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# Maternal Light Environment During Seed Development Affects Lettuce Seed Weight, Germinability, and Storability

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***Abstract.*** **Seed germinability and storability are important aspects of seed quality determined by the genotype and environment of seed development. Lettuce (*Lactuca sativa* L.) is produced commercially in most temperate and subtropical areas of the world. The objective of this study was to determine how photoperiod and light quality of the mother plant environment affects lettuce seed quality. Seeds of cv. Tango were produced in growth chambers under one of two treatments: a) short day (SD), consisting of 8 hours of fluorescent light (≈310 $\mu mol \cdot m^{-2} \cdot s^{-1}$) plus 16 hours of darkness daily, and b) long day (LD), consisting of 4 hours of incandescent light (≈21 $\mu mol \cdot m^{-2} \cdot s^{-1}$), 8 hours of fluorescent light, 4 hours of incandescent light, and 8 hours of darkness daily. The red to far-red ratio was ≈6.8 and 1.0 for the fluorescent and incandescent light, respectively. In both treatments, the temperature was 23 °C. The LD treatment produced significantly heavier seeds; however, germination at optimal conditions (20 °C-light) was similar for both treatments. Germinability (percentage and rates) at suboptimal conditions (30 °C, 20 °C with different external ABA concentrations, negative osmotic potentials, or dark) was higher for seeds produced under the LD treatment. On the other hand, seeds produced under the LD treatment presented better storability (evaluated by the accelerated aging test and standard germination after storage at 30 °C and 74% RH). The critical period for light environment effects was also studied. Seed weight patterns were determined early in seed development, during the first 6 days after flowering. Conversely, light environment effects on seed germinability and storability were determined at the end of seed development, after physiological maturity, which occurred by 11 days after flowering. These results show that lettuce seed germinability and storability may be modified by management of light conditions during seed production and provide useful information for seed producers, seed companies, and seed conservation institutions.**

Lettuce (*Lactuca sativa* L.) is one of the most important vegetables in the world. In the United States, between 2001 and 2006, lettuce was cultivated on over 121,000 ha per year with an annual crop value of ≈2 billion dollars, which makes it the most important fresh vegetable in the country (USDA, 2007). Lettuce seed quality is important because it affects seedling emergence and uniformity of growth, which is fundamental for attaining high yield and quality in a single harvest (Smith et al., 1973b; Wien, 1997; Wurr and Fellows, 1985). Thermoinhibition (sensitivity to high temperatures) and photodormancy (lack of germination in dark) are two characteristics frequently found in some lettuce cultivars that present reduced speed and uniformity in seed germination and seedling emergence in the field (Ryder, 1999; Wien, 1997). A common approach to overcome germination problems in lettuce has been to treat the seeds before sowing. For instance, seed priming improves germination and emergence of lettuce seeds under high temperatures (Cantliffe et al., 1981; Valdés et al., 1985). Still, these enhancement treatments represent a cost and additional manipulation of the harvested seeds. A superior approach would be to produce more vigorous or less dormant seeds in the field.

There are several reports about the effects of the maternal environment on different aspects of seed quality, including germinability, dormancy, size, and composition (Baskin and Baskin, 1998; Fenner, 1991, 1992; Gutterman, 2000; Hilhorst and Toorop, 1997). Some of the frequently studied environmental factors are temperature, water availability, light (quality and photoperiod), altitude, and mineral nutrition. In most studies where photoperiod effects on seed production were addressed, seeds produced under shorter days had higher germinability, e.g., *Ononis sicula* Guss. and lettuce (Gutterman, 1973), *Beta vulgaris* L. var. *crassa* Mansf. (Heide et al., 1976), *Portulaca oleracea* L. (Gutterman, 1974), *Amaranthus retroflexus* L. (Kigel et al., 1977), and *Chenopodium album* L. (Karssen, 1970). In fewer cases, shorter days resulted in the production of seeds with lower germinability, e.g., lettuce (Koller, 1962), *Carrichtera annua* L. (Gutterman, 1973), and *Polypogon monspeliensis* L. (Gutterman, 2000). Light quality, specifically the red to far-red (R:FR) ratio, during seed development affected the light requirements for seed germination in *Arabidopsis thaliana* L. (Hayes and Klein, 1974; McCullough and Shropshire, 1970), *Bidens pilosa* L. (Fenner, 1980), *Cucumis sativus* L. and *Cucumis prophetarum* L. (Gutterman and Porath, 1975), and *Piper auritum* Kunth (Orozco-Segovia et al., 1993). Cresswell and Grime (1981) studied light requirements for seed germination of 21 species, and concluded that light quality during seed drying strongly affect light requirements for germination.

In spite of the importance that high-quality seed production has for agriculture in general and horticulture in particular, the mechanisms operating during seed development that control germinability in the mature seed are still poorly understood (Fenner, 1991; Gutterman, 2000; Hilhorst and Toorop, 1997), and the management of particular environmental conditions, such as photoperiod or light quality, for specific improvement of some aspects of seed quality is not a frequent practice in seed production for most species.

Storability or longevity may be defined as the ability of the seed to survive long periods of time until the initiation of germination. In contrast to dormancy, storability represents a desirable seed trait for agronomic, vegetable, and ornamental crops and is commonly included as an attribute of seed quality. Although dormancy and storability often occur coincidently in the same seed, it is not clear if a cause–effect relationship exists between them. This knowledge is important for management of seed stocks by seed companies and producers, preservation of target genotypes in gene banks, and management of natural seed banks of weeds and wild species.

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The main objectives of this study were: a) to determine how photoperiod and light quality of the mother plant environment affects lettuce seed quality, and b) to assess the relationship between lettuce seed germinability and storability.

