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Effects of Storage Duration and Seed Treatment on Germination and Vigor of Soybean Seeds

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ABSTRACT

This experiment was carried out to investigate soybean seed vigor at natural ageing conditions and also the possibility of priming methods application for improving the efficiency of aged seeds. First the seeds of cultivars Williams and L17 which were produced in Moghan region in 2011, 2012 and 2013 and had 85% germination ability and standard moisture content (lower than 12%) were stored at controlled storage. Then different priming treatments were applied to improve efficiency and quality of deteriorated seeds. In order to conduct osmo-priming test, an experiment was conducted as factorial based on completely randomized design. The treatments were composed of seed storage duration (6, 18 and 30 months) and osmo-priming by PEG with 3 concentrations (-8, -10 and -12 bar) for 6, 12, 18 and 24 hours. The results indicated that The best osmo-priming treatment for seeds with different storage durations was -8 bars, but the required time for the most efficient osmo-priming treatment was different for various storage durations; such a way that for 6 months stored seeds, 12 hours osmo-priming showed the best effectiveness. In the case of 18 months stored seeds, the treatment -8 bars for 18 and 24 hours and also in 30 months stored seeds the treatment -8 bar for 24 hours, caused increase of germination efficiency in comparison with control. It can be concluded that high osmotic potentials had a negative effect on germination of studied seeds, probably due to oxygen deficiency and inadequacy of water adsorption and -8 bars osmo-priming is a proper treatment for stored soybean seeds.
KEYWORDS: natural ageing, priming, soybean seed, storage, vigor**.**

1. INTRODUCTION

Complicated systems are developed in the nature to optimize the seed life, including defensive protection, scavenging and amendment. Defensive protection expresses the role of seed coat and its structure and composition in seed protection. Some seed coat's layers contained flavonoides that play an important role in recycling of Oxygen radicals and control of oxidative stress, also are an essential mechanical obstacle against microorganism contamination; they are impermeable to solutes and prevent from leakage.

The seeds also develop a scavenging mechanism including some antioxidant enzymes such as superoxide dismutase, ascorbat peroxidase, glutathione peroxidase to control free radicals injuries [3, 4]. Also the amendment mechanisms that are induced during seed strengthening treatments (priming) can improve the seed germination conditions [13]. Some procedures are recently recognized such as seed priming and seed coating which improve seed quality and its sowing and storage efficiency. In this study the effect of different osmo-priming treatments was investigated on quality of soybean seeds which were deteriorated during several years of storage.

Seed priming is used to improve the seed germination uniformity, its rate and percentage. The primed seeds often indicate increased germination rate, more uniformity and final germination percent enhancement [5, 6, 7, 11, 12]. The increase of germination rate and uniformity is resulted from metabolic activities during solute absorption by seed [10]. The water is somewhat absorbed from osmotic solutions by seed that radicle can't emerge and seed priming is more effective on II stage of germination and seed germination is kept at lag phase [20].

The seeds are placed in some solutions like PEG (Polyethylene Glycol), KCL (Potassium Chloride), KH_2PO_4 (Potassium Hydrophosphate), $CaCl_2$ (Calcium Chloride), KNO_3 (Potassium Nitrate) and etc. These solutions control water absorption and inhibit from radicle emergence, also increase germination, seedling emergence and vigor. After priming, seeds are washed, desiccated and stored till application [5, 6, 9, 16, 18]. The salts and Manitol have been used greatly up to now, but both of them are absorbable by seeds, consequently have toxic effect on seeds. Polyethylene Glycol (PEG) is more preferred as an osmotic solution rather than others and has high molecular weight (6000-8000 Da) and because of large size of molecules do not penetrate in to seed [8].

The effect of priming by PEG was studied on plasma membrane's ATPase and mitochondria activity in soybean seeds. Priming with PEG can improve cold resistance of soybean seeds. Priming by PEG caused hydrolytic activity of ATPase up to 12 hours. Then the activity reduced gradually, but it was more than control [14]. Soybean

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seeds were primed at three times of 6, 12 and18 hours at 0, 100, 200, 300 and 400 gram/liter and dried seeds (unprimed) were used as a control. The relative growth rate increased with enhancement of seed priming duration. Also crop growth rate increased by enhancing PEG concentration from 0 to 300 gram/liter [17]. The impact of different PEG concentrations on seeds of two soybean cultivars was assessed, Proline content of hypocotyls decreased and protein content increased, also the seed germination percent of two soybean cultivars significantly increased. This reduction of proline content and enhancement of protein content is related to lipids peroxidation, membrane amendment and metabolism [22].

