Red and Far-red Light Treatments to Modify Thermoinhibition, Photoblasticity, and Longevity in Lettuce Seeds

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Additional Index Words. *Lactuca sativa*, light-emitting diode, photosensitivity, phytochrome, seed water content, storability

Summary. Germination in lettuce (*Lactuca sativa*) seeds can be inhibited by high temperatures (thermoinhibition) or darkness (positive photoblasticity). Priming is commonly used as a seed treatment to avoid these problems. However, this treatment is complicated, expensive and has detrimental effect on seed longevity. The objectives of this study were to 1) develop a simple alternative treatment to priming, based on red light irradiations and 2) to develop a treatment to extend seed longevity. Lettuce seeds from cultivars Tango, Milanesa, Ideal Cos, and Gallega de Invierno were partially hydrated in containers with 98% relative humidity (RH) and irradiated with red (R) light for 24 hours, or far-red (FR) light for 216 hours. Throughout the treatment, seeds reached 16% water content and, once finished, they were dried with air at 30 °C. Untreated (control) and primed seeds were also evaluated. Germination was evaluated in light and darkness at temperatures between 12 and 36 °C in a thermogradiant table. Effects in longevity were estimated by evaluating germination after various aging periods at 45 °C and 75% RH. The R-light treatment improved germination in the dark at temperatures up to 25 °C, but did not have a significant effect on seed germination under light. Germination after accelerated aging showed that R- and FR-light treatments extended seed longevity when compared with control seeds. Therefore, this effect would not be associated to photoblasticity alleviation or imposition. Primed seeds deteriorated faster than the control. Compared with priming, the R-light treatment was simpler and improved seed longevity. However, priming effects on alleviation of seed photoblasticity and thermoinhibition at temperatures over 25 °C were greater.

Lettuce, one of the most important vegetable crops in the world, is established through direct sowing or the transplant of seedlings; in both cases, high seed quality is essential to achieve a successful crop as low or slow germination results in poor and irregular plant populations (Smith et al., 1973). However, seeds of many lettuce cultivars present thermoinhibition (reduction of germination at temperatures over 25 °C) and/or positive photoblasticity (requirement of light for germination); two types of physiological dormancy that may delay and reduce field emergence, especially when sown in warm environments (Nascimento, 2003; Wien, 1997). Seed priming, a treatment of controlled seed hydration and drying, has proved to reduce lettuce seed photoblasticity and thermoinhibition, improving speed and uniformity of emergence (Sung et al., 2008; Valdes and Bradford, 1987). However, an undesired consequence of priming is reduced seed longevity (Chojnowski et al., 1997; Powell et al., 2000; Tarquis and Bradford, 1992). In addition, protocols for the treatment usually are specific to each genotype and lot, and the application of a wrong protocol may cause a reduction in seed quality (Capron et al., 2000; Coolbear et al., 1992). As a result, priming treatments are usually expensive, need to be carried out by specialized companies, and are not available for farmers of many countries. Consequently, the development of a simpler treatment, able to improve seed germination under unfavorable conditions without affecting seed longevity, is highly desirable.

Given that germination of photoblastic lettuce seeds is under light control, this stimulus may be managed to promote or retard germination. Red light promotes germination in positive photoblastic lettuce seeds, while FR may inhibit germination and induce secondary dormancy in lots that did not have positive photoblasticity (Contreras et al., 2008; Górska et al., 2013; Kendrick and Russell, 1975). Seeds sense light through phytochromes, proteins that may be found in two interconvertible forms: the R-light absorbing form (Pr) and the FR-light absorbing form (Pfr); Pr converts to Pfr when absorbs R-light, while Pfr converts to Pr after absorption of FR-light. Pfr is the biologically active form and a high Pfr to Pr ratio in positive photoblastic seeds will trigger germination (Kendrick and Russell, 1975; Seo et al., 2009). Different studies suggest that seeds matured in environments with higher R to FR ratio (R:FR), such as in direct sun light, will have a higher fraction of Pfr when dry, thus germinating better in darkness than seeds matured in environments with lower R:FR, such as under canopy of neighboring plants (Contreras et al., 2009; Cresswell and Grime, 1981; Hayes and Klein, 1974). Contreras et al. (2008, 2009) compared seeds produced in environments with contrasting light quality and observed that lettuce seeds developed in the environment with the higher R:FR ratio showed lower photoblasticity, thermoinhibition, and longevity. These authors observed that the effect of light environment in seed dormancy and longevity was produced at the last part of seed development, during the phase of maturation and drying, when seed decreases its water content from 40% to 8%.

