 5 ml of freshly prepared 0.5% TTC solution having pH 7.0. After shaking, the mixture was incubated at a temperature of 35°C for 2 hours. Then it was centrifuged at 10,000 rpm for 3 minutes and the supernatant was poured off. The formazan was extracted with 10 ml acetone for 16 hours at room temperature. It was then centrifuged for 3 minutes at 10,000 rpm and acetone solution containing formazan was transferred to the cuvette. The absorbance reading of the solution was taken at 520 nm wavelength using Systronics spectrophotometer 169.

Seedling length (cm)

The measurement of linear growth of five randomly selected normal seedlings taken from each seed lot of individual genotypes was measured in centimeters in all three replications at the termination of standard germination test period.

Seedling dry weight (mg)

Normal seedlings selected for measuring their length were kept further for taking dry weight. These were dried in hot air oven at 80°C for 24 hours and then seedling dry weight was recorded in milligrams. The average weight of five seedlings was taken for further calculations.

1000 seed weight (g)

Three replications of 1000-seeds each were weighed individually and the mean of these observations was recorded as 1000-seed weight.

Moisture content (%)

Precent moisture of seeds was calculated according to ISTA (1999) by hot air oven method Ten gram seeds from each categories were kept in moisture box in oven at 130± 1 °c for 2 hrs. the samples were taken out and cooled in desiccators before weighing. Moisture content was calculated as given below: -

$$\text{Moisture percentage} = \frac{M_2 - M_1}{M_2 - M_1} \times 100$$

Where

M_1 – is the weight of empty bottle and its lid .

M_2 – is the weight of bottle and its lid and its content before drying.

M_3 – is the weight of bottle, its lid and its content after drying.

Vigor index

Seedling vigor indices were calculated according to methods suggested by Anderson and Abdul-baki (1973).

Vigor index-I = Standard germination (%) x seedling length (cm)

Vigor index-II = Standard germination (%) x seedling dry weight (mg)

RESULT AND DISCUSSION

In seed priming seed water uptake is the first step as soon as seed is exposed to water and in present experiment the amount water uptake after each priming treatment was measured in fresh and aged seed lot. The rate of water uptake was faster when priming was done using plain water as compared to aqueous solution of PEG in both seed lots. Within a priming experiment aged seeds were slower to absorb the moisture as compared to fresh seeds. In water primed seeds the water uptake continues till 12 hrs of priming

and then slow down due to control hydration in case of PEG.

1. Standard germination

In table (1, 2, 3 and 4) Effect of various priming treatments was observed on standard germination of fresh and aged seed lot of both cultivars viz., BH-75 and BH-393.It was observed that fresh seed of both cultivars was quite healthy and showed 98% germination, whereas aged seed lot was deteriorated and standard germination was observed as 68%in BH-75 and 69% in BH-393.

Priming treatment to fresh seed lot of both the cultivars did not show any significant improvement in germination whereas, priming of aged seed lot of both the cultivars showed improvement up to significant level. The improvement in aged seed lot of both cultivars was recorded significantly higher in PEG primed seed lots. Among the varieties BH-393 showed 30% improvement as compared to 9% in BH-75 when primed with PEG.

With in the time treatment 12 hours treatment was observed best among all the treatments. In this treatment aged seed lot of BH-393, BH-75 showed 30% and 16% improvement in standard germination respectively. Effect of various priming treatments was observed on standard germination of fresh and aged seed lot of both cultivars viz., BH-75, and BH-393. It was observed that priming treatment to fresh seed lot of both cultivars did not show any significant improvement in germination. Similar response of fresh seed of Indian tomato to priming has been reported by Pandita and Nagarajan (2000) whereas priming of aged seed lot of both the cultivars showed improvement upto significant level. This type of response has been reported by various workers (Tarquis & Bradford (1992) in lettuce, Goel et al. (2003) in cotton, and Benamar et al., (2003) in pea seed). Nagarajan & Pandita (2001) reported that osmo priming can delay the onset of deterioration caused by accelerated ageing in tomato.

