EFFECTS OF MATERNAL PLANT ENVIRONMENT ON LETTUCE (*Lactuca sativa* L.) SEED DORMANCY, GERMINABILITY, AND STORABILITY

DISSERTATION

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Samuel A. Contreras, B.S., M.S.

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Dissertation Committee:

Dr. Mark A. Bennett, Advisor

Dr. Miller B. McDonald

Dr. James D. Metzger

Dr. Erich Grotewold

Approved by

_____

Advisor
Graduate Program in Horticulture

ABSTRACT

Seed dormancy, germinability and storability are important aspects of seed quality determined by the genotype and environment during seed development. While there have been several reports of maternal plant environment effects on seed germinability and dormancy, the mechanisms governing these effects are poorly understood, and modification of environmental conditions for improving specific aspects of seed quality during seed production is not a common practice among seed producers. Lettuce is one of the most important vegetables in the world and high quality seed is required for successful crop establishment. However, seed thermoinhibition (sensitivity to high temperatures) and photodormancy (lack of germination in dark) are two properties commonly affecting germination speed and uniformity of many lettuce genotypes. The main objectives of this study were to determine the effects that maternal plant conditions have on different aspects of lettuce seed quality such as germinability and storability. In separated experiments, 'Tango' lettuce seeds were produced in contrasting conditions for the following environmental factors: *i)* water availability, *ii)* day-length, *iii)* light quality (red to far-red [R:FR] ratio), and *iv)* temperature. In each experiment different aspects of seed quality were evaluated, including: *i)* seed weight, *ii)* standard germination (production of normal seedlings under optimal conditions), *iii)* germination (radicle

emergence) in a wide range of conditions, *iv)* vigor (accelerated aging test and seedling growth), *v)* storability (accelerated aging test and germination after different periods of storage), and *vi)* seed abscisic acid (ABA) content.

Restricted water availability during lettuce seed production had little effects on most aspects of seed quality, although a significant increase in seed weight and the production of fewer seeds per plant were observed. Additionally, water productivity (seed yield per volume of water consumed) increased significantly in response to restricted water availability, which is especially important for lettuce seed producers which are located mainly in arid regions.

The aspect of the maternal plant light environment that had the greatest effects on seed quality was the R:FR ratio. Seeds produced under higher R:FR ratios had higher germinability, poorer storability, lower sensitivity to external ABA, and lower ABA concentrations. These effects occurred during the last phase of seed development (maturation drying, after physiological maturity) and I hypothesized that higher accumulation of the active form of phytochrome (Pfr) in seeds drying under red-rich light would be involved in these responses. My results suggest that seed production under light environments with higher R:FR ratios represents a novel approach to the production of lettuce seed with lower levels of thermoinhibition and photodormancy, although reduction on seed storability is an undesired consequence that should be considered.

Lettuce ‘Tango’ seeds produced at higher temperatures (30/20°C vs. 20/10°C) had higher storability and lower thermoinhibition, photodormancy, and dry weight. Temperature effects on seed germinability occurred during the first phase of seed development (cell division and histo-differentiation), while effects on seed storability occurred at the last phase of seed development (maturation drying). I observed a sharp peak of seed ABA content at midpoint of seed development, and I hypothesized that effects of higher temperatures in improving seed germinability are mediated by a reduction in this ABA peak.

Dedicated to Alejandra

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VITA

September 10, 1974……... Born – Santiago, Chile

1998……..…. BS. Pontificia Universidad Catolica de Chile (PUC)

2000…… M.S. PUC

2001 to present…….. Auxiliary Professor, PUC

2003 to present…….. Graduate Research Associate
The Ohio State University

PUBLICATIONS

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FIELD OF STUDY

Major Field: Horticulture

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

CHAPTER 1

INTRODUCTION

Seeds represent a fundamental input for the establishment of agronomic, horticultural and forestry crops. Additionally, seeds are essential structures for plant reproduction, colonization and survival of many natural plant populations (Fenner and Thompson, 2005). Seeds are also used for germplasm conservation of many species because of their ability to survive for relatively long periods of time in storage. Germinability, dormancy, vigor, and storability are some of the attributes that determine the value of a seed to achieve these functions. Germinability may be defined as the potential of a seed or seed population to germinate under defined conditions (Black et al., 2006), whereas dormancy is the absence of germination of a viable seed under otherwise optimal conditions (Hilhorst and Toorop, 1997). The importance of favoring germinability over dormancy in crop species is essential and breeding programs have succeeded in eliminating or reducing seed dormancy of most major agronomic crops; nevertheless, many vegetable and ornamental species still exhibit forms of seed

dormancy that complicate crop management (Geneve, 1998). Germinability and dormancy are also important in weed and native plant species, where understanding of the issue contributes to sustainable management and preservation strategies.

Seeds are a delivery mechanism for breeding and biotechnological advances and, as a result, their commercial value has dramatically increased in recent decades (ISF, 2007). Consequently, crop producers demand the best performance possible for the seeds they buy, and seed vigor has become especially important. According to the Association of Official Seed Analysts (AOSA, 1983), "seed vigor compromises those properties which determine the potential for rapid, uniform emergence and development of normal seedlings under wide range of field conditions". Therefore, the presence of any level of dormancy in a seed lot represents a quality problem that affects its value. Many treatments have been designed to improve seed vigor; priming is a clear example of one of them (Halmer, 2004). Primed seeds of many species have shown improved vigor, and, in some cases (e.g. lettuce), the treatment has been useful in overcoming dormancy problems (Cantliffe et al., 1981; Valdes et al., 1985). Additionally, several other methods exist to overcome seed dormancy, such as stratification or chilling, scarification, and various chemical compounds (e.g. gibberellic acid, KNO_3) (Copeland and McDonald, 2001). Nevertheless, independent of the effectiveness of these treatments, they represent additional cost and manipulation of the harvested seeds, and producing more vigorous or less dormant seed in the field would be a superior approach.

Another important aspect of seed quality is its storability or ability to be stored for a prolonged period of time without loss of viability. This seed characteristic is important not just for the management of stocks by seed companies and growers, but also for the

preservation of target species in gene banks. Since dormancy is a mechanism whereby a species ensures survival and germination over time, it seems rational that dormant seeds should present better storability than non-dormant seeds. Several studies suggest that when seed dormancy is imposed by physical impermeability of seed coats (hardseededness), the storability of the seed is improved (Flood, 1978; Longer and Degago, 1996; Leggese and Powell, 1996; Debeaujon et al., 2000). On the other hand, a causal relationship has not been established between other types of dormancy and seed longevity. Studies performed over the last two decades provide some evidence for a possible causal relationship between certain forms of physiological dormancy and seed storability, although results are still not conclusive (Duan and Ma, 1991; Hilhorst and Toorop, 1997; Zarbakhsh et al., 1999; Tesnier et al., 2002; Fueyo et al., 2003). Because of the importance of the issue, especially regarding economic impact, germplasm conservation and understanding the ecology of seed banks, more specific research on possible causal relationships between seed storability and physiological forms of seed dormancy should be conducted.

There are several reports about the effects of the maternal environment on different aspects of seed quality, including germinability, dormancy, size, and composition (Fenner, 1991, 1992; Hilhorst and Toorop, 1997; Baskin and Baskin, 1998; Gutterman, 2000). These reports highlight results from a wide range of species and are presented in a diverse set of publications from agricultural, horticultural, forestry, ecology and physiological perspectives (Fenner, 1991, 1992). Some of the environmental factors that have been frequently analyzed are temperature, water availability, light (quality and photoperiod), altitude, and mineral nutrition. Although the response to each

factor will differ among species, some common tendencies have been observed. For example, high temperature, drought and short days during seed development are usually associated with higher germinability or lower levels of seed dormancy (Fenner, 1991; Fenner and Thompson, 2005). Despite this evidence, the issue has been scarcely reported on vegetables or ornamental species of economic importance, and the mechanisms affecting the response of seeds to the maternal environment are still poorly understood (Fenner, 1991; Hilhorst and Toorop, 1997; Gutterman, 2000; Fenner and Thompson, 2005). Given that there are differences in the nature of the endogenous mechanisms causing dormancy among species, it is expected that the same environmental factor may have different effects on dormancy depending on the species (Fenner, 1991). Even with these differences, some generalizations and speculations about the mechanisms involved have been stated (Fenner and Thompson, 2005). For example, high temperatures during seed development are generally associated with lower dormancy levels (e.g. lettuce [Drew and Brocklehurst, 1990]; *Avena fatua* [Sawhney et al. 1985]), which could be caused by reduced synthesis of inhibitory compounds at high temperature (e.g., abscisic acid), or greater synthesis of promoting substances (e.g., gibberellins). However, there are also examples of species in which dormancy increases with higher temperatures during seed development; for instance, high temperatures were conducive to development of thicker soybean seed coats and, therefore, more dormant seeds (Keigley and Mullen, 1986). In this case, the same factor (temperature) affects germinability differently depending on the dormancy mechanism (physiological vs. physical).

In the case of water availability, the type of response to drought conditions during seed development also seems dependent on the kind of dormancy involved. When dormancy is imposed mechanically by a thick seed coat, drought usually increases its thickness, thereby contributing to reduced germinability (Fenner, 1991; Baskin and Baskin, 1998). On the other hand, drought typically causes a reduction of seed dormancy when it is imposed by biochemical means, possibly by interfering with synthesis of inhibitors or promoters of germination (Fenner, 1991).

Regarding the light of the maternal plant environment, day-length and light quality (wavelength composition) have been reported to influence germinability during seed development (Fenner, 1991; Baskin and Baskin, 1998; Gutterman, 2000). Day-length has been more studied, and in most cases longer days have been associated with decreased germinability and higher dormancy, although exceptions have been reported (Fenner, 1991; Baskin and Baskin, 1998). When light quality has been studied, seed developed under light environments with lower red to far-red ratios has been observed to have lower germination in dark, compared with seed developed under environments relatively richer in red light (McCullough and Shrosphire, 1970; Hayes and Klein, 1974).

Despite the existing evidence of how the maternal environment affects seed germinability, and the importance of high quality seed production 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; Hilhorst and Toorop, 1997; Gutterman, 2000; Fenner and Thompson, 2005). Modification of environmental conditions for improving specific aspects of seed quality during seed production is not a common practice among seed producers.

Mechanisms that cause seed dormancy, independent of the seed production environment, are much better understood. However, they can vary depending on the species and the type of dormancy, and several aspects remain unknown. One possible classification for the kind of dormancy and causal mechanisms is the distinction between exogenous and endogenous dormancy (Copeland and McDonald, 2001). In the first case, dormancy is generally attributed to physical properties of the seed coat, which can act as a mechanical barrier to growth of the embryo, affect water uptake or gas exchange, supply inhibitors to the embryo or prevent their leaching (Bewley and Black, 1994). Seed coverings that impose exogenous dormancy are the endosperm, perisperm, seed coat integuments, or fruit pericarp (Geneve, 1998). The most common form of exogenous dormancy occurs when seed coats become suberized and impermeable to water, which is commonly known as hardseededness and is typical of many species from families such as Fabaceae, Malvaceae, Chenopodiaceae and Liliaceae (Geneve, 1998; Copeland and McDonald, 2001). Dormancy has also been attributed to a physical impediment by the seed coat to the enlarging embryo (Copeland and McDonald, 2001). Most of the evidence for this comes from the observation that in many species embryos from dormant seeds are able to germinate when seed coats are removed. Nevertheless, because seed coats often are the source of growth inhibitors or impede the leaching of those substances (Copeland and McDonald, 2001; Geneve, 1998), the real cause of dormancy could be the existence of inhibitors and not the mechanical restriction of the seed coats. Additionally, measurements of the force needed to puncture the seed coat indicated that there is no clear correlation between the "strength" (or resistance to puncturing) of the seed coats and level of dormancy (Bewley and Black, 1994). This information suggests that

dormancy could be caused by the lack of growth potential by the embryo, or its inability to produce compounds that weaken the seed coat. If that is the case, this form of dormancy should be classified as endogenous rather than exogenous.

Endogenous dormancy is the most common dormancy type found in seeds and is generally believed to be regulated by the existence of growth inhibitors, promoters or the balance between them (Copeland and McDonald, 2001; Finch-Savage and Leubner-Metzger, 2006). In the past two decades many studies were carried out that led to a better comprehension of the growth compounds associated with seed dormancy establishment and alleviation, and the mechanisms involved in synthesis and action. Among the compounds that would be acting to induce seed dormancy, abscisic acid (ABA) appears to play an important role, both by its presence in the seed or by sensitivity of the embryo to its action (Kucera et al., 2005; Finch-Savage and Leubner-Metzger, 2006). The use of ABA-deficient mutants and inhibitors of ABA synthesis has been helpful to demonstrate the importance of this phytohormone in dormancy in several crops. One of the first studies was conducted on *Arabidopsis* (Karssen et al., 1983), which established that the onset of dormancy correlated well with the presence of a fraction of ABA produced by the embryo around 14 days after pollination. In sunflower, Le Page-Degivry et al. (1990) found that endogenous ABA levels, which increased sharply in the first half of the seed development period, fell at precisely the same moment as when embryo dormancy became established. In this study, blocking the synthesis of ABA by the use of fluridone was effective in preventing embryo dormancy when applied before the period of ABA increase, but not when applied later. The authors concluded that dormancy must be induced by ABA during seed maturation. In tomato, seeds from an ABA-deficient line

did not exhibit dormancy, showed higher rates of germination, and were able to germinate under more negative water potential conditions in comparison with seeds of the wild type genotype (Groot and Karssen, 1992; Ni and Bradford, 1993). Several other studies suggest that gibberellins (GA) are a promoter of germination (Kucera et al., 2005; Finch-Savage and Leubner-Metzger, 2006). This has been observed, for instance, in GA-deficient lines of *Arabidopsis* and tomato that are not able to germinate without the addition of external GA (Tesnier et al., 2002; Ni and Bradford, 1993). Similar behavior has been observed with the use of GA-synthesis inhibitors such as paclobutrazol (Steinbach et al., 1997; White et al., 2000; White and Rivin, 2000). Additionally, some studies suggest that seed dormancy is governed by the intrinsic balance of ABA and GA (ABA:GA ratio) during seed development (Steinbach et al., 1997; White et al., 2000; White and Rivin, 2000; Cadman et al., 2006). It appears that seed dormancy and the control of germination are controlled by the ABA:GA ratio rather than the absolute hormone content (Finch-Savage and Leubner-Metzger, 2006).

An interesting aspect of the information summarized above is that less dormant seed (e.g. ABA-deficient or ABA-insensitive genotypes) not only exhibited a higher percentage of germination under optimal conditions (germination as defined by AOSA and ISTA rules), but also were able to germinate faster and under a wider range of conditions (e.g. under sub-optimal osmotic mediums), which corresponds to seed vigor. From this perspective, the lack of germination of a live seed under sub-optimal conditions could be attributed to the presence of some level of dormancy. By the same reasoning, the presence of dormancy should not be evaluated just by seed germination under optimal conditions, but by the analysis of germination rates and percentage under a range of sub-

optimal conditions (which corresponds to seed vigor). An important aspect of this approach is that the understanding of how environmental factors during seed development affect seed dormancy will not only facilitate the production of less dormant seed, but also the production of more vigorous seed. Since seeds of many vegetable and ornamental crops are a high value product, especially in the case of some hybrids, modification of specific environmental factors during seed production (e.g., water availability, temperature or light) should be a reasonable strategy if the result is the production of seeds with improved quality.

Lettuce (*Lactuca sativa* L.) is an important commercial vegetable crop cultivated worldwide in a diverse range of environments, with USA, Spain, Italy, Japan and France as the main producer countries (Ryder, 2006). In the USA, between 2001 and 2006, lettuce was cultivated on over 121,000 ha per year with an annual crop value of approximately 2 billion dollars, which makes it the most important fresh vegetable in the country (USDA, 2007). Establishment of the crop may be performed by direct planting the seeds in the field or by producing lettuce seedlings in plugs that later are transplanted in the field (Ryder, 2006). In both cases, 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; Wurr and Fellows, 1985; Wien, 1997). Botanically, lettuce seed is actually an achene (i.e. a dry, indehiscent, one-seeded fruit), that consists of an elongated dicotyledonous embryo surrounded by the three successive layers: endosperm, integument (seed coat), and pericarp (fruit wall) (Esau, 1976; Ryder, 2006). Thermoinhibition (sensitivity to high temperatures) and photodormancy (lack of germination in dark) commonly affect the germination speed and

uniformity of many lettuce genotypes (Wien, 1997; Ryder, 1999), and seed priming is a common practice to overcome these problems (Ryder, 2006). The levels of lettuce seed thermoinhibition and photodormancy have been observed to vary not only among the genotype (Gray, 1975; Kozarewa et al., 2006), but also among seedlots from a given genotype (Wurr et al., 1986), indicating a strong effect of the mother plant environmental conditions during seed production (Wurr et al., 1986). For example, several studies have shown that producing lettuce seed at higher temperatures (e.g. 30/20°C vs. 20/10°C) has a positive effect on reducing seed thermoinhibition (Koller, 1962; Gray et al., 1988; Drew and Brocklehurst, 1990; Sung et al., 1998; Kozarewa et al., 2006). The effects of water availability or maternal light environment during seed production have been less studied. Izzeldin and co-workers (1980) reported that lettuce plants cultivated under severe water stress (-0.5 MPa) produced heavier and more vigorous (measured as seedling radicle elongation) seeds than plants under reduced (-0.03 MPa) or moderate (-0.08 MPa) water deficit. Gutterman (1973) reported that lettuce seeds from plants grown under 8 h of light had higher germination in dark than seeds from plants grown under 16 h of light. Despite the importance of lettuce as a vegetable crop, the physiological mechanisms governing the effects of maternal environment on seed quality are still poorly understood, and the effects of each factor on different aspects of seed quality such as size, germinability, and storability, along with the relationships among these factors have not been documented.

The main objectives of this study were to determine the effects that maternal conditions have on different aspects of lettuce seed quality. The environmental factors that were studied are: i) water availability, ii) day-length, iii) light quality, and iv) temperature. For each factor, effects on different aspects of seed quality were studied,

including: i) seed weight, ii) standard germination (production of normal seedlings under optimal conditions), iii) germination (radicle emergence) in a wide range of conditions, iv) vigor (accelerated aging test and seedling growth), and v) storability (accelerated aging test and germination after different periods of storage). The relationship between different aspects of seed quality were also studied, with special emphasis on a possible causal-effect relationship between seed dormancy and storability.

Potential physiological mechanisms governing the effects of the different environmental factors were studied by determining the critical period during seed development when each environmental factor is affecting seed quality and relating this information with physiological events occurring during seed development. Determining any possible involvement of ABA was of special interest due to the importance that this phytohormone has for seed development, germinability and dormancy.

Among the reasons for selecting lettuce as a model species for this study are:

i) The economic importance of the species as a vegetable crop.

ii) The common occurrence of thermoinhibition and photodormancy affecting lettuce seed germination and emergence.

iii) The feasibility of plant cultivation and seed production in the greenhouse and growth chambers (e.g. relatively compact size; easy flower induction; abundant flowering and seed production; self-pollination without requiring external agents such as wind or insects; easy identification of flowering day; and relatively short period between flowering and seed maturity).

iv) The fact that lettuce belongs to the Asteraceae family, which includes numerous species of economic importance as crops (agronomic, ornamental, and pharmaceutical) or weeds. This increases the chances that my results can be applied to improving the knowledge about the seed biology of other species.

Lettuce cv. 'Tango' was selected because it is a commercial genotype with marked seed thermoinhibition and photodormancy.

CHAPTER 2

DEVELOPMENT OF GERMINABILITY AND DESICCATION TOLERANCE IN LETTUCE SEEDS

ABSTRACT

Germinability and desiccation tolerance are important attributes that seeds acquire during their development. Exploring the timing of the acquisition of these characteristics is important to better understand how different environmental conditions affecting the mother plant influence seed quality. Lettuce (*Lactuca sativa* L. cv. 'Tango') plants were cultivated in the greenhouse. Seed germination, under light and darkness, was evaluated in fresh and desiccated seeds at 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 25, and 31 days after flowering (DAF). Desiccation was performed ≈ 1 hour after harvest by placing the seeds at 25°C and 57%RH. Seeds achieved maximum dry weight (physiological maturity, PM) 13 DAF. In fresh seeds, 100% germination was observed 7 DAF. Onset of desiccation tolerance occurred 9 DAF, shortly after a peak of seed abscisic acid (ABA) concentration and coincident with the presence of abundant oil bodies in the cotyledonal cells. Dark germination of fresh seeds increased from 0% 11 DAF to 86% 17 DAF and then

decreased to less than 20% 21 DAF. In general, desiccated seeds had lower dark germination than fresh seeds. Onset of lettuce seed photodormancy during the maturation desiccation phase was coincident with a sharp loss of seed water content from 37 to 11% between 17 and 19 DAF. My results support the involvement of ABA in storage reserve deposition and desiccation tolerance during lettuce seed development, and suggest that changes in seed water content during the maturation desiccation phase are associated with the onset of photodormancy in lettuce seeds.

INTRODUCTION

Seed development has been described to occur in three phases: *i) histodifferentiation*, characterized by cell division, differentiation, and elongation; *ii) expansion*, which is a period of rapid gain in dry weight due to reserve deposition; and *iii) maturation drying*, where seed fresh weight and water content decrease (Bewley and Black, 1994). The moment of maximum dry weight, at the end of the expansion phase, has been termed *physiological maturity* (PM; Shaw and Loomis, 1950) or *mass maturity* (Ellis and Pieta-Filho, 1992). PM represents an important landmark in seed development because it has been proposed as the moment when seeds reach maximum seed quality (TeKrony and Egli, 1997; Black et al., 2006). Onset of seed germinability and desiccation tolerance are other important events of seed development, each of which may occur coincidently or at different moments than PM. In some species it has been observed that, at early stages of seed development, slow seed desiccation is required to promote

germination (Kermode and Bewley, 1985), while in other species germination of immature fresh seeds occurs without requiring desiccation or even at moments when desiccation is still not tolerated (Bartels et al., 1988).