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In addition to reports in lettuce by Contreras et al. (2008, 2009), a relationship between seed photoblasticity and longevity has been observed in several other species. Gorski and Rybicki (1985) reported that treating seeds of loose silky bentgrass (Agrostis spica-venti), redroot pigweed (Amaranthus retroflexus), and prickly lettuce (Lactuca serriola) with FR light induced dormancy and prolonged longevity of soil-buried seeds. In a similar experiment, Doroszewski (1997) observed improvement of longevity in FR-treated seeds in five of six species studied. More recently, Gorski et al. (2013) reported that lettuce seeds of cultivar Cud Vorburgu treated with FR-light acquired secondary dormancy and had a significantly extended longevity when compared with untreated seeds, both stored at room temperature in dark. Despite these observations, it remains unclear if there is a cause–effect relationship between seed photoblasticity and longevity. A treatment to increase seed longevity would be useful to gene banks, seed companies, and seed users in general, so is a matter that deserve been studied.

Because the photoconversion reaction in lettuce seeds takes place with a minimum seed water content close to 8% (Vertucci et al., 1987), we hypothesize that treatment of these seeds with R-light should decrease in photoblasticity and improve germination. Accordingly, the first objective of this study was to develop an alternative treatment to priming based on irradiation with R-light of seeds with controlled water contents. Since this can be achieved at lower seed water contents, this treatment should be simpler, less risky, and with a lower impact in seed longevity than priming. Additionally, a cause effect relation has been suggested between seed photoblasticity and longevity; therefore, we hypothesize that treating seeds with FR-light should be effective to induce photoblasticity and improve longevity, and the second objective was to develop a treatment to increase seed longevity based on irradiation with FR-light.

**Materials and methods**

**Plant material**

Four lettuce cultivars were used in this study: Tango cutting lettuce (Lactuca sativa var. acephala), Ideal Cos cos lettuce (L. sativa var. longifolia), and Gallega de Invierno and Milanesa butterhead lettuce (L. sativa var. capitata). Seed was obtained by self-production and used within 1 year from harvest. The amount of ‘Milanesa’ seed was restricted, so this cultivar was not included in some of the evaluations.

**EXPT. 1. Protocol determination for light treatments.** Preliminary tests were conducted to choose the best light treatment for improvement of lettuce seed quality, with RH and treatment durations selected from the best alternatives evaluated in prior experiments (Pimentel, 2013). The general protocol consisted of placing seeds inside a transparent plastic container (330 mL) with 98% RH, under a known source of light for a determined time (Fig. 1). Inside each container, a saturated potassium sulfate (K$_2$SO$_4$) solution was used to reach equilibrium of 98% RH (Winston and Bates, 1960). Seeds were placed on a grid that prevented their contact with the salt solution (Fig. 1). Following the light exposure, seeds were taken to a 30 °C air dryer for 90 min and then stored in hermetic plastic bags at 20 °C until evaluation.

Light-emitting diodes (LED) were used for the R-light seed conditioning, with a R:FR of 140 and 6 μmol·m$^{-2}$·s$^{-1}$ photosynthetically active radiation (PAR). The PAR and R:FR quantifications were determined using a portable light meter (LI-250A; LI-COR Biosciences, Lincoln, NE) and a light meter (SpectroSence 2; Skye Instruments, Llandrindod Wells, UK) with a 660/730 sensor (SKR 110, Skye Instruments), respectively. R-light treatments were carried out in a 20 °C room over a fabric covered shelf to avoid the possible interference of other light sources.

To determine the appropriate treatment duration, ‘Tango’ seeds were exposed for 24 and 144 h to R light at 98% RH; physiological seed germination was evaluated in dark at different temperatures.

To evaluate if other light source and temperature led to the same results as the R light at 20 °C treatment, irradiations with R and fluorescent light at different temperatures were performed in ‘Tango’, ‘Ideal Cos’, and ‘Gallega de Invierno’. For fluorescent-light treatments, fluorescent tubes with 2.8 R:FR and 13 μmol·m$^{-2}$·s$^{-1}$ PAR were used. Seeds were treated for 24 h and 98% RH in a 20 °C growth chamber or at room temperature (28 °C average, with 25 and 31 °C, minimum and maximum, respectively). Two replications of 200 seeds each were used per cultivar. Physiological seed germination was evaluated in dark at 25 °C.

