



The Impact of Seed Size and Aging on Physiological Performance of Lentil under Water Stress

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Abstract

A sub-sample of lentil (*Lens culinaris* Medik. cv. Kimia) seeds was kept as bulk (S_1) and another sample was separated to large (S_2) and small (S_3) seeds. A sub-sample of each size was kept as control or high vigor seed lot (A_1) and the two other sub-samples were artificially aged for 2 and 4 days (A_2 and A_3 , respectively). Field performance and some physiological traits of plants from these seeds were evaluated during 2011. Relative water content (RWC), ground cover and grain yield per unit area significantly decreased with decreasing water availability, but mean leaf temperature and proline content increased as water stress severed. Seed aging reduced RWC, MSI, ground cover and consequently grain yield per unit area. The superiority of plants from large seeds in ground cover and grain yield was increased with increasing seed aging and water limitation. It was, therefore, concluded that planting large seeds may reduce the deleterious effects of water stress and seed aging on field performance of lentil.

Keywords: Lentil; Seed aging; Seed size; Water stress

Introduction

Crop yields are restricted by water shortages in many parts of the world (Austin 1989). The physiological responses of plants to water stress and their relative importance for crop productivity vary with species, soil type, nutrients and climate. Decreasing water content is accompanied by loss of turgor and wilting, cessation of cell enlargement, closure of stomata, reduction in photosynthesis and interference with many other basic metabolic processes (Kramer and Boyer 1995). Relative water content is a good sign for water status in plants and spots it better than water potential (Patakas *et al.* 2002). Membrane stability index (MSI) and relative water content (RWC) have been decreased as a result of water deficit (Bayoumi *et al.* 2008). Tolerant cultivars have more RWC in

comparison with sensitive cultivars under drought stress (Patakas *et al.* 2002).

Plants can partly protect themselves against mild drought stress by accumulating osmolytes. Proline is one of the most common compatible osmolytes in drought stressed plants. For example, proline content of pea increased under drought stress (Alexieva *et al.* 2001). Other stresses such as salinity can also induce proline accumulation (Sairam *et al.* 2002; Ghassemi-Golezani *et al.* 2011). Proline metabolism in plants, however, has mainly been studied in response to osmotic stress (Verbruggen and Hermans, 2008). The accumulation of proline in plant tissue is, therefore, a clear marker for drought and salt stresses. Proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio *et al.* 2002). Thus,

increasing proline concentration can be used as an evaluating parameter for irrigation scheduling and for screening drought resistant varieties (Bates *et al.* 1973; Gunes *et al.* 2008).

Maximum seed quality is obtained at or slightly after mass maturity and thereafter seeds begin to deteriorate on mother plant (Ghassemi-Golezani and Mazloomi-Oskooyi 2008; Ghassemi-Golezani and Hosseinzadeh-Mahootchy 2009) and during storage, loose vigor and viability (Ellis and Roberts 1981). Several biochemical and physiological changes have been observed in seeds during aging, resulting in a progressive decline in seed quality (Marcos-Filho and McDonald 1998). When seed aging increases, germination rate and uniformity and tolerance to environmental stresses and consequently seedling emergence and post emergence seedling growth decrease (Khan *et al.* 2003). Seed size is an important characteristic of many plant life histories (Harper *et al.* 1970), since it is generally proportional to the amount of food reserves that will be destined to the embryo (Lloret *et al.* 1999). Larger seeds have a better performance than small seeds, especially under competitive conditions (Moles and Westoby 2004). Results of some researchers clarified that seed size notably affected seedling establishment, plant height, seed weight and number of seeds per spike in wheat (Royo *et al.* 2006). Also, larger seeds with well-developed root systems of seedlings

may gain an advantage by allowing to reach the soil moisture at deeper levels (Leishman and Westoby 1994). Since the interaction of seed size and aging on field performance of lentil is not documented, this research was carried out to investigate these interactive effects on some physiological characteristics and yield of lentil under different irrigation treatments.

