

Examination the effects of hydro-priming and priming by salicylic acid on lentil aged seeds

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ABSTRACT: A new harvested seeds of lentil were aged under moisture of 100% and temperature of 40 °C for 3, 6 and 9 days. A group of non- aged seeds were selected as control seeds. The seeds were then floated in different concentrations of salicylic acid (control or no priming, 0 or hydropriming, 500, 1000, 1500 and 2000 µM). Aging of seeds for 6 and 9 days led to reduction in all characteristics. By increasing of aging period seed deterioration significantly increased. In 9 days of aging treatment all seeds lost their vitality and any seed could not to germination. In 3 days aging treatment, however, germination percent and index increased compare to control seeds. It seems that in fresh seeds of lentil a period of time is needed to enhance germination capability. Highest ratio of plumule to radicle and the highest ratio of plumule to radicle dry weight were observed after 6 days of aging that shows a higher rate of growth in plumule compared to radicle which is not considered as a favorable characteristic for the seedlings. Seed priming by distilled water or salicylic acid not only had not a positive effect on improvement of seed vigor both in aged and non-aged seeds, but also led to asignificant decrease. However, the unfavorable effects of hydro-priming were considerably higher than that of priming with salicylic acid. It seems that priming is not considered as a favorable procedure for decreasing negative effects of seed aging in lentil seeds.

Keywords: germination; lentil; seed pre-treatment seed vigor; shoot growth; root growth.

Abbreviations: Salicylic acid (SA).

INTRODUCTION

Lentil is considered as an important legume crop in developing countries and used as a complimentary with cereals in nutritional diet for people. Lentil is a main source of vitamins such as riboflavin, B and carotene and is an appropriate source of necessary amino acids like as lysine that are lacking in cereals (Hwang et al., 1994). Lentil cultivation area in Iran is approximately 120000 ha with seed yield 73000 T which is mostly in the form of dry farming (70200 T) (FAO, 2014).

Salicylic acid (SA) or ortho-hydroxy benzoic acid and similar compounds are belongs to phenolic compounds. This hormone plays a key role in regulation of a variety of physiological procedures such as growth and development of plants, ion uptake, photosynthesis and seed germination (Murugu et al, 2004; El-Tayeb, 2005). The reports show that priming with SA results in increased germination percentage, speed and homogeneity. Pre-treatment of borage seeds with salicylic acid resulted in increased percentage and speed of seed emergence under field condition (Shekari et al., 2010). They observed a significant correlation between emergence speed and dry weight of the borage seedlings.

One of the factors to achievement higher yield per area is higher and rapid seedling emergence and their establishment. It is reasonable higher and rapid establishment led to effective utilization of environmental resources such as light, water and nutrients (Foti et al., 2002). Various reports have revealed that priming leads to increased percentage, speed and homogeneity of seed germination and seedling growth (Demir Kaya, 2006; Mohammadi et al., 2011; Miarsadegi et al., 2011). Pre-treatment of rapeseed seeds by salicylic acid improved emergence percentage and emergence index and led to increase seedling and young plant establishment and traits (Miarsadeghi et al., 2011).

Seed quality is determined due to several factors. However the most important factors are germination capability and seed vigor. Evidently seed vigor has a considerable effect on plant establishment (Tekrony and Egli., 1991). Also, seed vigor determines rapid seedling emergence and seed tolerance in a range of environmental factors (Pary, 1972). Genetic structure, environment and maternal plant nutrition, seed nutrients, stage of ripening, harvesting time, virulence factors and seed aging are the most important factors in seed vigor. It is stated that seed aging have the most significant effects on seed vigor (Roberts and Osei-Bonsu, 1988; Agrawal et al., 2005). Temperature, relative humidity of the environment and seed moisture content are

of the most important factors in maintenance of seed vigor in storage period. Seed aging is a physiologic phenomenon after harvesting that starts if the temperature, humidity and oxygen pressure of storage environment is high, and gradually leads to degradation of DNA and ribosomal RNA (Mc Donald, 1999), enhance enzyme activity, respiration and penetrability of cell membranes that all together lead to decreased seed germinability and seed and seedlings vigor and actually decreases yield (Hampton, 2003).