A sharp rise in seed abscisic acid (ABA) concentration during seed development of *Arabidopsis thaliana* (Karssen et al., 1983), sunflower (Le Page-Degivry et al., 1990), sorghum (Steinbach et al., 1997), and maize (White et al., 2000) has been observed approximately half-way to PM. This phytohormone is known to be involved in several important processes during seed development such as: *i)* prevention of precocious germination (vivipary), *ii)* storage reserve deposition, *iii)* acquisition of desiccation tolerance, and *iv)* induction of primary dormancy (Kermode, 2005).

Lettuce (*Lactuca sativa* L.) is one of the most important vegetables in the world. In the USA, between 2001 and 2006, lettuce was cultivated on over 121,000 ha per year with an annual crop value of approximately 2 billion dollars, which makes it the most important fresh vegetable in the country (USDA, 2007). Lettuce seeds (achenes) are produced inside flower heads that contain 12 to 20 perfect and self-fertile flowers (Black et al. 2006). Before flower heads open, the receptive pistil elongates through a tube of dehiscent anthers ensuring self pollination (Jones, 1927), and seed maturation is accomplished during the next 12 to 17 days (Ryder, 1999). Lettuce seed quality is affected by the environmental conditions during seed development (Koller, 1962; Izzeldin et al., 1980; Gray et al., 1988; Sung et al., 1998; Kozarewa et al. 2006). Knowing the moment during seed development for the acquisition of attributes such as germinability and desiccation tolerance, and relationship with morphological and physiological changes of the seeds is important for a better comprehension of the possible

mechanisms by which the maternal environment may affect seed quality. The main objectives of this study were: *i)* to determine the timing of the acquisition of germinability, photodormancy and desiccation tolerance during lettuce seed development, *ii)* to characterize the variations in seed ABA concentration during lettuce seed development, and *iii)* to examine the possible relationship between the occurrence of seed quality attributes and morphological or physiological events happening in the lettuce seed during development.

MATERIALS AND METHODS

Plant material. Forty five lettuce (cv. 'Tango') plants were produced in the greenhouse in 1.75 L plastic pots filled with a soilless growing media (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). Plants were grouped in three blocks with 15 plants each. On June 2, 2007, ≈ 10 d after flower initiation, 30 to 40 flower heads per plant were labeled with color strings to indicate flowering day. Thirty labeled flower heads from each block (≈ 2 per plant) were harvested 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 25, and 31 days after flowering (DAF). In the greenhouse, during the 31 d sampling period, the average maximum and minimum temperatures were 35.1 and 19.9°C, respectively, and the overall temperature and relative humidity averages were 26.6°C and 57.6% respectively. Immediately after harvest, seeds (achenes) were manually extracted from each flower head for evaluation.

Seed evaluation. Fresh and dry weight was determined on 50 seeds per block by weighing the seeds before and after drying in an oven at 103°C for 48 h.

Seed germination (radicle emergence) tests were conducted at 20°C using 50 seeds per block planted over two layers of blotters (Anchor Paper Co., St. Louis, MO) saturated in distilled water and placed in 9 cm Petri dishes. Germination in light was evaluated daily to 10 d and the germination index (GI) was calculated as the algebraic sum of the ratio of germinated seeds and days after sowing at the count moment. Germination in dark (Petri dishes wrapped in aluminum foil) was evaluated 10 d after sowing. Germination in light and dark was evaluated on fresh (immediately after harvest) and desiccated seeds. Seed desiccation was accomplished by placing ≈ 200 fresh seeds at 25°C inside plastic boxes (11 x 11 x 4 cm) containing a saturated NaBr solution. The seeds were placed in aluminum pots over a mesh that prevented direct contact between the seeds and the NaBr solution. The relative humidity inside the boxes, measured with a data logger (HOBO U12-012, Onset, Bourne, MA), was ≈ 57%. After 72 h of desiccation, seed germination in dark and light was evaluated as previously described. Weight of desiccated seeds was determined in a sample of 50 seeds before and after drying (48 h at 103°C).

Data are presented as the average and the standard error of the average calculated from three samples (blocks) for each stage of development.

Light microscopy. Ten seeds from each sampling date and block were fixed by at least 48 h in a solution 3:1 of ethanol (70%) and glacial acetic acid. Then, seeds were dehydrated by transferring them through the following sequence of solutions (24 h at 0°C in each solution): ethanol 80%, ethanol 90%, ethanol 100%, propanol, and butanol.

Dehydrated specimens were imbibed in a monomer mixture consisting of: 94.5% purified glycol methacrylate, 0.5% 2.2'-azobis[2-methylpropionitrile], and 5% polyethylene glycol 400 placed in gelatin capsules according to the method described by Feder and O'Brien (1968). After imbibition was complete (3 to 4 days) the capsules were sectioned using a microtome and the sections mounted on slides. The staining was performed using the periodic acid/Schiff's reaction and counterstained with toluidine blue as described by O'Brien and McCully (1981). Sections were observed in a light microscope.

Abscisic acid (ABA) determinations. Seeds used for determining seed ABA concentrations during development were produced in a different set of plants. Fifteen lettuce plants, cv. Tango, were cultivated in similar conditions as previously described, and a total of 600 flower heads were labeled the day of flowering on June 27, 2007. Between 13 and 5 flower heads (decreasing from the first to the last sampling) were harvested from 3 DAF at similar intervals as previously described. The average maximum and minimum temperatures during the sampling period were 30.6 and 19.7°C, respectively, and the overall temperature and relative humidity averages were 24.6°C and 64.6% respectively. Seeds were extracted immediately after harvest and separated in three sub-samples of 80 seeds each to determine fresh and dry weight, and three sub-samples, equivalent to 30-60 mg dry weight each, that were frozen in liquid nitrogen and stored at -80°C until ABA analysis. ABA extraction and determination was performed as described by Roth-Bejerano et al. (1999) with some modifications. 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 was determined from this supernatant by using anti-ABA monoclonal specific antibodies and competitive ELISA test according to instructions from Phytodetek® ABA Test Kit (Agdia, Elkhart, IN, USA). Data are presented as the average and the standard error of the average calculated from three sub-samples for each moment of development.

RESULTS

Changes in seed fresh and dry weight during development are shown in Fig. 2.1. Seed physiological maturity (PM), determined by an iterative regression analysis procedure (Pieta-Filho and Ellis, 1991), occurred 12.7 ±0.4 DAF, when seeds reached a maximum dry weight of 0.79 ±0.02 mg·seed^{-1} (Fig. 2.1). Seed water content (SWC) at PM was ≈ 40% and remained over 38% until 17 DAF, after which it decreased to less than 12%, 19 DAF (Fig. 2.1). When seed were desiccated, the equilibrium SWC of the seeds under the desiccation conditions (25°C, 57% RH) varied with seed age (Fig. 2.2). From 3 to 9 DAF the equilibrium SWC under desiccation decreased from 11 to 7%, whereas between 7 and 31 DAF this SWC did not vary significantly and averaged 7.1 ±0.1% (Fig. 2.2).

Fresh seeds did not germinate 3 DAF, however by 5 and 7 DAF germination increased to 18 and 100%, respectively (Fig. 2.3a). Germination decreased from 100% at 15 DAF to 76% at 17 DAF, after which it increased steadily until reaching 100% again at 25 DAF (Fig. 2.3a). Germination index for fresh seeds reached a peak of 0.8 at 13 DAF, after which it decreased and started increasing again to reach a new peak of 0.93 at 31

DAF (Fig. 2.3a). When germination was tested after seed desiccation, no germination was observed 3 and 7 DAF, but by 9 DAF germination was 100% (Fig. 2.3b). From 9 DAF the germination pattern for desiccated seeds was similar to that observed for fresh seeds (Fig. 2.3a), with a decrease in germination 17 DAF and recovery to 100% germination at 25 DAF (Fig. 2.3b). Similarly, two peaks for GI were observed for desiccated seeds, 1.0 (i.e. full germination after 1 d) at 13 DAF and 0.87 at 31 DAF (Fig. 2.3b).

Dark germination of fresh lettuce seeds reached a first peak of 18% at 7 DAF, decreased to 0% at 9 and 11 DAF, increased to a new peak of 85% at 17 DAF, and finally decreased to stabilize around 15% between 21 and 31 DAF (Fig. 2.4). Dark germination of desiccated seeds had a transient peak of 7% at 9 DAF, decreased to 0% at 11 DAF, increased to 52% at 13 DAF, and then decreased to stabilize around 10% between 19 and 31 DAF (Fig. 2.4).

Examination of seed sections using light microscopy showed that by 3 DAF the embryo had already developed to the heart stage and cotyledon elongation had begun (Fig. 2.5a). By 5 DAF the embryo was in the torpedo stage, with the two cotyledons clearly distinguishable (Fig. 2.5b). By 7 DAF the embryo elongation was essentially complete (Fig. 2.5c), the endosperm was reduced to a layer of cells lying between the embryo and the integuments (Fig. 2.5d), and storage granules began to appear in the embryonic cells (Figs. 2.5d and 2.5e). By 9 DAF seed coats started to change color from white to brown (data not shown), whereas in the cells of the embryo, especially the cotyledons, storage granules become abundant (Fig. 2.5f). The image quality of seed

sections from 11 DAF was not good because of poor penetration of the glycol methacrylate mixture, although they showed the embryo increasing in size and in anatomical details (data not shown).

Lettuce seeds from the plants cultivated to obtain curves of ABA accumulation had a similar pattern of development to that shown in Fig. 2.1. However in this case PM was estimated at 10.8 DAF and the average maximum dry weight was 0.69 mg per seed (data not shown). The seed ABA concentration was around 2 $ng \cdot mg^{-1}$ dry weight (DW) at 3 and 5 DAF, and had a peak of 11.8 $ng \cdot mg^{-1}$ at 7 DAF after which it decreased to a level around 0.2 $ng \cdot mg^{-1}$ between 13 and 33 DAF (Fig. 2.6).

DISCUSSION

Full germination of fresh seeds at 7 DAF (Fig. 2.3a) was coincident with the moment in which the embryo has elongated and occupied the space initially filled by the endosperm (tissue that was now reduced to a two cell layer surrounding the embryo) (Figs. 2.5c). Although fresh seeds were able to germinate 100% ≈ 6 d before PM, the maximum rate of germination, expressed as GI, was coincident with PM. One possible explanation is the observed reduction in ABA accumulated in the seed. ABA concentrations reached a peak 7 DAF (Fig. 2.6), which corresponds to approximately 65% of the time needed to attain PM, and decreased until reaching its minimum level around PM (Fig. 2.6). In the study of seed germinability, 65% of the time to PM is equivalent to between 7 and 9 DAF, so the GI increase for fresh seeds (Fig. 2.3a) would be coincident with the reduction of the ABA concentrations on the seeds (Fig. 2.6). In other species (e.g. arabidopsis and sorghum), the early peak of seed ABA concentration

has been observed to impede germination of fresh seed at early stages of development, and the onset of germination has been coincident with the reduction of ABA concentration in the seeds (Karssen et al., 1983; Steinbach et al., 1997). Based on these results, high ABA concentration at early stages of lettuce seed development did not impede germination of seed separated from the mother plant but reduced germination rates. Another possible reason for the increase in GI from 7 to 13 DAF is the accumulation of reserve compounds in the embryo during the expansion phase, which would facilitate the quick growth of the radicle to complete germination.

The equilibrium SWC under the desiccation conditions varied with the stage of lettuce seed development, decreasing from 11% at 3 DAF to 7% at 9 DAF (Fig. 2.2). This difference may be explained by changes in seed chemical composition, especially lipid content. At the same relative humidity, seeds with higher lipid content are expected to have lower SWC than seeds with lower lipid content (Taylor, 1997). Oil bodies in the cells of the lettuce embryos were observed 7 DAF (Fig. 2.7e). Accumulation of these reserves, especially in cotyledon cells, became abundant 9 DAF (Fig. 2.7f), which coincides with the stabilization of the equilibrium SWC around 7% (Fig. 2.2). This is also the moment when onset of desiccation tolerance occurred (Fig. 2.3b). The peak of ABA concentrations in lettuce seeds during development (7 DAF, ≈ 65% to PM) was coincident with the onset of desiccation tolerance (9 DAF, ≈ 70% to PM) and deposition of storage reserves (7 to 9 DAF, 55 to 70% to PM). ABA has been suggested to be involved with desiccation tolerance and reserve deposition in the embryo (Kermode, 2005).

In some species, germination at early stages of seed development has been observed only after seed desiccation (Dasgupta et al., 1982; Kermode and Bewley, 1985). Additionally, desiccation of immature seeds has also shown to improve germination rates (Kermode et al., 1986). Metabolic changes that increase ABA breakdown or decrease embryo sensitivity to this phytohormone have been proposed as possible mechanisms by which desiccation would promote germination (Kermode et al. 1986). In the case of lettuce, desiccation at early stages of seed development was not required for promoting germination, since fresh seeds germinated 100% at 7 DAF (Fig 2.3a), at which time the seeds were still unable to tolerate desiccation (Fig. 2.3b). Additionally, dark germination of fresh seeds was in general higher than for desiccated seeds (Fig. 2.4). Only at the moment of PM (13 DAF), seed desiccation had a positive effect on the GI (0.8 and 1.0 in fresh and desiccated seeds, respectively; Fig. 2.3), and dark germination (25 and 52% in fresh and desiccated seeds, respectively; Fig. 2.4). These results contrast those reported by Globerson (1981), who observed that in 'Gran Rapids' lettuce seeds harvested 9 and 10 DAF (PM was at 12 DAF), germination percentage in dark and in dark plus red light breaks was higher in desiccated (1 week at room temperature) than in fresh seeds. Possible reasons for these discrepancies are the difference in genotypes and desiccation methods.

After PM, germination of fresh and desiccated lettuce seeds decreased from 100% at 15 DAF to ≈ 70% at 17 DAF (Fig 2.3). In both cases, 100% germination was restored 25 DAF (Fig. 2.3), which implies that transitory reductions in seed germination were not caused by a loss of viability but by some type of primary dormancy. Reductions of 10 to 20% in lettuce seed germination after PM were reported by Globerson (1981) in two lines

of cv. ‘Grand Rapids’; in both cases, full germination was restored after 3 months of storage. ABA is typically associated with the onset of seed dormancy, however after PM seed ABA concentration reached lower levels (≈ 0.2 ng/g DW; Fig. 2.6) that did not change along with germination patterns. Therefore, the transitory reduction in lettuce seed germination between 15 and 25 DAF (Fig. 2.3) was not explained by differences in seed ABA concentration. However, it may be explained by differences in ABA sensitivity of the embryo or in GA concentration or sensitivity, aspects that were not analyzed in the present study.

Photodormancy or lack of germination in dark is a problem commonly found in some lettuce cultivars that present difficulties in achieving rapid and uniform seed germination and seedling emergence on the field (Wien, 1997; Ryder, 1999). The degree of light sensitivity in lettuce varies among cultivars and ‘Tango’ is described as a photosensitive genotype (H.J. Hill, personal communication). In general, fresh seeds germinated better in dark than desiccated seeds (Fig. 2.4). At 11 DAF and earlier, photodormancy was present in both fresh and desiccated seeds (Fig. 2.4), suppressing germination of seeds that under light germinated at 100% (Fig. 2.3). This developmental period of maximum photodormancy coincides with the peak in seed ABA concentration (Fig. 2.6). An increase in the GA to ABA balance, mediated by the phytochrome action, has been suggested as the mechanism by which light induces germination in photodormant seeds (Toyomasu et al., 1998; Seo et al., 2006). In this study, the strong photodormancy observed 11 DAF and earlier could be explained by a low GA to ABA balance and the absence of activated phytochrome (Pfr) to revert this situation. In fresh lettuce seeds photodormancy practically disappeared 15 and 17 DAF, and desiccated

seeds reached maximum dark germination of 52 and 44% at 13 and 15 DAF, respectively (Fig. 2.4). My results support those reported by Globerson (1981), who observed a peak in lettuce dark germination at or soon after PM, suggesting that germination at this phase of development is not under phytochrome control. Photodormancy imposition in fresh seeds between 17 DAF (86% germination) and 19 DAF (38% germination) coincides with a sharp reduction in seed water content from 37% at 17 DAF to 11% at 19 DAF (Figs. 2.1 and 2.4). No significant differences in seed ABA concentrations were observed during this period (Fig. 2.6), so this parameter would not explain photodormancy acquisition during the maturation drying phase of lettuce seeds. However, there are other parameters that could change during this phase and explain photodormancy acquisition, such as increase in GA levels or sensitivity, decrease in ABA sensitivity, or decrease in Pfr level or sensitivity. It is known that desiccation induces changes in membrane physico-chemical properties (Hoekstra et al., 2001) and cellular membrane properties have been proposed to be involved in regulation of seed dormancy (Hilhorst, 1998). Because the onset of photodormancy occurred in a period of drastic changes in seed water content and it was exacerbated by artificial seed desiccation, the possibility of changes in sensitivity to ABA, GA, or Pfr mediated by changes in cellular membrane properties seems particularly feasible.

In conclusion, under my experimental conditions, fresh 'Tango' lettuce seeds onset of germination occurred 7 DAF, half-way to seed PM. Acquisition of desiccation tolerance occurred 9 DAF (70% to PM), following a sharp peak of seed ABA concentration and coincident with abundant accumulation of storage reserves (oil bodies) in cotyledonal cells. The ABA peak half-way to PM did not impede seed germination of

fresh and desiccated seeds, but was associated with lower germination rates and photodormancy prior to PM. Additionally, my results support the suggested involvement of ABA on storage reserve deposition and acquisition of desiccation tolerance during lettuce seed development. No light was required for fresh seed germination 2 to 4 d after PM, and the onset of photodormancy coincided with a loss of seed water content at the maturation drying phase.

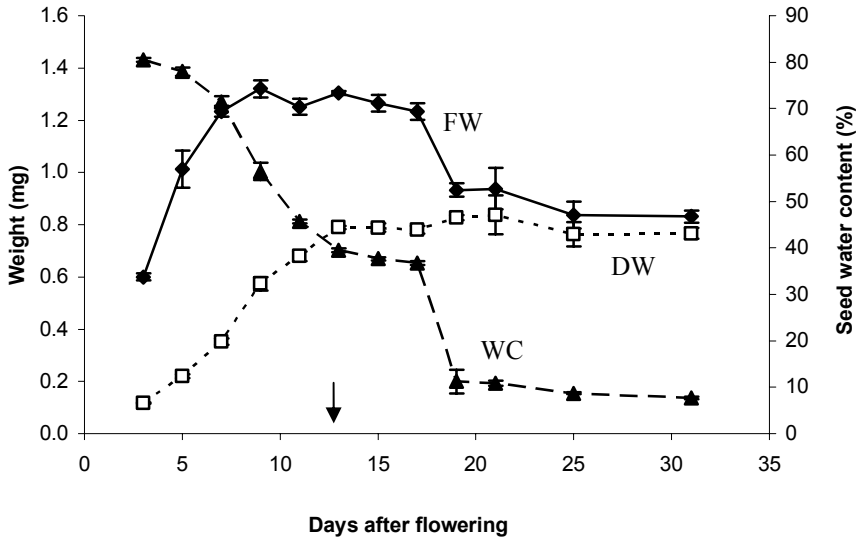


Figure 2.1: Lettuce seed fresh weight (FW, diamonds), dry weight (DW, squares), and water content (WC, triangles) at different developmental stages. The arrow indicates when physiological maturity (maximum dry weight) was achieved. Data are the average ±SE from three replications.

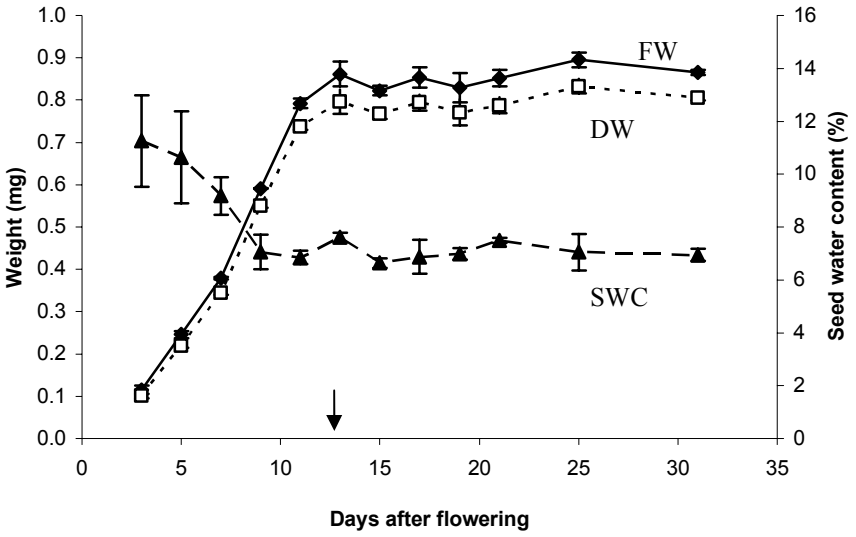


Figure 2.2: Fresh weight (FW, diamonds; immediately after desiccated), dry weight (DW, squares), and water content (WC, triangles) of lettuce seeds desiccated (3 d at 25°C and 57% RH) after harvest at different moments during development. The arrow indicates when physiological maturity (maximum dry weight) was achieved. Data are the average ±SE from three replications.