## Materials and Methods

Two experiments were performed to determine: a) effects of maternal light environment on lettuce seed quality, and b) the critical period during lettuce seed development for light environment effects.

### Expt. 1: Maternal light environment effects on lettuce seed quality

'Tango' lettuce plants were produced in the greenhouse in 1.75-L plastic pots filled with a soilless growing medium (Metromix 360, Scotts, Marysville, OH). Plants were irrigated daily and each pot was fertilized weekly with 50 mL of a solution containing 35 mg N, 15 mg P, and 29 mg K (Peters Professional, Scotts, Marysville, OH). After bolting and before flowering, plants were transferred into growth chambers representing one of two treatments: a) short day (SD), consisting of 8 h fluorescent light plus 16 h of darkness daily, or b) long day (LD), consisting of 4 h incandescent light, 8 h fluorescent light, 4 h of incandescent light, and 8 h of darkness daily. The photosynthetic photon flux (*PPF*), measured by using a portable light meter (LI-189, LI-COR Biosciences, Lincoln, NE), was ≈310 and 21 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the fluorescent and incandescent light, respectively. The R:FR ratio, calculated as the sum of wavelengths between 656 and 664 nm divided by the sum of wavelengths between 726 and 734 nm, was ≈6.8 and 1.0 for the fluorescent and incandescent light, respectively. Light spectral irradiance was measured by using a portable spectroradiometer (LI-1800, LI-COR Biosciences). In both treatments, the temperature was a constant 23 °C. The experiment was repeated three times using plants from different sowing dates. Each replication was considered a block and consisted of 10 plants randomly assigned to each chamber (randomized complete block design). Seeds (achenes) were harvested by manually extracting only fully matured flower heads (dry and open, with visible seeds of ≈8.5% water content on a wet basis) of each plant. Seeds were cleaned and stored in paper envelopes inside a storage room at 4 °C and 25% RH until evaluation. The equilibrium seed water content during storage was 4.7% ± 0.2% (wet basis).

*Seed evaluation.* For seed fresh and dry weight determination, three groups of 100 seeds each were extracted from each replication and weighed before and after drying in an oven at 103 °C for 48 h.

The standard germination (SG) test (ISTA, 1999) was conducted in two groups of 50 seeds for each replication. Seeds were planted over two layers of blotter paper (Anchor Paper Co., St. Paul, MN) saturated in distilled water and placed in square transparent plastic boxes (11 × 11 × 4 cm). These boxes were placed in a germination chamber at 20 °C and constant light. After 4 and 7 d, only normal seedlings were counted as germinated (ISTA, 1999).

Other germination tests were conducted using two groups of 50 seeds per replication, planted over two layers of blotters saturated in 10 mL of distilled water, a solution of (±) abscisic acid (ABA; Sigma-Aldrich, St. Louis, MO) or polyethylene glycol (PEG 8000, Sigma-Aldrich) and placed in 9-cm petri dishes. The PEG concentrations were calculated to obtain water potentials of –0.15, –0.30, –0.45, and –0.60 MPa according to Michel (1983). Germination tests at different ABA and PEG concentrations were performed at 20 °C and constant light, with daily counts of germinated seeds (radicle emergence) to 14 d. Germination at 30 °C and constant light was evaluated daily to 7 d. The germination index (GI) was calculated according with the following equation (adapted from Maguire, 1962):

$$GI = (\text{ratio of germinated seeds day } 1)/1 + \cdots + (\text{ratio of germinated seeds day ``X'')/``X'' + \cdots + (\text{ratio of germinated seeds last count})/\text{days to final count.}$$

Germination in dark was performed using black-painted petri dishes placed on a thermogradient table (Series #16065, Seed Processing Holland B.V., Enkhuizen, Netherlands) at 14, 19, 24, or 29 °C; germination was evaluated 4 d after sowing.

For the accelerated aging (AA) test, lettuce seeds were aged at 41 °C and ≈100% RH for 72 h and then germinated following the SG protocol. Normal seedlings (ISTA, 1999) were evaluated 10 d after planting.

*Seed storage.* Seeds were stored in square plastic boxes (11 × 11 × 4 cm) containing 100 mL of a saturated NaCl solution; the seeds were placed inside aluminum pots over a mesh tray so there was no direct contact between seeds and the salt solution. The boxes, containing the seeds, were placed inside plastic bags and put in a dark chamber at 30 °C. The relative humidity inside the boxes, measured with a data logger (HOBO U12-012, Onset, Bourne, MA), was ≈74% and the seed water content under these storage conditions was 7.2% ± 0.8% (wet basis). Seed samples were extracted after 2, 4, 6, 8, and 10 months of storage, and SG was evaluated.

*Abscisic acid extraction and determinations.* ABA extraction and determination from mature lettuce seeds were performed as described by Roth-Bejerano et al. (1999) with some modifications. Sixty seeds were frozen in liquid nitrogen and stored at –80 °C. After freeze-drying (lyophilization), the seeds were ground to powder in liquid nitrogen and then weighed. Methanol containing 0.5 $g \cdot L^{-1}$ citric acid monohydrate and 100 $mg \cdot L^{-1}$ butylated hydroxytoluene was added at a ratio of 1.0 mL for each 10 mg of dry tissue. The suspension was stirred at 4 °C in dark for at least 20 h and then centrifuged at 1500 g for 10 min. ABA content was determined from this supernatant by using anti-ABA monoclonal specific antibodies and competitive ELISA test according to instructions by Phytodetek® ABA Test Kit (Agdia, Elkhart, IN).

Vigor index and average radicle length measurements were determined on 80 seeds per replication (two groups of 40) using the Seedling Vigor Imaging System (SVIS, Ohio State University, Columbus, OH) according to methodology described by Sako et al. (2001). Before being placed in germination boxes, seeds were imbibed 8 h in light to alleviate photodormancy. Seedlings were scanned 72 h after initiating imbibition.