In Iran lentil mainly is cultivated as rainfed crop. Farmers usually use lentil seeds from poorly maintained farmhouses for cropping that in turns leads to decreased seed quality. This study was conducted in order to evaluate the effects of aging on performance of aged seeds and to compare it with that of control seeds, and also by using seed priming with salicylic acid and priming by distilled water (hydropriming) in order to decrease aging effects.

MATERIALS AND METHODS

A factorial based on completely random design was conducted in three replications in seed and plant physiology laboratory of University of Zanjan in order to evaluate the influence of seed priming of aged seeds exposed to salicylic acid and distilled water on some seedling characteristics in lentil. The lentil seeds were obtained from a recently harvested lentil crop in the village of Bilverdi located 60 km away from Tabriz. The seeds were divided into 4 equal groups to prepare for accelerated aging test. The first group was put into a bag and kept in temperature of 3 to 5°C in a refrigerator as the control treatment. The other three groups were used for the first aging stage (3days), second aging stage (6days) and the third aging stage (9 days) and put into separate containers and placed over water containers. The bags were then fastened up and moved into an incubator of 40°C and then the first bag, second bag and the third bag were moved out of the incubator after 72, 144 and 216 hours, respectively; and the Standard Germination test in 20°C was conducted on them and the control seeds.

After preparation of different concentrations of SA, the aged lentil seeds were exposed to different treatments of SA for 4 hours in room temperature. Different levels of Salicylic Acid involved 6 levels of control or non-treated seeds, distilled water or hydro-priming, 500, 1000, 1500 and 2000 µM. Seeds were placed simultaneously in petri dishes and rolled towels. The germinated seeds were counted in the petri dishes for 10 following days. GP (Germination Percent) was estimated in the petri dishes after the period of 10 days from dividing the overall number of germinated seeds by total seeds planted.

$$GP = 100 (n_i / N)$$

GI, Germination Index was calculated using the equation of Benech Arnold et al. (1991) as follows:

$$GI = (d_{n-1} \times G_1) + (d_{n-2} \times G_2) + (d_{n-3} \times G_3) + \dots$$

Where, d_n is the last day that all seeds germinated and G_n is number of germinated seeds in the day 'n'.

At the end of this 10 days period length of radial (mm), length of plumule (mm), radicle dry weight (mg), plumule dry weight (mg), rate of plumule to radicle length, rate of plumule to radicle dry weight and vigor index (seedling length x germination percentage) (Islam et al., 2009) were all measured.

The analysis of variance was evaluated for the data by use of the Factorial Test in completely randomized design and the Duncan multiple range tests (5%). Before analysis of variance, data tested for normal distribution. The MSTST-C software was used for statistic measurements.

RESULTS AND DISCUSSION

Seed aging, priming and interactions between these two characters had a considerably significant effect on all characters (table 1).

Table 1. Analysis of variance was assessed during germination of lentil under different levels of salicylic acid and aging.

S.Q.V	df	Germination percent	Germination index	Vigor index	Root length	shoot length	Root dry weight	shoot dry weight
Aging(A)	3	6890.656**	88638.004**	101581697.593**	5719.089**	26542.768**	1750.539**	7723.63**
SA(B)	5	3832.694**	46285.345**	75537320.484**	718.777**	3758.294**	773.606**	3027.441**
(AxB)	15	702.74**	8602.462**	18433108.929**	239.442**	1144.084**	137.379**	664.197**
Error	48	3.561	51.7	648881	72.572	221.448	0.37	0.698
C.V.(%)		10.81	11.87	35.5	29.5	26.4	4.65	3.01

* and **: Significant at the 5% and 1% levels of probability.

Germination percent

The highest number of germinated seeds observed in non-primed seeds in treatment with three days of aging. After this treatment, non-primed and non-aged seeds showed the highest number of germinated seeds. The lowest number of germination was observed in the treatment of 9 day aging which no germination was observed and all lentil seeds had lost their germinability (fig. 1).