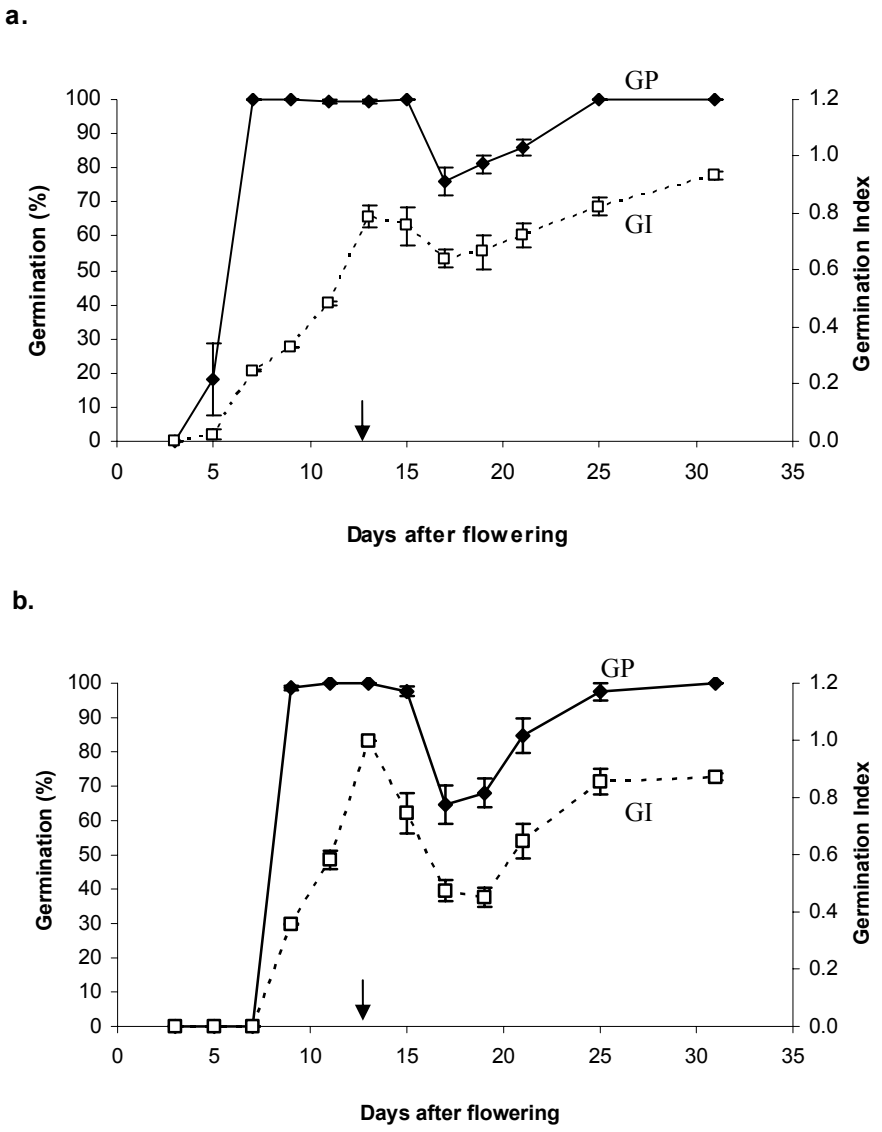


Figure 2.3: Germination percentage (GP, diamonds) and germination index (GI, squares) under light at 20°C of fresh (**a**; immediately after harvest), and desiccated (**b**; 3 d at 25°C and 57% RH) lettuce seeds after harvest at different stages during development. The arrow indicates when physiological maturity (maximum dry weight) was achieved. Data are the average ±SE from three replications.

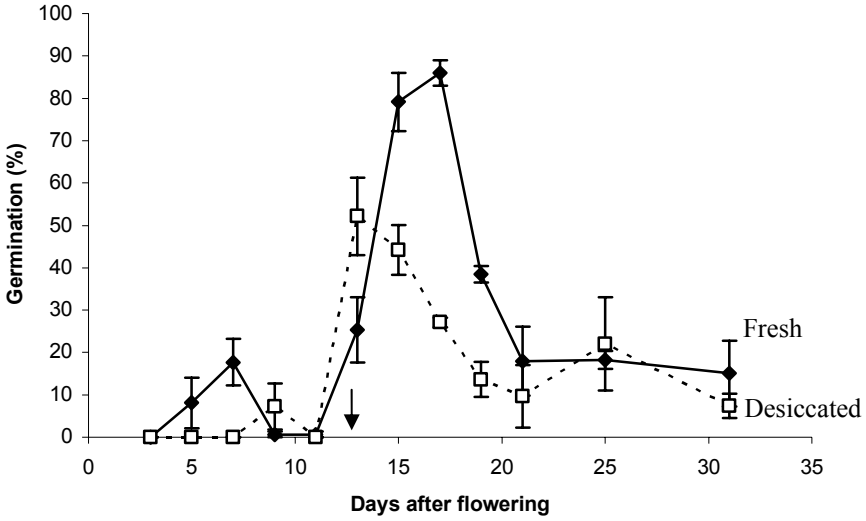


Figure 2.4: Dark germination of fresh (diamonds) and desiccated (squares; 3 d at 25°C and 57% RH) lettuce seeds at different developmental stages. The arrow indicates when physiological maturity (maximum dry weight) was achieved. Data are the average ±SE from three replications.

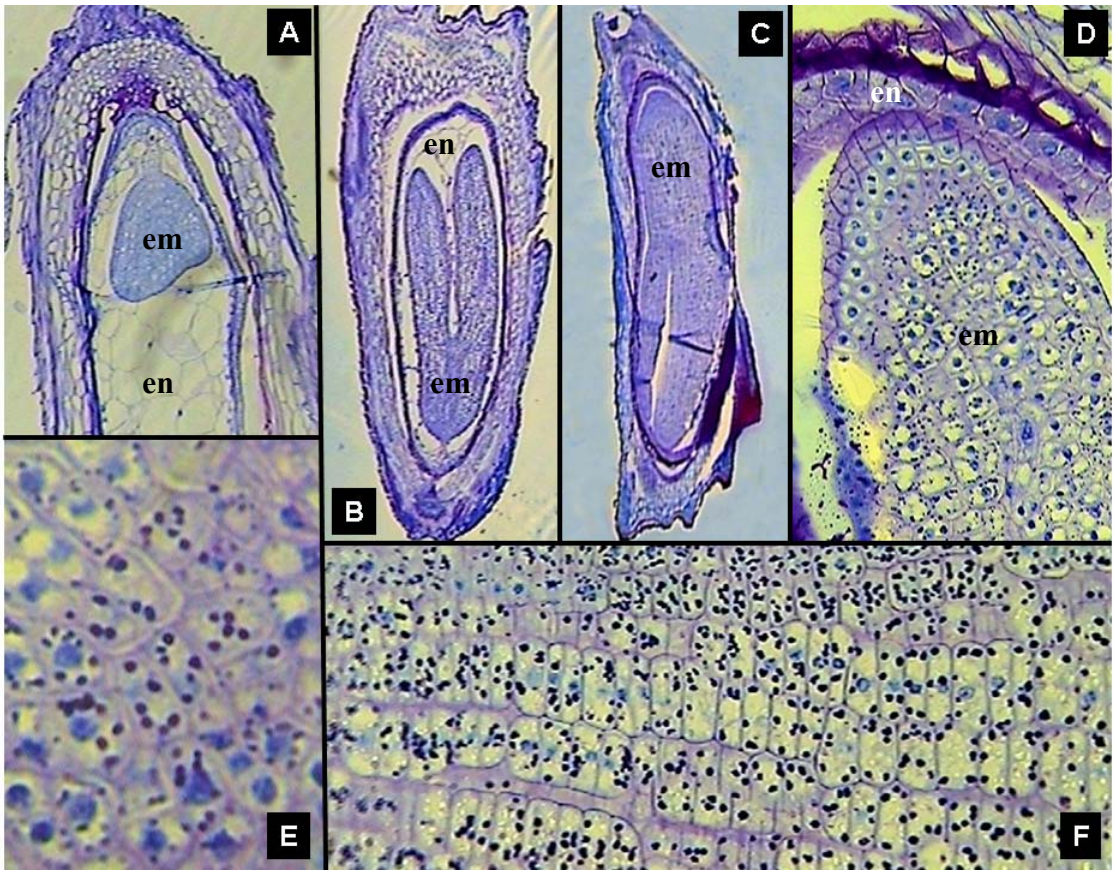


Figure 2.5: Lettuce embryo 3 (A), 5 (B), and 7 (C) days after flowering (DAF). Radicle tip (D) and cell detail (E) of a lettuce embryo 7 DAF. Cotyledonal cell detail (E) in lettuce embryo 9 DAF. em: embryo, en: endosperm.

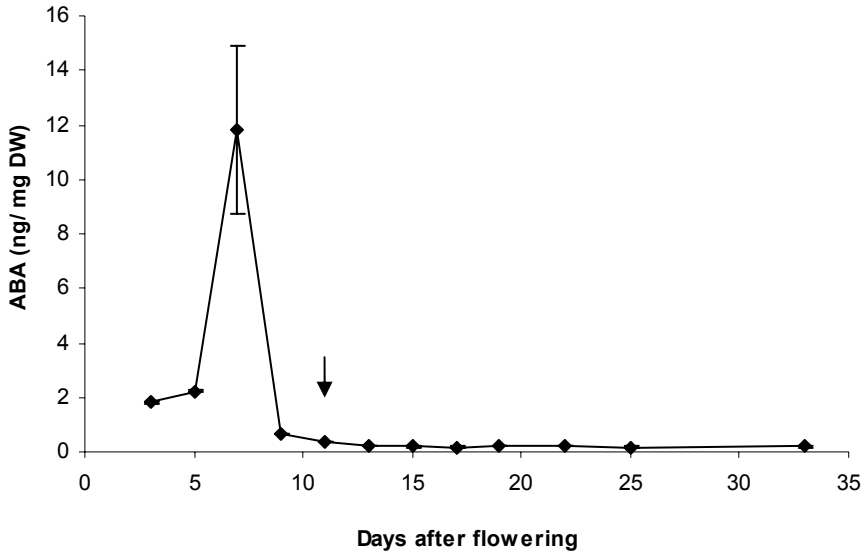


Figure 2.6: Abscisic acid (ABA) concentration in lettuce seeds at different moments during development. The arrow indicates the moment of physiological maturity (maximum dry weight). Data are the average ±SE from three sub-samples.

CHAPTER 3

EFFECTS OF WATER AVAILABILITY DURING LETTUCE SEED DEVELOPMENT

ABSTRACT

Availability of water by the mother plant during seed production is important because it may affect seed yield and quality. Lettuce is one of the most important vegetables in the world and its seed is produced mainly in semi-arid regions. The objective of this study was to determine how water availability during seed development affects lettuce seed production. Three experiments were performed in the greenhouse and growth chambers using lettuce (cv. 'Tango') cultivated in pots. When watering volumes and evapotranspiration were restricted (dry treatment) from bolting to seed harvest to 54 and 58% of a well-watered control (wet treatment), plants were smaller in height, with reduced dry weight and produced fewer and heavier seeds. Yield (mg seed·plant^{-1}) and harvest index were similar for both treatments; however, water productivity (seed yield·watering volume^{-1}) was nearly 50% higher in the dry treatment. Seeds from the dry treatment had a modest improvement in seed vigor (assessed by seedling growth) and

decreased germinability (higher sensitivity to ABA and water potential) compared to the wet treatment. In another experiment, water stress was applied abruptly to well-hydrated lettuce plants with developing seeds. Seeds that were at 1/3 and 2/3 of physiological maturity when water was withheld had lower germinability and greater storability than seeds with no water restrictions. These results are valuable information for developing rationale improvement strategies of management practices for lettuce seed production in semi-arid conditions.

INTRODUCTION

The availability of water by the mother plant during seed production is important because it affects seed yield and quality of many species. In general, most of the research on water requirements of different crops has focused on optimizing yield of commodity products and only rarely considered seed yield or quality parameters (George, 1999). Optimal water management for seed production may be different from best management practices for crop production, especially in species for which the final products are not grains (e.g., many vegetables and forages).

Effects of water stress on seed yield depend on the crop, and will vary depending on the intensity, duration and timing of the water deficit (Izzeldin et al., 1980). In general, water stress before and during flowering has been observed to affect seed yield by reducing the number of seeds produced per plant (Bartels and Caesar, 1987; Oliva et al., 1994; Champolivier and Merrien, 1996; Zebrauskiene et al., 2005), while individual seed weight has been affected by water deficit after flowering (Ludlow et al., 1990; Ramamoorthy and Basu, 1996; Champolivier and Merrien, 1996; Fougereux et al., 1997;

Castañeda et al., 2006). However, there are cases in which moderate water deficits have increased seed yield and/or seed size. For instance, Shock et al. (2007) reported that moderate deficit irrigation from flowering to seed maturity improved yield and individual weight of alfalfa (*Medicago sativa* L.) seeds. Santos et al. (2006) observed that seeds of *Macroptilium lathyroides* were heavier when produced under restricted water availability (60-70% soil field capacity). Less research has examined the effects of water stress on other aspects of seed quality such as germinability and storability. In general, the consensus is that water deficiency during seed development reduces dormancy and improves germination of wild species (Fenner, 1991; Hilhorst and Toorop, 1997; Gutterman, 2000). Water deficit had no effect on seed germinability and vigor of onion (*Allium cepa* L.) (Zebrauskiene et al., 2005), peanut (*Arachis hypogaea* L.) (Ramamoorthy and Basu, 1996), and maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L) Moench.) (Ghassemi-Golezani et al., 1997). Zhao *et al*. (1993) observed that seeds from water stressed cotton (*Gossypium hirsutum* L.) plants had faster germination and higher vigor. Ramamoorthy and Basu (1996) and Sinniah et al. (1998) reported that water deficit during seed production improved storability of peanut and rapid-cycling brassica (*Brassica campestris* [*rapa*] L.) seeds, respectively.

Lettuce (*Lactuca sativa* L.) is one of the most important vegetables in the world. In the USA, between 2001 and 2006, lettuce was cultivated on over 121,000 ha per year with an annual crop value of approximately 2 billion dollars, which makes it the most important fresh vegetable in the country (USDA, 2007). Lettuce seed quality is important because it affects establishment of the crop, along with final yield and quality (Smith et al., 1973b; Wurr and Fellows, 1985). Working with pot-grown lettuce plants in the

greenhouse, Soffer and Smith (1974) observed that withholding watering and nutrients during the last half (20-25 days) of the seed production period did not affect seed yield per plant, seed weight distribution, or seedling vigor (measured as seedling root length). Izzeldin et al. (1980) studied the effects of water stress on lettuce seed yield and quality by applying three levels of soil moisture deficit (-0.03, -0.08 and -0.5 MPa) at different growth stages of plants grown in a greenhouse. These authors reported that lettuce plants under moderate water deficit produced the highest seed yields; however, the highest quality (size and vigor measured as seedling radicle elongation) occurred for seeds produced under severe water stress treatments.

The objective of this study was to determine how water availability during seed development affects lettuce seed production, especially seed germinability.

MATERIALS AND METHODS

Experiment 1

Lettuce plants (cv. 'Tango') were produced in the greenhouse in 1.75 L plastic pots filled with a soilless growing media (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). At bolting, plants were randomly assigned to one of three treatments: i) *wet*, ii) *dry*, and iii) *very dry*. Daily watering volume for plants in the *wet* treatment was between 300 and 450 mL, depending on daily temperatures, while plants from the *dry* and *very dry* treatment received 200 and 100 mL of water per day, respectively. Plants were grouped in four blocks or replications, each with three plants per treatment.

Average air temperature in the greenhouse from early flowering (25 June) up to the last harvest (31 July) was 22.4°C, with maximums that ranged between 29.4 and 21.6°C and averaged 25.0°C, and minimums that ranged from 23.1 to 18.1°C and averaged 20.0°C. Plant evapotranspiration (ET) was measured on 2 July (24.2°C average temperature) as the plant (including pot) weight lost after irrigation in a 24 h period. Plant transpiration was measured similarly, but evaporation was suppressed by covering the soilless mix surface of the pot with a layer of aluminum foil. For each treatment, ET and transpiration measurements were performed using one plant per replication. Volumetric soil water content was measured on each pot on 30 June and 3 July by using a time-domain reflectometer (Soilmoisture Equipment Corp., Santa Barbara, CA).

Several lettuce seed harvests were conducted manually between 16 and 31 July, cutting only fully mature flower heads (dry with exposed seeds) with scissors. Once harvested and cleaned, seeds were desiccated to 8.4% water content using anhydrous $CaSO_4$ in a closed container and then stored in hermetic ally-sealed plastic bags at 10°C until evaluation. Seed weight and water content were calculated using 100 seeds per replication and drying the seeds in an oven at 103°C for 48 h. After the last harvest, plant dry weight (excluding roots) was determined by drying the plants for 48 h at 60°C.

Seed evaluation. The standard germination (SG) test was conducted with 50 seeds from each replication. Seeds were planted over two layers of blotter paper (Anchor Paper Co., St. Paul, MN), saturated in distilled water and placed in transparent plastic boxes (11x11x4 cm). The boxes were then 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 50 seeds per replication, planted over two layers of blotters saturated with 10 mL distilled water, or 10 mL of solution containing various concentrations of (±) abscisic acid (ABA; Sigma-Aldrich, St. Louis, MO) or polyethylene glycol (PEG 8000, Sigma-Aldrich, St. Louis, MO) and placed in Petri dishes (9 cm diameter). The PEG concentrations used were calculated to obtain water potentials of –0.15, -0.30, -0.45, and -0.60 MPa (Michel, 1983). Germination tests at different ABA and PEG concentrations were performed at 20°C and constant light, with counts of germinated seeds (radicle emergence) at 2, 4, 6, 8, 10 and 12 d. The germination index (GI) was calculated as the algebraic sum of the ratio of germinated seeds and days after sowing at the count moment. Germination at 30°C-light and 20°C-dark was evaluated at 7 and 4 d after sowing respectively.

Vigor Index and average radicle length measurements were determined on 50 seeds per replication using the Seedling Vigor Image System© (SVIS) according to methodology described by Sako et al. (2001).

The data were analyzed as a randomized complete block design using the ANOVA procedure of SAS (SAS Institute, Cary, N.C.). Before the analysis, germination percentages and GI values were transformed to the *arcsin* of the square root of the fraction value. When significant differences existed ($p<0.05$), the least significant difference (LSD; $\alpha=0.05$) was calculated to establish differences among treatments.

Experiment 2

Vegetative stage lettuce plants were cultivated in the greenhouse as described for Exp. 1. At bolting, plants were assigned randomly to one of two treatments: i) *wet*, and ii) *dry*. From bolting to flowering, plants in the *wet* and *dry* treatments were irrigated with 300 and 150 mL of water, respectively. Plants were grouped in four blocks or replications, each with six plants per treatment. After flower initiation, two plants per replication (including pot) were weighed each morning before watering. Evapotranspiration was calculated as the plant weight difference immediately after irrigation and before irrigation the next day. In this way, the watering volume for each treatment was determined daily as equivalent to the ET of the previous day.

Approximately 10 flower heads per plant were labeled with a colored string at the day of flowering (10 August). Six flower heads per replication were sampled at 4, 6, 8, 10, 12, and 14 d after flowering (DAF), and fresh and dry weight of seeds determined.

Three harvests were conducted manually, cutting only mature flower heads, on 12 and 23 August, and 1 September. In each harvest, the number of seeds per flower head was calculated from a sample of 20 heads per replication. Desiccation, storage and weight evaluation of the seeds was accomplished as described in Exp. 1. After the last harvest, plant height and dry weight (excluding roots) were determined. For each replication, the harvest index was calculated as the fraction between the dry weight of seeds harvested and dry weight of the plants (including seeds).

Seed evaluation. Only seeds from the second harvest (23 August) were used for germination assessment. Seed evaluation and data analysis were performed according to methodology described in Exp. 1. 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 were evaluated 11 d after planting.

Experiment 3

Twelve lettuce plants were cultivated in the greenhouse as previously described and moved to a growth chamber when bolting occurred. Air temperature in the chamber was 25 and 15°C for day (12 h, fluorescent light, ≈ 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and night, respectively. Each plant was provided 200 mL water daily until the treatments started. About 10 d after flower initiation, ≈ 35 flower heads per plant were labeled with a colored string on the day of flowering. Four plants were randomly assigned to one of three treatments: i) *no water restriction* (200 mL water per plant), ii) *no watering from 8 DAF* (last watering 7 DAF with 100 mL water), and iii) *no watering from 4 DAF* (last watering 3 DAF with 100 mL water). For the control treatment (no water restriction), seed weight accumulation was determined by sampling flower heads at different times after flowering as described for Exp. 2. For each treatment, only labeled flower heads were harvested 27 DAF, when flower heads were dry and open, and the seeds had less than 8% water content. Seeds were cleaned and placed in paper envelopes at 4°C and 25% RH until evaluation.

Seed evaluation. Seed dry weight and germination data were collected using four sub-samples of 50 seeds per treatment. All germination tests were performed as previously described. Normal seedling and dark germination were evaluated 7 and 4 d after planting, respectively. All other germination evaluations were conducted daily until 7 d after planting. The AA test was performed as described in Exp. 2. Data are presented as the average and standard error values from the four sub-samples of 50 seeds each.

RESULTS

Experiment 1. Water available to the lettuce plants differed for the treatments employed, with volumetric soil water content for the *wet* treatment more than two times greater than that for the *dry* and *very dry* treatments (Fig. 3.1A). For the three treatments, small differences between evapotranspiration and transpiration volumes were noted (Fig. 3.1B), which could be due to the basal leaves of the lettuce plants covering most of the upper surface of the pots, thereby reducing the direct water evaporation from the soilless mix. Water evapotranspiration from pots of each treatment was likely related to the respective daily watering volumes; i.e. for the *dry* and *very dry* treatments, the values were approximately 200 and 100 mL per day, respectively (Fig. 3.1B).

Plant dry weight at the end of seed harvest was significantly affected by treatment and was directly related to the watering volume of each treatment (Table 3.1). However, individual seed dry weight was inversely related with the watering volume, and was significantly higher in seeds from the *very dry* treatment, followed by the *dry* and *wet* treatments (Table 3.1). Despite the differences in weight, no significant differences in seed germinability were observed among the treatments, regardless of the temperature or

light condition (Table 3.1). The vigor index and seedling radicle length values from the SVIS analysis tended to increase with decreasing watering volumes during seed production, however the differences were only significant for radicle length (Table 3.1). When seed germination was evaluated at different ABA concentrations (Fig. 3.2A) or water potentials (Fig. 3.2B), seeds from the *wet* and *dry* treatments responded similarly, while seeds from the *very dry* treatment tended to have lower germination percentages and rates (expressed as GI).

Experiment 2. The application of treatments varied slightly from Exp. 1. Because of variation in daily evapotranspiration associated with the greenhouse temperature, the watering volume of each treatment was determined daily according to the evapotranspiration of the previous day (Fig. 3.3). The average daily evapotranspiration and watering volumes for lettuce plants from the *dry* treatment were 58 and 54%, respectively, of the values for the *wet* treatment.