The seeds of soybean cv. 033 were subjected to priming with different PEG 6000 concentrations (-0.4, -0.8, -1.2, -1.6 and -2 MPa) and water for 6, 12, 24 and 48 hours and then were placed at growth chamber at 25˚C to germinate. The results indicated that -1.2 MPa concentration of PEG led to increased germination percent, germination index and seedling vigor, while it decreased mean time of germination, mean time to 50% germination and electrical conductivity. Also 12 hours priming duration was better than other treatments [19].

This research was conducted to assess soybean's seed vigor at natural aging conditions and to identify the proper concentration of osmotic solution and priming duration.

2. MATERIALS AND METHODS

This study was performed as completely randomized design in laboratory. The treatments were consisted of seed storage periods (6, 18 and 30 months) and different concentrations of osmo-priming treatments by PEG (-8, -10 and -12 bar) at 6, 12, 18 and 24 hours. The obtained osmotic potential levels from polyethylene glycol were calculated by Michel and Kaufmann (1973).

Water potential= $-(1.18 \times 10^{-2})\,C - (1.18 \times 10^{-4})\,C^2 + (2.67 \times 10^{-4})\,CT + (8.39 \times 10^{-7})\,C^2T$

That C is polyethylene glycol concentration (gram per kilogram water) and T is defined in centigrade degree.

First the seeds of cv. Williams with 85% germination ability and standard moisture (below 12%) that were produced in Moghan, were stored in a controlled storage. First after the fields of cv. Williams were harvested, the seed cleaning, processing and packaging was carried out in 2010, 2011 and 2012. Then seed sampling was performed according to ISTA rules and seed germination test for seed samples was conducted in Iran seed quality Analysis laboratory and the samples with 85% germination ability and 11-12% moisture contents were selected for assessment of natural aging and also long term seed storage at controlled conditions. This operation was performed during 3 years and at the mid of December of each year the seeds with above mentioned germination and moisture content were transferred to a controlled storage of seed and plant certification and registration institute. The storage had cooling system and temperature range was 14-17˚C and relative humidity was 40-50%. Then different priming treatments were exerted to improve the efficiency and quality of deteriorated seeds. After determined durations of priming, the seeds were washed three times with distillated water and then were desiccated at 25˚C for 24 hours. Afterwards the standard germination test was performed and some traits including normal seedlings percent, abnormal seedlings percent, germination rate and seedling vigor index were measured.

The standard germination test

This test was performed as between paper method according to ISTA rules. First 50 seeds were selected from each sample and then were planted in 3 layers towel paper [2]. The papers were moistened before planting. Then sown papers were transferred to air tight plastic containers and were placed in growth chamber at 25˚C temperature in light for 7 days. During this period, daily recording of germinated seeds was done and seeds with at least 2 mm length of radicle were considered as germinated seeds.

The normal and abnormal seedlings were determined at the end of test duration, then 10 normal seedlings were selected randomly, seedling length, radicle and shoot length were measured by ruler with 1 mm precision, also seedlings dry weight was measured by a particular scale with 0.001 g accuracy after placing the seeds in oven at 71˚C for 48 hours. The number of normal seedlings was regarded as germination percent and was reported in percent. The number of germinated seeds was daily recorded for determining the germination rate. By counting of germinated seeds, some germination indices which are related to seedling vigor were calculated as below:

Germination rate

The germination rate was calculated by ISTA (2009) formula which is a specification of germination speed and is obtained by below relation:

Germination rate: $\sum_{i}^{n} \frac{Si}{Di}$

Which Si is germinated seeds number in each counting, Di is the number of days to n^{th} counting and n is counting times.