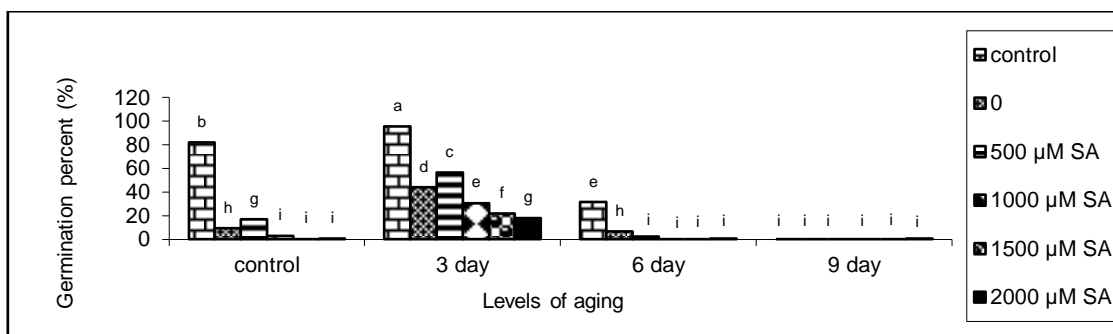


Figure1. The interaction between priming and aging on lentil seed germination percent.

Priming did not have a positive effect on seed germination in lentil, neither hydro-priming nor seed treatment with salicylic acid hormone. Priming led to enhanced seed aging and reduced germinated seeds. Also, in no aged and three days aged seeds, hydro-priming had a greater negative effect than that of priming with 500 μM salicylic acid, which is consistent with findings of GholamiTilebani et al. (2013) that conducted the same experiment on rice seeds. Higher concentrations of SA had a more unfavorable effect compared to that of hydro-priming. In the treatment of 6 day aging after non-treated seeds, the hydro-primed seeds and then primed seeds with 500 μM SA respectively placed. In the seeds treated with 1000 μM salicylic acid germination were close to 1% and seeds primed with 1500 μM and 2000 μM were under 1%.

The results showed that priming of lentil seeds not only have not any positive effect and efficiency on increasing seed vigor, but resulted in decreased seed vigor. Also, hydropriming had a more undesirable effects compared to priming with SA which led to more reduction in seed germination. In contrast, seed aging for 3 days led to a significantly increase in germination percent in non-treated seeds compared to control treatment. It seems that in fresh seeds of lentil a period of time is needed to enhance seed germination.

Seed vigor decreases along with seed aging and following this, seed germinability diminish, too (De Figueiredo et al., 2003). Seed germination percent (KhajeHoseini et al., 2003), seedling emergence and growth (Basra et al., 2003) have been reported to decrease significantly due to seed aging. Cotton seed germination percent decreased after increasing seed aging period (Basra et al., 2003). Acacia seed germination was much lower in aged seeds compared to that of control (Rehman et al., 1999). A positive effect of seed pre-treatment has been reported for some plants such as chickpea (Satvir et al., 2003). Hydro-priming in seeds of lentil, soybean, French bean and broad bean has been reported to improve germination and initial growth (Yazdani et al., 2011). Priming of wheat seeds by 0.5 μM salicylic acid resulted to increase seed germination rate (Ellias et al., 2006), however, priming with 1 μM resulted to decrease seed germination.

Decreased integrity in plasma membrane, structural changes in nucleic acids and decreased activity of enzymes are some of the most important changes on seeds as a result of aging (Justic and Bass, 1979). These changes lead to decreased seed quality, decreased seed germination percent and rate, slower growth of the plant, increased sensitivity to environmental stresses and sometimes decreased yield, too (Kalpana and Rao, 1995; Coin et al., 1995).

Germination index

The graph of germination index was very similar to that of seed germination percent. In all levels of seed aging the highest germination index was reported for non-primed seeds, and seed priming significantly decreased germination speed, in both hydro-primed and SA primed seeds (Fig. 2).