Rates of development and desiccation for lettuce seeds from both treatments were similar (Fig. 3.4). Seed physiological maturity (PM, maximum dry weight), determined by an iterative regression analysis procedure (Pieta-Filho and Ellis, 1991), occurred 10.8 ±0.6 and 10.8 ±0.3 DAF for plants from the *wet* and *dry* treatments, respectively; however, individual seeds from the *dry* treatment were significantly heavier ($p < 0.001$) (Fig. 3.4, Table 3.1). Plants from the *dry* treatment were more compact, with height and dry weight significantly lower than for the *wet* treatment (Table 3.2). Although plants from the *wet* treatment produced more seeds per flower head and more seeds (g dry weight) per plant (Table 3.2), these differences were not statistically significant ($p = 0.05$). The total number of seeds produced per plant was significantly higher for plants from the

wet treatment (Table 3.2), which was the product of more flower heads per plant and a higher number of seeds per flower head. The harvest index was similar for plants from both experiments; however the water productivity (seed yield per unit of water used; Oweis and Hachum 2004) was higher for plants from the *dry* treatment (Table 3.2). As was observed in the first experiment, seed germinability was similar for both treatments, despite differences in seed size (Table 3.2). The vigor index and radicle length were higher for seedlings from the *dry* treatment seed; however, these differences were not significant (Table 3.2). Germination percentages and rates from both treatments were affected by exogenous ABA (Fig. 3.5A) and reduced water potentials (Fig. 3.5B). However, seeds from the *wet* treatment were less sensitive to these conditions.

Experiment 3. Lettuce seeds produced by plants under water stress tended to be heavier than seeds from plants without water restriction (Table 3.3). Percentage of normal seedlings at 20°C was higher for seeds from plants without water restriction, although the total germination (percentage and rate) at this temperature (light or dark) was similar for seed from the three treatments. Seeds produced without water restriction performed better when germinated at 30°C (light or dark) and with exogenous ABA; however, at a -0.4 MPa water potential, seeds had lower percentage and rate of germination (Table 3.3). After 72 h of accelerated aging at 41°C and ≈ 100%RH, seeds from plants under water stress, especially the no watering from 8 DAF treatment, performed better.

DISCUSSION

Experiments 1 and 2

In these experiments, the watering treatments were initiated at bolting and reductions in water availability and plant evapotranspiration were observed during the seed production period (Figs. 3.1, 3.3). Consequently, lettuce plants under water deficit had a significant reduction in dry matter accumulation (Tables 3.1, 3.2). This reduction in growth may be explained by the acclimation mechanisms activated in plants as a response to water stress, which include reduction in leaf area, enhancement of root extension, stomata closure, and osmotic adjustment of cells (Taiz and Zeiger, 2002). Izzeldin et al. (1980) observed similar reductions in size and weight of lettuce plants grown under severe water deficit conditions (soil moisture -0.5 MPa) during vegetative and/or reproductive growth.

Despite the reduced growth, lettuce plants from restricted watering treatments produced heavier seeds in both experiments (Tables 3.1, 3.2). According to Wery (2005), the effects of water stress on seed yield and quality may be explained by the relationship between vegetative sources and reproductive sinks. In my experiments, lettuce plants (cv. 'Tango') under water deficit had reduced growth and produced fewer seeds (Table 3.2). Consequently, plants grown under water deficit had fewer reproductive sinks (seeds), reducing competition and increasing the resources available for growth of each seed compared with plants from the *wet* treatment. Similar results have been observed in seed production of lettuce (Izzeldin et al., 1980), alfalfa (Shock et al., 2007), and *Macroptilium lathyroides* (Santos et al., 2006).

In Exp. 2, plants from the *dry* treatment produced nearly 20% fewer seeds (g) per plant, but the difference was not statistically significant (p= 0.051; Table 3.2). However, this difference was sufficient to biologically compensate for the reduction in size of *dry* plants and cause the *wet* and *dry* treated lettuce plants to have similar harvest indices (Table 3.2). When evaluating crop management strategies in dry areas, water productivity may be more important than yield per unit area (Oweis and Hachum, 2004). The water productivity in lettuce plants from the *dry* treatment was ≈ 50% higher than for plants from the *wet* treatment. Globally, the principal areas of lettuce seed production are located in the semi-arid regions of California (USA) and New South Wales (Australia) (Ryder, 1999). Because irrigation is required under these conditions, the substantial increase in water productivity attained by restricting watering represents important information for the development of better management practices during lettuce seed production.

Seed weight has been positively correlated with seed vigor (Smith et al. 1973a) and seedling growth after emergence (Smith et al., 1973b). In this study, lettuce seed performance was evaluated as the ability to produce normal seedlings under optimal conditions (SG), to germinate at different sub-optimal conditions, by seedling growth and uniformity (vigor index and radicle length from SVIS), and by the AA test. Despite the differences in seed weight, there were no significant differences in the percentage normal seedlings after SG and the germinability at different temperatures and light conditions for seeds from the different treatments in Exps. 1 and 2. Germination of 'Tango' lettuce seed

was drastically reduced in the dark for treatments from Exps. 1 and 2. Although seed produced by plants under water deficit tended to have higher dark germination, the differences were not significant (Tables 3.1, 3.2).

The average seedling radicle length after 3 days of germination has been used for vigor evaluation of lettuce seeds (Smith et al., 1973a), and a good correlation of this parameter with lettuce field emergence and yield has been observed (Smith et al., 1973b; Wurr and Fellows, 1985). The SVIS integrates parameters of seedling growth (radicle and hypocotyl) and uniformity (standard deviation from seedling lengths) to produce a vigor index from 0 (minimum vigor) to 1000 (maximum vigor) (Sako et al., 2001). When used to evaluate lettuce seed vigor, the vigor index from SVIS has shown good correlation with field emergence (Contreras and Barros, 2003). In my experiments, the seed vigor index and average radicle length tended to be higher in seeds from plants under water deficit; however, significant differences were only observed for radicle length in Exp. 1. Seeds from the *very dry* treatment produced seedlings with significantly longer radicles than seeds from the *wet* and *dry* treatments. A similar result was reported by Izzeldin et al. (1980) who observed that lettuce seeds from plants under severe water stress were heavier and produced seedlings with longer radicle lengths than seeds from plants with moderate or no water deficit. The AA test has been used for evaluation of seed storability and vigor (Copeland and McDonald, 2001). Treatments from Exp. 2 did not differ significantly for the AA test results (p= 0.085), although seeds from the dry treatment performed better after the aging (Table 3.2). This result, along with the results

from the SVIS, support the idea that lettuce seeds produced by plants under water deficit are heavier and more vigorous, which is consistent with the results reported by Izzeldin et al. (1980) for lettuce cv 'Calmar'.

Seed germination (percentage and rate) was reduced by increased exogenous ABA and decreased water potential, and seeds from the *very dry* and *dry* treatments were more affected in Exps. 1 and 2, respectively (Figs. 3.2, 3.5). Seed dormancy has been positively related with ABA presence or sensitivity of seeds to this phytohormone (Benech-Arnold et al., 1991; Ni and Bradford, 1993; Yogeesha et al., 2006; Finch-Savage and Leubner-Metzger, 2006), and also sensitivity of germination to water potential (Ni and Bradford, 1993). Thus, seeds from plants with restricted irrigation would be more dormant than those from well-hydrated plants. These results differed from the reported trend that associates water stress with production of less dormant seeds (Fenner, 1991; Hilhorst and Toorop, 1997; Gutterman, 2000), which could be explained by the effective acclimation of the plants to reduced availability of water before the seed production period. In Exps. 1 and 2, the differences in watering volumes started at bolting, and plants adjusted to the water available by reducing their size and producing fewer seeds per plant (Tables 3.1, 3.2). In this way availability of water and nutrients would not be restricted for the growing seeds during the period of seed development; in fact, seeds from the treatments with reduced water availability were able to accumulate more dry matter and tended to be more vigorous than those from the wet treatments (Tables 3.1, 3.2).

Experiment 3

The methodology used here differed from Exps. 1 and 2 because water stress was applied abruptly to well-hydrated lettuce plants grown in growth chambers and the effects were evaluated only on seeds at 4 or 8 DAF when water was withheld. Curves of dry matter accumulation for developing seeds from the control plants (no water restriction) were performed (data not shown), and PM was estimated to occur 13 DAF. Therefore, 4 and 8 DAF represented approximately 1/3 and 2/3 of seed PM, respectively.

Dry weight tended to be higher for individual lettuce seeds from plants with water restriction, which could be explained by more flowering and competition for resources in plants without water deficit. Seeds from lettuce plants that received the last watering 4 DAF were smaller than those that received the last watering 8 DAF, which could be due to less resources being available for filling seeds at the earlier water deficit.

When SG was evaluated, seeds from the treatments without watering from 8 and 4 DAF produced 94% normal seedlings, which was lower than the control which had 99% normal seedlings (Table 3.3). This difference in SG results may be interpreted as a reduction in seed quality due to the applied water stress. The control also outperformed the water stress treatments in germination index at 30°C, dark germination percentage at 30°C, and germination percentage and index at 50 μM ABA (Table 3.3). These results suggest a reduction in seed germinability as a consequence of reduced water availability to the mother plant during seed development, which is in contrast to the tendency observed in other studies (Fenner, 1991; Benech-Arnold *et al*., 1991; Vogler and

Bahnisch, 2006). However, seed germinability (percentage and rate) at -0.4 MPa water potential was better for seeds from the water deficit treatments than for seeds from the control.

Light requirement for lettuce seed germination has been reported and extensively studied (Ikuma and Thimann, 1964; McArthur, 1978; van der Woude et al., 1980; Toyomasu et al., 1998). The degree of light sensitivity in lettuce varies among cultivars and 'Tango' is described as a very photosensitive genotype (H.J. Hill, Seed Dynamics Inc., personal communication). In fact, in Exps. 1 and 2 lettuce seeds did not germinate more than 25% in the dark (Tables 3.1, 3.2). However, in Exp. 3, dark germination at 20°C was close to 100% for seed from all the treatments (Table 3.3). Seed production in Exp. 3 was performed in growth chambers with artificial fluorescent light, which has a red to far-red (R:FR) ratio over 6, which is much higher than the natural light used in the greenhouse during seed production in Exp. 1 and 2 (R:FR ratio between 0.9 and 1.2). Dark germination of *Arabidopsis thaliana* (L.) Heynh. seeds varied depending on light quality during seed production, and seed developed under light with high R:FR ratio germinated better in the dark (Hayes and Klein, 1974). A similar result was observed with 'Tango' lettuce seeds (Chapters 4, 5, 6), and the greater dark germination of seeds in this experiment can be explained by the modified light quality inside the growth chambers compared to the greenhouse seed production environment.

Interestingly, results from the AA test showed that lettuce seeds from the water stress treatments performed better than seeds from the control (Table 3.3). In other experiments, the AA test was a good predictor for storability of 'Tango' lettuce seed (Chapter 4), suggesting that seeds from water stress treatments had better storability.

Similar results have been reported for seeds of peanut (Ramamoorthy and Basu, 1996) and rapid-cycling brassica (Sinniah et al., 1998), who found than seed produced under water stress had better storability than seed from well-hydrated controls. From an ecological point of view, producing seed with better storability under conditions of stress could ensure survival of the seeds in the soil for prolonged periods of time until more favorable emergence conditions are present.

In summary, the effects of restricted water availability during lettuce seed development depended on how the water deficit occurred. When the volume of water available to the plants was reduced before flowering, plants produced fewer but heavier seeds. The increase in individual seed weight was accompanied by a slight improvement in seed vigor (assessed by seedling growth) and a decrease in germinability (expressed as a higher sensitivity to ABA and water potential). Additionally, an important increase in water productivity (lettuce seed yield per volume of water applied) can be attained by restricting watering volumes. Lettuce plants under water deficit were more compact and produced fewer seeds per plant, suggesting that field plant populations should be increased when restricted watering occurs. The feasibility of applying these practices for lettuce seed production still requires evaluation in field experiments.

When water stress was applied abruptly to lettuce plants with seeds in the early stages of development, germinability was negatively affected. Total and sudden withholding of watering to lettuce plants during seed production should be avoided because of the potential reduction in quality that can affect individual seeds.

Parameter	Treatment			*p*-value[1]	LSD[2] (α=0.05)
	Wet	Dry	Very dry		
Plant dry weight (g·plant^{-1})	23.4	13.7	7.3	<0.001	2.03
Seed dry weight (mg·seed^{-1})	0.71	0.83	1.02	<0.001	0.05
Normal seedlings at 20°C-light (%)	99.0	99.0	100.0	0.125	
Germination at 20°C-light (%)	100.0	100.0	100.0	---	
Germination at 25°C-light (%)	100.0	100.0	100.0	---	
Germination at 30°C-light (%)	94.5	99.0	99.0	0.210	
Germination at 20°C-dark (%)	5.5	15.5	15.5	0.428	
Vigor Index[3]	514.5	564.5	598.25	0.156	
Radicle length (pixels·seedling^{-1}) [3]	213.8	216.1	226.8	0.009	8.37

1: *p*-value from analysis of variance
2: Least significant difference
3: Values from SVIS© (Seedling Vigor Image System)

Table 3.1: Parameters of growth and seed quality for lettuce plants under three daily watering treatments: *wet* (300 to 450 mL per plant), *dry* (200 mL per plant), and *very dry* (100 mL per plant) (Exp. 1).

Parameter	Treatment		*p*-value[1]	% dry respect to wet
	Wet	Dry		
Growth				
Plant height (cm)	121.4	109.7	0.021	90.4
Plant dry weight (g·plant^{-1})	30.8	23.5	0.010	76.4
Seed Yield				
Seeds per flower head	19.4	17.7	0.052	91.2
Estimated number of seeds per plant	8211	5587	0.010	68.0
Seed dry weight (mg·seed^{-1})	0.64	0.77	<0.001	119.1
Seeds per plant (g dry weight·plant^{-1})	5.27	4.29	0.051	81.4
Harvest Index[2]	0.17	0.18	0.474	105.9
Water productivity (g/Lt)[3]	0.61	0.92	0.006	150.8
Seed Germination and vigor				
At 20°C- light (%)	100.0	99.5	0.391	99.5
At 25°C- light (%)	99.0	99.5	0.391	100.5
At 30°C- light (%)	98.5	97.5	0.664	99.0
At 20°C- dark (%)	12.0	23.3	0.050	194.2
Normal seedlings after AA[4](%)	96.6	98.5	0.085	101.9
Vigor Index[5]	697.3	721.8	0.518	103.5
Radicle length (pixels·seedling^{-1}) [5]	261.7	290.3	0.212	110.9

1: *p*-value from analysis of variance
2: Harvest Index= (total seed dry weight per plant)·(plant dry weight + total seed dry weight per plant) $^{-1}$
3: Water productivity= (total seed dry weight per plant, grams)·(total watering volume per plant, liters) $^{-1}$
4: AA, accelerated aging of the seeds at 41°C and ~100%RH for 72 h.
5: Values from SVIS© (Seedling Vigor Image System).

Table 3.2: Parameters of growth, seed yield and seed quality for lettuce plants under different treatments of water availability: *wet* (daily watering volume equivalent to evapotranspiration volume), and *dry* (watering volume ≈ 54% of wet treatment)(Exp. 2).

Parameter	Treatment		
	No water restriction	No watering from 8 DAF[1]	No watering from 4 DAF
Seed dry weight (mg·seed^{-1})	0.782 ±0.005	0.854 ±0.001	0.819 ±0.008
Normal seedlings at 20°C (%)	98.6 ±0.5	94.3 ±2.0	94.3 ±1.7
Germination at 20°C (%)	100.0 ±0.0	99.5 ±0.5	100.0 ±0.0
Germination Index at 20°C	1.00 ±0.00	0.98 ±0.01	0.99 ±0.01
Germination at 30°C (%)	100.0 ±0.0	99.0 ±1.0	98.5 ±1.0
Germination Index at 30°C	1.00 ±0.00	0.90 ±0.02	0.90 ±0.02
Dark germination at 20°C (%)	100.0 ±0.00	98.5 ±0.5	99.5 ±0.5
Dark germination at 30°C (%)	88.4 ±3.7	31.0 ±5.1	15.0 ±1.3
Normal seedlings after AA[2] (%)	2.0 ±1.2	77.0 ±2.4	49.0 ±6.6
Germination at 20°C with ABA, 50 µM (%)	97.5 ±1.3	78.9 ±3.6	74.8 ±2.6
Germination Index at 20°C with ABA, 50µM	0.67 ±0.03	0.48 ±0.04	0.57 ±0.03
Germination at 20°C in -0.4 MPa osmotic solution (%)	78.3 ±5.0	94.5 ±0.9	91.5 ±2.2
Germination Index at 20°C in -0.4 MPa osmotic solution	0.58 ±0.04	0.64 ±0.01	0.75 ±0.02

[1]: DAF, days after flowering
[2]: AA, accelerated aging of the seeds at 41°C and ~100%RH for 72 h.

Table 3.3: Parameters of quality for lettuce seeds produced under different water restriction treatments (Exp. 3). Data are presented as average ± standard error values from four sub-samples of 50 seeds each.

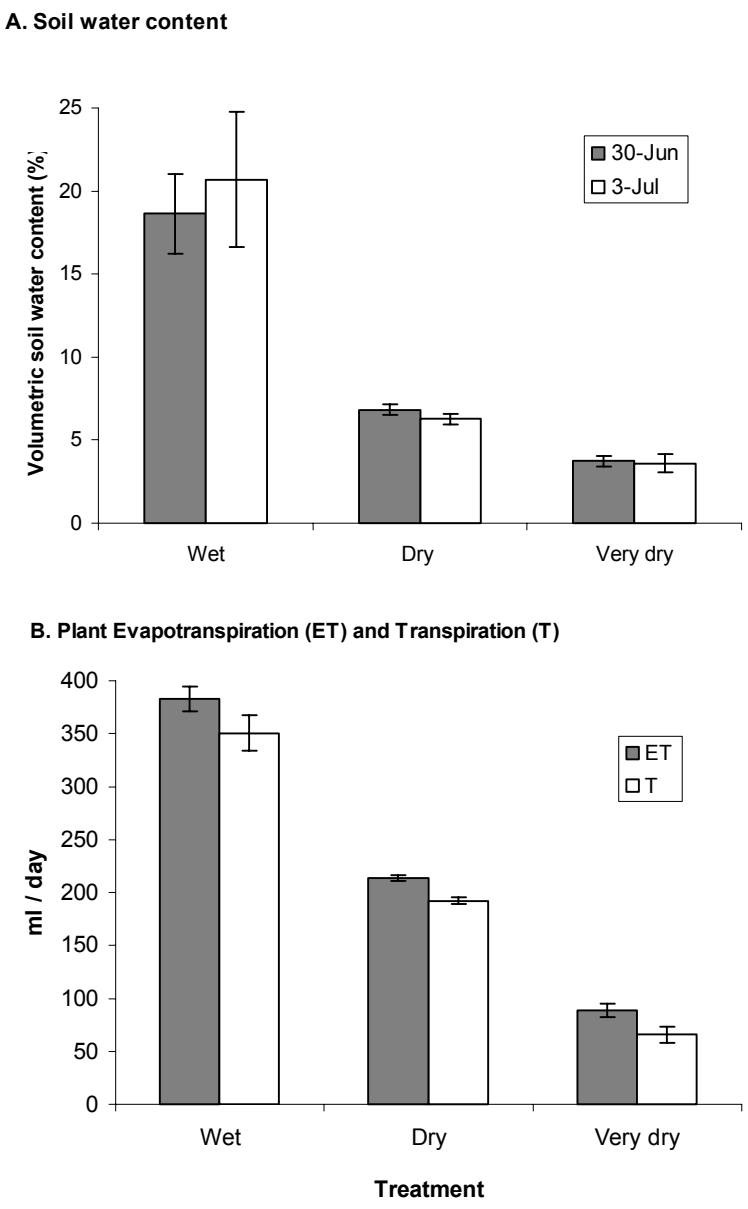


Figure 3.1: A) Soil water content (volume of water/ volume of soil) for three treatments (Exp. 1) on June 30 and July 3. B) Lettuce plant evapotranspiration (ET) and transpiration (T) calculated for each treatment from July 2 to July 3. Data are means +/- SE of four replications.

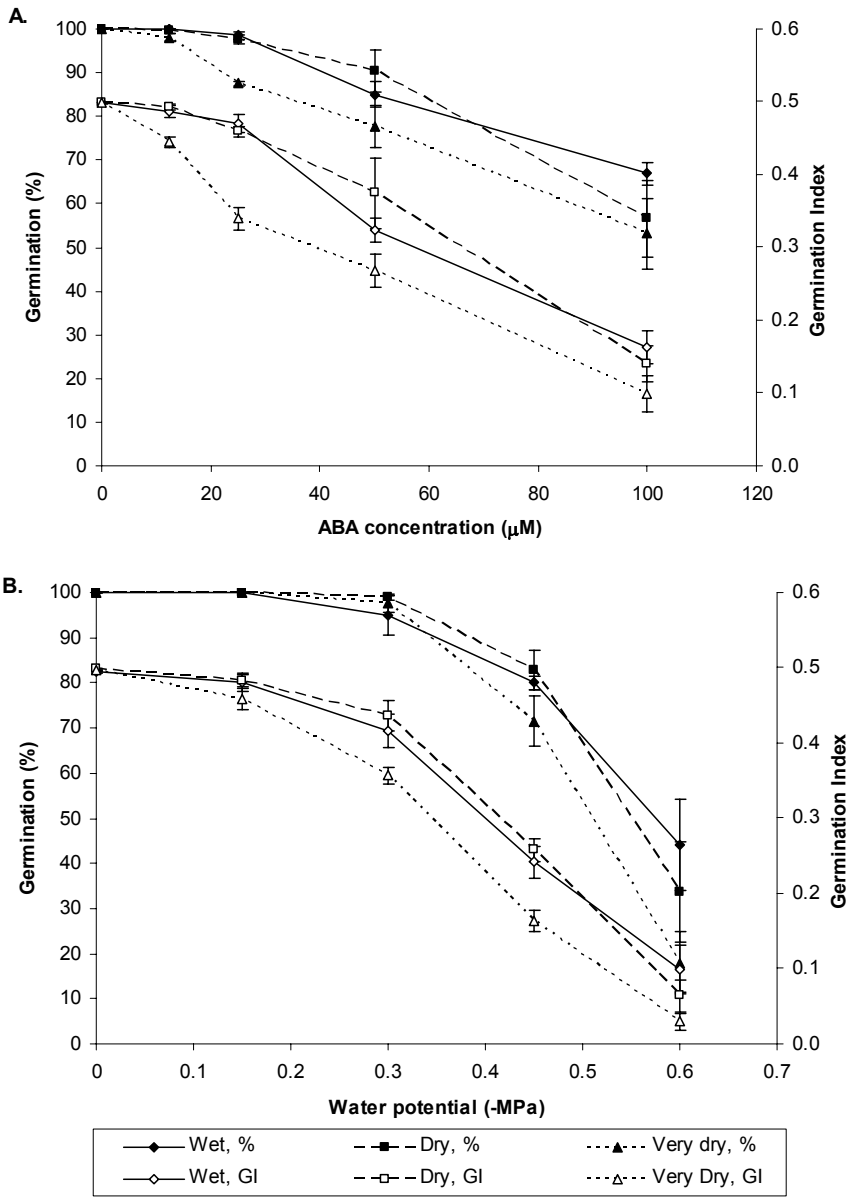


Figure 3.2: Germination percentage (%) and germination index (GI) of lettuce seeds from wet (diamond), dry (square), and very dry (triangle) treatments (Exp. 1) at five ABA concentrations (A) and five water potentials (B). Data are means +/- SE of four replications of 50 seeds each.