The effects of brief interruptions with far-red (FR) light on dark germination at 20 °C were investigated on two subsamples of 50 seeds per replication. FR breaks for 4 min at 2, 4, 6, 8, and 24 h after sowing were provided by light-emitting diodes (Quantum Devices, Barneveld, WI) with a wavelength peak at 732 nm, a photon flux of 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a R:FR ratio of 0.01. Seed germination was evaluated 4 d after sowing.

The data were analyzed by the ANOVA procedure. Before the analysis, germination percentages and GI values were transformed to the arcsin of the square root of the fraction value. Correlation coefficients between different parameters of germinability and storability were calculated.

### Expt. 2: Critical period for light environment effects

Four lettuce plants (cv. Tango) with ≈25 flower heads each labeled by flowering day were assigned to one of each of the following six treatments: L (LD throughout seed development), S (SD throughout seed development), L6 (6 d in LD, then SD), S6 (6 d in SD, then LD), L12 (12 d in LD, then SD), and S12 (12 d in SD, then LD). From 3 d after flowering (DAF) and each alternating day, five flower heads from plants in L and S treatments were sampled to determine seed wet and dry weight accumulation. For all treatments, management of the plants was performed as described in Expt. 1. Seed harvest was performed manually by extracting only labeled and fully matured flower heads of each plant. The experiment was repeated two times.

*Seed evaluation and data analysis.* Seed dry weight, germination at 30 °C, germination in 50 μM ABA solution, dark germination at 20 °C, and germination of normal seedlings after AA were evaluated as described for Expt. 1. Differences among treatments were analyzed using ANOVA and significantly different means separated using least significant difference (LSD, $\alpha = 0.05$). Specific groups of treatments were compared by contrast analysis. Statistical analysis of Expts. 1 and 2 were conducted using SAS (SAS Institute, Cary, NC).

## Results and Discussion

Heavier seeds are commonly believed to perform better in most seedling establishment

environments (Fenner, 1992; Wulff, 1995), although exceptions have been reported (Bennett, 2004). In lettuce, seed weight has been positively correlated with seed vigor (Smith et al., 1973a) and seedling growth after emergence (Smith et al., 1973b). In Expt. 1, seed performance was evaluated by a) SG test or the ability to produce normal seedlings under optimal (20 °C-light) conditions, b) germinability (radicle emergence) at different optimal and suboptimal conditions, c) seedling growth and uniformity (vigor index and radicle length from SVIS), and d) the AA test. Seeds produced under LD were significantly heavier than seeds from SD; however, no differences were observed in SG or germination at 20 °C (Table 1). At 20 °C, germination rates, expressed as GI, were slightly ($P = 0.041$) higher for seed from SD (Table 1), which could be due to faster imbibition associated with smaller seed size. Similar associations between smaller seeds and faster germination were reported for *Triticum aestivum* L. (Lafond and Baker, 1986), *Zea mays* L. (Bennett et al., 1988; Muchena and Grogan, 1977; Shieh and McDonald, 1982), *Erodium brachycarpum* Godr. (Stamp, 1990), and *Pastinaca sativa* L. (Hendrix, 1984).

Average seedling radicle length after 3 d of germination has been used for vigor evaluation of lettuce seeds (Smith et al., 1973a), and a positive correlation of this parameter with lettuce field emergence and yield has been observed (Contreras and Barros, 2005; Smith et al., 1973b; Wurr and Fellows, 1985). The SVIS integrates parameters of seedling growth (radicle and hypocotyl length) and uniformity (standard deviation from seedling lengths) to produce a vigor index from 0 (minimum vigor) to 1000 (maximum vigor) (Sako et al., 2001). In our experiments, no differences between seeds from SD and LD treatments were observed for the vigor index and average radicle length (Table 1). Seeds from LD were heavier than seeds from SD, thus production of larger seedlings would be expected. On the other hand, seeds from SD were smaller but germinated faster, and this could explain why at seedling evaluation (3 d after sowing) no differences in seedling growth were observed between treatments.

For both treatments, germination percentage and rate (expressed as GI) were lower at 30 °C; nevertheless, seeds from the SD treatment were less affected and had significantly higher germination percentage than seeds from the LD treatment (Table 1). Thermoinhibition of lettuce seed germination at high temperature (≥25–30 °C) is one of the most important problems affecting lettuce seedling establishment (Wien, 1997), and seeds from cv. Tango are known to be sensitive to high temperatures (H.J. Hill, pers. comm.). Different levels of thermoinhibition during seed germination have been observed among lettuce types and cultivars (Gray, 1975; Kozarewa et al., 2006). In addition to differences in high temperature germination among lettuce types and cultivars, differences among seedlots within cultivars have also been observed (Wurr et al., 1986). Several reports have documented the effect of producing lettuce seed at higher temperatures (e.g., 30/20 °C compared with 20/10 °C, day/night) in reducing seed thermoinhibition (Drew and Brocklehurst, 1990; Gray et al., 1988; Koller, 1962; Kozarewa et al., 2006; Sung et al., 1998); however, the effect of maternal light environment on lettuce seed thermoinhibition has rarely been studied. Koller (1962) compared germination at 20, 23, and 26 °C of lettuce seed produced under 8 or 24 h of light and found that lettuce plants had poor seed production under constant light. But the available data indicate that germinability at any of the three temperatures evaluated was better for seed produced under longer days. Koller's data contradict our results, where SD during lettuce seed production improved seed germination at higher temperatures compared with LD (Table 1). Possible reasons for this may be differences in lettuce cultivars, treatment application, or evaluation methodologies.

