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EFFECT OF SEED PRIMING ON LETTUCE SEED STORAGE.

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ABSTRACT

Lettuce seeds of white paris cultivar were primed in -1 MPa of 7 different agents for 6 days at 20 °C then stored at 7 °C for 8 months and germinated at 20 °C. Lettuce seeds showed high response to seed priming after storage period; all priming treatments had significant differences compared with control (non primed seeds). Poly ethylene glycol (PEG) recorded the highest values of increasing germination percentage, coefficient of velocity, germination performance index, seedling fresh and dry weight and seedling length and reducing mean time to germination, time to 50% germination and (25%-75%) germination, and abnormal seedling.

INTRODUCTION

Lettuce (*Lactuca sativa*, L.) is one of the most important vegetable salad crops in the world and Egypt. Its seeds have low germination percentage 70% which returns to metabolic blocks which associated with immature seeds and secondary dormancy (Hassan 1991). Lettuce seeds show orthodox storage behavior, is unaffected by loss of moisture and by exposing seeds to chilling temperatures. Seeds are commonly used in commercial propagation and for long-term genetic conservation (Doijode 2001). There are many successful methods have been used to enhance germination parameters in lettuce seeds and keeping its benefit effects during storage. Seed priming is the most successful method in this regard. Seed priming is the presowing treatment in which seeds are soaked in an osmotic solution that allows them to imbibe water and go through the first stages of germination, but does not permit radical protrusion through the seed coat (Heydecker1973). Seeds usually dried to original moisture contents and stored for some time before planting after seed priming treatment.

Several studies have reported that priming did not affect the storage life of seeds such as carrot when stored for 450 days at 10 °C (Dearmen *et al*, 1987), spinach after 30 days of storage at 5 °C (Atherton and Farooque,1983). On other hand some researchers reported that priming treatment increase storage life; Valdes and Bradford (1987) reported that osmotically primed seeds retained the capacity for germination at high temperature after seed coating and storage, providing an effective means of improving stand establishment of direct-seeded lettuce in hot weather.

Corbineau and Come (1990) mentioned that germination of untreated *Valerianella olitoria* seeds was unaffected by length of storage. Primed seeds lost their germinability after 2 months, but maintained their improved germinability up to that time. Nascimento and Cantliffe (1998) reported that seed priming effectively improved seed germination at supra-optimal temperature but storage conditions are optimized in order to prolong seed

viability of primed seeds of certain genotypes. Kim *el al*. (2000) found that seed priming, regardless of company, did not affect seed viability or seed vigour after 9 months storage of two lettuce genotypes, but germination at 30 °C of pelleted and film-coated seeds lacking the priming treatment was reduced before and after 9 months storage.

Tamanini *et al*. (2001) noted that primed and coated lettuce seeds maintain their quality for at least 24 months when stored at controlled conditions (15 °C and 40% moisture) regardless of the packaging material used. Korkmaz and Pill (2003) found that storing the primed seeds of Cortina' and'Greenlakes' cultivars of lettuce for one month had little or no effect on the viability final germination percentage and germination rate 50%, but slightly reduced the germination synchrony.

Pazdera (2003) noted that osmotic priming of ten lots of lettuce seeds for 24 and 72 h had positive effect on seed parameters after 300 days of storage. These seed lots had significantly higher germination percentage and germination energy and significantly shorter mean time of germination than the stored untreated control. Prehydration treatment affected the storability of the treated seeds negatively and longer durations of hydration had more negative influence on the storability than shorter durations. Korkmaz (2006) recorded that priming seeds in 10 micro M ACC incorporated into the KH_2PO_4 solution improved high temperature performance of lettuce seeds and these seeds can be stored for two months at 4 °C and still exhibit improved germination performance at 35 °C. Hill *et al*. (2007) reported that primed lettuce seeds were more sensitive to the adverse effects of higher seed moisture content than nonprimed seeds during storage at elevated temperatures. Aazami and Mohammadi (2008) recorded that using the best material for pre-priming, along with suitable drying management with appropriate quality and good conditions of the storage is important.

Thus, this study aimed to investigate how long lettuce seeds can keep benefits effect of seed priming technique.

MATERIAL AND METHODS

Two laboratory experiments were carried out during year 2008 in seed laboratory testing of seed vegetable and technology Department, Horticulture Research Institute (HRI) and Horticulture Department, Faculty of Agriculture, Suez Canal University. This study aimed to investigate benefit effects of seed priming during storage period of lettuce seeds.

