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INFLUENCE OF SEED SIZE AND AGING ON SEEDLING GROWTH AND FIELD ESTABLISHMENT OF LENTIL (*LENS CULINARIS* MEDIK)

ABSTRACT

A sub-sample of lentil (*Lens culinaris* Medik. cv. Kimia) seeds was kept as bulk (S_1) and the other seeds were separated by a sieve with four millimeters diameter. Consequently, three seed lots including bulk (S_1), large (S_2) and small (S_3) seeds were obtained. Seeds of each size were divided into three sub-samples. A sub-sample was kept as control or high vigor seed lot (A_1). The other two sub-samples with about 20% moisture content were artificially aged at 40°C for 2 and 4 days (A_2 and A_3, respectively). These seeds were evaluated in laboratory and field during 2011 and 2012 as factorial experiments on the bases of RCB design. The lowest mean germination and emergence times and the highest germination percentage, seedling dry weight and emergence percentage were recorded for high vigor seed lot. Large seeds produced the largest seedlings in comparison with bulk and small seeds. Although, seedling dry weight was reduced with increasing seed aging, the lowest reduction was recorded for seedlings from large seeds. The most deteriorated large seeds germinated earlier than those of bulk and small seeds. Early germination of the most deteriorated large seeds resulted in rapid emergence of seedlings in the field. This is also reflected in the highest positive correlation of mean germination time with mean emergence time. It was concluded that some deleterious effects of seed aging can be reduced by increasing seed size.

Key words: aging, field establishment, germination, lentil, seed size

INTRODUCTION

One of the main problems observed in the field is poor establishment, which is influenced by seed quality, adverse climatic conditions and poor field management (Maiti and Carrillogutierrez, 1989). Seed quality includes several attributes that lead to near maximum germination capacity to produce

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seedlings, which emerge rapidly from the seedbed and continue to grow uniformly thereafter (Harrington, 1971). Seed quality, as measured by its vigor and viability, plays a major role in establishment of seedling as well as higher crop yield (Ghassemi-Golezani *et al.*, 2010, 2011, 2012). Seed is seldom planted immediately after harvesting; it is stored for a certain period of time before sowing. After harvest, seeds start deteriorating, moving inexorably towards loosing viability (Gregg *et al.*, 1994). During deterioration, vigor is the first component of seed quality, which is lost; this is followed by a loss of germination capacity and viability (Trawatha *et al.*, 1995). The deterioration rhythm of the seed could be slow or rapid and it depends both on the genetic structure of the material and the treatments applied to the seed, but mostly, on the environmental factors during the storage. Thus, a seed can have an accelerated ageing at a few days or weeks only, but it can be still young enough after a few years of storage.

Many other physiological processes have been linked to seed ageing; e.g. in aged seeds membrane phospholipid content decreased and fatty acid levels increased, even though no extensive lipid peroxidation occurred (Bruggink *et al.*, 1991). Both genetic damage and loss of membrane integrity may cause changes in protein synthesis during germination (Gidrol *et al.*, 1990) and result in delayed germination, abnormal growth and finally, loss of viability (Ellis and Roberts, 1981). Seed deterioration has been reported to reduce field emergence and growth of wheat (Ganguli and Sen-Mandi, 1990), winter oilseed rape (Ghassemi-Golezani *et al.*, 2010), maize (Ghassemi-Golezani *et al.*, 2011) and chickpea (Ghassemi-Golezani *et al.*, 2012).

Another important factor that may influence germination (Pearson *et al.*, 2002), emergence (Castro, 1999), growth and survival of seedlings (Baraloto *et al.*, 2005) is seed size. The effect of seed size on germination and following seedling emergence have been investigated by many researchers in various crop species such as spring wheat (Lafond and Baker, 1986), rice (Roy *et al.*, 1996) and oat (Willenborg *et al.*, 2005). However, these results varied widely between species. Large seeds may be favored because they produce larger and more vigorous seedlings with better chances of survival than small seeds (Moles and Westoby, 2004).