**EXPT. 2. R-light treatment as alternative to improve germination.** Seeds were treated with R light for 24 h at 20 °C and 98% RH. Three replications of 400 seeds of ‘Milanesa’ and four replications of 700 seeds of ‘Tango’, ‘Ideal Cos’, and ‘Gallega de Invierno’ were used. The experimental unit corresponded to single containers, which were arranged in a completely randomized design.

**Priming**

Protocol for priming was adapted from Schwember and Bradford (2005). Seeds were treated in 9-cm-diameter petri dishes with three filter papers (90 mm, 87 g·m$^{-2}$; Munktell Filter, Falun, Sweden) with 10 mL of a polyethylene glycol [PEG (PEG 8000, Sigma-Aldrich, St. Louis, MO)] solution at $\gamma_s$ of ~1.25 MPa and then sealed with laboratory film (parafilm M; Bemis Flexible Packaging, Neenah, WI). The PEG concentration was calculated according to the formula proposed by Michel (1983). Dishes with seeds were taken to a 20 °C room with fluorescent light for 48 h. Then, seeds were rinsed with distilled water and dried for 90 min in a 30 °C air dryer. Each dish represented an experimental unit and there were four replications arranged in a completely randomized design.

Physiological germination of control (untreated) and treated seeds was evaluated at a range of temperatures (12 to 36 °C) in light or darkness.

**EXPT. 3. FR-light treatment to improve seed longevity.** Seeds were exposed to FR light during 216 h at 20 °C and 98% RH. Source of FR light corresponded to a LED lighting system (SNAP-LITE; Quantum Devices, Barneveld, WI) with 0.039 R:FR and 0.1 μmol·m$^{-2}$·s$^{-1}$ PAR. The containers were kept in a fabric covered box to avoid the entrance of light with different wavelength. The light source was at the top of the box. Three replications were used for ‘Milanesa’ and four for ‘Tango’,
Germinated seeds day 1)/1+

from Maguire, 1962): GI = (ratio of germinated seeds day 1)/1+

of germinated seeds day X)/X...+(ratio of germinated seeds day 7)/7.

SEED LONGEVITY. Salt-saturated-accelerated aging (SSAA) was carried out to estimate seed longevity. Seeds were placed in hermetically closed 330-mL square plastic containers with saturated sodium chloride (NaCl) solution (75% RH) at 45 °C in a water-jacketed incubator (model 3015, Sheldon Manufacturing, Cornelius, OR). Inside the container, 100 to 250 seeds per replication were placed over an aluminum plate placed on a grid, so contact between seeds and salt solution was avoided. After aging, standard germination was evaluated at 20 °C and light in 25 to 50 seeds per replication; normal seedlings were evaluated 7 d after planting according to ISTA rules (ISTA, 2011).

DATA ANALYSIS. Seed evaluation results were analyzed with an analysis of variance. When significant differences among treatments (P < 0.05) existed, differences were analyzed through a least significant difference test (α = 0.05). Before analysis, germination percentages and index were transformed to arcsine of the square root of the proportion of the number.

Results

Expt. 1. Moisture content reached by ‘Tango’ seeds after 18 h in a closed container at 20 °C and 98% RH was ≈16%. Figure 2 shows that ‘Tango’ seeds treated at 20 °C for 24 h with R light reached higher dark germination percentages than untreated seeds or those treated for 144 h, so subsequent treatment of the seeds were done for 24 h.

Seed treated with fluorescent light and/or at room temperature also presented a significant germination improvement at 25 °C and dark (Table 1). However, the magnitude of the effect depended on the cultivar and was lower than that of the treatment using the combination of R light and 20 °C.

Expt. 2. When evaluated under light at temperatures between 12 and 36 °C, R-light-treated seeds did not improve their germination compared with untreated seeds (Fig. 3). Seeds from all evaluated cultivars reached about 100% germination at temperatures between 12 and 30 °C; Figure 3 presents results for ‘Tango’ and ‘Milanesa’, similar tendencies were observed for ‘Ideal Cos’ and ‘Gallega de Invierno’.