Lettuce seed germinability was also assessed by germination in dark at 14, 19, 24, and 29 °C. Germination of seeds from both treatments was affected by the absence of light; however, seed from LD had significantly higher germination percentage than seed from SD at 14 ($P = 0.018$), 19 ($P = 0.019$), and 24 °C ($P = 0.039$) (Fig. 1). Along with thermoinhibition, photodormancy is a common problem affecting lettuce seed emergence and crop establishment (Wien, 1997) and has been extensively studied (Ikuma and Thimann, 1964; McArthur, 1978; Toyomasu et al., 1998; Van der Woude and Toole, 1980). The degree of light sensitivity in lettuce varies among cultivars, and Tango is described as a photosensitive genotype (H.J. Hill, pers. comm.), coincident with our results. Additionally, light requirements for germination of photosensitive lettuce genotypes increase with temperature (Ikuma and Thimann, 1964; Sung et al., 1998; Van der Woude and Toole, 1980), which explains

Table 1. Quality attributes for 'Tango' lettuce seed produced under one of two light treatments.[z]

| Parameter | LD | SD | *P* value[y] |
|---|---|---|---|
| Dry weight (mg/seed) | 0.84 | 0.73 | 0.001 |
| Normal seedlings at 20 °C (%) | 99.7 | 98.7 | 0.423 |
| Germination at 20 °C (%) | 100.0 | 100.0 | — |
| Germination index at 20 °C | 0.98 | 1.00 | 0.041 |
| Germination at 30 °C (%) | 21 | 60 | 0.034 |
| Germination index at 30 °C | 0.05 | 0.35 | 0.066 |
| Normal seedlings after AA[x] (%) | 66 | 5 | 0.025 |
| Vigor index[w] | 785 | 778 | 0.793 |
| Radicle length (pixels/seedling)[w] | 357 | 387 | 0.085 |
| Seed ABA content (pg/mg dry weight) | 84 | 37 | 0.038 |

[z]Short day (SD; 8 h fluorescent light + 16 h darkness daily) or long day (LD; 4 h incandescent light + 8 h fluorescent light + 4 h of incandescent light + 8 h of darkness daily).
[y]Calculated from analysis of variance. In the case of germination percentage and germination index *P* values were calculated with transformed data (arcsin of the square root of the fraction value).
[x]AA, accelerated aging of the seeds at 41 °C and ≈100% RH for 72 h.
[w]Values from SVIS (Seed Vigor Image System).

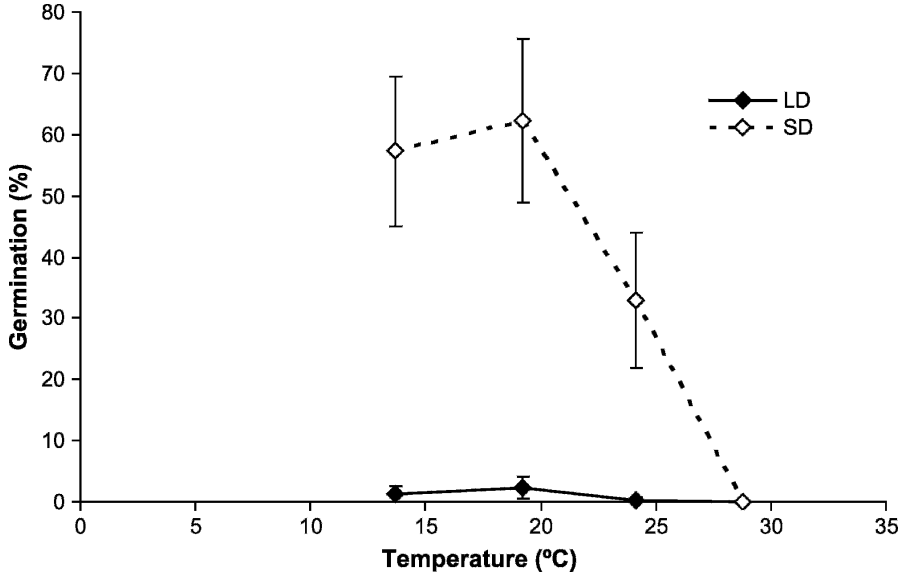


Fig. 1. Germination percentage after 4 d at different temperatures in dark of 'Tango' lettuce seed produced under one of two light treatments: short day (SD; 8 h fluorescent light + 16 h darkness daily) or long day (LD; 4 h incandescent light + 8 h fluorescent light + 4 h of incandescent light + 8 h of darkness daily). Data are means ±SE of three replications.

the lack of germination observed at 29 °C (Fig. 1). Higher germinability in dark of seed produced by lettuce plants under short days was also observed by Gutterman (1973), who reported that lettuce seeds from plants grown under 8 h of light had higher germination than seeds from plants under 16 h of light (germination was evaluated after 48 h at 26 °C in dark with one light break of 5 min of white light 1.5 h after sowing). Improvement of dark germination in seeds produced under shorter days has also been reported in *Chenopodium album* L. (Karssen, 1970) and *Amaranthus retroflexus* L. (Kigel et al., 1977).

Seeds produced under SD had lower sensitivity to increased exogenous ABA (Fig. 2a) and decreased water potentials (Fig. 2b) during germination. Seed dormancy has been positively related with ABA presence or sensitivity of seeds to this phytohormone (Benech-Arnold et al., 1991; Finch-Savage and Leubner-Metzger, 2006; Ni and Bradford, 1993; Yogeesha et al., 2006), and also sensitivity of germination to water potential (Ni and Bradford, 1993). Thus, it may be said that seeds from the LD treatment are more dormant than those from the SD treatment. Additional evidence supporting this concept is the significantly higher ABA content in mature seeds from the LD treatment (Table 1). Variations in seed ABA concentration have been observed among different genotypes of the same species (Goldbach and Michael, 1976; Groot and Karssen, 1992; Steinbach et al., 1995; Yogeesha et al., 2006) and for the same genotype produced at different temperatures (Goldbach and Michael, 1976) or water treatments (Benech-Arnold et al., 1991). However, no report about variation in seed ABA content associated with different maternal light environment was found. When variations in seed ABA accumulation and final content have been observed, higher ABA concentrations usually have been associated with lower germinability and higher dormancy (Ni and Bradford, 1993; Steinbach et al., 1995; Yogeesha et al., 2006). Our results suggest that the higher germinability observed in seeds from SD could be explained, in part, by lower seed ABA sensitivity and content.

