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RESEARCH PAPER

Improving the germination characteristics of aged lentil (*Lens culinaris* L.) seeds using the ascorbic acid priming method

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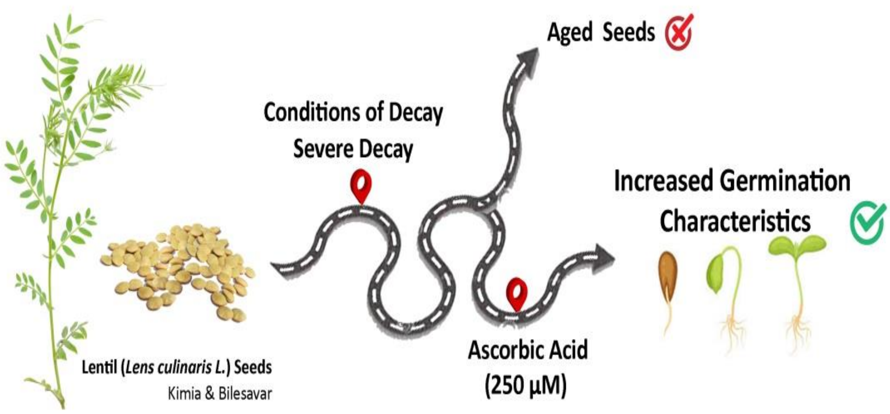
Highlights

- The success of breeding programs to produce hybrid seed depends on the ability of the reformer to identify high-yielding hybrid parental lines.
- To study the heritability of various agronomic and physiological traits in the progeny of six chickpea cultivars using generation mean analysis to determine the effects of genes.
- The estimation of the genetic parameters of the genes and finally the determination of the breeding methods was appropriate.

Graphical Abstract



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Abstract

Lentils are one of the most important legumes in the world and Iran. Seeds age during storage. The longer the seed is stored and the more unsuitable the storage conditions are, the faster it ages and the shorter the life of the seed. In recent years, much attention has been paid to the use of antioxidants to improve aged seed. In this study, the effect of ascorbic acid on aged seed recovery was investigated in two lentil cultivars. Seeds of two lentil cultivars (Kimia and Bilesavar) were subjected to different conditions of decay (no decay, mild decay, and severe decay). The deteriorated seeds were inoculated with different concentrations of ascorbic acid (0, 250, 500, and 750 µM) and evaluated in a standard germination test. The results showed that as the seed deteriorated, germination decreased in both lentil cultivars. Under conditions of no deterioration and mild deterioration, priming the seed with different concentrations of ascorbic acid had no significant effect on the germination characteristics of seeds and seedlings. However, under conditions of severe deterioration of germination, priming seeds with 250 µM ascorbic acid significantly increased germination characteristics. According to the results, it is possible to improve aged lentil seeds by priming with a concentration of 250 µM ascorbic acid.

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1. Introduction

Lentils are a valuable source of protein. This feature, as well as the plant's ability to grow in depleted soils and diverse environmental conditions, has led to the cultivation of this plant as a valuable species to this day. Seed quality is very important in crop production. Seed quality is derived from several factors, but the criteria of germination, vigor, viability and seed health are the most important aspects of seed quality. Seed quality is a set of factors that cause proper seedling germination and the establishment in the field. High seed quality is an essential factor in ensuring proper germination and establishment of crops. Therefore, the seeds used during sowing should be of high quality (Mukasa et al., 2003). Seeds mature during the storage period, which leads to a decrease in seed quality. The longer the storage period and the worse the storage conditions, especially in terms of heat and humidity, the faster the seed matures and the shorter the seed life. Ascorbic acid, as an important antioxidant, is involved in neutralizing reactive oxygen radicals, including superoxide ions (Noctor and Foyer, 1998). Seed pretreatment is a pre-seed strategy that affects seedling development by altering metabolic activities before root emergence and generally increasing germination and seedling emergence (Taylor and Harman, 1990).

Seed priming is a pre-planting treatment that is often used to improve yield (Bradford, 1986). Priming with ascorbic acid inhibits reactive oxygen species, increases photosynthesis capacity, improves cold, heat, salinity and drought tolerance (Mohammadi et al., 2014). Ascorbic acid along with glutathione and several other antioxidant enzymes are involved in neutralizing reactive oxygen radicals, including superoxide ions from a variety of abiotic stresses (Burguieres et al., 2007). Activated oxygen species damage cell membranes, DNA and proteins and produce toxic compounds in the plant's antioxidant system (Bailly, 2004). The antioxidant system contains enzymes and antioxidant metabolites that eliminate active types of oxygen. Catalase, peroxidase, and other antioxidant enzymes break down hydrogen peroxide to convert it to water and oxygen, eliminating and inactivating reactive oxygen species (Mittler, 2002). McDonald (1999) showed that seed pretreatment with compounds such as ascorbic acid, cinnamic acid and alpha-tocopherol before artificial and natural erosion, seed vigor characteristics and shelf life of seeds of rice, corn, canola, sunflower, Improved French beans, peas, lentils, millet and jute (McDonald, 1999). Recent research on crop seeds has shown that it is possible to improve mature seeds by using some antioxidants. In general, seed priming increases the speed and uniformity of germination (Pessarakli et al., 2004). This experiment aimed to study the possibility of improving seed deterioration using seed priming by different concentrations of ascorbic acid in two lentil cultivars.