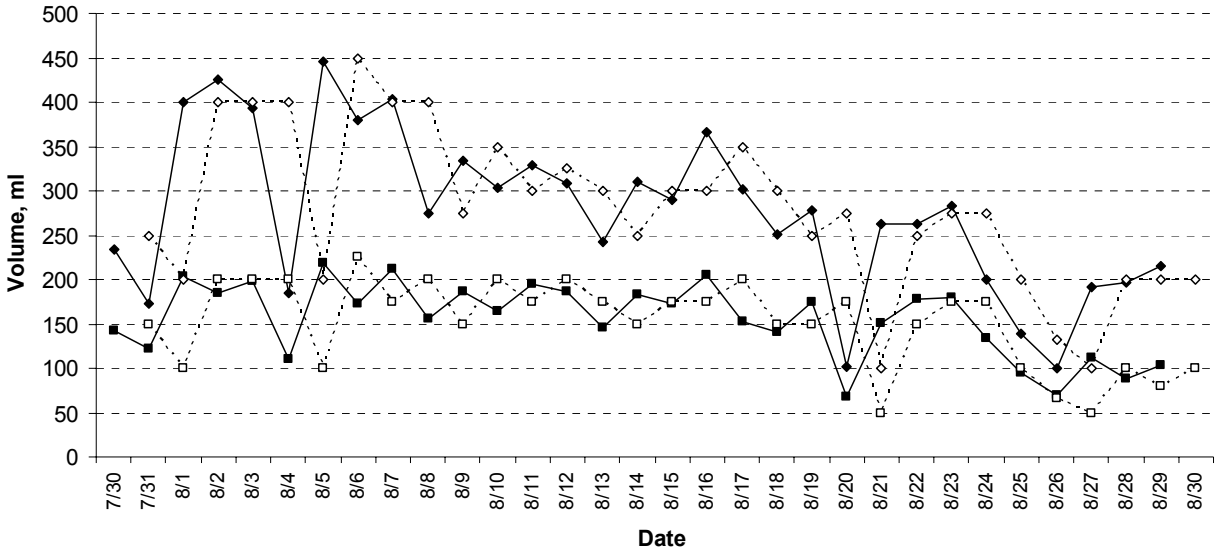


Figure 3.3: Daily evapotranspiration (solid line) and watering (broken line) volumes for lettuce plants from *wet* (diamonds) and *dry* (squares) treatments from early flowering to last seed harvest (Exp. 2).

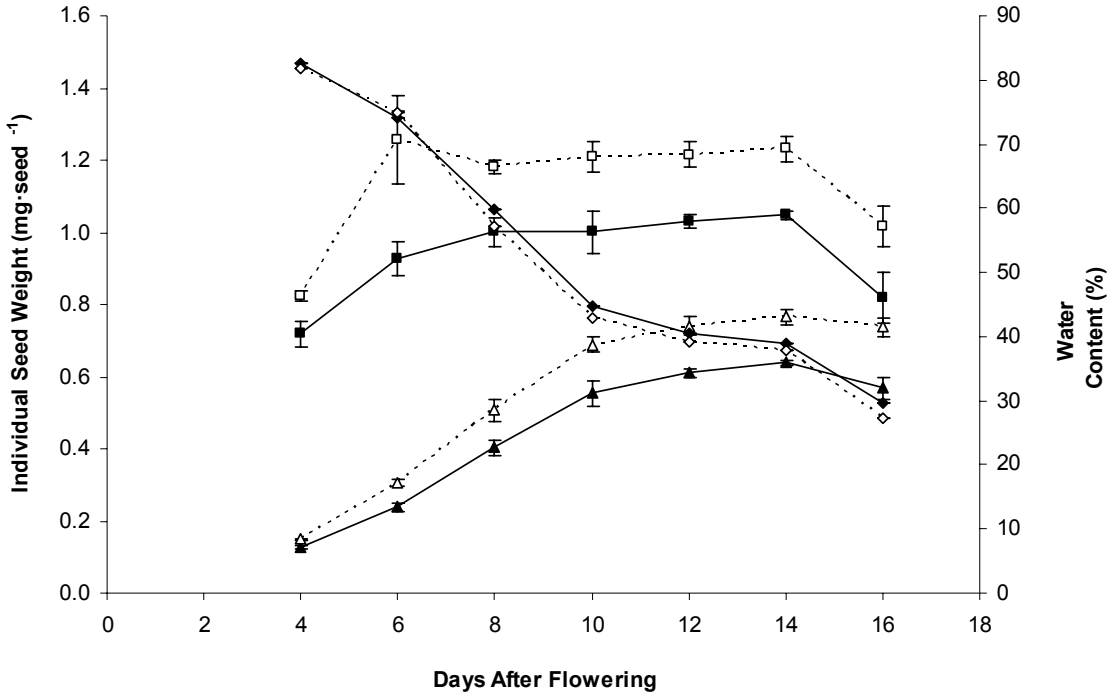


Figure 3.4: Fresh weight (squares), dry weight (triangles), and water content (diamonds) of individual lettuce seeds from wet (solid line) and dry (broken line) treatments during seed development from 4 to 16 DAF (Exp. 2). Data are means +/- SE calculated from four replications (seeds from six flower heads were evaluated in each replication).

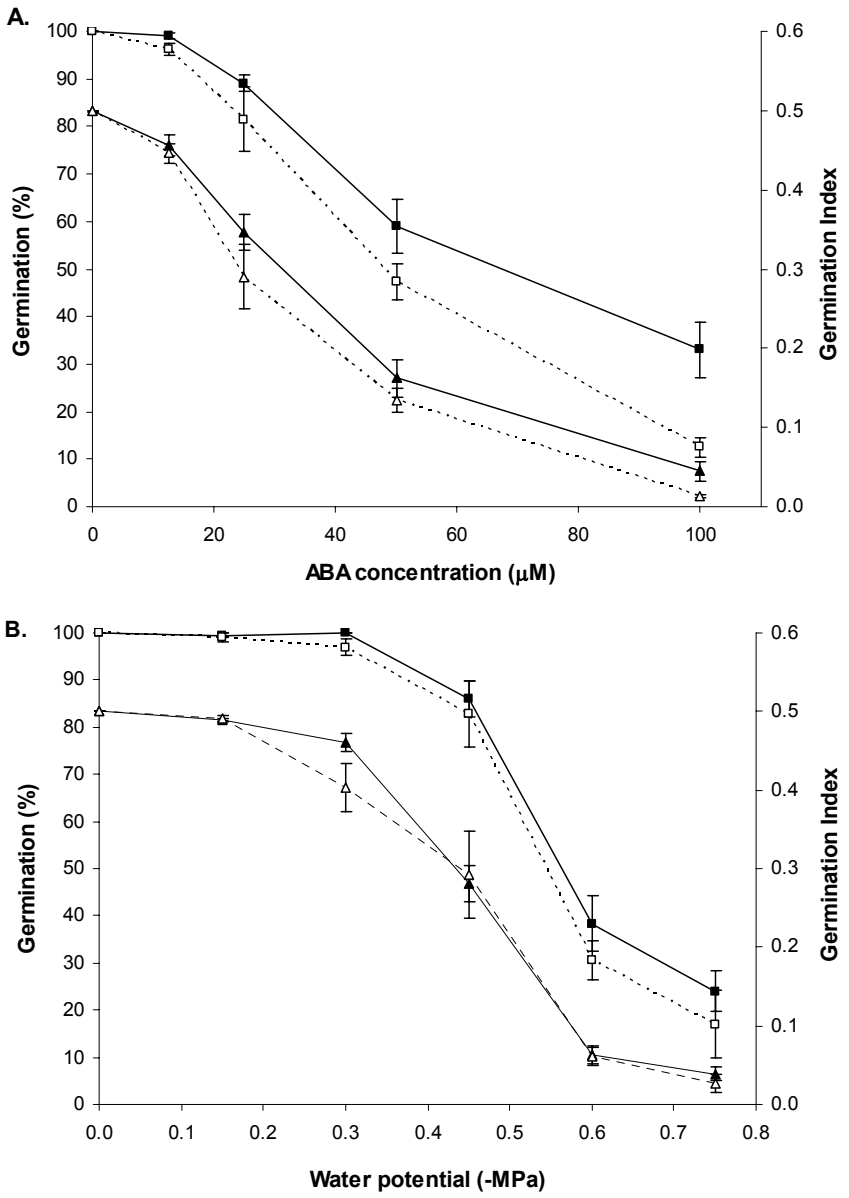


Figure 3.5: Germination percentage (squares) and germination index (triangles) of lettuce seeds from wet (solid line) and dry (broken line) treatments (Exp. 2) at five ABA concentrations (A) and five water potentials (B). Data are means +/- SE of four replications of 50 seeds each.

CHAPTER 4

DAY-LENGTH DURING SEED DEVELOPMENT AFFECTS LETTUCE SEED WEIGHT, GERMINABILITY AND STORABILITY

ABSTRACT

Seed germinability and storability are important aspects of seed quality determined by the genotype and environment of seed development. Lettuce is produced commercially in most temperate and subtropical areas of the world. The objective of this study was to determine how day-length of the mother plant environment affects lettuce seed quality. Seeds of cv. Tango were produced in growth chambers under one of two treatments: i) short day (SD), consisting of 8 h of fluorescent light (≈ 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$) plus 16 h of darkness daily, and ii) long day (LD), consisting of 4 h incandescent light (≈ 21 $\mu mol \cdot m^{-2} \cdot s^{-1}$), 8 h fluorescent light, 4 h of incandescent light, and 8 h of darkness daily. 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. At suboptimal conditions (30°C, 20°C with different external ABA concentrations, negative osmotic potentials, or dark), seed germinability (% and

rates) from the SD treatment was higher. After accelerated aging (41°C, ≈ 100% RH, 72 h), germination of normal seedlings was higher for seeds from LD. Seed germination was also evaluated after 2, 4, 6, 8 and 10 months of storage at 30°C, 74% RH. Stored seed had a progressive and significant reduction in germinability for both treatments; however, seeds from SD were more influenced by these conditions. The results indicated that day-length during seed development affected lettuce seed weight, germinability, and storability. Germinability and storability were inversely related. The critical period for day-length effects was also studied. Seed weight patterns were determined early in seed development, during the first 6 d after flowering. Conversely, day-length effects on seed germinability and storability were determined at the end of seed development, after physiological maturity, which occurred by 11 d 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.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most important vegetables in the world. In the USA, between 2001 and 2006, lettuce was cultivated on over 121,000 ha per year with an annual crop value of approximately 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; Wurr and

Fellows, 1985; Wien, 1997). Thermoinhibition (sensitivity to high temperatures) and photodormancy (lack of germination in dark) are two characteristics frequently found in some lettuce cultivars that reduce speed and uniformity in seed germination and seedling emergence in the field (Wien, 1997; Ryder, 1999). A common approach to overcome germination problems in lettuce has been to treat the seeds prior to sowing. For instance, seed priming improves germination and emergence of lettuce seeds under high temperatures (Cantliffe et al., 1981; Valdes 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 (Fenner, 1991, 1992; Hilhorst and Toorop, 1997; Baskin and Baskin, 1998; Gutterman, 2000). Some of the frequently studied environmental factors are temperature, water availability, light (quality and photoperiod), altitude, and mineral nutrition. In most studies where day-length 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 a few 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). 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; Hilhorst and Toorop, 1997; Gutterman, 2000) and the management of particular environmental conditions, such as day-length, 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. Despite the fact that 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.

The main objectives of this study were *i)* to investigate wether day-length of the mother plant affects lettuce seed quality, and *ii)* assess the relationship between lettuce seed germinability and storability.

MATERIALS AND METHODS

Two experiments were performed to determine *i)* effects of day-length on lettuce seed quality, and *ii)* critical period during lettuce seed development for day-length effects.

Experiment 1, effects of day-length

Lettuce plants (cv. 'Tango') 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: i) *short day* (**SD**), consisting of 8 h fluorescent light (photosynthetic photon flux (PPF)≈ 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$) plus 16 h of darkness daily, and ii) *long day* (**LD**), consisting of 4 h incandescent light (PPF≈ 21 $\mu mol \cdot m^{-2} \cdot s^{-1}$), 8 h fluorescent light, 4 h of incandescent light, and 8 h of darkness daily. 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 approximately 8.5% water content) 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% (fresh weight 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 x 11 x 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 distilled water, or 10 mL of solution containing various concentrations of (±) abscisic acid (ABA; Sigma-Aldrich, St. Louis, MO) or polyethylene glycol (PEG 8000, Sigma-Aldrich, St. Louis, MO) 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 as the algebraic sum of the ratio of germinated seeds and days after sowing at the count moment. Germination in dark was performed using black 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 x 11 x 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 approximately 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 0.5g/L citric acid monohydrate and 100 mg/L 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 1500g 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) 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 sub-samples 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. The R:FR ratio was calculated as the sum of wavelengths between 656 and 664 nm divided by the sum of wavelengths between 726 and 734 nm. Light spectral irradiance was measured by using a portable spectroradiometer (LI-1800, LI-COR Biosciences, Lincoln, NE). 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.

Experiment 2, critical moment determination

Four lettuce (cv. 'Tango') plants 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; ***S12***, 12 d in SD, then LD. From 3 days after flowering (DAF) and each alternate 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 Exp. 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 Exp. 1. Differences among treatments were analyzed using ANOVA and significantly different means separated using least significant difference (LSD, α= 0.05). Specific groups of treatments were compared by contrast analysis.

RESULTS

Experiment 1

Lettuce seeds produced under LD were significantly heavier than those produced under SD conditions (Table 4.1). At 20°C seed from SD and LD treatments had 100% germination and produced similar percentages of normal seedlings, however the GI was significantly higher for seeds from the SD treatment (Table 4.1). 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 4.1). Conversely, seed from the LD treatment outperformed seed from the SD treatment in the AA test, producing a significantly higher percentage of normal seedlings after aging (Table 4.1). When vigor was assessed by SVIS, the vigor index and radicle length values were similar for lettuce seedlings from SD and LD treatments (Table 4.1). ABA concentration in mature seeds was significantly higher in seeds from the LD treatment (Table 4.1).

Day-length during lettuce seed development affected dark germination at three of the four evaluated temperatures. Seeds from SD had a significantly higher dark germination at 14 (p= 0.018), 19 (p= 0.019), and 24°C (p= 0.039). However, at 29°C seeds from both treatments had 0% dark germination (Fig. 4.1). The day-length treatments also affected lettuce seed germinability at increased exogenous ABA levels and reduced water potentials (Fig. 4.2). In both treatments, germination percentage and GI were reduced by higher ABA concentrations (Fig. 4.2a) or lower water potentials (Fig. 4.2b), although seeds from LD had greater reductions in these values than seeds from SD.

When SG was evaluated 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 four months of storage, produced an average of 91% normal seedlings compared with 22% from the SD seeds (Fig. 4.3). The results from the AA test had a strong correlation (r= 0.94; p= 0.005) with SG after four months storage (Table 4.2). The correlation coefficient (r-value) also was calculated between the values of lettuce seed performance after four months storage (normal seedlings percentage and GI) and lettuce seed germinability under different conditions; some of the r-values and their significance (p-value) are presented in Table 4.2. In general, seed germinability and storability

parameters were inversely correlated (negative *r*-values); however, the only parameter of germinability that had a significant correlation with the three parameters used for storability was dark germination (Table 4.2).

Dark germination at 20°C of seeds from the SD treatment was reduced from 69 to 10% by successive FR light breaks during the first 24 h of germination (Table 4.3).

Experiment 2

Although LD and SD treatments differed in the amount of dry matter accumulated by lettuce seeds, the pattern of development was similar (Fig. 4.4). Seed physiological maturity (PM, maximum dry weight), 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. 4.4). Consequently, 6 DAF (time of plant transfer for the *L6* and *S6* treatments) represented approximately half of PM, whereas 12 DAF (moment of plant movement for the *L12* and *S12* treatments) represented approximately one day 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. 4.5a). When the average seed weight of *L*, *L6* and *L12* was compared to 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 day-length treatment on individual seed dry weight was produced early in seed development, during the first 6 DAF or first half of the PM process. Seed germinability was assessed as dark germination at 20°C (Fig. 4.5b),

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 of seed from *S*, *L6*, and *L12* was compared to 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, day-length effect on seed storability (assessed by the AA test) was produced during the latter stages 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 to those of seeds from *S*, *L6*, and *L12* treatments (Fig. 4.5c).

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 Exp. 1, seed performance was evaluated by *i)* SG test or the ability to produce normal seedlings under optimal (20°C-light) conditions, *ii)* germinability (radicle emergence) at different optimal and sub-optimal conditions, *iii)* seedling growth and uniformity (vigor index and radicle length from SVIS), and *iv)* the AA test. Seeds produced under LD were

significantly heavier than seeds from SD; however, no differences were observed in SG (Table 4.1). At 20°C, germination rates, expressed as GI, were slightly but significantly higher for seed from SD (Table 4.1), 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. (Muchena and Grogan, 1977; Shieh and McDonald, 1982; Bennett et al., 1988), *Erodium brachycarpum* Godr. (Stamp, 1990) and *Pastinaca sativa* L. (Hendrix, 1984).

Seeds from SD showed significantly better germination (percentage and GI) at 30°C (Table 4.1). Thermoinhibition of lettuce seed germination at high temperature (≥ 30°C) is one of the most important problems affecting lettuce seedling establishment (Wien, 1997). Different levels of thermoinhibition during seed germination have been observed among lettuce types and cultivars (Gray, 1975; Kozarewa et al., 2006). Seeds from cv. 'Tango' are sensitive to high temperatures during germination (H.J. Hill, personal communication). In fact, seed from SD and LD had poorer germination when germinated at 30°C compared to 20°C, but seeds from LD were significantly more affected than seeds from SD (Table 4.1). 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 to 20/10°C, day/night) in reducing seed thermoinhibition (Koller, 1962; Gray et al., 1988; Drew and Brocklehurst, 1990; Sung et al., 1998; Kozarewa et al., 2006); however, the effect of day-length 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. The few data available indicate that germinability at any of the three temperatures evaluated was better for seed produced under longer days. His data contradict my results, where SD during lettuce seed production improved seed germination at higher temperatures compared to LD (Table 4.1). 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, 19, and 24°C (Figure 4.1). 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; van der Woude and Toole, 1980; Toyomasu et al., 1998). The degree of light sensitivity in lettuce varies among cultivars and 'Tango' is described as a photosensitive genotype (H.J. Hill, personal communication), consistent with these results. Additionally, light requirements for germination of photosensitive lettuce genotypes increase with temperature (Ikuma and Thimann, 1964; van der Woude and Toole, 1980; Sung et al. 1998), which explains the lack of germination observed at 29°C (Fig. 4.1). Higher germinability in dark of seed produced by lettuce plants under SD 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 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. 4.2a) and decreased water potentials (Fig. 4.2b) during germination. Seed dormancy has been positively related with ABA presence or sensitivity of seeds to this phytohormone (Benech-Arnold et al., 1991; Ni and Bradford, 1993; Yogeesha et al., 2006; Finch-Savage and Leubner-Metzger, 2006), and also sensitivity of germination to water potential (Ni and Bradford, 1993). Thus, seeds from plants under LD would be expected to be 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 4.1). 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 day-length 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). My results suggest that the higher germinability observed in seeds from SD could be explained, in part, by lower seed ABA sensitivity and content.

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 (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). When used to evaluate lettuce seed vigor, the vigor index from SVIS has produced positive correlations with field emergence (Contreras and Barros, 2003). In my experiments, no differences between seeds from SD and LD treatments were observed for the vigor index and average radicle length (Table 4.1). 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 (three days after sowing) no differences in seedling growth were observed between treatments. The AA test has been used for evaluation of seed storability and vigor (Copeland and McDonald, 2001). Lettuce ('Tango') seeds from LD performed better after AA, producing a greater number of normal seedlings than seed from SD conditions (Table 4.1). These results suggest that seeds from LD, despite their lower germinability, are more vigorous and longer lived than seeds from SD. This conclusion is supported by the evaluation of SG after different periods of storage at 30°C and 74% RH (Fig. 4.3). Based on these results, day-length treatments during lettuce seed development not only affected seed germinability, but also seed storability. A causal relationship between seed storability and some forms of physiological dormancy has been previously suggested (Hilhorst and Toorop, 1997; Zarbakhsh et al., 1999; Tesnier et al., 2002).

However, this hypothesis remains controversial (Fueyo et al., 2003). In my experiments, the level of photodormancy exhibited by 'Tango' lettuce seeds was significantly correlated to seed storability (Table 4.2). 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, longevity 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 my study between lettuce seed dormancy 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.