The AA test has been used for evaluation of seed storability and vigor (Copeland and McDonald, 2001). 'Tango' lettuce seeds from LD performed better after AA, producing a greater number of normal seedlings than seed from SD conditions (Table 1). These results suggest that seeds from LD, despite their lower germinability, are more vigorous and longer-lived than seeds from SD. This assumption was corroborated by the evaluation of SG after different periods of storage at 30 °C and 74% RH. Seeds from the SD treatment deteriorated faster than seeds from the LD treatment, which, after 4 months of storage, produced an average of 91% normal seedlings compared with 22% from the SD seeds (Fig. 3). Based on these results, the maternal light environment during lettuce seed development affected not only seed

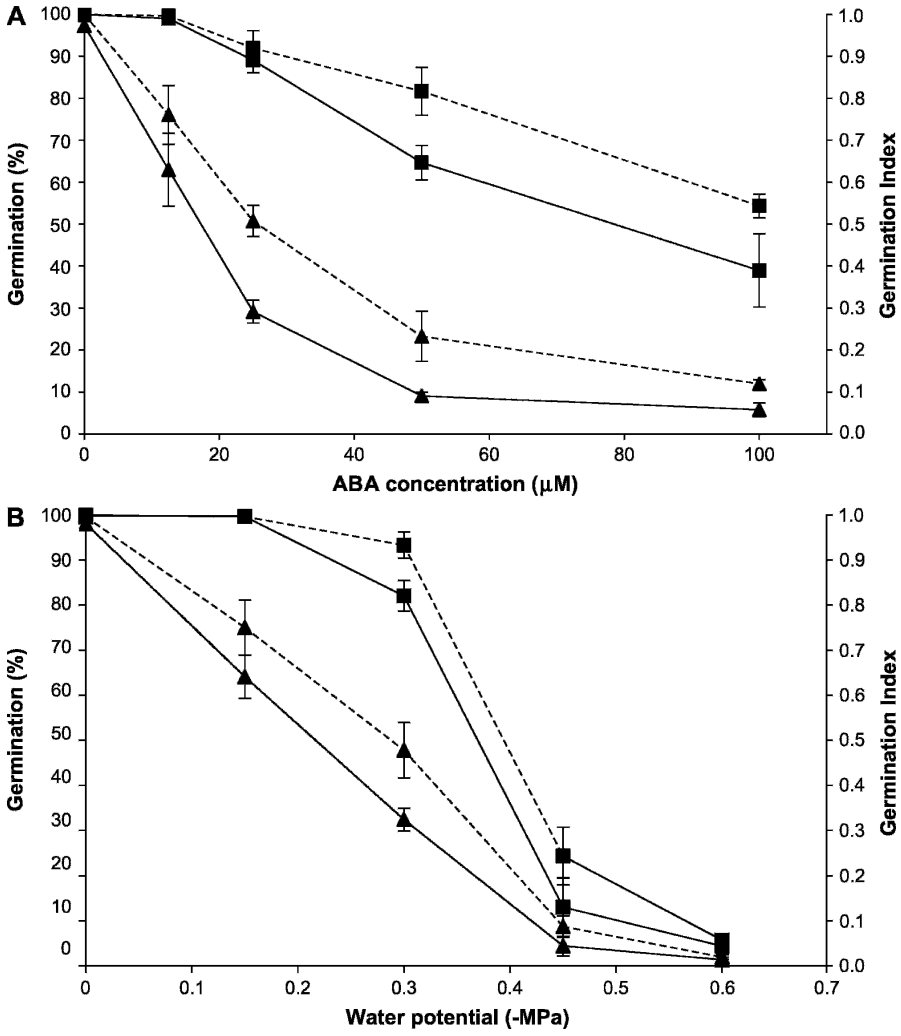


Fig. 2. Germination percentage (square) and germination index (triangle) at different external abscisic acid (ABA) concentrations (**A**) and water potential (**B**) of 'Tango' lettuce seed produced under one of two light treatments: short day (8 h fluorescent light + 16 h darkness daily; broken line) or long day (4 h incandescent light + 8 h fluorescent light + 4 h of incandescent light + 8 h of darkness daily; solid line). Data are means ±SE of three replications.