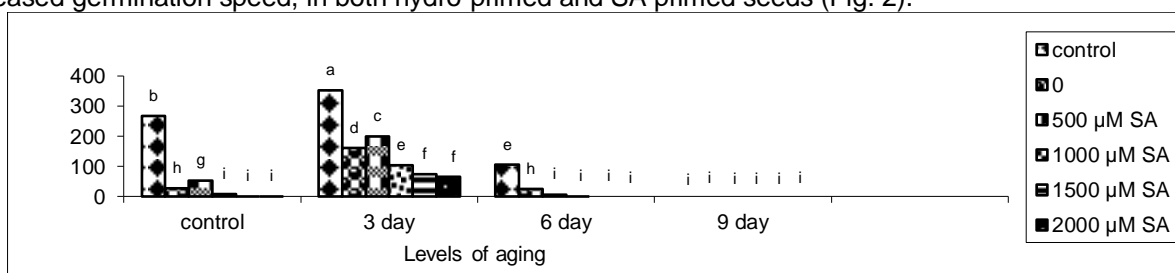


Figure2. The interaction between priming and aging on lentil seed germination index.

On the other hand and similar to germination percent, seed aging for 3 days not only increased number of germinated seeds, but increased number of initially germinated seeds that in turn resulted in higher germination index. In contrast, aging of seeds for 6 days, similar to germination percent, decreased germination index and speed considerably. Seed germination index is, in general, one of the most important parameters in determination of germination speed that had a direct relationship with seed quality and vigor. In other words, the better quality of seeds is the more seed germination percentage and the number of germinated seeds especially during the initial day's increases and consequently germination index. Ghorbani et al., (2006) reported by decreasing number of normal germinated seeds of wheat germination index diminishes too.

Vigor index

A similar trend was seen in the vigor index (fig. 3). This shows that seeds with higher rate of germination result in longer seedlings and had a higher vigor index. It was reported that when germination speed is high, both plumule and radical length may be high (Nascimento, 2003). Aging occurs in ripening, harvesting and storing depending on temperature and humidity (Marshall and Lewis, 2004).

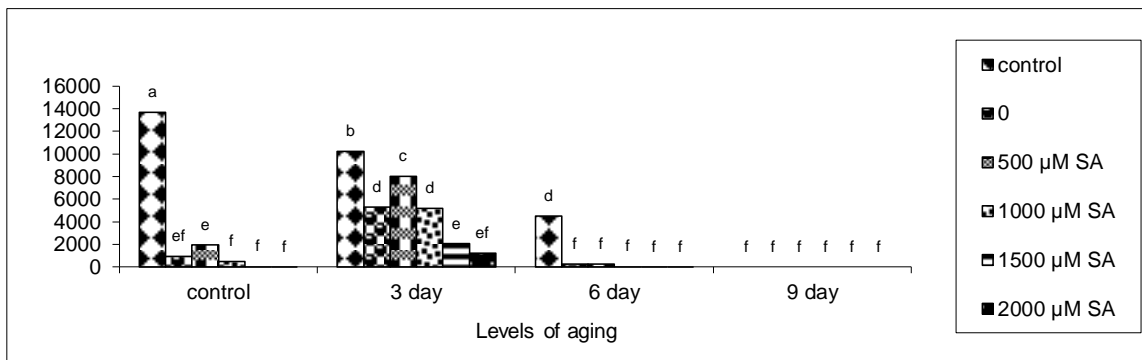


Figure3. The interaction between priming and aging on lentil seed vigor index.

In another study on effects of aging on seed endosperm resources and heterotrophic growth of wheat seedlings showed that aged seeds compared to that of control, have lower germination speed and by each one day increase in aging period this speed decreases 0.9 percent (Soltani et al., 2008). Aged turnip seeds showed an increase in dehydrogenase and peroxidase activity, seed leakage and electrical conductance; and decrease in seed germination speed (Verma et al., 2003). Pre-treatment of lentil seeds using PEG did not influenced seed germination and both treated and non-treated seeds showed equal germination speed (Hosseini and Nassiri Mahalati, 2007).

Radical and plumule length

The highest radicle length (63.05 mm) and plumule length (115.6 mm) was seen in non-primed seeds and primed seeds with 1000 μM SA in non-aging condition which showed a significant difference with other levels of priming (figs. 4 and 5). Treatment with three days of aging and concentration of 1000 μM SA resulted in the longest radicle and plumule length compared to that of other treatments (figs. 4 and 5).