2. Materials and Methods

This research was conducted on the campus of agriculture and natural resources of Razi University during the years 2018-19. First, the seeds underwent deterioration. They were then primed with different concentrations of ascorbic acid. Therefore, the research was conducted as a factorial experiment in a completely randomized design. Factors included different levels of seed deterioration (no deterioration, mild deterioration and severe deterioration) and seed priming with ascorbic acid antioxidant (at four concentrations of 0, 250, 500 and 750 μM) and seeds of two lentils cultivars (Kimia and Bilesavar). After deterioration, the seeds were subjected to different priming treatments. Finally, the seeds were evaluated in the standard germination test according to the rules and regulations of the International Seed Testing Association. In the standard germination test, various characteristics related to seed germination were measured as follows:

$$\text{Final germination percentage: FGP}=\frac{\text{Number of seeds germinated}}{\text{Total number of seeds planted}}\times 100 \quad (1)$$

It is noteworthy that seedlings with roots larger than 2 mm were considered as germinated.

$$\text{Normal seedlings percentage: NSP}=\frac{\text{Normal number of germinated seeds}}{\text{Total number of seeds planted}}\times 100 \quad (2)$$

It should be noted that seedlings without stems or with very short roots or stems or with abnormal roots and stems were considered as abnormal seedlings. The number of abnormal seedlings was deducted from the number of germinated seeds and counted as the number of normal seedlings.

Seedling length: SL= Stem length + Root length (3)
Seedling weight: SW= Stem weight + Root weight (4)

After data collection, MSTAT-C statistical software was used to normalize the data, analyze the variance and compare the means. The means were compared by LSD method at the level of 5%.

3. Results and discussion

3.1. Final germination percentage and Normal seedlings percentage

Analysis of variance showed that the simple effects of cultivar, seed deterioration, priming by ascorbic acid concentrations on Final germination percentage and Normal seedlings percentage were significant. Also, the interaction of cultivar × seed deterioration and the interaction of seed deterioration × priming by ascorbic acid concentrations on Final germination percentage and Normal seedlings percentage were significant. But the interaction of cultivar × priming by ascorbic acid concentrations was not significant (Table 1). Comparison of the mean interaction of cultivar × seed deterioration showed that in both Kimia and Bilesavar cultivars, with the intensification of seed deterioration, the values of Final germination percentage and Normal seedlings percentage gradually decreased. The effect of severe deterioration was greater in Bilesavar cultivar than the Kimia cultivar (Table 2). In severe deterioration conditions in Kimia and Bilesavar cultivars, the final germination percentage was 74 and 54% and Normal seedlings percentages were 63 and 47%, respectively. Comparison of the mean interaction of seed deterioration × priming by ascorbic acid concentrations shows that in conditions without mild deterioration (control) and mild deterioration, seed priming at different concentrations of ascorbic acid did not cause much change in Final germination percentage and Normal seedlings percentage. But in severe deterioration conditions, with increasing ascorbic acid concentration from 0 to 250, 500, and 750 μM, germination percentage from 57 to 72, 62, and 64 Final germination percentage and Normal seedlings percentage from 42 to 63, 55, and 54% reached, respectively. Therefore, in conditions of severe seed deterioration, seed priming with ascorbic acid, especially at a concentration of 250 μM, increased and improved the traits of Final germination percentage and Normal seedlings percentage in lentils.

Table 1. Analysis of variance (mean squares) Effect of cultivar, deterioration and seed priming on ascorbic acid concentrations on seed and seedling characteristics of lentils.

S.O.V	Degrees of freedom (df)	Final germination percentage	Normal seedlings percentage	Seedling length	Seedling weight
Cultivar	1	910.222**	308.347**	11.392**	12.185**
Deterioration	2	7418.0973**	11088.097**	299.777**	126.041**
Interaction	2	738.014**	659.431**	1.108 ns	4.004**
Ascorbic acid concentration	3	94.833**	78.681*	0.927 ns	0.601 ns
Cultivar × Deterioration	3	34.185 ns	68.792 ns	0.572 ns	0.106 ns
Deterioration × Concentration	6	78.931**	63.875*	2.539**	1.106*
Cultivar × Deterioration × Concentration	6	37.477 ns	51.764 ns	1.282 ns	0.578 ns
Error	48	17.764	27.806**	0.768	0.468
Coefficient of variation	-	5.09	6.97	5.68	7.04

ns, * and ** are non-significant and significant at the 5% and 1% probability levels, respectively.