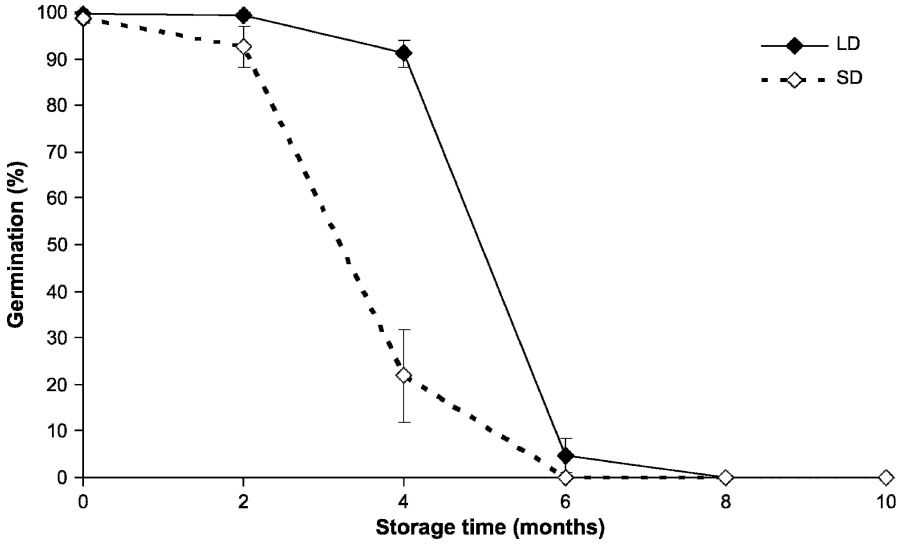


Fig. 3. Lettuce seed germination percentage of normal seedlings after different storage periods at 30 °C and 74% RH of Tango' lettuce seed produced under one of two light treatments: short day (SD; 8 h fluorescent light + 16 h darkness daily) or long day (LD; 4 h incandescent light + 8 h fluorescent light + 4 h of incandescent light + 8 h of darkness daily). Data are means ±SE of three replications.

germinability but also seed storability. A causal relationship between seed storability and some forms of physiological dormancy has been suggested (Hilhorst and Toorop, 1997; Tesnier et al., 2002; Zarbakhsh et al., 1999). However, this hypothesis remains controversial (Fueyo et al., 2003). The results from the AA test had a strong correlation ($r$ = 0.94; $P$ = 0.005) with SG after 4 months of storage (Table 2). The correlation coefficient ($r$ value) also was calculated between the values of lettuce seed performance after 4 months of storage (normal seedlings percentage and GI) and lettuce seed germinability under different conditions; some of the $r$ values and their significance ($P$ values) are presented in Table 2. In general, seed germinability and storability parameters were inversely related (negative $r$ values); however, the only parameter of germinability that had a significant ($P < 0.05$) correlation with the three parameters used for storability was dark germination (Table 2). According to these results, the type of physiological dormancy exhibited by 'Tango' lettuce seeds is significant and inversely related to seed storability. From an ecological perspective, this correlation makes sense because seeds with lower germinability (i.e., higher dormancy) will remain in the soil for longer periods, until optimal conditions permit germination. Thus, the ability to survive is more important for dormant seeds than for seeds with high germinability that likely will germinate shortly after being shed from the mother plant. The significant correlation observed in our study between lettuce seed photodormancy and storability may assist in the management of seed stocks by germplasm centers and seed companies. Careful evaluation of dark germination would permit the identification of seed lots most suitable for storage. Further research should be directed to corroborate the existence of this correlation in other lettuce cultivars, as well as in other species, especially those of the Asteraceae family.

Table 2. Correlation coefficients ($r$ value) between 'Tango' lettuce seed germinability and storability parameters.

| Storability parameter | Germination after AA[y] | Germinability parameter: Dark germination at 19 °C | Germinability parameter: Germination in ABA, 50 µM | Germinability parameter: Germination at 30 °C |
|---|---|---|---|---|
| Normal seedlings after 4 mo. of storage[z] | 0.940 (0.005)[x] | –0.983 (<0.001) | –0.586 (0.221) | –0.687 (0.132) |
| Germination index after 4 mo. of storage | 0.821 (0.045) | –0.865 (0.026) | –0.262 (0.616) | –0.401 (0.431) |
| Germination after AA | — | –0.984 (<0.001) | –0.375 (0.463) | –0.469 (0.348) |

[z]Storage at 30 °C and 74% RH.
[y]AA = accelerated aging: 72 h at 41 °C and ≈100% RH.
[x]$P$ value for the correlation.

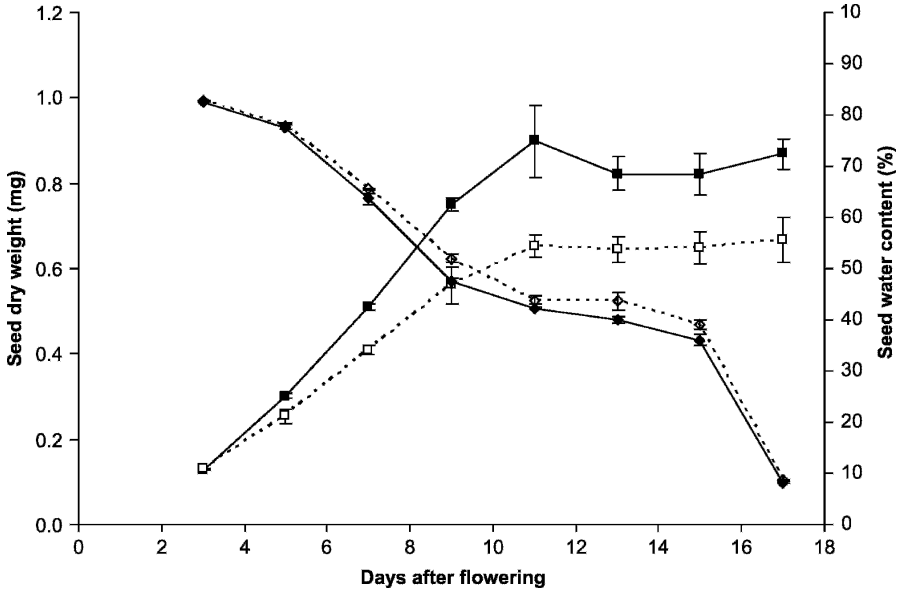


Fig. 4. Dry weight accumulation (squares) and seed water content (diamonds) during development of Tango' lettuce seed produced under one of two light treatments: short day (8 h fluorescent light + 16 h darkness daily; broken line) or long day (4 h incandescent light + 8 h fluorescent light + 4 h of incandescent light + 8 h of darkness daily; solid line). Data are means ± SE of two replications.