Materials and Methods

Seeds of lentil (*Lens culinaris* Medik. cv. Kimia) were obtained from Dryland Agricultural Research Center, Kermanshah, Iran. A sub-sample of the seeds was kept as bulk (S_1) with 1000 grain weight of 42 g. 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) with 1000 grain weight of 50 g and those passed the sieve were considered as small (S_3) seeds with 1000 grain weight of 35 g. Seeds of each size were divided into three sub-samples. A sub-sample was kept as control or high vigor seed lot with 97.2% normal germination (A_1). The two other sub-samples with about 20% moisture content were artificially aged, using controlled deterioration test (ISTA 2010) at 40°C for 2 and 4 days, reducing germination to 90.8% and 82.3% (A_2 and A_3 , respectively). So, three seed lots with different levels of aging were provided for laboratory tests and field experiment.

The field experiment was conducted at the Research Farm of the University of Tabriz (Latitude 38°05' N, Longitude 46°17' E, Altitude 1360 m above the sea level) in 2011. 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. Each plot consisted of 6 rows with 4 m length, spaced 25 cm apart. The experiment was arranged as split plot factorial, based on randomized complete block design with three replications. All plots were irrigated immediately after sowing and subsequent irrigations were carried out after 70 (I₁), 120 (I₂) and 170 (I₃) mm evaporation from class A pan. Weeds were removed by hand during crop growth and development.

Relative water content was determined according to Barr and Weatherley (1962). Fresh weight of the youngest fully expanded leaves of five plants from each plot was determined. Then, leaves were soaked in distilled water for 24 h. After that, the water on the leaves was quickly and carefully removed by tissue paper and subsequently turgid weight was recorded. Leaf dry weight was obtained after drying the samples for 48 h at 75°C. Relative water content was calculated by the following equation:

$$\text{RWC} = \frac{[(\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})] \times 100}$$

Leaf temperature (°C) was directly measured by an infra-red thermometer (TES-

1327) at the flowering stage. Fresh leaf samples (0.1 g) from each plot were taken in 10 ml double-distilled water in glass vials and kept at 40°C for 10 min. Initial conductivity (C₁) was recorded with a conductivity meter after transferring the sample to 25°C. The samples were kept at 100°C for 30 min and cooled at 25°C. Final conductivity (C₂) was measured according to Sairam (1994). The membrane stability index (MSI) was calculated as:

$$\text{MSI} = [1 - (C_1/C_2)] \times 100$$

Proline content was determined according to Bates *et al.* (1973). Ground cover was measured every week by viewing the canopy through wooden frame (50 × 50 cm dimensions) divided into 100 equal sections. The sections were counted when more than half filled with crop green area. Finally, plants of 1 m² in the middle part of each plot were harvested and grain yield per unit area was determined. Analyses of variance of the data based on the experimental design and comparison of means at P≤0.05 were carried out, using MSTATC software.

Results

Analyses of variance of the data showed significant effects of irrigation treatments on RWC, leaf temperature, proline, ground cover and grain yield per unit area. Ground cover and grain yield per unit area significantly affected by seed size and relative water content (RWC), leaf temperature, membrane

stability index (MSI), ground cover and grain yield were significantly influenced by seed aging (Table 1). Interactions of irrigation \times seed size for ground cover and grain yield per

unit area, irrigation \times seed aging for relative water content (RWC) and membrane stability index (MSI) and seed size \times seed aging for ground cover were also significant (Table 1).

Table 1. Analysis of variance of the effects of seed size and aging on some physiological traits of lentil under different irrigation treatments

S.O.V	d.f	MS					
		RWC	Leaf temperature	MSI	Proline	Ground cover	Grain yield
Replication	2	7.415	1.593	26.580	0.955	7.676	158.330
Irrigation (I)	2	357.953**	254.333**	136.967	34.851**	700.589**	12499.652 **
Error	4	10.075	10.815	46.324	0.849	1.398	25.885
Seed size (S)	2	0.850	1.037	72.801	0.971	467.551**	1048.865**
I \times S	4	1.174	7.981	61.843	0.112	20.223*	772.786*
Seed aging (A)	2	592.878**	33.593**	604.081**	0.496	1233.109**	607.090*
I \times A	4	34.019**	6.648	209.668*	0.356	7.975	228.807
S \times A	4	1.444	1.852	79.100	0.092	28.498*	164.335
I \times S \times A	8	1.179	7.213	66.552	0.301	8.405	122.432
Error	48	7.350	6.463	72.216	0.516	8.143	184.091
CV%	-	4.25	10.53	11.09	15.28	5.86	19.05

*, **: Statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively
RWC: Relative water content, MSI: Membrane stability index

Relative water content (RWC), ground cover and grain yield per unit area significantly decreased with decreasing water availability, but mean leaf temperature and

proline content increased as water stress severed. The highest RWC, MSI, ground cover and grain yield were recorded for plants from the non-aged seed lot (A_1) (Table 2).