Table 2. Comparison of mean cultivar × seed deterioration on seed and seedling characteristics of lentils.

	The severity of seed deterioration	Final germination percentage	Normal seedlings percentage	Seedling length (cm)	Seedling weight (mg)
Kimia	No Deterioration	99.66[a]	98.83 [a]	19.03 [a]	11.90 [a]
	Mild Deterioration	85.33 [b]	70.83 [c]	1609[a]	10.35 [b]
	Severe Deterioration	74.25 [c]	63.5 [d]	12.30 [a]	8.14 [d]
Bilesavar	No Deterioration	98.83[a]	97.83 [a]	18.73 [a]	11.71 [a]
	Mild Deterioration	84.75 [b]	75.25 [b]	14.98 [a]	9.82 [c]
	Severe Deterioration	54.33 [d]	47.66 [e]	11.33[a]	6.39 [e]

In each column, the averages with similar letters have no significant difference at the 5% level.

3.2. Seedling length and weight

In the analysis of variance, simple effects of cultivar and seed deterioration on the seedling length and weight were significant. The interaction of cultivar × seed deterioration was not significant on seedling length but was significant on seedling weight. Also, the interaction effect of seed deterioration seed priming by ascorbic acid concentrations on the seedling length and weight was significant (Table 1). Comparison of means showed that in both Kimia and Bilesavar cultivars, seed deterioration reduced seedling length and weight. The effect of seed deterioration, especially severe deterioration on the seedling length and weight in the Bilesavar cultivar was greater than the Kimia cultivar (Table 2). Basra et al., (2003) reported that seedling length and fresh weight decreased with increasing early maturation time in cotton seeds (Basra et al., 2003).

Comparison of the mean interaction of seed deterioration × priming by ascorbic acid concentrations showed that in non-worn seeds (control), seed priming by ascorbic acid concentrations did not increase seedling length and weight (Table 3). Under mild seed deterioration, priming at any concentration of ascorbic acid could not improve seedling length and weight. However, under severe deterioration, seed priming at 250 μM ascorbic acid increased seedling length and weight, but 500 and 750 μM did not. Therefore, seed priming at a concentration of 250 μM was effective in improving the deterioration of lentil seeds in terms of seedling length and weight. Ascorbic acid is responsible for regulating the redox oxidation potential during germination (Copeland and McDonald, 1999). Priming with ascorbic acid increased germination rate, final germination percentage, root and shoot length, reduced germination time and fifty percent germination time in rice seeds (Farooq et al., 2006).

Table 3. Comparison of mean deterioration × seed priming by ascorbic acid on seed and seedling characteristics of lentils.

The severity of seed deterioration	Ascorbic acid concentration (μ M)	Final germination percentage	Normal seedlings percentage	Seedling length (cm)	Seedling weight (mg)
No Deterioration	0	98.66 [a]	98.00 [a]	19.32 [a]	12.07 [a]
	250	99.00 [a]	98.00 [a]	18.41[a]	11.62 [ab]
	500	100 [a]	99.66 [a]	19.03 [a]	11.66 [a]
	750	99.33 [a]	97.66 [a]	18.76 [a]	11.87 [a]
Mild Deterioration	0	85.33[b]	71.50 [b]	16.42 [b]	10.85 [b]
	250	86.00 [b]	73.16 [b]	15.63[bc]	9.74 [c]
	500	83.00 [b]	72.83 [b]	14.91 [c]	9.85[c]
	750	85.83 [b]	74.66 [b]	15.20[c]	9.90[c]
Severe Deterioration	0	75.66[e]	42.50[d]	11.15[e]	6.98 [e]
	250	72.83[c]	63.16 [c]	12.69[d]	7.85 [d]
	500	62.66[d]	55.00 [d]	11.82 [de]	7.17 [de]
	750	64.00[d]	54.66 [d]	11.59 [e]	7.05 [e]

4. Conclusions

In conditions without seed deterioration as well as mild seed deterioration, seed priming with different concentrations of ascorbic acid had no significant effect on seed and seedling germination characteristics. However, in severe deterioration conditions, seed priming with a concentration of 250 μM ascorbic acid significantly increased the final germination percentage, normal seedlings percentage, seedling length and weight. Therefore, it seems possible to improve aged lentil seeds by seed priming at a concentration of 250 μM ascorbic acid.

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