Although LD and SD treatments differed in the amount of dry matter accumulated by lettuce seeds, the pattern of development was similar (Fig. 4). Seed physiological maturity (PM, defined as the moment of maximum seed dry weight accumulation), determined by an iterative regression analysis procedure (Pieta-Filho and Ellis, 1991), occurred 10.4 ± 0.4 and 10.7 ± 0.1 DAF for LD and SD plants, respectively (Fig. 4). Consequently, 6 DAF (time when the plants from L6 and S6 treatments where moved from one light treatment to another) represented about half of PM, whereas 12 DAF (time of plant movement for the L12 and S12 treatments) represented about 1 d after PM.

Individual seed dry weights increased in seeds from S6 and S12 in relation to S; however, the greatest values were for seeds from the L, L6, and L12 treatments (Fig. 5a). When the average seed weight of L, L6 and L12 was compared with the average value of S, S6, and S12 by a contrast analysis, a significant difference ($P < 0.001$) was observed, indicating that the effect of the light treatment on individual seed dry weight was produced early in seed development, during the first 6 DAF or first half of the PM process (Figs. 4 and 5a). It was previously observed that longer days affected flowering and the number of seeds produced by lettuce plants (Koller, 1962). Additionally, the number of seeds produced per lettuce plant has an inverse relationship with individual seed weight (Contreras, 2007; Izzeldin et al., 1980), because of the higher competition for resources among seeds during seed filling. Thus, it may be that the difference in dry weight between 'Tango' seeds from SD and LD treatments was caused by the production of more flower heads and seeds in plants under SD. However, this hypothesis cannot be supported or rejected by our results because the number of flower heads or seeds per plant were not evaluated.

In Expt. 2, seed germinability was assessed as dark germination at 20 °C (Fig. 5b), germination in light at 30 °C (data not shown), and germination with light in 50 µM ABA solution (data not shown). When the average dark germination at 20 °C of seed from S, L6, and L12 was compared with the average from L, S6, and S12 by contrast analysis, the difference was significant ($P < 0.001$). Differences were also detected when similar contrast analyses were performed for germination percentage at 30 °C ($P$ = 0.013), GI at 30 °C ($P$ = 0.002), and GI in 50 µM ABA solution ($P$ = 0.005). Based on these results, the effect of the day-length treatments on lettuce seed germinability occurred in the last portion of seed development, after PM. Similar to the results observed for germinability, maternal light environment effect on seed storability (assessed by the AA test) was produced during the last part of seed development, after PM. The percentage of normal lettuce seedlings after AA of seeds from L, S6, and S12 differed ($P$ = 0.002) when compared with those of seeds from S, L6, and L12 treatments (Fig. 5c). Maximum seed quality during seed development is believed to coincide with PM, or the moment of maximum seed dry weight accumulation, after which viability and vigor would decrease (Abdul-Baki and Anderson, 1972; Harrington, 1972). However, there is evidence that in some species seed quality may increase after PM (Demir and Ellis, 1992a, 1992b; Sinniah et al., 1998; Welbaum, 1999).