Based on results from Exp. 2, the effect of day-length treatments on lettuce seed weight occurs early in seed development, during the first half of PM (Fig. 4.5a). 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 (Izzeldin et al., 1980; Chapter 3), 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 my results since the number of flower heads or seeds per plant were not evaluated.

Effects of day-length treatments on 'Tango' lettuce seed germinability and storability were produced at the end of seed development, after PM (Figs. 4.5b, c). Maximum seed quality during seed development is believed to coincide with PM, or the moment of maximum dry weight, after which viability and vigor 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 and b; Sinniah et al., 1998; Welbaum, 1999). Based on my results, 'Tango' lettuce seed germinability and storability may increase or decrease after PM depending on the day-length of the mother plant environment (Fig. 4.5). 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 my experiments, lettuce seeds were approximately 40% water content at PM, and above 35% for at least 4 d following PM; 6 d after PM, seeds were approximately 8.5% water content, which was maintained until harvest (Fig.4.4).

The light requirement for lettuce seed germination is mediated by the action of phytochrome, a soluble protein found in two interconvertible forms: *Pr*, which is the red light absorbing form, and *Pfr*, which absorbs far red light (Taiz and Zeiger, 2002). *Pfr* is the active form of phytochrome, required for occurrence of phytochrome controlled events, such as lettuce seed germination. 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 *preexistent Pfr* will depend on the light quality and intensity to which seeds were exposed at the end of seed development and dehydration. In my experiments, the SD treatment consisted only of fluorescent light, which is relatively rich in red light (R:FR ≈ 6.8; Chapter 5). 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; Chapter 5). These differences in light quality favor higher accumulation of *Pfr* in seeds from plants under SD compared to 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 4.3) supports the idea that seeds from SD had a higher content of *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. Light quality during seed development affected the light requirements for seed germination in *Arabidopsis thaliana* L. (McCullough and Shropshire, 1970; Hayes and Klein, 1974), *Bidens pilosa* L. (Fenner, 1980), *Cucumis sativus* L. and *C. 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 conditions during seed drying strongly affect light requirements for germination. I have conducted additional experiments to test the hypothesis that light quality during lettuce seed production affects seed germinability and storability (Chapter 5).

There is evidence that *Pfr* would promote seed germination by promoting gibberellin biosynthesis and suppressing ABA formation (Toyomasu et al., 1998; Roth-Bejerano et al., 1999). If this is the case, differences in *Pfr* concentration could explain higher ABA concentration observed in mature lettuce seeds produced under the LD conditions applied in my experiments (i.e. day extension with a FR-rich light). Differences in *Pfr* would also indirectly explain the higher germinability and low storability of 'Tango' lettuce seeds produced under the SD conditions. Further research is required to confirm possible cause-effect relationships between *Pfr* presence and lettuce seed storability.

In conclusion, the day-length treatments applied in these experiments affected the weight, germinability and storability of lettuce seeds cv 'Tango'. Effects on seed weight were determined during the first 6 days of seed development (first half of the time needed to reach PM), while day-length 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 my 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, I hypothesize that light quality during lettuce seed production plays a critical role in seed germinability and storability and this hypothesis will be tested in the next chapter.

Parameter	LD	SD	*p*- value[1]
Dry weight (mg·seed^{-1})	0.84	0.73	0.001
Normal seedlings at 20°C, %	99.7	98.7	0.423
Germination at 20°C, %	100	100	-
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[2], %	66	5	0.025
Vigor index[3]	785	778	0.793
Radicle length (pixels·seedling^{-1})[3]	357	387	0.085
Seed ABA content (pg·mg dry weight^{-1})	84	37	0.038

[1]: 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)
[2]: AA, accelerated aging of the seeds at 41°C and ~100%RH for 72h
[3]: Values from SVIS© (Seed Vigor Image System)

Table 4.1: Quality attributes for lettuce seed produced under long (LD, 16 h light) and short (SD, 8 h light) days.

Storability Parameter	Germination after AA[2]	Germinability parameter		
		Dark germination at 19°C	Germination in ABA, 50 µM	Germination at 30°C
Normal seedlings after 4 months storage[1]	0.940 (0.005)	-0.983 (<0.001)[3]	-0.586 (0.221)	-0.687 (0.132)
Germination Index after 4 months 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)

[1]: storage at 30°C and 74% RH
[2]: AA= accelerated aging, 72 h at 41°C and ~100%RH
[3]: *p*-value for the correlation

Table 4.2: Correlation coefficients between lettuce seed germinability and storability parameters.

Germination condition	Day-length treatment		*p*-value[1]
	LD	SD	
Dark	2.3 ± 1.5	69.3 ± 12.4	0.010
Dark + FR	1.0 ± 0.6	10.3 ± 7.33	0.147

[1]: calculated from analysis of variance with transformed data (arcsin of the square root of the fraction value)

Table 4.3: Germination percentage at 20°C in continuous dark and dark plus FR breaks for lettuce seeds produced in long (LD, 16 h) and short (SD, 8 h) days. Data are means ± SE of three replications.

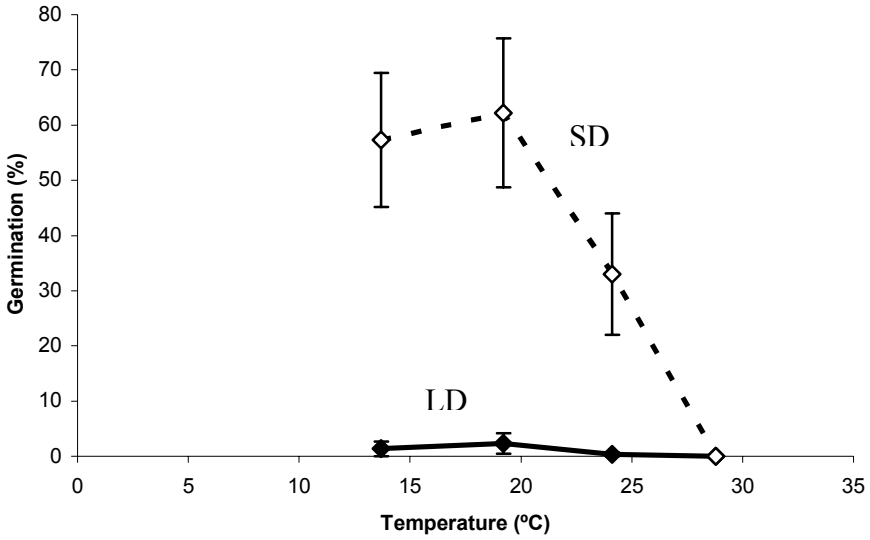


Figure 4.1: Germination percentage at different temperatures in dark of lettuce seeds produced in long (LD, 16 h; solid line) and short (SD, 8 h; broken line) days. Data are means ± SE of three replications.

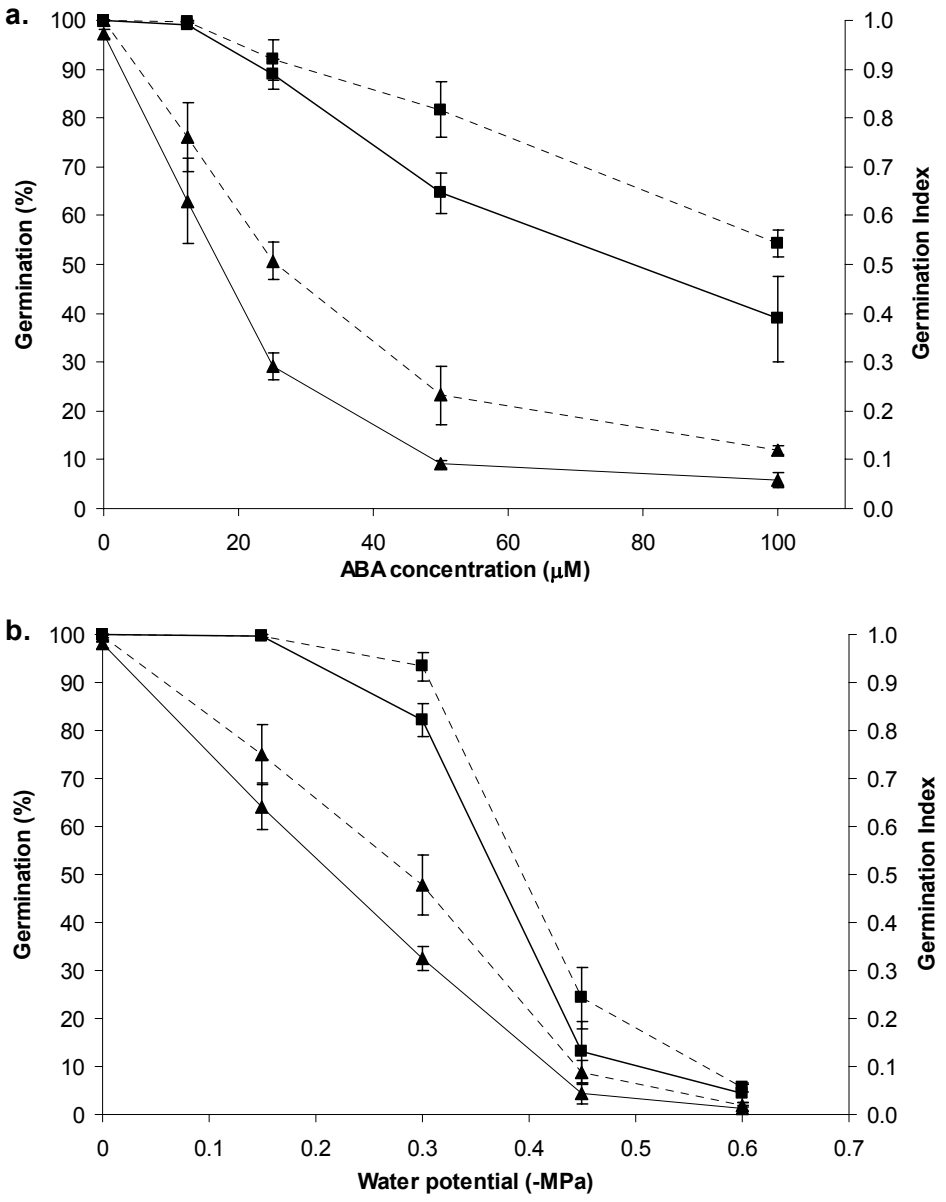


Figure 4.2: Germination percentage (square) and germination index (triangle) under 20°C-light at different external abscisic acid (ABA) concentrations (a) and water potential (b) of lettuce seeds produced under long (16 h, solid line) and short (8 h, broken line) days. Data are means ± SE of three replications.

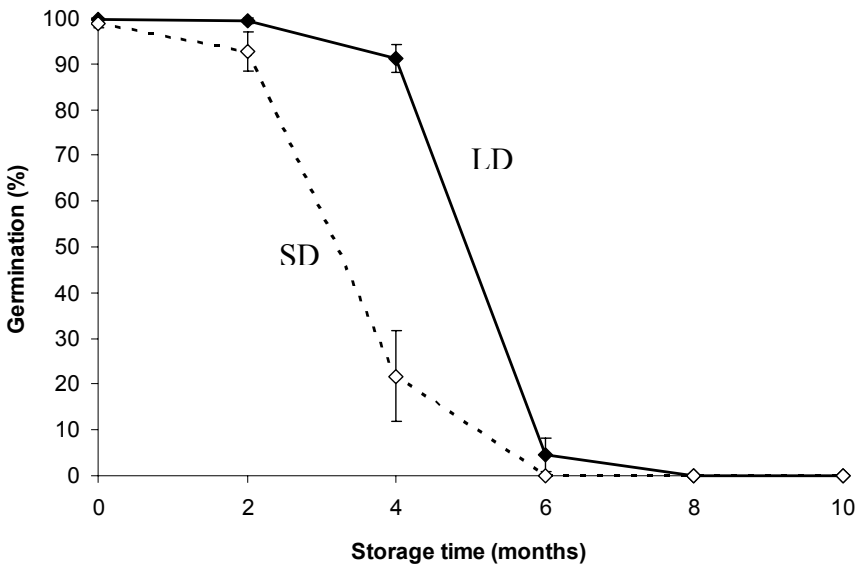


Figure 4.3: Lettuce seed germination percentage of normal seedlings after different storage periods at 30°C and 74% RH of seeds from long (LD, 16 h, solid line) and short (SD, 8 h, broken line) day treatments. Data are means ± SE of three replications.

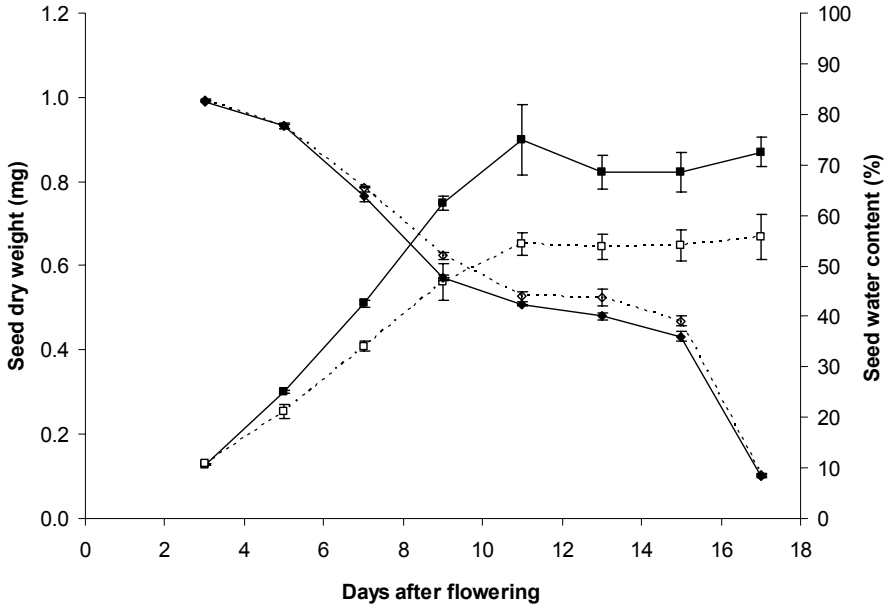
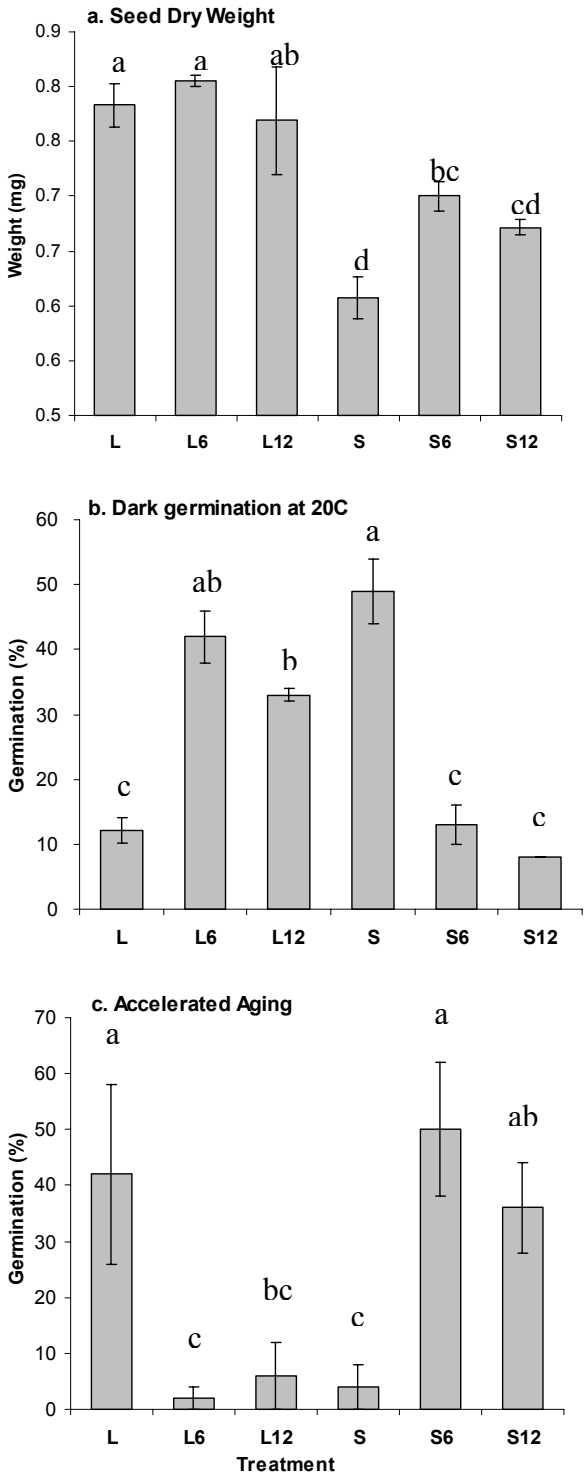


Figure 4.4: Dry weight accumulation (squares) and seed water content (diamonds) during development of lettuce seeds produced under long (16 h, solid line) and short (8 h, broken line) days. Data are means ± SE of two replications.

Figure 4.5: Seed dry weight (a), dark germination at 20°C (b), and normal seedling after accelerated aging (c) from lettuce seeds produced under different combinations of long (LD, 16 h) and short (LD, 8 h) days: **L**, LD throughout seed development; **S**, SD throughout seed development; **L6**, 6 days in LD, then SD; **S6**, 6 days in SD, then LD; **L12**, 12 days in LD, then SD; **S12**, 12 day in SD, then LD. Data are means ± SE. In the same graphic, treatments with different letters are significantly different (LSD, a= 0.05).


a. Seed Dry Weight
Weight (mg)
0.9
0.8
0.8
0.7
0.7
0.6
0.6
0.5
a
a
ab
d
bc
cd
L
L6
L12
S
S6
S12
b. Dark germination at 20C
Germination (%)
60
50
40
30
20
10
0
c
ab
b
a
c
c
L
L6
L12
S
S6
S12
c. Accelerated Aging
Germination (%)
70
60
50
40
30
20
10
0
a
c
bc
c
a
ab
L
L6
L12
S
S6
S12
Treatment

CHAPTER 5

RED TO FAR-RED RATIO DURING SEED DEVELOPMENT AFFECTS LETTUCE SEED GERMINABILITY AND STORABILITY

ABSTRACT

Lettuce is one of the most important vegetable crops in the world. Thermoinhibition and photodormancy are two characteristics of lettuce seed that frequently reduce germination and seedling emergence in the field. In addition to germinability, storability is an important aspect of lettuce seed quality. The main objective of this study was to evaluate the effects of producing lettuce seeds under light with contrasting red to far-red ratios (R:FR) on seed germinability and storability. 'Tango' lettuce seeds were produced in growth chambers under one of two treatments: i) Red-rich light (R-treatment), and ii) Far-red-rich light (FR-treatment). Seeds produced under the FR-treatment were 5% heavier than seeds from the R-treatment, but in both cases the percentage normal seedlings germinated at 20°C-light was approximately 100%. When germinated in the dark, seeds from the R-treatment germinated 100% between 12 and 23°C, and over 50% at 30°C, while seeds from the FR-treatment

germinated less than 35% between 12 and 23°C and less than 5% at 30°C. When germinating under light, seeds from the R-treatment had higher germination percentages and rates under a broader range of temperatures, having less thermoinhibition than seeds from the FR-treatment. Seeds from the R-treatment had lower abscisic acid (ABA) content and were better able to germinate when exposed to external ABA concentrations and reduced water potentials than seeds from the FR-treatment. Seed storability as assessed by the accelerated aging test was higher in seeds from the FR-treatment. My results suggest that seed production under environments with higher R:FR light represents a novel approach to the production of lettuce seeds with lower thermoinhibition and photodormancy; however, reduction in seed size and storability are two undesired consequences.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is the most important fresh vegetable in the USA; between 2001 and 2006, it was cultivated on over 121,000 ha per year with an annual crop value of approximately 2 billion dollars (USDA, 2007). Lettuce seed thermoinhibition (sensitivity to high temperatures) and photodormancy (lack of germination in dark) are two characteristics that frequently affect speed and uniformity of seedling emergence, making it difficult to attain successful crop establishment in the field (Wien, 1997; Ryder, 1999). Seed treatments, such as priming, have been successful in improving lettuce seed germination and seedling emergence under unfavorable conditions (Cantliffe et al., 1981; Valdes et al., 1985). However, these treatments

represent additional costs and manipulation of the harvested seeds, as well as a potential reduction in storability of primed seed lots (Tarquis and Bradford, 1992). A better approach would be to produce more vigorous or less dormant seeds in the field.

Levels of thermoinhibition and photodormancy vary not only among lettuce genotypes (Gray, 1975; Sung et al., 1998; Kozarewa et al., 2006), but also among seed lots of the same cultivar (Wurr et al. 1986; Sung et al., 1998). Differences in germinability among seedlots within cultivars are mainly due to the particular environmental conditions under which each seedlot was produced (Wurr et al., 1986). For example, Izzeldin et al. (1980) reported significant increases in lettuce seed vigor (measured as seedling radicle elongation) from seeds that developed from plants under water stress, and a positive correlation between lettuce seed germinability and temperature of the maternal environment has been extensively reported (Koller, 1962; Gray et al., 1988; Drew and Brocklehurst, 1990; Sung et al., 1998; Kozarewa et al. 2006). Light is another environmental factor affecting lettuce seed quality during production. Koller (1962) reported that seeds produced under 24 h of light had higher germination at 20, 23, and 26°C than seeds produced under 8 h of light. Gutterman (1973) reported that lettuce seeds from plants grown under 8 h of light had higher germination than seeds from plants grown under 16 h of light (germination was evaluated after 48 h at 26°C in dark with one light break of 5 min white light 1.5 h after sowing). In a previous work (Chapter 4), I observed that ‘Tango’ lettuce seeds produced under short days (SD: 8 h fluorescent light) had reduced thermoinhibition, photodormancy, and storability compared with seeds produced under long days (LD: 4 h incandescent light + 8 h fluorescent light + 4 h incandescent light). The effects of the day-length treatments were

observed to occur at the end of seed development, after physiological maturity (maximum dry weight accumulation). Because of the importance of phytochrome in regulating photodormancy and the methodology used in these experiments (extension of day-length with a far-red-rich source of light), I hypothesized that light quality, rather than hours of light, would be the critical factor explaining differences in germinability and storability between lettuce seeds produced under SD vs. LD treatments. Light quality during seed development affected the light requirements for seed germination in *Arabidopsis thaliana* (McCullough and Shropshire, 1970; Hayes and Klein, 1974) and *Piper auritum* (Orozco-Segovia et al., 1993). Cresswell and Grime (1981) studied light requirements for the germination of 21 species and concluded that light conditions during seed drying strongly affect light requirements for germination.