Seedling vigor index (SVI)

After determining the normal and abnormal seedlings, 10 normal seedlings were randomly selected, then were placed in oven at 75˚C for 24 hours, after that the seedling dry weight was measured by precise scale. The seedling vigor index [1] was specified by below relation:

Seedling weight vigor index= Seedling dry weight ×final germination percent

3. RESULTS AND DISCUSSION

The effect of osmo-priming on quality of stored seed

This experiment was conducted to evaluate osmotic potential levels and osmo-priming duration on quality of stored soybean seed. The results of variance analysis indicated that normal seedlings percent, abnormal seedling percent and germination rate of stored seeds at different periods were influenced by osmo-priming treatments and the interaction of osmo-priming duration and concentration on seeds that were stored for different periods, was significant (table 1).

The mean comparisons for investigating the best osmo-priming levels and potentials on stored seeds for 6 months (figure 1) specified that -8 bar osmo-priming treatment for 12 hours significantly had higher normal seedlings than control (87.5% versus 82.75%). However -10 and -12 bar osmo-priming for 18 and 24 hours had negative effect on 6 months stored seeds. The abnormal seedlings percent (figure 2) also was significantly lower than control in -8 bar treatment for 12 and 18 hours and it seems that amendment mechanisms caused reduction of abnormal seedlings number and increase of normal seedlings in these treatments. Huang *et al.*, (2008) suggested that amendment mechanisms can improve seed germination percent that is induced during priming treatments. Also germination rate (figure 3) was significantly higher in these two treatments. Study on the effect of osmo-priming treatments on quality of 6 months stored seeds revealed that -8 bar osmo-priming for 12 and 18 hours led to improper result on germination indices and their seed quality was significantly lower than control. Therefore it can be concluded that -8 bar osmo-priming for 12 and 18 hours improves seed efficiency and higher concentrations of polyethylene glycol for 18 and 24 hours has negative effect on 6 months stored seeds.

Burgass and Powell (1984) concluded that increase of germination rate and its uniformity is resulted from metabolic activities of seeds during solution absorption.

Table 1: variance analysis of related traits to osmo-priming test

		Mean squares								
		Priming after 6 months			Priming after 18 months			Priming after 30 months		
sov	df	GR	NS	AS	GR	NS	AS	GR	NS	AS
treatment	12	77.5**	66.8**	67.9**	161.3**	130.9**	68.5**	331.1**	256.8**	131.5**
Control versus primed seed	1	7.8**	14.4 ns	13.1 ns	53.6 ns	11.8 ns	0.003 ns	50.7 ns	50.7 ns	13.0 ns
Priming duration	3	88.0**	49.7**	39.7**	51.2**	45.6**	72.6**	426.8**	298.3**	134.6**
Osmotic potential	2	223.2**	208.5**	205.5**	488.3**	280.5**	165.1**	763.7**	469.5**	365.9**
Priming duration× Osmotic potential	6	35.3**	36.8**	45.3**	125.4**	143.5**	45.7**	185.9**	199.4**	71.6**
Error	27	10.13	5.71	7.48	9.81	7.68	4.32	13.95	7.08	2.9
c.v		3.91	21.77	8.58	4.19	17.27	7.82	5.81	13.67	7.34

ns: non significant ; ** significant at 1% level; GR=Germination Rate; NS= Normal Seedlings; AS= Abnormal Seedlings.

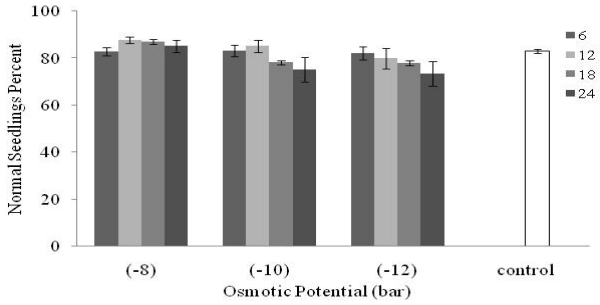

Figure 1: The mean comparison of osmotic potential and priming duration interaction on normal seedlings percent of stored seeds for 6 months. LSD (0.05) =4.392