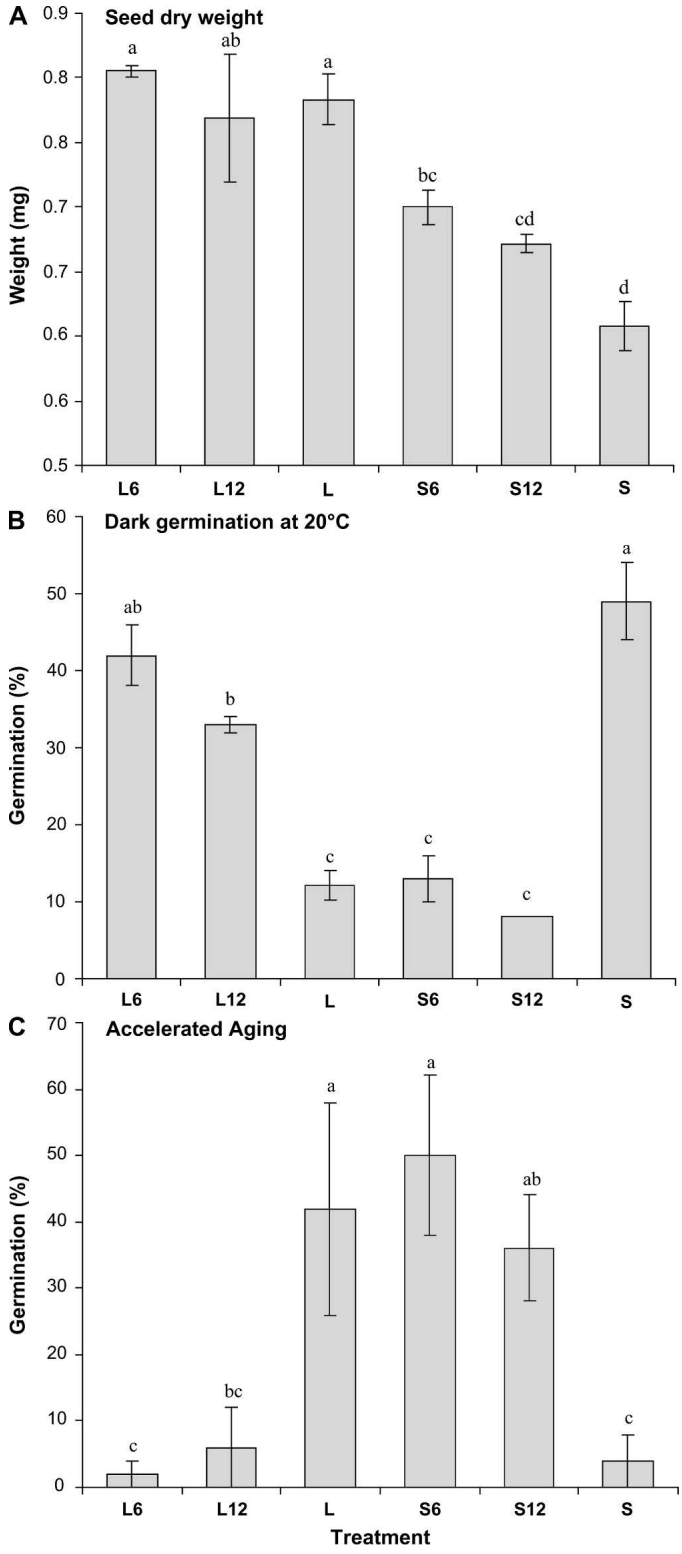


Fig. 5. Seed dry weight (**A**), dark germination at 20 °C (**B**), and normal seedling after accelerated aging (**C**) from 'Tango' lettuce seed produced under different combinations of long day (LD; 4 h incandescent light + 8 h fluorescent light + 4 h of incandescent light + 8 h of darkness daily) and short day (SD; 8 h fluorescent light + 16 h darkness daily) light conditions: L (LD throughout seed development), S (SD throughout seed development), L6 (6 d in LD, then SD), S6 (6 d in SD, then LD), L12 (12 d in LD, then SD), and S12 (12 d in SD, then LD). Data are means ± SE. In the same graphic, treatments with different letters are significantly different (LSD, $\alpha = 0.05$).

Based on our results, 'Tango' lettuce seed germinability and storability may increase or decrease after PM depending on the maternal light environment (Fig. 5). This phase of seed development is known as maturation drying (Bewley and Black, 1994) and is characterized by fresh weight loss and a decline in seed water content. During this period, there is no further accumulation of dry matter in the seed, but seed water content is sufficient to allow metabolic activity. In our experiments, lettuce seeds were ≈40% water content (wet basis) at PM, and above 35% for at least 4 d following PM; 6 d after PM, seeds were ≈8.5% water content, which was maintained until harvest (Fig. 4).

The light requirement for lettuce seed germination is mediated by the action of phytochrome, a soluble protein found in two interconvertible forms: *Pr* (red light absorbing, biologically inactive) and *Pfr* (far-red light absorbing, biologically active) (Shinomura, 1997). According to Vertucci et al. (1987), phytochrome photoconversion occurs when seed water contents are over 8% in lettuce, so conversion is possible in the seed during desiccation and until harvest. *Pfr* (or some stable intermediate able to yield *Pfr* in dark) may persist in the seed after final maturation and dehydration (Taylorson, 1982). The amount of this *pre-existent Pfr* will depend on the light quality and intensity to which seeds were exposed at the end of seed development and dehydration (Taylorson, 1982). In our experiments, the SD treatment consisted only of fluorescent light, which is relatively rich in red light (R:FR ≈ 6.8). Conversely, incandescent light, which was used to extend day-length in the LD treatment, is relatively rich in far-red light (R:FR ≈ 1.0). These differences in light quality favor higher accumulation of *pre-existent Pfr* in seeds from plants under SD compared with seeds from LD treatment, which would explain the higher dark germination of seeds from SD treatment and why the effect was produced at the end of seed development. The suppression of dark germination of seeds from SD treatments by breaks of FR light (Table 3) supports the idea that seeds from SD had a higher content of *pre-existent Pfr* than seeds from LD treatments. Thus, light quality, and not hours of light, would be the critical factor explaining differences in 'Tango' lettuce seed germinability and storability for seeds produced under SD vs. LD treatments. This hypothesis is supported by results from other authors that have reported reductions in photodormancy caused by seed development under environments with higher R:FR ratios (Cresswell and Grime,1981; Hayes and Klein, 1974; McCullough and Shropshire, 1970).

There is evidence that *Pfr* would promote seed germination by promoting gibberellin biosynthesis and suppressing ABA formation (Roth-Bejerano et al., 1999; Toyomasu et al., 1998). Additional research should be conducted to determine if differences in ABA concentration of mature lettuces seeds developed under different light environments is mediated by *Pfr* action. Possible cause–effect relationships between *pre-existent Pfr* and lettuce seed germinability or storability should be also studied.

In conclusion, the light treatments applied in these experiments affected the weight, germinability and storability of lettuce seeds cv. Tango. Effects on seed weight were

Table 3. Germination percentage after 4 d at 20 °C in continuous dark and dark plus far-red light breaks (FR; 4 min. at 2, 4, 6, 8, and 24 h after sowing) for 'Tango' lettuce seed produced under one of two light treatments.[z]

| | Day-length treatment | | |
|---|---|---|---|
| Germination condition | LD | SD | *P* value[y] |
| Dark | 2.3 ± 1.5 | 69.3 ± 12.4 | 0.010 |
| Dark + FR | 1.0 ± 0.6 | 10.3 ± 7.33 | 0.147 |

[z]Short day (SD; 8 h fluorescent light + 16 h darkness daily) or long day (LD; 4 h incandescent light + 8 h fluorescent light + 4 h of incandescent light + 8 h of darkness daily).
[y]Calculated from analysis of variance with transformed data (arcsin of the square root of the fraction value).

determined during the first 6 d of seed development (first half of the time needed to reach PM), while maternal light environment effects on seed germinability and storability occurred after PM, during the phase of seed maturation and drying. Lettuce seed storability had an inverse and significant correlation with dark germination. These results are of practical interest for seed producers, and contribute to our knowledge about the factors affecting quality during seed production and the relationship between different aspects of seed quality. Based on the results of these experiments, we hypothesize that light quality (R:FR ratio) during lettuce seed production plays a critical role in seed germinability and storability.

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