When germination was evaluated on the thermogradient table in darkness, different degrees of positive photoblasticity were observed (Fig. 4). Untreated seeds of ‘Tango’ did not exceed 20% germination at their optimum temperature (≈21 °C) and were the most sensitive cultivar to the lack of light (Fig. 4A). Untreated seeds of ‘Milanesa’ and ‘Gallega de Invierno’ germinated over 80% at 21 °C, but decreased significantly at lower or higher temperatures (Fig. 4B and D). ‘Ideal Cos’ seeds showed the lower photoblasticity, they reached about 100% germination from 12 to 21 °C, but germination decreased thereafter (Fig. 4C). R-light treatments had a positive effect in photoblasticity alleviation, which was more marked in ‘Tango’ seeds (Fig. 4A), and less in ‘Ideal Cos’ (Fig. 4C). However, the effect of priming in alleviation of photoblasticity was greater than that of R-light treatment in the four evaluated cultivars (Fig. 4).

Expt. 3. After 5 and 7 d of SSAA, untreated and light (R or FR) treated seeds performed better than the primed seeds, producing a significantly higher percentage of normal seedlings (Table 2). In ‘Tango’, light treated seeds produced higher percentages of normal seedlings than untreated seeds; however, these differences were not significant. ‘Ideal Cos’ seeds that received light treatments had a significantly better performance than the control ones after 5 and 7 d of SSAA, while in ‘Gallega de Invierno’, this difference was evidenced after 7 d of SSAA (Table 2).

Figure 5 shows the decrease of physiological germination percentages in treated and untreated seeds of ‘Tango’, ‘Gallega de Invierno’, and
‘Ideal Cos’ after different periods of SSAA. Once again, primed seeds were most affected by the SSAA, followed by untreated seeds. In this evaluation, a “dark treatment” was included, consisting of treating the seeds at 98% RH for 24 h (similar to R-light treatment), but without light. In the three cultivars evaluated, a consistent improvement of longevity was observed in R-light-, FR-light-, and dark-treated seeds. Only in ‘Tango’ seeds, the FR-light treatment was better than the R-light and dark treatment (Fig. 5A).

**Discussion**

Thermoinhibition and positive photoblasticity are problems commonly present in many lettuce cultivars (Cantliffe et al., 2000; Wien, 1997). When germinated in light, the four cultivars used in this study were able to reach about 100% germination at temperatures until 30 °C. However, different levels of positive photoblasticity were observed in the dark (Fig. 4). The proposed R-light treatment did not have an effect when seeds germinated in light (Fig. 3), but a significant improvement of germination in dark was observed (Fig. 4). The magnitude of the R-light-treatment benefit depended on the germination temperature and cultivar, being greater in Tango, the cultivar with the highest level of photoblasticity, and lower in Ideal Cos, genotype with the lowest photoblasticity. When compared with priming, R-light-treatment benefit was similar at germinations in dark between 16 and 25 °C (29 °C in the case of ‘Ideal Cos’); however, at higher temperatures priming benefit on seed germination surpassed that of R-light treatment. One of the main benefits of priming is increasing germination speed and uniformity, in addition to overcome thermoinhibition in lettuce seeds (Heydecker et al., 1973; Hill, 1999; Valdes et al., 1985); none of these benefits were observed in R-light-treated seeds when germination was evaluated in light, so benefits of R-light treatment would be restricted to photoblasticity alleviation.

Phytochrome photoactivation (i.e., conversion from Pr to Pfr) is progressively induced by R-light as seed moisture content increases from 8% to 18% (Hsiao and Vidaver, 1971; Vertucci et al., 1987). Therefore, 16% water content reached by lettuce seeds during the R-light treatment at 98% RH would be enough to permit this photoactivation, and a higher proportion of Pfr in R-light treated seeds...
would explain its improvement of germination in the dark. However, effects of the R-light treatment were limited in comparison with priming, which would be explained by the different water content reached by seeds during each treatment. During priming, seeds reach about a 40% water content, allowing the occurrence of metabolic processes such as DNA and mitochondrial repair, and transcription and translation of new proteins (Bewley et al., 2013), along with changes in the expression of key genes affecting synthesis of abscisic acid, gibberellins, and ethylene (Schwember and Bradford, 2010), thus favoring germination and alleviation of dormancy. Because of the lower seed water content reached during R-light treatment (16%), these events would not occur, which may explain why the treatment benefits did not increase when its duration was extended from 24 to 144 h and the difference of benefits compared with priming. R-light treatment effect would be restricted to conversion of \textit{Pr} to \textit{Pfr}.