After germination, larger seeds retain a greater proportion of their seed reserves, which can then be mobilized for seedling growth, maintenance and repair (Green and Juniper, 2004). The seedling-size effect foresees that larger seeds produce larger seedlings, which are more robust and better able to escape size-dependent mortality. In addition to being able to emerge from deeper soil layers, the larger seedlings have a larger shoot, which can overtop neighboring seedlings and capture more light (Foster, 1986). Larger seedlings also form deeper and more extensive roots, which can capture more soil water during the dry season (Lloret *et al.*, 1999). Little information is available regarding the combined effects of seed size and deterioration on seedling vigor and field establishment of lentil, which are investigated in this research.

MATERIALS AND METHODS

Providing seeds with different sizes

Seeds of lentil (*Lens culinaris* Medik. cv. Kimia) were obtained from Research Center of Dry-land, Kermanshah, Iran. A sub-sample of the seeds was kept as bulk (S_1) and the other seeds were separated by a sieve with four millimeters diameter. The seeds that remained on the sieve were considered as large (S_2) and those passed the sieve were considered as small (S_3) seeds.

Providing seeds with different levels of vigor

Seeds of each size were divided into three sub-samples. A sub-sample was kept as control or high vigor seed lot (A_1). The two other sub-samples with about 20% moisture content were artificially aged at 40°C for 2 and 4 days (A_2 and A_3, respectively). So, three seed lots with different levels of vigor were provided for laboratory tests and field experiment.

Laboratory tests

Laboratory tests were carried out in 2 years, 2011 and 2012 as factorial based on RCB design with four replications at the Seed Technology Laboratory of the University of Tabriz, Iran. Four repli-cates of 25 seeds were placed between moist filter papers and germinated in an incubator adjusted on 20°C for 10 days. Germination (protrusion of radicle by 2 mm) was recorded in daily intervals. At the end, percentage of normal seedlings and seedling dry weight were determined. Mean germination time (MGT) was calculated according to Ellis and Roberts (1980):

$$MGT = \frac{\sum(D \times n)}{\sum n}$$

Where *n* is the number of seeds germinated on day *D* and *D* is the number of days counted from the beginning of the test.

Field experiments

The field experiment was conducted at the Research Farm of the University of Tabriz (Latitude 38˚05’ N, Lon-gitude 46˚17’ E, Altitude 1360 m above sea level) in 2 years, 2011 and 2012. All the seeds were treated with Benomyl at a rate of 2 g × kg^{-1} before sowing. Seeds were hand sown in about 5 cm depth with a density of 100 seeds m^{-2} on 5th May 2011 and 14th May 2012.

Each plot consisted of 6 rows with 4 m length in 2011 and with 3 m length in 2012, spaced 25 cm apart. The experiment was arranged as factorial, based on RCB design with three replications in. All plots were irrigated immediately after sowing. Subsequent irrigations were carried out as necessary. Weeds were controlled by hand during crop growth and development. Seedling emergence was recorded in daily intervals up to final establishment in each plot. Subsequently, mean emergence time and percentage of seedling emergence were calculated. Combined analyses of variance of the data based on the experimental de-sign and comparison of means at $P \leq 0.05$ were carried out, using MSTATC software.

RESULTS

The analysis of variance of the laboratory data showed significant effects of seed aging on mean germination time, germination percentage and seedling dry weight ($P \leq 0.01$), but the effect of seed size was only significant on seedling dry weight ($P \leq 0.01$). Interaction of seed size × aging was also significant for mean germination time and seedling dry weight ($P \leq 0.01$).

The lowest mean germination time was obtained for the high vigor seed lot (A_1) and this trait was increased with increasing seed aging, indicating that seeds of A_1 germinated earlier than those of A_2 and A_3. High seed vigor also resulted in high germination percentage and seedling dry weight. These traits were significantly decreased with increasing seed aging (Table 1).