Understanding the influence of the maternal environment light quality on seed germinability and storability could assist in the production and handling of high quality lettuce seeds. The main objective of this study was to test the hypothesis that lettuce seeds produced under light with a high red to far-red (R:FR) ratio have better germinability and poorer storability than seeds produced under light with lower R:FR ratio.

MATERIALS AND METHODS

Two experiments were performed to determine the effects of the spectral composition on lettuce seed quality: *i)* effects during lettuce seed production (intact plants), and *ii)* effects during artificial seed desiccation (flower heads removed from the mother plant).

Experiment 1, light quality effects during seed production

Lettuce (*Lactuca sativa* L. cv. 'Tango') plants were produced in the greenhouse in 1.75 L plastic pots filled with a soilless growing media (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: i) *Red-rich light* (**R-treatment**) consisting of a main light period of 10 h fluorescent light with a photosynthetic photon flux (PPF) of 224 ± 27 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a R:FR = 6.8, plus a supplement of 4 h fluorescent light with a PPF = 29 ± 2 and R:FR = 8.4, and ii) *Far-red-rich light* (**FR-treatment**) consisting of a main light period of 10 h fluorescent and incandescent light with a PPF = 227 ±31 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a R:FR = 1.5, plus a supplement of 4 h incandescent light with a PPF = 23 ± 2 and R:FR = 1.0. In both treatments there were 10 h of dark and the 4 h supplement light period was split using 2 h before and 2 h after the main light period. The temperature was 25°C during the main and complementary light periods and 15°C during dark. The R:FR ratio was calculated as the sum of wavelengths between 656 and 664 nm divided by the sum of wavelengths between 726 and 734 nm. Light spectral irradiance was measured by using a portable spectroradiometer (LI-1800, LI-COR Biosciences, Lincoln, NE). There were 12 plants randomly assigned to each treatment (chamber). Seeds were harvested manually by extracting only fully matured flower heads (dry and open, with visible seeds of approximately 8.5% water content, fresh weight

basis) of each plant. Seeds were cleaned and then stored in paper envelopes inside a storage room at 4°C and 25% RH until evaluation. The equilibrium seed water content during storage was 5.1 ± 0.2% (fresh weight basis).

Seed evaluation. For fresh and dry weight determinations, three samples of 50 seeds per treatment were weighed before and after drying in an oven at 103°C for 48 h.

The standard germination (SG) test was conducted using four samples of 50 seeds for each treatment. 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 x 11 x 4 cm). These boxes were placed in a germination chamber at 20°C and constant light. After 4 and 7 d normal seedlings were counted as germinated (ISTA, 1999).

Other germination tests were conducted using four samples of 50 seeds per replication, planted over two layers of blotters saturated in 10 ml distilled water, or 10 mL of solution containing various concentrations of (±) abscisic acid (ABA; Sigma-Aldrich, St. Louis, MO) or polyethylene glycol (PEG 8000, Sigma-Aldrich, St. Louis, MO) and placed in Petri dishes (9 cm diameter). The PEG concentrations were calculated to obtain water potentials of -0.15, -0.30, -0.45, -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 7 d. Germination at 30°C and constant light was evaluated daily to 7 d. The germination index (GI) was calculated as the algebraic sum of the ratio of germinated seeds and days after sowing (DAS) at the count moment. Germination in dark was performed using black

Petri dishes on a thermogradient table (Series #16065, Seed Processing Holland B.V., Enkhuizen, Netherlands) at 12.4, 17.3, 23.4, or 29.7°C; germination was evaluated 4 DAS.

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 11 DAS.

Abscisic acid extraction and determinations. ABA extraction and determination from mature 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 lyophilization, the seeds were ground to powder in liquid nitrogen and then weighed. Methanol containing 0.5g·L^{-1} citric acid monohydrate and 100 mg·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 was determined from this supernatant by using anti-ABA monoclonal specific antibodies and competitive ELISA test according to instructions from Phytodetek® ABA Test Kit (Agdia, Elkhart, IN, USA).

The effects of brief interruptions with far-red (FR) light on dark germination at 20°C were investigated on four sub-samples of 50 seeds per replication. FR light was provided by light emitting diodes (Quantum Devices, Barneveld, WI) with a wavelength peak at 732 nm, a photon flux of 160 μmol·m^{-2}·s^{-1}, and a R:FR ratio of 0.01. One FR break of 5 min was applied at 30, 60, 120, or 240 min after sowing, and repeated FR

breaks of 4 min each were applied at 2, 4, 6, 8, and 24 h after sowing. Seeds were also germinated at 20°C in dark, under constant white light (R:FR= 11.1, photon flux= 24 $\mu mol \cdot m^{-2} \cdot s^{-1}$), or under constant FR light (R:FR= 0.01, photon flux= 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

The data are presented as average values ± standard error (SE) of the average.

Experiment 2, effects during artificial seed desiccation

Nine lettuce plants (cv. 'Tango') were cultivated in the greenhouse as described for Exp. 1. Approximately 14 flower heads per plant were labeled the day of flowering and harvested manually 14 days later. Immediately after harvest, 30 flower heads were randomly assigned to each of the following desiccation treatments: i) *dark*, ii) *total light*, iii) *red light*, and iv) *far-red light*. Seed water content at harvest was calculated on seeds from five flower heads. Seeds from the flower heads assigned to the *dark*, *red light*, and *far-red light* treatments were extracted in a dark room illuminated with green light (from light emitting diodes with a peak at 575 nm), while seeds from flower heads assigned to the *total light* treatment were extracted under fluorescent light in the laboratory. After extraction, the seeds were placed inside square plastic boxes (11 x 11 x 4 cm) containing 100 mL saturated NaBr solution. Seeds were placed on a mesh layer suspended in the plastic boxes to prevent direct contact between the seeds and NaBr solution. All boxes containing the seeds were placed in a chamber at 25°C with constant fluorescent and incandescent light. Boxes of the dark treatment were wrapped with aluminum foil to prevent light from reaching the seeds. The sides and bottom of all other boxes were also wrapped with aluminum foil and only the top cover was transparent. Seeds from the *total*

light treatment received a mix of fluorescent and incandescent light with a R:FR = 1.11. By using filters located over the box covers, seeds from the *red* and *far-red* light treatment were under light with R:FR of 45.53 and 0.01, respectively. The relative humidity inside the boxes was approximately 57%. After three days, a sample of 48 seeds (12 seeds from each treatment) was used for seed water content determination and the rest of the seeds, separated by treatment, were placed inside paper envelopes at 4°C and 25% RH until evaluation. This experiment was repeated four times.

Seed evaluation and data analysis. Dark germination at 20°C, germination at 30°C, and AA tests were conducted using 50 seeds from each replication and similar methodology as described for Exp. 1. Differences among treatments were analyzed using ANOVA and the least significant difference (LSD, α= 0.05) procedure. Percentages and GI values were transformed to the arcsin of the square root of the fraction value before statistical analysis.

RESULTS

Experiment 1

Lettuce seeds produced under the FR-treatment were approximately 5% heavier than seeds from the R-treatment; however, SG was similar for seeds produced under both light conditions (Table 5.1). After 72 h AA at 41°C and ≈ 100%RH, SG of seeds from the R-treatment was more affected, producing less than 11% normal seedlings compared to 98% normal seedlings from the FR-treatment (Table 5.1). ABA content in mature seeds from the FR-treatment was 65% higher than in seeds from the R-treatment (Table 5.1).

Seeds produced under the R-treatment did not require light to germinate between 12.4 and 23.4°C and had greater than 50% germination in dark at 29.7°C, whereas seeds from the FR-treatment germinated no more than 35% in dark between 12.4 and 23.4°C and less than 5% in dark at 29.7°C (Table 5.2, Fig. 5.1). At 20°C under constant FR light, seeds from the R-treatment germinated only 6%, while 0% germination was observed in seeds from the FR-treatment (Table 5.2). Independent of the time during dark germination when one FR light break of 5 min was applied, seeds from the R-treatment were less affected and germinated over 95%, compared with seeds from the FR-treatment that did not germinate more than 10% (Table 5.2). When repeated FR light breaks of 4 min were applied during dark germination, seeds from the FR-treatment were more affected, decreasing from 28 to 1% germination, compared to seeds from the R-treatment where germination decreased from 99 to 86% (Table 5.2).

When germinating under light at temperatures between 13.0 and 33.0°C, germination was close to 100% for seeds from both treatments, but between 34.5 and 38.0°C germination of seeds from the FR-treatment was more affected than for seeds from the R-treatment, which were able to germinate over 94 and 81% at 36.5 and 38.0°C, respectively (Fig. 5.2a). Seeds from the R-treatment had a GI close to 1.0 (complete germination during the first 24 h) between 19.4 and 34.5°C, and over 0.8 at 13.0 and 36.5°C (Fig. 5.2b). On the other hand, seeds from the R-treatment had a GI close to 1.0 at 24.4 and 28.1°C, but were more affected by extreme temperatures than seeds from the R-treatment, having a GI lower than 0.5 at 13.0°C or temperatures over 34.5°C (Fig. 5.2b).

Spectral composition during lettuce seed production also affected seed sensitivity to exogenous ABA levels and reduced water potentials (Fig. 5.3). In both treatments, germination percentage and GI were reduced by higher ABA concentrations (Fig. 5.3a) or lower water potentials (Fig. 5.3b), but seeds from the FR-treatment were more sensitive than seeds from the R-treatment.

Experiment 2

Average seed water content (fresh weight basis) at harvest (14 days after flowering) and after desiccation was 37.8 ± 0.5 and 6.7 ± 0.4%, respectively. The light condition during desiccation affected seed germination in dark; seeds desiccated under red light had 67% germination, significantly more than seeds from the other three treatments (Table 5.3). Production of normal seedlings after AA was no more than 27% for any of the desiccation treatments and seeds from the dark treatment had significantly lower performance than seeds from the total light and far-red light treatments (Table 5.3). Germination at 30°C was poor (≤ 12%) for seeds from any of the desiccation treatments, with no significant differences among treatments (Table 5.3).

DISCUSSION

Previously, I reported that lettuce seeds produced under a LD treatment were heavier, had lower germinability, better storability and higher ABA content than seeds produced under a SD treatment (Chapter 4). In those experiments, the light extension of the LD treatment was attained by using incandescent light, which is rich in FR wavelengths, and I hypothesized that the observed differences were primarily caused by

differences in the R:FR ratio between the SD (rich in R light) and LD (rich in FR light) treatments. Based on this hypothesis, the R- and FR- treatments are equivalent to the SD and LD treatments, respectively. Thus, if my hypothesis is correct, seeds from the FR- treatment should be heavier, have lower germinability, better storability and higher ABA content than seeds from the R-treatment.

Seeds from the FR-treatment were 5% heavier than seeds from the R-treatment (Table 5.1), which is consistent with my hypothesis. Previously (Chapter 4), it was suggested that the differences in seed weight could be associated with the presence of fewer seeds in plants growing under FR-rich light, so the seeds would be heavier due to lower competition for photosynthates. The number of seeds per plant is a function of the number of flower heads per plant and the number of seeds per flower head. In this experiment, no differences in the number of seeds per flower head were observed (data not shown). If the use of light treatments were intended to be used during lettuce seed production, it would be important to test their effects on seed yield, specifically the number of seeds per plant.

Seeds from both treatments had SG close to 100% (Table 5.1). However, when germination was evaluated at sub-optimal conditions, seeds produced under R-treatment had better germinability than seeds from the FR-treatment (Figs. 5.1, 5.2, 5.3). Photodormancy and thermoinhibition are two lettuce seeds characteristics that frequently impede rapid and uniform emergence of seedlings in the field (Wien, 1997; Ryder, 1999), which is essential for attaining high yield and quality in a single harvest (Wurr and Fellows, 1985; Wien, 1997). Seeds produced under R-treatment did not have photodormancy and reached full germination in dark between 12 and 23°C, while seeds

from the FR-treatment did not germinate more than 35% under similar conditions (Fig. 5.1). 'Tango' is a lettuce cultivar characterized as being very photosensitive or photodormant (Chapter 4; H.J. Hill, personal communication) and my results confirm that the dependence of light for seed germination may be modified by the light conditions of the maternal environment. In addition to my previous report (Chapter 4), reduction in photodormancy caused by seed development under environments with higher R:FR has also been observed in *Arabidopsis thaliana* (McCullough and Shropshire, 1970; Hayes and Klein, 1974). At 30°C, dark germination of seeds from both treatments was affected; however, seeds from the R-treatment germinated over 50% compared to only 3% for seeds from the FR-treatment (Fig. 5.1). These results confirm the increase of photodormancy at higher temperatures reported by others (Ikuma and Thimann, 1964; van der Woude and Toole, 1980; Fielding et al., 1992).

When germinating under light, seeds from the R-treatment achieved full and rapid germination over a wider range of temperatures than seeds from the FR-treatment (Fig. 5.2). These results concur with the results presented in Chapter 4 and confirm that maternal environments with higher R:FR during seed development and maturation significantly reduce the thermoinhibition of 'Tango' lettuce seeds. This genotype is characterized by the high thermoinhibition of its seeds, which frequently require seedlots to be primed to ensure successful establishment of the crop (H.J. Hill, personal communication). Several reports have documented the effect of producing lettuce seeds at higher temperatures (e.g., 30/20°C compared to 20/10°C, day/night) in reducing seed thermoinhibition (Koller, 1962; Gray et al., 1988; Drew and Brocklehurst, 1990; Sung et al., 1998; Kozarewa et al., 2006); however, the effects of maternal light quality

environment on lettuce seed thermoinhibition has rarely been studied. Seed production under modified light conditions represents a novel approach to the production of lettuce seeds with improved germinability.

Seeds from the R-treatment had lower ABA content (Table 5.1) and germinated better under elevated external ABA concentrations (Fig. 5.3a) and reduced water potentials (Fig. 5.3b) than seeds from the FR-treatment. These results support the idea that seeds produced under higher R:FR light are less dormant, suggesting that the higher germinability observed in seeds from the R-treatment would be explained, in part, by lower ABA sensitivity and content (Chapter 4). Relief of photodormancy in lettuce seeds is mediated by phytochrome, a soluble protein synthesized as *Pr*, the biologically inactive form that converts into *Pfr* by absorbing R light (Shinomura, 1997). *Pfr* is the biologically active form of phytochrome, which may re-convert into *Pr* by absorbing FR light, and is required for the occurrence of phytochrome controlled events, such as lettuce seed germination. Previously, I reported that the effects of SD and LD treatments on seed germinability and storability occurred after PM, during seed maturation and drying (Chapter 4). At this phase, the water of the seeds varied from ≈ 43% at PM to ≈ 8% at harvest (Chapter 4), which is sufficient for phytochrome photoconversion in lettuce seeds (Vertucci et al., 1987). *Pfr* or some stable intermediate able to yield *Pfr* in the dark may persist in the seed after desiccation and harvest, and the amount of this *preexistent Pfr* will depend on the R:FR ratio and intensity to which seeds were exposed at the end of seed development and dehydration (Taylorson, 1982). Accordingly, at harvest, seeds from the R-treatment would have a higher amount of preexisting *Pfr* than seeds from the FR-treatment, which would explain the observed differences in photodormancy (Fig.

5.1). This explanation is supported by the report of Cresswell and Grime (1981), who studied light requirements for germination of 21 species, and observed that seeds which matured and dried within green tissues required light for germination. These authors concluded that green tissue acts as a light filter which reduces the R:FR ratio of the light that reaches the seeds, so seeds surrounded by green tissue would have most of their phytochrome in the inactive form (*Pr*).

I investigated germination in dark, constant FR-light, or dark plus one or several FR-light breaks to test the hypothesis that differences in photodormancy between seeds from R- and FR-treatments are explained by higher amounts of preexisting *Pfr* in seeds from the R-treatment. When germinated under constant FR-light, seed germination decreased to 6 and 0% in seeds from the R- and FR-treatments, respectively (Table 5.2), which supports my hypothesis. Additionally, after the FR breaks, dark germination of seeds from the FR-treatment decreased from 29 to between 10 and 1% (Table 2), depending on number of FR breaks (repeated breaks were more effective than a single break) and the timing of the break (a single break at 30 min was more effective than a single break at 2 or 4 h). However, a single FR break reduced dark germination of seeds from the R-treatment only marginally (not more than 5%), while repeated FR breaks reduced it to 86% (Table 5.2). The reason why in seed from the FR-treatment breaks of FR-light are relatively ineffective in reducing dark germination compared to constant FR-light is unclear.

It is well documented that seed germination is regulated by the balance of two phytohormones with antagonistic effects: *i)* ABA, which inhibits germination, and *ii)* gibberellin (GA), which induces germination (Kucera et al., 2005; Finch-Savage and

Leubner-Metzger, 2006). In species like lettuce (Toyomasu et al., 1998), and *Arabidopsis* (Yamaguchi et al., 1998), light induces germination through promotion of GA synthesis by *Pfr*. Additionally, Roth-Bejerano et al. (1999) observed that in photodormant lettuce seeds, 2 h of red light during imbibition reduced the ABA accumulated in seeds compared to seeds imbibed in complete darkness. More recently, Seo et al. (2006) reported that in *Arabidopsis* seeds the metabolism (biosynthesis and inactivation) of both GA and ABA was regulated by light (via phytochrome) and suggested that ABA suppresses GA biosynthesis during seed development and germination. Based on this information and the results from my previous (Chapter 4) and current study, I speculate that maternal environments with a high R:FR light improve germinability of lettuce seeds not only by favoring *Pfr* accumulation in the mature seed, but also by inducing phytochrome mediated responses in the seed, such as the promotion of GA accumulation and inhibition of ABA biosynthesis during seed maturation and drying. A significant *Pfr* mediated control of ABA and GA metabolism during seed maturation and drying warrants further research.

Based on the results presented in Chapter 4, where it was shown effects of the SD and LD treatments on seed germinability and storability occurred at the end of seed development (after PM), the main objective of this second experiment was to test if R:FR ratio during artificial seed desiccation of lettuce seeds would have similar effects on seed germinability and storability. In this experiment, seeds were removed from the flower heads when they had ≈ 38% seed water content, after PM and when the flower heads were still green and fully covering the seeds. Desiccation occurred at a constant 25°C and 57% RH under different light conditions. As expected, the light treatment during

desiccation had significant effects on the seed photodormancy (Table 5.3). The highest dark germination percentage was for seeds desiccated under red light, while the lowest was for seeds desiccated under far-red light (Table 5.3). However, dark germination for seeds desiccated under red light was lower than seeds from the R-treatment (Exp. 1) germinated in dark at the same temperature (66.5 vs. 99.5% germination; Tables 5.2, 5.3). The light treatment during desiccation did not affect seed germination at 30°C (Table 5.3), and, seeds from the four treatments had higher levels of thermoinhibition than seeds from Exp. 1 (Fig. 5.2, Table 5.3). These results differed from what was expected based on the data from Exp. 1 and my previous study (Chapter 4). A possible explanation for these differences is that the desiccation rates between seeds that dry in the flower heads (attached to the mother plant) and naked seeds are not the same, which would affect the time available for metabolic changes associated with *Pfr* differences. Previously, I observed that decreases in lettuce seed water content from PM (≈ 40%) to harvest (≈ 8%) occurred over a period of approximately 6 d (Chapter 4). Under the desiccation conditions of Exp. 2, seed water content decreased from 38 to 7% in less than 24 h (data not shown). Additionally, when desiccated in the flower heads, the seeds remain exposed to the light of the maternal environment for a variable time (from a few days to 3 or 4 weeks) before harvesting. During this period, seeds contain approximately 8% water content, which is the limit for phytochrome photoconversion in lettuce seeds (Vertucci et al., 1987); thus additional accumulation of *Pfr* may still occur in the seeds, which was not the case for seeds desiccated artificially in Exp. 2.

Storability, or the ability of the seeds to survive long periods of storage, is another important aspect of seed quality. Previously, I observed that 'Tango' lettuce seed storability was significantly and inversely correlated with dark germination, and that it was significantly improved when a LD treatment was applied to the mother plant between PM and harvest (compared with a SD treatment applied at the same time; Chapter 4). Additionally, lettuce seed storability (germination after 4 months of storage at 30°C and 74% RH) was correlated with results from the AA test (Chapter 4). Thus, the AA test was used to evaluate lettuce seed storability. As expected, lettuce seeds produced under R-treatment had lower storability than seeds from the FR-treatment and there was an inverse relationship between seed storability and dark germination (Table 5.1). In Exp. 2, although there were significant differences in results from the AA test, the values were below 27% normal seedlings for all treatments and the type of differences were not expected (i.e. lower storability for seed desiccated under red light compared to seeds from far-red light; Table 5.3). In this case, these results could be explained by the methodology used in Exp. 2, as was already discussed. These results suggest a possible causal relationship between *Pfr* action and/or presence in seeds and seed storability. Because the importance of proving this relationship for management of seed stocks by seed companies and germplasm conservation, and for understanding seed bank dynamics in native and weed plant populations, it should be further studied in lettuce and other species, especially from the Asteraceae family.

In conclusion, my results provide evidence for the hypothesis that light quality of the maternal environment affects seed germinability and storability of 'Tango' lettuce seeds. Higher R:FR ratios promoted the production of lettuce seeds with poorer

storability and lower levels of thermoinhibition and photodormancy. Modification of the light environment during production of horticultural species has been suggested as a feasible practice for improvement of yield and quality of different types of crops (Clifford et al., 2004; Paul et al., 2005; Paul and Moore, 2006). Producing lettuce seeds under environments with higher R:FR ratio represents a novel approach to the production of lettuce seeds that are able to germinate rapidly and uniformly in a broader range of field conditions. However, undesired reductions in seed size and storability are two negative effects that should also be recognized and studied. Further research should examine the feasibility of modifying light quality conditions as a measure to improve seed quality during the production of other lettuce cultivars and species.