Table 2. Means of physiological traits and grain yield of lentil affected by irrigation treatments and seed aging

Treatments	RWC (%)	Leaf temperature (°C)	MSI (%)	Proline (mg.g ⁻¹ fresh weight)	Ground cover	Grain yield (g/m ²)
Irrigation						
I ₁	67.41a	21.26c	77.63a	3.484c	53.67a	92.01a
I ₂	63.71b	23.81b	77.53a	4.878b	49.00b	72.65b
I ₃	60.13c	27.37a	74.74a	5.735a	43.49c	49.05c
Seed aging						
A ₁	68.13a	23.19b	81.17a	4.85a	56.21a	76.41a
A ₂	64.31b	23.89b	77.00a	4.63a	46.86b	70.19ab
A ₃	58.81c	25.37a	71.73b	4.60a	43.08c	67.11b

Different letters in each column for each treatment indicate significant difference at $p \leq 0.05$
I₁, I₂ and I₃: Irrigations after 70,120 and 170 mm evaporation from class A pan, respectively
A₁, A₂ and A₃: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively
RWC: Relative water content, MSI: Membrane stability index

Seed aging decreased RWC and MSI of the resultant plants under all irrigation treatments. This decline for the A₃ plants under severe

water limitation was larger than that under other irrigation treatments (Figure 1).

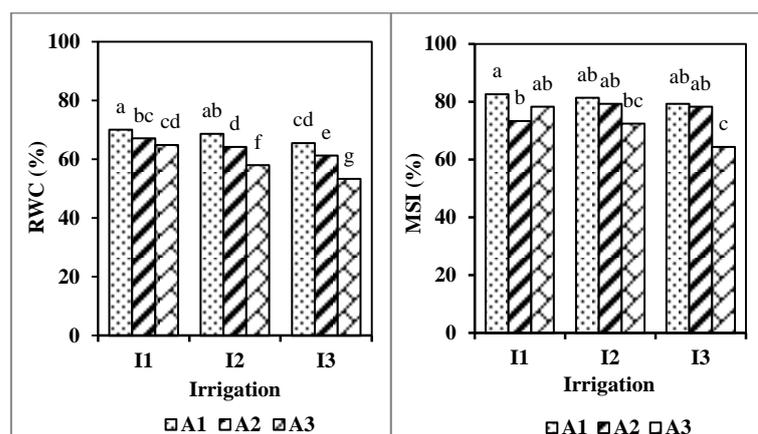


Figure 1. Means of relative water content (RWC) and membrane stability index (MSI) for plants from differentially aged seed lots of lentil under different irrigation treatments

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

I₁, I₂ and I₃: Irrigation after 70, 120 and 170 mm evaporation from class A pan, respectively

A₁, A₂ and A₃: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively

Ground cover was reduced with increasing seed aging; the highest reduction was recorded for plants from the most aged small seeds (Figure 2a). Plants from large seeds were superior in ground cover and grain yield per unit area under limited irrigation conditions (I₂ and I₃). However, these traits for the plants from bulk and large seeds under well watering were statistically similar. Plants from small seeds produced the lowest ground cover and grain yield under all irrigation treatments (Figures 2b, 2c).

Discussion

The decrease in leaf RWC as a result of water stress (Table 2) could be related to low water uptake and more transpiration rate under stress conditions (Lourtie *et al.* 1995). Lower RWC of plants from most aged seeds (A₃) due to water deficit (Figure 1) was the result of delayed seedling emergence and less rooting. However, vigorous plants can tolerate drought stress, because of expanded rooting system and better water uptake (Ghassemi-Golezani *et al.* 2012).