Table 1

Means of germination time, germination percentage and seedling dry weight of lentil affected by seed aging

Treatments	MGT [day]	Germination [%]	Seedling dry weight [g]
A_1	3.183 c	97.17 a	0.2646 a
A_2	3.940 b	90.83 b	0.2237 b
A_3	5.327 a	82.33 c	0.1838 c

Different letters at each column indicate significant difference at $p \leq 0.05$

A_1, A_2 and A_3: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively

Large seeds (S_2) produced the largest seedlings in comparison with bulk (S_1) and small seeds (S_3). Seedling dry weight for small seeds was significantly lower than that for bulk and large seeds (Fig. 1.). Mean germination time of lentil seeds with different levels of seed size increased as seed aging increased. However, the most deteriorated large seeds (S_2A_3) germinated earlier than those of bulk (S_1A_3) and small (S_3A_3) seeds (Fig.2-A.). Although, seedling dry weight was reduced with increasing seed aging, the lowest reduction was recorded for seedlings from large seeds (Fig. 2-B.).

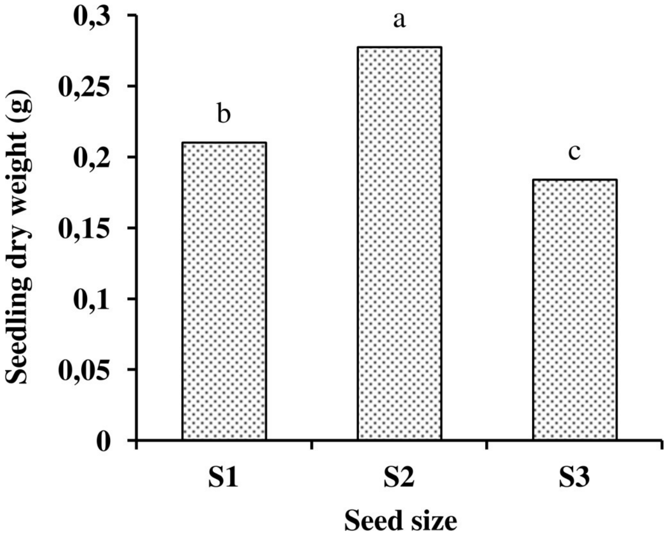

Fig. 1. Seedling dry weight of lentil for different seed sizes
Different letters indicate significant difference at $p \leq 0.05$. S_1, S_2 and S_3: Bulk, large and small seeds of lentil, respectively

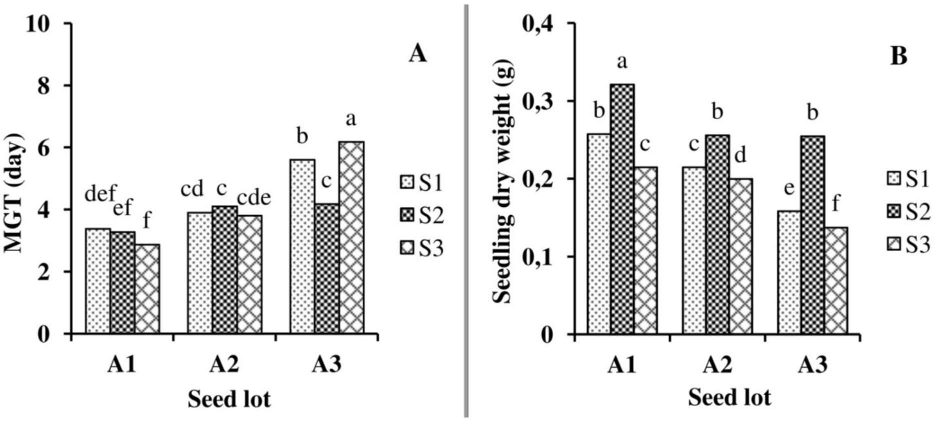

Fig. 2. Mean germination time and seedling dry weight for differentially deteriorated seeds of different sizes in lentil. Different letters indicate significant difference at $p \leq 0.05$
A_1, A_2 and A_3: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively
S_1, S_2 and S_3: Bulk, large and small seeds of lentil, respectively

Seed aging had significant effects on mean emergence time and seedling emergence percentage ($P \leq 0.01$), but seed size had no significant effect on these traits. The interaction of seed size × aging for mean emergence time and the interaction of year × aging for seedling emergence percentage were also significant ($P \leq 0.01$).