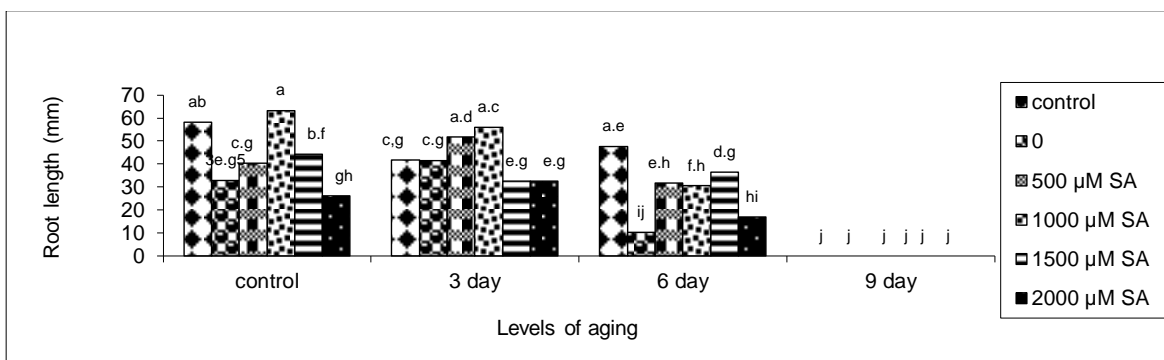


Figure 4. The interaction between priming and aging on lentil root length.

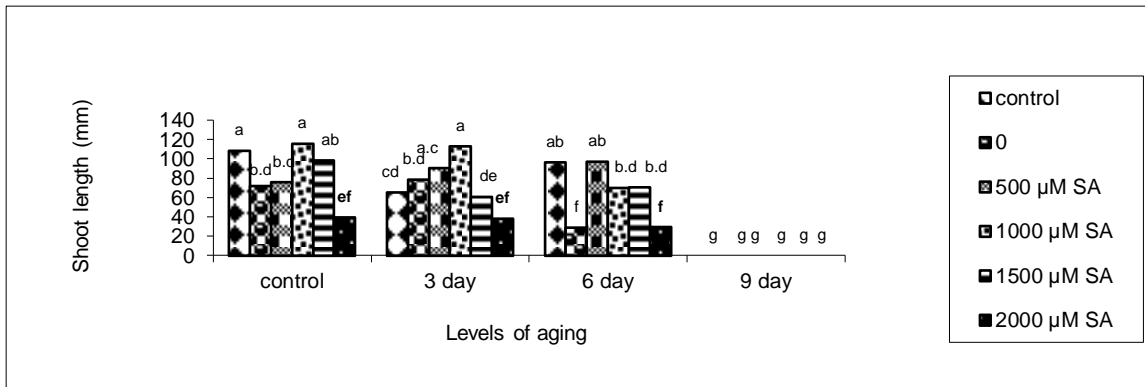


Figure 5. The interaction between priming and aging on lentil shoot length.

The lowest radicle length was reported for 6 days of aging and hydro-primed seeds and 2000 μ M SA. In general, by increasing SA concentration and aging days a downward trend was observed for radicle length (fig. 4). A similar trend was observed for plumule length, too (fig. 5). This shows that priming with salicylic acid in 6 days of aging not only did not increased length of radicle and plumule, but decreased it in higher concentrations of salicylic acid.

Root length is one of the most important and common parameters for explaining efficient root systems and predicting reactions of plant to environmental changes. Also this character is an efficient indicator of water and nutrient uptake in root (Shekari et al., 2010). Treatment of plants with salicylic acid increased cell division in central meristem of root and increased root growth in plants (Shakirova et al., 2003). It has been reported that treatment of wheat seeds not only improved germination percentage and speed, but increased vigor through increased length of roots and shoots, fresh weight and dry weight of the seedlings and number of roots (Sakhabutdinova, 2003). Aging of turnip seeds decreased seedling length (plumule and radicle length) considerably (Verma et al., 2003). Salicylic acid increased lengths of both root and shoot (figs. 4 and 5) which is consistent with Shakirova et al (2003) and Faridoddin (2003).

Radicle and plumule dry weight

The highest dry weight for radicle (50.7 mg) and plumule (104.3 mg) was observed for non-primed control seeds (non- aged) (Figs. 7 and 6).