White paris cultivar (Romine type) seeds had been soaked in 7 solutions of PEG, KNO_3, K_2HPO_4, NaCl, KCl, Sucrose and Na_2SO_4 for 6 days at 20 °C then stored in paper envelopes for 8 months at 7 ˚C then germinated under 20 ˚C using whatman filter papers number 1 on plastic Petri dishes 12 cm diameter with four replications (50 seeds for each replication).

These treatments were arranged in a completely randomized design with four replicates for each treatment. The treatments mean were compared using the duncan multiple Range test (Duncan, 1965).

The following data recorded:

1. **Germination percentage (GP)** was measured according to ISTA rules **(ISTA 1999).**
2. **Mean time to germination in days (MGT)** was calculated according to formula MGT= nd/N where n is the number of germinated seed on each day, d is the number of days from the beginning of the test, and N is the total number of germinated seeds (Edwards and Sundstrom, 1987).
3. **Coefficient of velocity** was calculated according to the formula Coefficient of velocity = 1/MGT X 100 where MGT is mean time to germination in days (Edwards and Sundstrom, 1987).
4. **Germination performance index (GPI)** was calculated according to formula GPI= GP/MGT where GP is germination percentage and MGT is mean time to germination in days (Pill and Fieldhouse, 1982).
5. **Time to reach 50% germination (T50)**
   Days required to 50% germination.
6. **Uniformity of germination**
   It is the time between 25% of germination and 75% of germination (T75 – T25).
7. **Seedling fresh weight (mg)** was measured on ten seedlings randomly taken from each replicate, weighted, and the average fresh weight per seedlings was calculated.
8. **Seedling dry weight (mg)** Measurement used the same seedlings taken for the determination of fresh weight. They were oven-dried at 70˚ C until constant weight was reached. The average weight per dried seedling was calculated.
9. **Abnormal seedlings percentage was calculated as follows:**
   Abnormal seedlings percentage = Abnormal seedlings/Total seedlings X 100.
10. **Seedling length (cm)** was measured on ten seedlings randomly taken from each replicate and the mean length of seedlings was calculated.

RESULTS AND DISCUSSION

1. Germination percentage (GP%)

Table 1 shows that primed seeds kept useful benefits of seed germination after 8 months of storage in white paris cultivar. The most effective results obtained from PEG and sucrose. There were significant differences among priming treatments and control. The lowest germination percentage was obtained from control (65.66, 65.66) in both experiments, respectively. The positive effects of priming in germination performance have been attributed to the induction of biochemical repair mechanisms. Metabolic events such as synthesis of protein, RNA and DNA are initiated within minutes of seed hydration (Osborne, 1983). Mitochondria increase in number during leek priming (Bray, 1995). The reactivation of metabolic activity during imbibition also restores cellular integrity through the synthesis of proteins, lipids and RNA (Bewley and Black, 1994). Repair type replicative DNA synthesis occurs in leek embryos during osmopriming without detectable cell division (Ashraf and Bary, 1993). During lettuce seed germination, the endo-

beta -mannanase activity in the primed lettuce seeds was higher in the micropylar region than in the lateral regions. In addition, activity was higher in primed than in the unprimed seeds. (Sung and Jiang, 2004).

2. Mean germination time (MGT) (hours)

Table 1 shows that PEG and KCl reduced value of mean germination time compared with other priming treatments. The highest value of MGT obtained from control. Priming decreases solute leakage during imbibition. Exposing seeds to limited amounts of water during priming may minimize imbibitional damage and allow repair of membrane damage (Bray, 1995) and that phenomena cause reduction of abnormal percentage of seedlings. Priming may increase oligosaccharide concentrations which preserve membrane integrity during desiccation (Horbowicz and Obendorf., 1994; Bernal-Lugo and Leopold, 1995). The quantity of membrane phospholipids also changes during priming (Basra *et al*., 1988).

3. Coefficient of velocity

Coefficient of velocity for germinated lettuce seeds tended to increase with priming treatments. There were significant differences among treatment compared with control. The highest values recorded with PEG (139.07, 142.66) followed by KCl (123.95, 110.82). The lowest values were obtained from (control) (50.08, 46.31) (Table 1).