Table 1: Effect of priming treatments on germination vigor index-I and vigor-II in fresh barley CV. BH-393

Priming treatments	**Germination (%)**	***Vigour index I***	**Vigour index II**
Water	96.267	2498.995	1110.993
PEG	95.067	2413.888	997.947
S.E. (m)			7.00740
C.D. at 5%	N.S.	N.S.	20.6767
Control	98.000	2810.640	1176.000
12 hrs	98.000	2737.800	1222.100
18 hrs	95.833	2538.633	1003.383
24 hrs	94.500	2255.466	970.133
30 hrs	92.000	1939.667	900.733
S.E. (m)	0.6791	100.7543	11.0797
C.D. at 5%	2.0037	297.2932	32.6927

Table 2: of priming treatments on germination vigor index-I and vigor-II in aged barley CV. BH-393

Priming treatments	Germination (%)	Vigour index I	Vigour index II
Water	86.00	1952.933	896.960
PEG	97.133	2081.493	968.760
S.E. (m)	0.3682	39.9167	24.8095
C.D. at 5%	1.0864	117.7813	8.4081
Control	69.833	1028.733	584.367
12 hrs	99.000	2604.000	1140.500
18 hrs	98.000	2250.800	1046.900
24 hrs	96.333	2242.533	977.700
30 hrs	94.667	1960.000	914833
S.E. (m)	0.5821	63.1139	13.2943
C.D. at 5%	1.7177	186.2286	39.2272

Table 3: Effect of priming treatments on germination vigor index-I and vigor-II in fresh barley CV. BH-75

Priming treatments	Germination (%)	Vigour index I	Vigour index II
Water	91.600	2248.747	1190.629
PEG	90.800	2119.140	1175.312
S.E. (m)			
C.D. at 5%	N.S.	N.S.	N.S.
Control	98.000	2658.667	1101.333
12 hrs	95.667	2403.850	1402.290
18 hrs	94.667	2220.133	1306.677
24 hrs	87.167	1948.000	1122.287
30 hrs	80.500	1689.067	982.267
S.E. (m)	1.5706	72.3140	23.3131
C.D. at 5%	4.6342	213.3750	68.7892

Table 4: Effect of priming treatments on germination vigor index-I and vigor-II in aged barley CV. BH-75

Priming treatments	Germination (%)	*Vigour index I*	Vigour index II
Water	74.200	1509.467	849.781
PEG	77.533	1523.760	994.807
S.E. (m)	0.7513		10.1632
C.D. at 5%	2.2168	N.S.	29.9883
Control	68.667	713.667	707.467
12 hrs	84.500	1979.000	1095.557
18 hrs	78.000	1829.500	979.333
24 hrs	74.333	1556.300	895.213
30 hrs	73.833	1504.600	933.900
S.E. (m)	1.1879	63.5037	16.0694
C.D. at 5%	3.5051	187.3787	47.4156

1. Vigor index –1 and II

It was recorded that fresh seed lot of both the varieties (BH-393, Bh-75) did not show any significant improvement in vigor index-1, as depicted by data in table (1, 2, 3and 4).But aged seed lot of both varieties showed significant improvement in vigor index-1. Maximum improvement was observed in case of PEG primed seed lot in both cultivars (BH-393, 2081 and BH-75, 1523) .Within the treatment of time interval, maximum improvement was recorded in aged seed lots of both cultivars of barley at 12 hrs treatments it was also recorded maximum as 2604.00 and 1979.000 in BH-393 andBH-75 respectively.