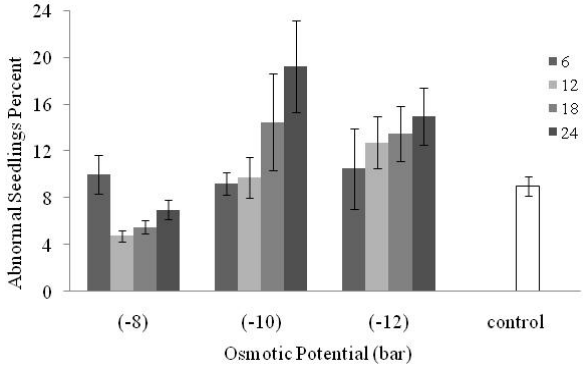

Figure 2: The mean comparison of osmotic potential and priming duration interaction on abnormal seedlings percent in stored seeds for 6 months. LSD (0.05) =3.301

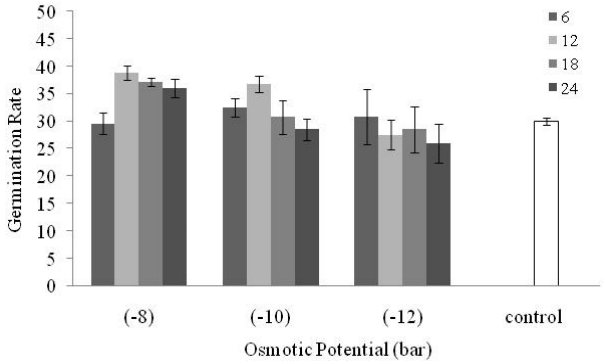

Figure 3: The mean comparison of interaction of osmotic potential and priming duration on germination rate of stored seeds for 6 months. LSD (0.05) =3.769

The mean comparisons for investigating the best osmo-priming treatment at 18 months stored seeds illustrated that -8 bar osmo-priming treatment for 18 and 24 hours resulted in a significant increase of normal seedlings compared to control (87.5% versus 82.75%) (figure 4). However -10 and -12 bar osmo-priming for 18 and 24 hours had negative effect on normal seedlings percent and led to a significant reduction compared to control (65-70%). -8 bar treatment for 18 hours (9.5%) and 24 hours (7.25%) caused significantly decrease in abnormal seedlings percent in comparison with control (figure 5), indicating priming amendment effects. The mean comparison for germination rate (figure 6) indicated that -8 bar osmo-priming for 24 hours (32), 18 hours (30) and 12 hours (29.5); and also -12 bar osmo-priming for 6 hours (30) resulted in significantly higher germination rate in comparison with control (26.6).

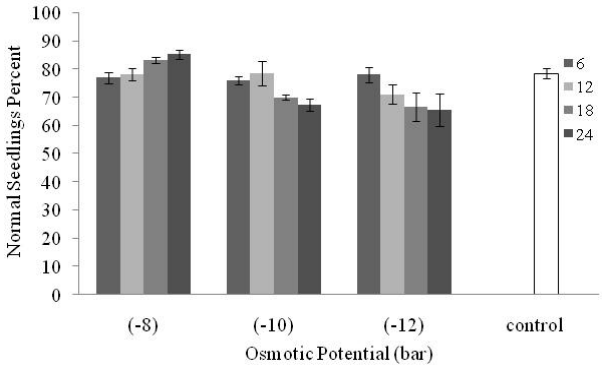

Figure 4: The mean comparison of osmotic potential and priming duration interaction on normal seedlings percent of 18 months stored seeds. LSD (0.05) =4.371

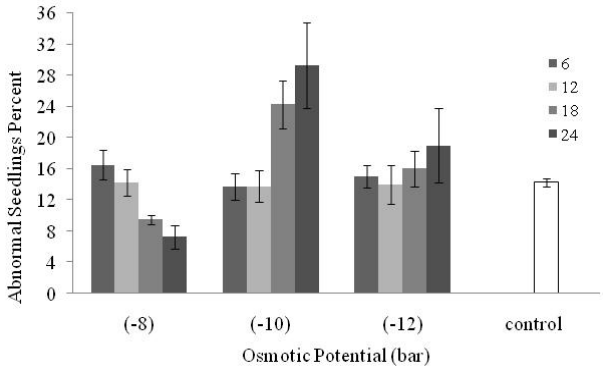

Figure 5: The mean comparison of osmotic potential and priming duration interaction on abnormal seedlings percent in stored seeds for 18 months. LSD (0.05) =3.813