4. Germination performance index (GPI)

Data recorded in Table 1 show that seed priming treatments significantly increased GPI in both experiments. PEG, KCl and Na_2SO_4 increased GPI almost 4- fold over the control. The lowest values obtained from priming lettuce seeds was from sucrose (57.80, 55.39) in both times, respectively.

5. Time in days required to reach 50% germination (T50)

T50 is the time in days needed to reach 50% of germination. The positive effect is when data show greater value of T50. Data in Table 1 reveal that seed priming caused a significant reduction in T50 as compared with control in both experiments. Seed priming was effective in reducing time of T50 of lettuce seeds germinated under laboratory conditions. It appears that highest value obtained from PEG (00.50) in both times. Other treatments means were similar to each other. The lowest values of T50 were obtained from $MgSO_4$ and control.

6. Uniformity of germination

Uniformity of germination defined as the time in days between 25 and 75% of germination. The higher value of uniformity value is the less uniformity, or more variability, occurred.

It is clear from data (Table 1) that time of uniformity was significantly increased in both experiments (was reduced) by priming lettuce seeds. All treatments were superior to the control. More uniformity (low uniformity index value) was obtained by priming lettuce seeds in PEG, NaCl and sucrose. The control seeds failed to reach 75% of germination.

7. Abnormal seedlings percentage

Table 2 shows that significant differences were obtained between control and seed priming treatments. The lowest values of abnormal seedlings

recorded by PEG, KNO_3, K_2HPO_4, KCl, NaCl and Na_2SO_4 (00.00) in both experiments, respectively. The highest value of abnormal seedlings obtained from control.

8. Seedling fresh weight (mg)

Data in Table 2 shows that there were no significant differences among all of treatments except control which gave the lowest vales of fresh weight.

9. Seedling dry weight (mg)

Table 2 shows that there were no significant differences among priming treatments themselves, in additional among priming treatments and control (non primed seeds).

10. Seedling length (cm)

Data recorded in Table 2 indicate the significant differences occurred between priming treatment means and control in seedling length. The most tested treatments (PEG, K2HPO4, NaCl, KCl, Sucrose and Na2SO4) were similar to each other followed by KNO_3. The lowest value was obtained from control treatment.

The principle of hydration treatments is based on the fact that it is possible to hydrate seeds in some ways at a moisture level sufficient to initiate the early events of germination but not sufficient to permit radical protrusion. (Taylor *et al*. 1998). Seed priming appears to be as effective physiological treatment to improve germination behavior. It also results in better homogeneity of germination. The differences of reaction cultivars towards seed priming were recorded by many researchers as (Guedes and Cantliffe 1979).

Seed priming have kept its useful benefits of germination after 8 months of storage. These equivocal results are in agreement with the results of other authors (Mauromicale and Cavallaro, 1995; Pazdera and Hosnedl, 2002; Kim et al 2000). The most effective priming solutions in this regard were PEG, KCl, sucrose, mannitol and $MgSO_4$.

Primed seeds can be stored following drying back and upon rehydration, they may still exhibit faster germination rates, greater tolerance to environmental stress, and reduced dormancy (Khan 1992). However, there are conflicting results on the effect of priming on the storage life of the seeds. Gurusinghe and Bradford (2001) found that there is correlative evidence indicating that sucrose and alpha-galactosy1-sucrose oligosaccharides (raffinose family of oligosaccharides, RFOs) may be involved in seed longevity. Priming treatments can improve short-term seed performance. RFOs are metabolized quickly following seed imbibition, loss of RFOs during priming could lead to more rapid deterioration in dry storage. Other suggested physiological factors could possibly be involved in the extension of seed longevity after priming, and exposing seeds to storage conditions might induce heat shock proteins (hsp). The abundance of BiP (78 KD Binding Protien), hsp 70 and class I small hsp in primed seeds subjected to post priming treatments was examined to assess this possibility. BiP MRNA and protein amounts increased during post-priming heat treatments that extended longevity of tomato seeds (Gurusinghe *et al*, 2002).