Seedlings of A_1 emerged earlier than those of A_2 and A_3, and mean emergence time for A_3 seeds was significantly higher than that for A_2 seeds (Table 2). The highest emergence percentage was obtained by A_1 seeds, followed by A_2 and A_3 seeds (Table 2). Seedling emergence percentage for A_1 seed lot was similar in both years, but for A_2 and A_3 seed lots it was significantly lower in second year (Fig. 3). Seedlings from the most deteriorated large seeds (S_2A_3) emerged earlier than those from the bulk (S_1A_3) and small (S_3A_3) seeds. However, emergence time for A_1 and A_2 seeds of different sizes was statistically similar (Fig. 4).

Table 2

Means of seedling emergence time and percentage of lentil affected by seed aging

Treatments	MET [day]	Seedling emergence [%]
A_1	10.35 c	81.94 a
A_2	14.07 b	54.78 b
A_3	18.99 a	41.89 c

Different letters at each column indicate significant difference at $p \leq 0.05$
A_1, A_2 and A_3: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively

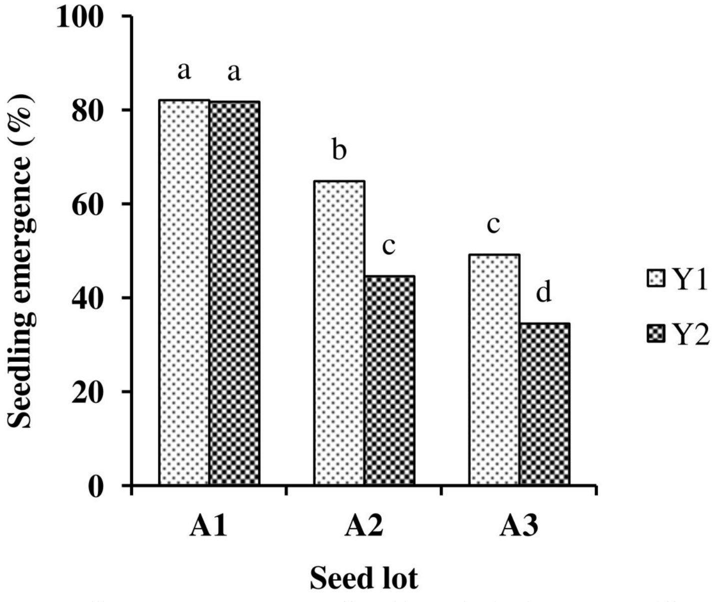

Fig. 3. Mean seedling emergence percentage affected by seed aging in two years. Different letters indicate significant difference at $p \leq 0.05$ A_1, A_2 and A_3: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively Y_1 and Y_2: First and second years, respectively