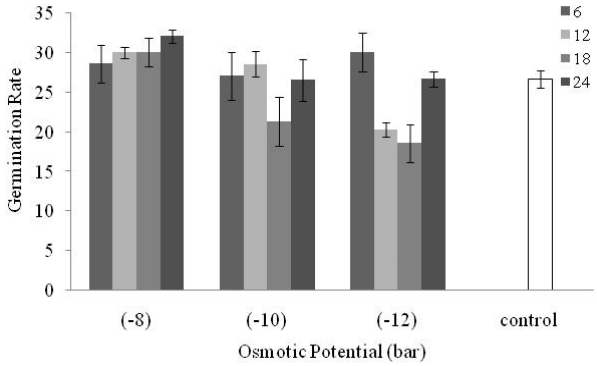

Figure 6: The mean comparison of osmotic potential and priming duration interaction on germination rate of stored seeds for 18 months. LSD (0.05) =2.892

The mean comparison results of determining the best osmo-priming treatment on 30 months stored seeds specified that -8 bar osmo-priming for 24 hours caused enhancement of normal seedling percent (figure 7) compared to control (74% versus 68%), but -10 and -12 bar osmo-priming for 18 and 24 hours, significantly reduced normal seedlings percent in comparison with control. the abnormal seedlings percent (figure 8) at -8 bar osmo-priming for 24 hours, showed a significant reduction compared to control (9.75 % versus 15.75 %). -8 and -12 bar osmo-priming for 18 and 24 hours caused a significant increase of abnormal seedlings percent compared to control. The results of mean comparison for germination rate of 30 months stored seeds (figure 9) indicated that -8 bar treatment for 24, 12 and 18 hours recorded the highest germination rate (30,28.6 and 28), respectively and had significantly higher germination rate than control (25.2). -12 and -10 bar osmo-priming treatments resulted in a significant reduction of germination rate compared to control.

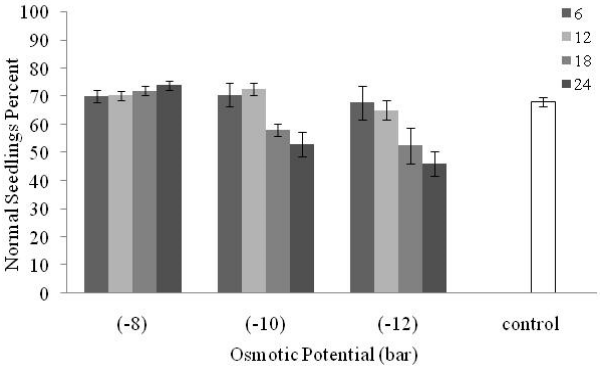

Figure 7: The mean comparison of osmotic potential and priming duration interaction on normal seedlings percent in stored seeds for 30 months. LSD (0.05) =5.174

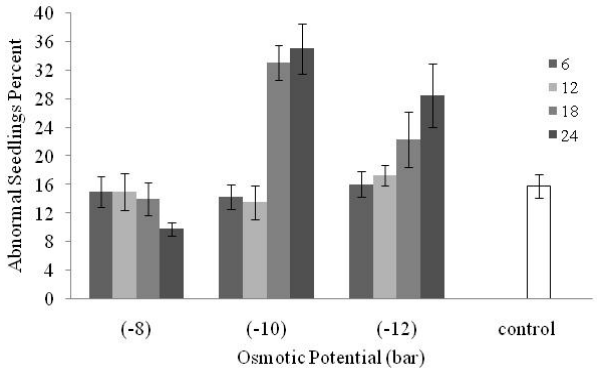

Figure 8: The mean comparison of osmotic potential and priming duration interaction on abnormal seedlings percent in stored seeds for 30 months. LSD (0.05) =3.719