In Case Vigor Index-II In table (1,2,3 and 4) Periodical data on vigor index-II showed significant improvement in fresh primed seed lot of BH-393. Maximum vigor index-11 was recorded in PEG primed treatment in cultivars BH-393 (1110.97), whereas BH-75 did not showed any improvement in vigor index-11.In case of aged primed seed lot it was significantly higher in both the cultivars. It was recorded maximum in PEG primed seed lot 968.760 in BH-393 and 994.807 in BH-75.In time interval treatment significant improvement in vigor index-11 was observed in fresh seed lot of BH-393 and recorded maximum improvement in 12 hrs treatment, while BH-75 did not exhibited any improvement. Significant improvement was observed in both the varieties of aged seed lot of barley. Maximum improvement was recorded in 12 hrs. The present experiment revealed that there is non significant improvement in fresh seed lot of both the cultivars and age seed lot of both the cultivars showed significant improvement. Maximum important was observed in case of PEG primed seed lot in both the cultivars. Nagarajan & Pandita (2001), reported that PEG priming reduces the deleterious effect of ageing and improved germination, speed of germination and vigor in tomato seed which were subjected to accelerated ageing. Nascimento et al. (2004) reported that priming increased the germination of seed of low vigor and response was cultivar dependent.

2. Rate of germination

Effect of various priming treatments was observed in rate of germination of fresh and aged seed lots of both cultivars BH-75 and BH-393. Quite significant improvement was observed in rate of germination in both the cultivars. In fresh seed lot It was significantly higher in water primed seed lot in both the varieties 23.613 in BH-393 and 20.891 in BH-75.whereas in aged seed lot of both the cultivars showed maximum improvement in PEG primed seed lot of BH-393 (22.493) and BH-75 (19.010) in table (5, 6, 7 and 8). In case of time interval maximum improvement was observed in both the cultivars in fresh primed seed lots 24.207 and 23.368 in BH-393 and BH-75 respectively. While in case of aged primed seed lots of barley maximum improvement was observed in 12 hrs treatments and was recorded as 24.177 in BH-393 and 19.880 in BH-75. Bailly et al. (2002) reported that accelerated ageing decreased seed germiabilly and slowdown the hypoctyl growth, whereas priming with PEG solution resulted in an increase in germination rate and seedling growth in sunflower.

Table 5: Effect of priming treatments on rate of germination, dehdyrogenase activity and electrical conductivity in fresh barley CV. BH-393

Priming treatments	Rate of germination (%)	Dehydrogenase activity	Electrical conductivity
Water	23.613	0. 189	42.396
PEG	21.909	0.169	44.287
S.E. (m)	0.1292	0.0012	NS
C.D. at 5%	0.37813	0.0034	
Control	21.000	0.105	31.153
12 hrs	24.207	0.213	36.760
18 hrs	23.522	0.210	42.903
24 hrs	23.233	0.176	50.070
30 hrs	21.842	0.191	55.820
S.E. (m)	0.2043	0.0018	1.2021
C.D. at 5%	0.6029	0.0054	3.5470

Table 6: Effect of priming treatments on rateof germination, dehdyrogenase activity and electrical conductivity in aged barley CV. BH-393

Priming treatments	Rate of germination (%)	Dehydrogenase activity	Electrical conductivity
Water	21.995	0.127	54.395
PEG	22.499	0.140	49.195
S.E. (m)	0.0916	0.0011	1.3241
C.D. at 5%	0.2703	0.0032	3.9071
Control	17.833	0.091	49.380
12 hrs	24.177	0.180	44.512
18 hrs	23.648	0.149	50.962
24 hrs	23.277	0.137	59.868
30 hrs	22.302	0.110	59.253
S.E. (m)	0.1449	0.0017	2.0936
C.D. at 5%	0.4274	0.0051	6.1777

Table 7: Effect of priming treatments on rateof germination, dehdyrogenase activity and electrical conductivity in fresh barley CV. BH-75