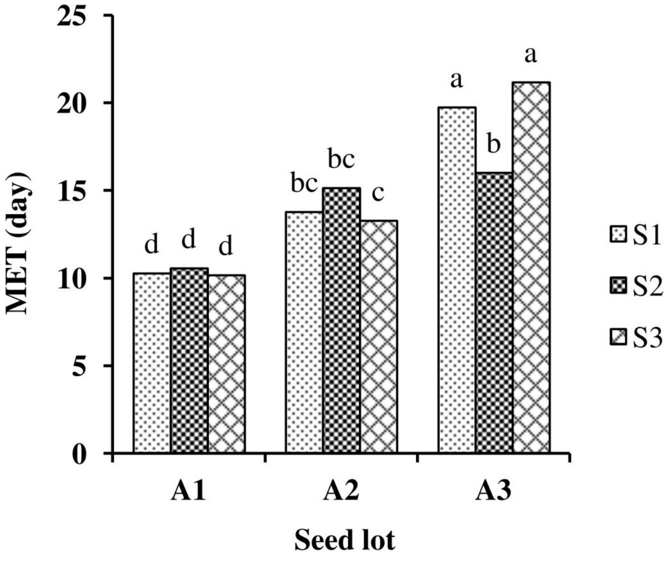

Fig. 4. Mean emergence time of seedlings from differentially deteriorated seeds of different sizes in lentil Different letters indicate significant difference at $p \leq 0.05$, A_1, A_2 and A_3: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively; S_1, S_2 and S_3: Bulk, large and small seeds of lentil, respectively

Mean germination time negatively correlated with germination percentage, seedling dry weight and emergence percentage, but positively correlated with mean seedling emergence time. Correlation of germination percentage with mean emergence time was negative and significant, but with emergence percentage was positive. Correlations between seedling dry weight and mean emergence time and also between mean emergence time and emergence percentage were negative and significant (Table 3).

Table 3

Correlation coefficients of different traits of lentil

Traits	1	2	3	4	5
1. Mean germination time [day]	1				
2. Germination [%]	-0.87**	1			
3. Seedling dry weight (g)	-0.72*	0.65	1		
4. Mean emergence time [day]	0.98**	-0.93**	-0.73*	1	
5. Seedling emergence [%]	-0.84**	0.95**	0.65	-0.91**	1

*, **: Statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

DISCUSSION

Low performance of the aged seeds during germination and seedling growth (Table 1) could be related to spontaneous oxidation of unsaturated fatty acids

(Pukacka and Kuiper, 1988). These results support the hypothesis that in some seed type's oxidation of unsaturated fatty acids represents a primary cause for seed damage by free radicals. It may also be suggested that mild aging resulted in the delayed germination. Copeland and McDonald (1995) reported that continual accumulation of free fatty acids culminates in a reduction of cellular pH and is detrimental to normal cellular metabolism. Furthermore, it denatures enzymes resulting in loss of their activity. Some studies have shown that peroxidative changes in fatty acid composition of membrane lipids lead to massive dysfunction of cellular membranes associated with increased viscosity and permeability of bilayers (Copland and McDonald, 1995). Lipid peroxidation results in the loss of intact membranes in the mitochondrial cristae thereby reducing ATP production during germination process (McDonald, 1999).

Production of the largest seedlings by large seeds (Fig. 1) can be attributed to the large carbohydrate reserve of these seeds (Singh and Pai, 1988). Other reports indicated that seedlings derived from larger seeds often have greater biomass than seedlings derived from smaller seeds (Wulff, 1986; Meyer and Carlson, 2001). Generally, the larger a seed is the greater the metabolic reserve it will have available to make up for lack of resources in the environment. This is known as the reserve effect. The reserve effect is believed to be the underlying mechanism for overcoming hazards to seedling survival and winning competition with other seedlings (Westoby *et al.*, 1997).

The physiological changes during seed aging resulted in poor stand establishment in the field (Table 2), particularly in the second year (Fig. 3). This is also supported by significant correlations of seed quality parameters with field emergence (Table 3). Since, there is a strong relationship between plant population density and grain yield of crops, declining seedling emergence to sub-optimal densities can reduce grain yield per unit area (Ghassemi-Golezani *et al.*, 2010, 2011, 2012). Therefore, optimum stand establishment is a pre-requirement to improve crop performance, particularly under adverse environmental conditions (Ghassemi-Golezani *et al.*, 2011).

Slow emergence of seedlings (Table 2) could lead to the produc-tion of weaker plants, that can reduce stress tolerance, increase pest damage and reduce crop yield (Harris *et al.*, 1999). This is also reflected in significant and negative correlation of mean germination time with seedling dry weight (Table 3). However, early germination of the most deteriorated large seeds (Fig. 2-A) resulted in rapid emergence of seedlings in the field (Fig. 4). These results clearly suggest that deleterious effects of seed aging to some extent can be reduced by increasing seed size.

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