Advantages that R-light treatments would have over priming are a simpler and less specific protocol, along with a lower or null effect on seed longevity. When light source and temperature were changed in the protocol of the R-light treatment, significant alleviation of photoblasticity at 25 °C still occurred, but magnitude of the benefit depended on the cultivar (Table 1). Results show that temperature control around 20 °C and a R-light source produce better results, although changes in these conditions still improve seed performance.

The effect of seed treatments on seed longevity was estimated by evaluation of germination after different periods of SSAA (Table 2, Fig. 5). As expected, priming showed to increase significantly deterioration rates of treated seeds (Tarquis and Bradford, 1992). R-light-treated seeds presented lower deterioration rates than the control, indicating an improvement in seed longevity. Contreras et al. (2008, 2009) found that seeds that completed their development in an environment with a lower R:FR had greater longevity and photoblasticity than seeds maturing at environments with higher R:FR. The authors speculated a possible cause–effect relation between seed photoblasticity and longevity. Additionally, Górski et al. (2013) reported that lettuce seeds imbibed by 6 d under natural-FR light [light filtered through dense canopy of rhubarb (\textit{Rheum rhabarbarum}) leaves] acquired secondary dormancy (positive photoblasticity) and presented extended longevity compared with control (nondormant) seeds. On the basis of those results, we hypothesized that irradiation of partially hydrated seeds with FR light would improve its longevity, thus a FR-light treatment was also evaluated. In this case, possible effects should not be limited to \textit{Pfr} conversion, but related to other metabolic events occurring in the seeds, so duration of treatment was extended. However, results showed that the effect of the FR-light treatment, although improved seed longevity, was not different with effect of the R-light treatment (Table 2). Therefore, a treatment with no light was incorporated in a new experiment to compare loss of physiological germination capacity after

![Fig. 3. Germination percentage (A and C) and germination index (B and D) at different temperatures in light of ‘Tango’ (A and B) and ‘Milanesa’ (C and D) lettuce seeds treated with red light during 24 h at 20 °C and 98% relative humidity (solid line) and untreated (dotted line). Data are means ± SE from four replicates of 20 (‘Milanesa’) or 25 (‘Tango’) seeds each; (1.8 × °C) + 32 = °F.](image-url)
different periods of accelerated aging (Fig. 5). Results of this experiment confirm the positive effect of R- and FR-light treatments on seed longevity. However, in general, the benefit did not differ with that of the dark treatment, indicating that the effect on longevity of this group of treatments would not be related with light, but with the controlled hydration and subsequent drying of the seed. The importance of seed drying on different aspects of seed quality, including longevity, has been reported (Hay and Probert, 1995; Navratil and Burris, 1984; Schwember and Bradford, 2005). In the case of lettuce, seed drying occurs in the field under uncontrolled conditions. Results of this study suggest that a partial hydration of seeds up to a 16% water content and drying at controlled conditions has an effect in extending seed longevity. Physiological mechanisms explaining this effect deserve future investigation.

In conclusion, the proposed treatment of irradiating partially hydrated seeds with R light has a positive effect on alleviation of photoblasticity in lettuce. Compared with priming, this is a simpler treatment that also improves seed longevity; however, priming effects on alleviation of seed photoblasticity and thermoinhibition at temperatures over 25 °C are greater.
Benefit of the R-light treatment in seed longevity was similar to the effect of similar treatments using FR light or no-light (in darkness), so this effect would not be associated to photoblasticity alleviation or imposition, but to the controlled hydration and drying of the seeds.

**Literature cited**


Fig. 5. Seed physiological germination at 20 °C (68.0 °F) and light of three lettuce cultivars after different periods of salt-saturated accelerated aging at 45 °C (113.0 °F) and 75% relative humidity (RH). Control = untreated seeds, R Light = seeds treated under red light at 98% RH for 24 h, FR Light = seeds treated under far-red light at 98% RH for 216 h, Dark = seeds treated at 98% RH in darkness for 24 h, Priming = seeds treated in -1.25 MPa (12.5 bar) solution with fluorescent light for 48 h. All treatments were performed at 20 °C. After each treatment, seeds were dried at 30 °C (86.0 °F) for 90 min. Data are means ± SE from four replicates of 25 seeds each.


Pimentel, I. 2013. Red and far-red light treatments to modify thermoinhibition, photoblasticity and longevity in lettuce seeds. Pontificia Universidad Católica de Chile, Santiago, Chile, MS Thesis.