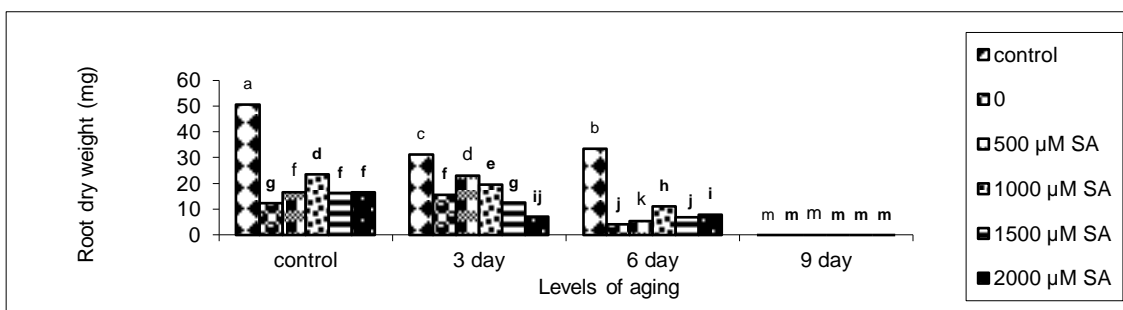


Figure 6. The interaction between priming and aging on lentil root dry weight.

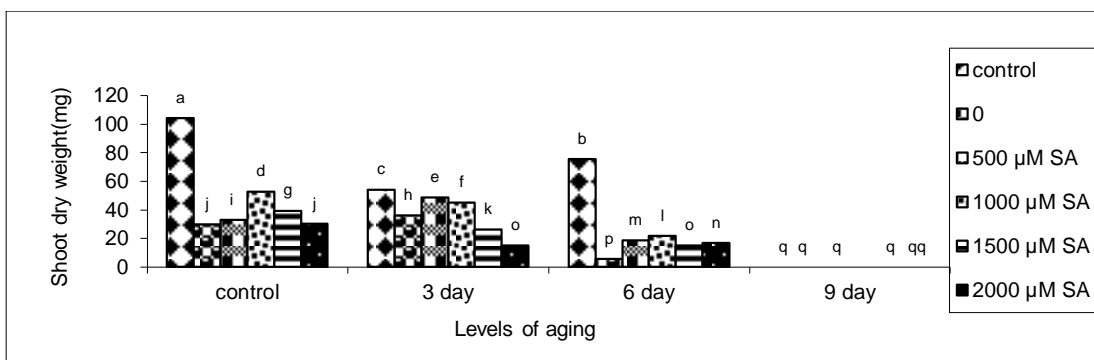


Figure 7. The interaction between priming and aging on lentil shoot dry weight.

Among different levels of salicylic acid, concentration of 1000 μ M SA showed the highest dry weight in different levels of aging (3 to 6 days). The high concentrations of salicylic acid did not have an improving effect

for 3 and 6 days of aging and could not improve the negative effects of aging. Seedling dry weight was observed to decrease significantly as a result of aging (Soltani et al., 2006) which is consistent with results of our study. Decreased dry weight of the seedlings might be due to decreased mobility of seed stored materials or due to decreased efficiency of reservoir conversion (Soltani et al., 2006). Although there are some reports have shown that use of salicylic acid increased dry weight in wheat (Singh and Yousha, 2003) or increased radicle and plumule dry weight in corn (Khodari, 2004), but in the present study no positive effect was detected for SA on lentil. In contrast with our research results, it has been reported when lentil seeds are pre-treated with PEG, dry radicle and plumule mass was approximately 5 mg more than that of control (Hosseini and NassiriMahalati, 2007).

CONCLUSION

The results indicated that by increasing aging level, different traits of seed germination such as germination index, vigor, seedling length and weight decreased, too. Seed priming did not have a positive effect on improving performance of the seedlings. Hydro-primed seeds showed a more considerable decrease compared to that of seeds primed by salicylic acid. Decreased dry weight of plumule and radicle in the present study could be due to decreased mobility of seed stored materials or decreased efficiency of alteration of mobile reservoir.

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