Priming treatments	Rate of germination (%)	Dehydrogenase activity	Electrical conductivity
Water	20.891	0.234	25.43
PEG	20.409	0.166	25.66
S.E. (m)	0.1384	0.0020	
C.D. at 5%	0.4083	0.0058	NS
Control	21.137	0.155	34.403
12 hrs	23.368	0.268	21.987
18 hrs	22.742	0.212	23.270
24 hrs	18.205	0.201	23.222
30 hrs	17.797	0.164	24.878
S.E. (m)	0.2188	0.0031	1.5240
C.D. at 5%	0.6455	0.0092	4.4969

Table 8: Effect of priming treatments on rate of germination, dehdyrogenase activity and electrical conductivity in aged barley CV. BH-75

Priming treatments	Rate of germination (%)	Dehydrogenase activity	Electrical conductivity
Water	17.205	0.138	55.131
PEG	19.010	0.149	54.581
S.E. (m)	0.0875	0.0013	
C.D. at 5%	0.2581	0.0040	NS
Control	15.233	0.088	55.477
12 hrs	19.880	0.220	50.195
18 hrs	19.400	0.175	55.178
24 hrs	18.572	0.136	56.812
30 hrs	17.453	0.098	58.620
S.E. (m)	0.1383	0.0021	0.7972
C.D. at 5%	0.4081	0.0063	2.3522

1. Dehydrogenase Activity

Effect of various priming treatment was observed on dehydrogenase activity in fresh and aged seed lots of both primed cultivars in table (5, 6, 7 and 8). It was recorded that dehydrogenase activity in fresh primed seed lot of both the varieties was significantly higher in water primed seed lot and was recorded (0.189) in BH-393 and (0.234) in BH-75.In aged seed lot of both the varieties it recorded significantly higher in PEG primed seed lot, of BH-393 (0.140) and in BH-75 (0.149).In case of time interval treatments 12 hour treatment is best among all the treatments. Dehydrogenase activity was observed significantly higher in both fresh primed and aged primed seed lot of both the cultivars. It was recorded 0.2137 and 0.268 in BH-393 and BH-75 in fresh seed lot respectively, while as in aged primed seed lot it was recorded 0.180 and 0.220 in BH-393 and BH-75 respectively.

Measurement of activity of specific enzymes was one of the earliest biochemical technique used to asses deterioration and to predict seed viability (AOSA, 1983), and was observed as optical density. DHA test is indirect and quick test to ascertain the viability and vigor of seed lot and have been shown to give correlation with field parameters like field emergence in sunflower (Gupta & Agrawal, 1980), Okra (Narwal, 1995), Wheat (Steiner et al., 1989).

2. Electrical conductivity test

Electrical conductivity was recorded as S/cm/ seed in table (5, 6, 7 and 8). It was recorded non-significant in fresh seed of both the cultivars, BH-393 and BH-75. It observed significantly different in seed lot of BH-393 in aged primed seed lot while as, it was significantly lower in water primed seed lot of BH-393 (49.195)

Within priming time interval it was observed non-significant in both fresh primed seed lots of both the cultivars, but in aged primed seed lot it recorded significantly lower in BH-393 (44.512) in 12 hours treatment. The estimates of electrical conductivity have been correlated with those of seed viability in same cases. The electrical conductivity of seed leachates have been reported to be higher in aged seed indicating deterioration of cell permeability and degradation of food reserves in seed (Ching 1973, Delouche & Baskin, 1978). Electrical conductivity of the seed leachates, increased progressively with artificial ageing in cotton (Goel et al., 2003). In present

experiment electrical conductivity was recorded as non significant in fresh seed lot of both cultivars. The same observation was recorded in sweet corn by Parera & Canttiffe (1994). Basra et al. (2003) recorded lower electrical conductivity after 24 hrs. priming in wheat in present experiment electrical conductivity was also recorded significantly lower. In aged primed seed lot of BH-393. Basra et al., 2003 recorded lowest electrical conductivity after 24 hrs. hydropriming in case of wheat.

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