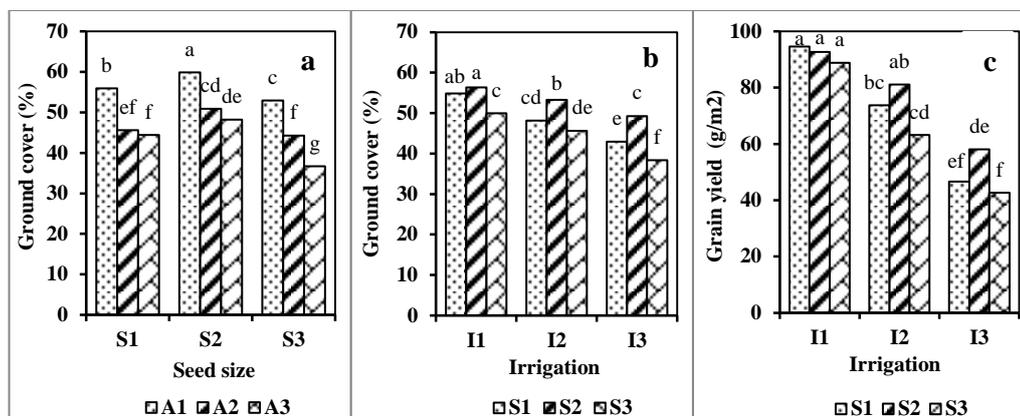


Figure 2. Means of ground green cover for lentil plants affected by seed size × aging (a) and seed size × irrigation treatments (b) and grain yield of plants from different seed sizes grown under different water supply (c)

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

I₁, I₂ and I₃: Irrigations after 70, 120 and 170 mm evaporation from class A pan, respectively

S₁, S₂ and S₃: Bulk, large and small seeds of lentil, respectively

A₁, A₂ and A₃: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively

Increasing leaf temperature due to water stress was associated with decreasing relative water content (Table 2) and consequently reduction in stomatal conductance and transpiration (Siddique *et al.* 2000). During drought, leaves are subjected to both heat and water deficiency stress. Reduction in transpiration rates of leaves can eventually increase temperature (Clarke *et al.* 1993).

Reduction in membrane stability index (MSI) of lentil leaves due to water limitation and seed aging (Figure 1) related to membrane lipids peroxidation, membrane damage and ion leakage. Oxidative reactions are responsible for the deteriorative changes observed in aged seeds, leading to delayed seedling emergence and production of weak plants which are susceptible to biotic and abiotic stresses (Sairam and Saxena 2002).

This may be the reason for lower leaf MSI of plants from aged seeds particularly under severe water stress.

A greater deal of effort has been made to develop plants that can withstand drought or production system that avoid water stress (Norwood 2001; Ghassemi-Golezani *et al.* 2008). Increasing leaf proline content with decreasing water supply (Table 2) means that an efficient mechanism for osmotic regulation, stabilizing sub-cellular structures and cellular adaptation to water stress was provided (Gunes *et al.* 2008). Proline accumulation is, therefore, believed to play adaptive roles in plant stress tolerance (Verbruggen and Hermans 2008).

Optimum stand establishment and early achievement of maximum ground cover are essential for the efficient use of resources like

water and light (Ghassemi-Golezani *et al.* 2010). Reduction in percentage ground green cover due to water stress (Table 2) may be attributed to competition of plants for water and nutrients (Ghassemi-Golezani *et al.* 2010) which can strongly reduce the absorption of incident PAR, either by drought-induced limitation of leaf area expansion or by temporary leaf wilting and early leaf senescence. Since, there is a linear relationship between ground cover and light interception (Burstall and Harris 1983), poor ground cover can potentially reduce photosynthesis and consequently yield of lentil.

Increasing leaf temperature and decreasing relative water content and ground green cover due to both seed aging and water stress led to significant reductions in grain yield per unit area (Table 2). This suggest that cultivation of vigorous seeds may somewhat reduce deleterious effects of water stress. Marcos-Filho and McDonald (1998) stated that the effects of water stress on soybean yield appeared to be related to limited availability of photosynthate and nitrogen for translocation to developing seed. Lower grain yield of plants from aged seed lots (Table 2) is related to slow emergence of seedlings from aged seeds, poor stand establishment

and delayed flowering of plants (Ghassemi-Golezani *et al.* 2010).

High ground cover and grain yield of plants from the most aged large seeds (Figure 2a), particularly under limited irrigations (Figures 2b, 2c), clearly suggest that high reserve seeds can help to improve field performance of aged seeds under stressful conditions. The reserve effect predicts that, after germination, larger seeds retain a greater proportion of their seed reserves, which can then be mobilized for seedling growth, maintenance and repair. Thus, the extra reserves in the large seeds improve the chances that the seedling can establish when environmental conditions are adverse (Green and Juniper 2004). 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 (Bond *et al.* 1999), 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).

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