T1-2

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تأثير مهيئات الأنبات على تخزين بذور الخس

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تم تهيئة بذور صنف هوايت باريس وذلك بالنقع فى ٧ مواد مختلفه بتركيز ١- ميجاباسكال لمدة ٦ ايام فى درجة حرارة ٢٠ مئوية ثم خزنت البذور لمدة ٨ أشهر فى درجة حرارة ٧ مئوية ثم جرى استنباتها فى درجة حرارة ٢٠ مئوية. أظهرت النتائج وجود أستجابه بذور الخس بصورة معنوية لمعاملات التهيئة للأنبات حيث كان هناك فارق معنوى بين كل معاملات التهيئة للأنبات بالمقارنة مع الكنترول و كان البولى ايثلين جليكول هو اكثر المواد تأثير فى زيادة نسبة الانبات و معامل سرعة الانبات تماثل الانبات والوزن الطازج والجاف وطول البادرات أضافة لتقليل الوقت الازم للأنبات الكامل و الوقت الازم للوصول الى ٥٠% أنبات و تقليل الفترة بين نسبة ٢٥% أنبات و ٧٥% أنبات (تماثل الأنبات) وتقليل النسبة المئوية للبادرات الشاذة.

قام بتحكيم البحث

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Table (1): Effect of priming solutions on germination percentage (GP%), mean time to germination in days, coefficient of velocity in lettuce, germination performance index (GPI), time to reach 50% germination (T50) and uniformity of germination in lettuce seeds after 8 months of storage.

Treatments	GP%		MGT		Coefficient of velocity		GPI		T 50		Uniformity (T25 -T75)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
PEG	88.00 a	88.00 a	0.72 e	0.70 g	139.07 a	142.66 a	122.30 a	125.54 a	00.50 c	00.50 d	00.50 cd	00.50 c
KNO_3	78.00 d	78.66 c	1.19 c	1.06 de	83.90 de	93.83 de	65.46 de	73.90 efg	1.00 b	1.00 c	1.66 a	00.83 b
K_2HPO_4	80.00 cd	80.66 bc	1.25 c	1.18 d	80.25 ef	84.19 e	64.12 e	67.92 fg	1.16 b	1.00 c	1.166 ab	1.33 a
KCl	80.66 cd	79.33 c	0.80 e	0.90 f	123.95 b	110.82 b	99.95 b	87.88 bcd	1.00 b	1.00 c	00.83 bc	00.50 c
NaCl	82.33 bc	80.66 bc	1.00 d	1.01 ef	100.04 c	99.45 cd	82.28 c	80.18 cde	1.00 b	1.00 c	00.50 cd	00.50 c
Na_2SO_4	85.33 ab	86.66 ab	1.02 d	0.92 ef	97.46 c	107.81 bc	83.36 c	93.50 b	1.00 b	1.00 c	00.83 bc	00.83 b
Sucrose	88.00 a	86.00 ab	1.52 b	1.55 b	65.69 g	64.41 f	57.80 e	55.39 h	1.00 b	1.00 c	00.50 cd	00.50 c
Control	65.66 e	65.66 d	2.00 a	2.16 a	50.08 h	46.31 g	32.92 f	30.41 i	1.50 a	1.50 a	-- - - d	-- - - d

Values within the same column followed by the same letters are not significantly different using Duncan's Multiple Range at 5% level.

Table (2): Effect of priming treatments on abnormal seedlings percentage (%), seedling fresh weight, seedling dry weight (mg) and seedlings length (cm) in lettuce seeds after 8 months of storage.

Treatments	Abnormal seedlings percentage (%)		Seedling fresh weight (mg)		Seedling dry weight (mg)		Seedlings Length (cm)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
PEG	00.00 d	00.00 e	00.42 ab	00.42 ab	00.01 bcd	00.02 a	12.40 a	12.52 abc
O_3	00.00 d	00.00 e	00.39 b	00.35 c	00.01 d	00.01 a	9.72 b	9.72 d
K_2HPO_4	00.00 d	00.00 e	00.42 ab	00.42 ab	00.01 cd	00.01 a	12.28 a	12.84 ab
KCl	00.00 d	00.00 e	00.44 a	00.45 a	00.01 bcd	00.02 a	13.06 a	13.54 a
NaCl	00.00 d	00.00 e	00.44 ab	00.43 a	00.02 bc	00.08 a	12.76 a	11.22 c
Na_2SO_4	00.00 cd	00.66 de	00.41 ab	00.41 ab	00.02 abc	00.10 a	11.90 a	11.82 bc
Sucrose	00.00 cd	2.66 c	00.41 ab	00.41 ab	00.03 a	00.02 a	12.64 a	11.84 bc
Control	14.50 a	15.33 a	00.25 c	00.25 d	00.01 d	00.01 a	7.72 c	7.46 e

Values within the same column followed by the same letters are not significantly different using Duncan's Multiple Range at 5% level.