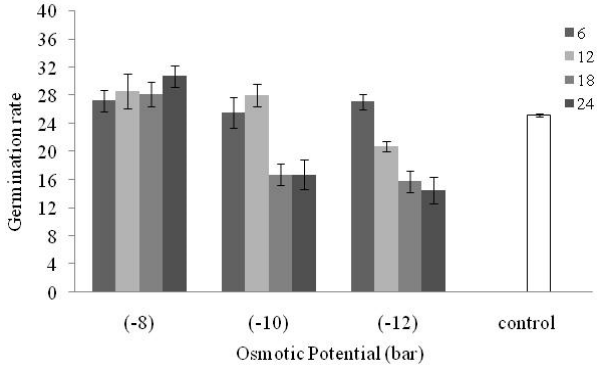

Figure 9: The mean comparison of osmotic potential and priming duration interaction on germination rate of stored seeds for 30 months. LSD (0.05) =2.356

The mean comparison of osmo-priming treatments on germination characteristics of stored seeds indicated that -8 bar osmo-priming treatment was the best treatment for stored seeds at different storage periods, but the required time for the best effectiveness of osmo-priming treatments was differed for seeds with various storage durations. 12 hours osmo-priming was the best treatment for 6 months stored seeds. For seeds with 18 months storage duration, 18 and 24 hours of -8 bar osmo-priming treatment and for 30 months stored seeds -8 bar treatment for 24 hours, led to increased germination efficiency compared to control. It can be concluded that high osmotic potentials had negative effect on seed germination, probably due to oxygen deficiency or impossibility of sufficient water adsorption and -8 bar osmo-priming was a proper treatment for stored seeds of soybean. According to Black and Bewley (2000) one disadvantage of PEG is reverse relation of solution's oxygen with its concentration [8]. On the other hand it seems that increase of storage duration causes delay in germination processes and water absorption, so more time required for seed priming. Therefore 18 and 30 months stored seeds showed the best effectiveness at 18 and 24 hours of seed priming.

It is accepted as a norm that amendment of deteriorated seeds (that lipid peroxidation is happen at them), is take place by hydration at seed priming stage that results in seed germination. Most of the studies have indicated that amendment is occurred in seeds by priming; however it may take place during seed priming or after that.

Generally it is supposed that water absorption stage by seed is along with activation of vital metabolisms for germination and regeneration of enzymes [8].

Many researches have indicated that seed priming will cause seeds to be repaired and be protected from deterioration injuries like mitochondria and enzymes inactivity, membrane damages during seed storage and aging [8]. With considering the priming effect on seed germination of crops, the results of researches imply that seed priming will cause early seed germination, higher germination percent, uniformity of seedling emergence and improvement of seedling establishment and vigor, such a way that a crop develops its root system properly and tolerates unfavorable environmental conditions better than other crops, the use efficiency of light, water and nutrients increases and ultimately sooner reaches to physiological maturity and also produces higher yield [21].

4. CONCLUSIONS

The best osmo-priming treatment for seeds with different storage periods was -8 bar treatment, but the required time for the best efficiency of osmo-priming treatment was differed for seeds with various storage durations. For 6 months stored seeds, 12 hours osmo-priming indicated the best efficiency. For 18 months stored seeds, 24 and 18 hours of -8 bars osmo-priming and also for 30 months stored seeds 24 hours of -8 bars osmo-priming treatment led to increased germination efficiency in comparison with control. It can be concluded that high osmotic potentials had a negative effect on germination of studied seeds, probably due to oxygen deficiency and inadequacy of water adsorption and -8 bars osmo-priming is a proper treatment for stored soybean seeds. On the other hands it seems that by increasing the storage duration, initiation of germination processes and water absorption will delay, so more time is required for osmo-priming treatment of seeds. Therefore 18 and 30 months stored seeds indicated the best efficiency at 18 and 24 hours of osmo-priming treatment.

Also Black and Bewley (2000) reported that seed priming will cause seeds to be repaired and be protected from deterioration injuries like mitochondria and enzymes inactivity, membrane damages during seed storage and aging. It's accepted as a generality that amendment of deteriorated seeds is occurred by hydration which results in seed germination of the researches have been indicated that with priming treatment, amendment is achieved in seeds; however it may be occurred during seed priming or after that.

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