Parameter	**R-treatment**	**FR-treatment**
Dry weight (mg·seed^{-1})	0.753 ±0.002	0.792 ±0.029
Standard germination, normal seedlings %	98.0 ±1.4	99.0 ±0.6
Normal seedlings after AA[(1)], %	10.5 ±1.0	98.0 ±1.3
Seed ABA content (pg·mg dry weight^{-1})	26.5 ±2.1	43.8 ±2.9

[1]: AA, accelerated aging of the seeds at 41°C and ~100% RH for 72 h
[2]: FR, far-red light with a wavelength peak at 732 nm and R:FR= 0.017 during 4 minutes at 2, 4, 6, 8, and 24 h after sowing

Table 5.1: Quality attributes for 'Tango' lettuce seed produced under two different light environments: i) R-treatment (10 h of light with R:FR= 6.8 and 4 h of light with R:FR= 8.4), and ii) FR-treatment (10 h of light with R:FR= 1.5 and 4 h of light with R:FR= 1.0). Data are means ± standard error from 4 samples of 50 seeds each.

Light condition during germination	R-treatment	FR-treatment
Constant white light	99.0 ±0.6	100.0 ±0.0
Constant dark	99.5 ±0.5	28.5 ±4.0
Constant FR[(1)] light	6.0 ±2.9	0.0 ±0.0
Dark + FR[(2)] after 0.5 h	94.5 ±1.5	5.0 ±1.3
Dark + FR after 1.0 h	97.0 ±0.6	6.5 ±2.1
Dark + FR after 2.0 h	95.0 ±1.7	9.5 ±4.3
Dark + FR after 4.0 h	97.5 ±1.0	9.5 ±1.3
Dark + repeated FR[(3)] breaks	86.0 ±3.4	1.0 ±1.0

[1]: Far-red light (R:FR= 0.01, photon flux= 10 μmol·m^{-2}·s^{-1})
[2]: Far-red light (R:FR= 0.01, photon flux= 160 μmol·m^{-2}·s^{-1}) during 5 min
[3]: Far-red light (R:FR= 0.01, photon flux= 160 μmol·m^{-2}·s^{-1}) during 4 min at 2, 4, 6, 8, and 24 h after sowing

Table 5.2: Germination percentage at 20°C under different light conditions for 'Tango' lettuce seeds produced under two different light environments: i) R-treatment (10 h of light with R:FR= 6.8 and 4 h of light with R:FR= 8.4), and ii) FR-treatment (10 h of light with R:FR= 1.5 and 4 h of light with R:FR= 1.0). Data are means ± standard error from 4 samples of 50 seeds each.

Treatment	Dark germination at 20°C, %	Normal seedlings after AA[1], %	Germination at 30°C, %	Germination Index at 30°C
Total light	14.0 b[2]	26.8 a	11.5	0.02
Dark	8.0 bc	6.0 b	7.0	0.02
Red	66.5 a	19.5 ab	8.5	0.02
Far red	1.5 c	21.6 a	5.0	0.01
***p*-value[3]**	<0.001	0.038	0.511	0.374

[1]: AA, accelerated aging of the seeds at 41°C and ~100% RH for 72 h
[2]: in a same column, difference between values with a same later is not significant according with LSD test ($\alpha < 0.05$)
[3]: calculated from analysis of variance

Table 5.3: Effect of spectral composition during artificial desiccation on quality attributes for 'Tango' lettuce seeds with 38% water content harvested 14 d after flowering and desiccated at 20°C, 57% RH and under different light treatments: i) total light (R:FR= 1.1), ii) dark, iii) red (R:FR= 45.5), and iv) far red (R:FR< 0.01).

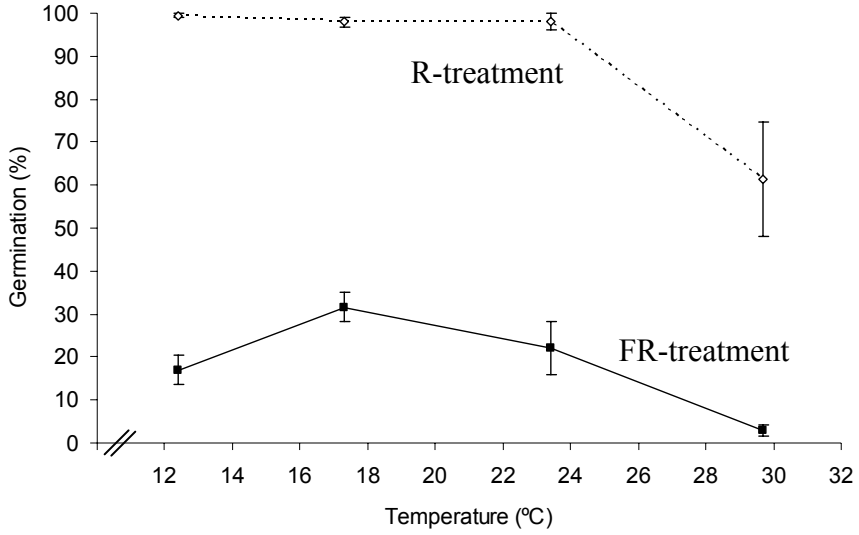


Figure 5.1: Germination percentages at different temperatures in dark of lettuce seeds produced under red-rich-light (R-treatment; broken line) or far-red-rich-light (FR-treatment; solid line). Data are means ±SE from four samples of 50 seeds each.

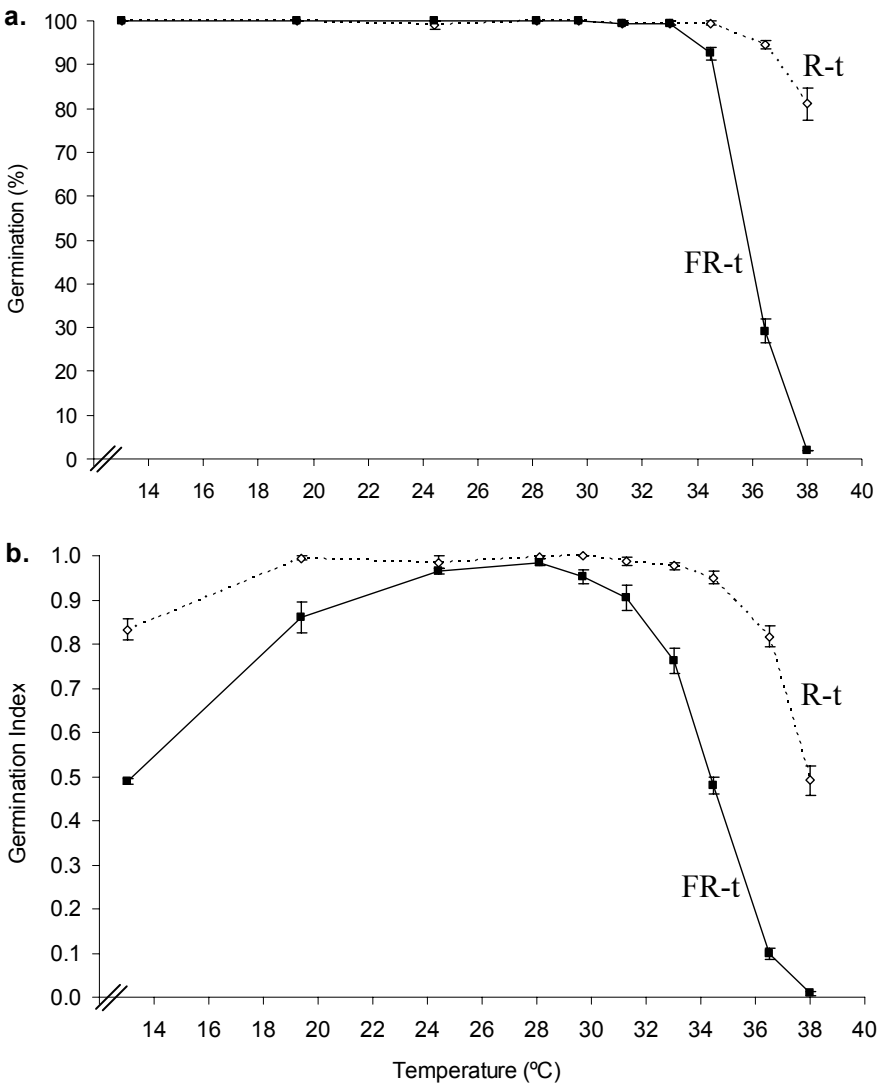


Figure 5.2: Germination percentage (a) and germination index (b) at different temperatures in light of lettuce seeds produced under red-rich-light (R-t; broken line) or far-red-rich-light (FR-t; solid line). Data are means ±SE from four samples of 50 seeds each.

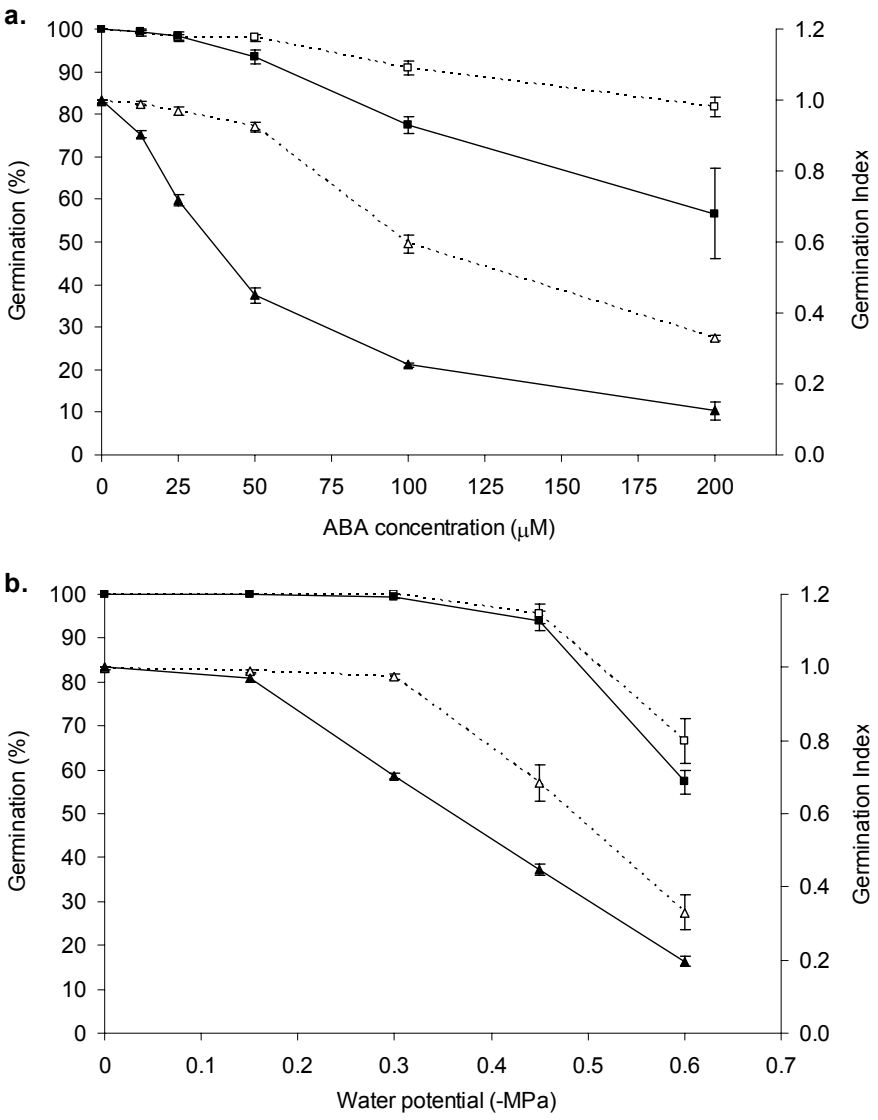


Figure 5.3: Germination percentage (square) and germination index (triangle) at different external abscisic acid (ABA) concentrations (a) and water potentials (b) of lettuce seeds produced under red-rich-light (broken line) or far-red-rich-light (solid line). Data are means ±SE from four samples of 50 seeds each.

CHAPTER 6

TEMPERATURE DURING SEED DEVELOPMENT AFFECTS WEIGHT, GERMINABILITY AND STORABILITY OF LETTUCE SEEDS

ABSTRACT

Seed germinability and storability are important aspects of lettuce seed quality determined by the genotype and environment of seed development. The objective of this study was to determine how temperature of the mother plant environment affects lettuce seed quality. Seeds of cv. Tango were produced in growth chambers under one of two treatments: *i)* high temperature (HT), with day/night temperatures of 30/20°C, respectively, and *ii)* low temperature (LT), with temperatures of 20/10°C. Seeds produced at LT were 37% heavier than seed from HT, however germination at optimal conditions (20°C-light) was similar for both treatments. Seeds from HT had higher dark germination at 18, 24 and 29°C. Germinability (% and rates) under light at temperatures between 20 and 30°C was similar for seeds from both treatments, however at temperatures between 30 and 40°C seeds from HT performed better than those from LT. When germinated in exogenous abscisic acid concentrations or negative osmotic potentials, germinability of

seed from HT was less affected than LT. After accelerated aging (41°C, ≈ 100%RH, 72 h), germination of normal seedlings was higher for seeds from HT. Germination after 1 and 2 months of storage at 30°C and 74% RH was better for seeds from HT. The critical moment for temperature effects was also studied. Seed weight, dark germination at 30°C and germination at low osmotic potential were shown to be determined earlier during seed development (before 5 and 4 d after flowering for seeds from LT and HT, respectively). On the other hand, seed storability was determined at the end of seed development, after physiological maturity (≈ 16 and 11 d after flowering for LT and HT seeds, respectively). In conclusion, higher 'Tango' lettuce seed germinability and storability results were attained when seeds were produced at higher temperatures.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) seed quality affects seedling emergence and uniformity of growth, which is fundamental to attain high yield and quality in a single harvest (Wurr and Fellows, 1985; Wien, 1997). Thermoinhibition (sensitivity to high temperatures) and photodormancy (lack of germination in dark) are two characteristics that frequently affect speed and uniformity of lettuce seed germination, making it difficult to attain successful crop establishment in the field (Wien, 1997; Ryder, 1999). Along with germinability, storability is another important aspect of lettuce seed quality because it facilitates management of seed stocks by seed companies and producers.

Seed quality attributes depend on the genotype and the seed production environment. There are several reports about the benefits that producing lettuce seeds at higher temperatures (e.g. 30/20°C vs. 20/20°C) has on reducing seed thermodormancy (Koller, 1962; Gray et al., 1988; Drew and Brocklehurst, 1990; Sung et al., 1998; Kozarewa et al., 2006). However, lettuce seed weight decreased when production temperature increased (Gray et al., 1988; Sung et al., 1998), and reduced seed vigor has been suggested as a consequence of the diminution in seed weight (Gray et al., 1988).

The critical period during seed development for the effects of production temperature in seed germinability, and the physiological mechanisms governing this response remain unclear. Additionally, the effects that temperature during production has on lettuce seed storability or the relationship between storability and other attributes of seed quality are aspects of practical importance for seed producers which have not been addressed.

The main objectives of this study were to: *i)* investigate the effects that temperature during lettuce seed production has on different attributes of seed quality, such as germinability, vigor and storability, and *ii)* determine the critical periods during lettuce seed development at which temperature effects occur.

MATERIALS AND METHODS

Two experiments were performed to determine *i)* effects of temperature on lettuce seed quality, and *ii)* critical periods during lettuce seed development for temperature effects.

Experiment 1, effects of temperature

Lettuce (cv. 'Tango') 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: *i)* high temperature (**HT**), with day (12 h)/night (12 h) temperatures of 30/20°C, and *ii)* low temperature (**LT**), with temperatures of 20/10°C. Light during day hours was provided with fluorescent light (≈ 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The experiment was repeated four 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). At least six flower heads per plant were labeled with colored strings at the day of flowering and five flower heads per replication were sampled every other day from 3 d after flowering (DAF) to 17 and 25 DAF for HT and LT treatments, respectively. Fresh and dry weight of the seeds (achenes) was determined.

For each replication, approximately 45 d after the initiation of plant flowering, seeds were harvested by manually extracting only fully matured flower heads (dry and open, with visible seeds of ≈ 8% water content, fresh weight basis) of each plant. Seeds were cleaned (air blower and hand) and stored in paper envelopes inside a storage room

at 4°C and 25% RH until evaluation. The equilibrium seed water content (fresh weight basis) during storage was 6.1 ±0.2 and 7.1 ±0.1% for seed from the LT and HT treatments, respectively.

Seed evaluation. For seed fresh and dry weight determinations, three groups of 50 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 using 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 x 11 x 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 distilled water, or 10 mL of solution containing various concentrations of (±) abscisic acid (ABA; Sigma-Aldrich, St. Louis, MO) or polyethylene glycol (PEG 8000, Sigma-Aldrich, St. Louis, MO) 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 7 d. A thermogradient table (Series #16065, Seed Processing Holland B.V., Enkhuizen, Netherlands) was used to evaluate germination at different temperatures under dark

(evaluation after 4 d) or light (daily evaluation to 7 d). The germination index (GI) was calculated as the algebraic sum of the ratio of germinated seeds and days after sowing (DAS) at the count moment.

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 DAS.

Vigor index and average radicle and hypocotyl length measurements were determined on 80 seeds per replication (two groups of 40) using the Seedling Vigor Imaging System© (SVIS) 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.

Breaks of far-red (FR) light during dark germination at 20°C were applied to 50 seeds per replication, and compared with seeds germinating under constant dark. Far-red breaks 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 red to far-red (R:FR) ratio of 0.01 during 4 min at 2, 4, 6, 8, and 24 h after sowing. The R:FR ratio was calculated as the sum of wavelengths between 656 and 664 nm divided by the sum of wavelengths between 726 and 734 nm. Seed germination was evaluated 4 d after sowing.

Seed storage. Seeds were stored in square plastic boxes (11x11x4 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%. Seed samples were extracted after 1, 2, and 3 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 0.5g/L citric acid monohydrate and 100 mg/L 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 1500g 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).

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. Untransformed data are presented in tables and figures. Correlation coefficients between different parameters of germinability and storability were calculated.

Experiment 2, critical moment determination

Flower heads (as many as were available) of four plants from each temperature treatment were labeled on the day of anthesis. Labeling of flower heads at HT was performed 0, 4, 8 and 12 days before movement of the plants to LT, and plants at LT were labeled 0, 5, 10, and 15 days before being moved to HT. There were eight treatment combinations: *i)* **LOW** (LT throughout seed development), *ii)* **3/4 LOW- 1/4 HIGH** (15 days in LT, then HT), *iii)* **2/4 LOW- 2/4 HIGH** (10 days in LT, then HT), *iv)* **1/4 LOW- 3/4 HIGH** (5 days in LT, then HT), *v)* **HIGH** (HT throughout seed development), *vi)* **3/4 HIGH- 1/4 LOW** (12 days in HT, then LT), *vii)* **2/4 HIGH- 2/4 LOW** (8 days in HT, then LT), and *viii)* **1/4 HIGH- 3/4 LOW** (4 days in HT, then LT). Plant management was performed similarly as in Exp. 1. Fully matured flower heads from each labeling moment were harvested manually.

Seed evaluation. Seed dry weight, dark germination at 30°C, germination at –0.5 MPa, and percentage of normal seedlings after AA were evaluated as described for Exp. 1. Four sub-samples of 50 seeds per treatment were used for each evaluation and the data are reported as the average and the standard error (SE) of the average.

RESULTS

Experiment 1

Lettuce seed physiological maturity (PM, maximum dry weight), determined by an iterative regression analysis procedure (Pieta-Filho and Ellis, 1991), occurred 16.3 ±0.5 and 10.6 ±0.2 DAF for LT and HT plants, respectively (Fig. 6.1), and the dry weight

of seeds produced under LT was 37% higher than for seeds from the HT treatment (Fig. 6.1, Table 6.1). The SG results or production of normal seedlings at 20°C-light were the same for seeds from both treatments (Table 6.1); however, seeds from the HT treatment performed significantly better than seeds from LT following the AA test. When vigor was evaluated by the SVIS no significant differences between treatments were found for vigor index, growth index, and radicle length; however seeds from the LT treatment outperformed seeds from HT in the uniformity index and hypocotyl length (Table 6.1). No significant differences in mature seed ABA concentration were found (Table 6.1).

When germinated under light, lettuce seeds from the HT treatment performed better than seeds from LT at temperatures between 30 and 40°C (Fig. 6.2a). Seeds from the HT treatment had ≈ 100% germination at temperatures between 20 and 35°C, whereas seeds from LT decreased from 100% germination at 31°C to 89 and 46% germination at 33 and 35°C respectively (Fig. 6.2a). Similarly, seeds from the HT treatment had a GI over 0.8 (i.e., ≈100% germination during the first two days) between 20 and 35°C, while the GI values of seeds from LT were lower than 0.5 at temperatures equal or higher to 33°C (Fig. 6.2a). When germinated in dark, seeds from both treatments were more affected by increases in temperature, however seeds produced under HT outperformed seeds from the LT treatment at all of the four temperatures evaluated (Fig. 6.2b). Temperature treatments during seed production also affected lettuce seed sensitivity to exogenous ABA and reduced water potentials (Fig. 6.3). Germination of seeds from both treatments was reduced by higher ABA concentrations (Fig. 6.3a) or lower water potentials; however, seeds from LT had greater reductions in these values than seeds from HT (Fig. 6.3b).

When SG was evaluated after different periods of storage at 30°C and 74% RH, seeds from the LT treatment deteriorated faster than seeds from the HT treatment (Fig. 6.4). After one and two months of storage seeds from the HT treatment produced respectively 83 and 25% normal seedling, compared to only 47 and 4% from the LT seeds (Fig. 6.4).

Successive FR light breaks during dark germination at 20°C reduced significantly the germination of seeds from the LT treatment; although germination of seeds from the HT treatment also was affected, the reduction was not significant (Table 6.2).

Experiment 2

Individual seed dry weight from seeds that developed the first 4, 8, or 12 DAF under HT conditions was similar to the weight of seeds from the HIGH treatment and lower than the weight of seeds from the LOW, 3/4 LOW-1/4 HIGH, 2/4 LOW-2/4 HIGH, and 1/4 LOW-3/4 HIGH treatments (Fig. 6.5a). Additionally, seeds that developed for at least the first 5 DAF at LT had a similar dry weight to seeds from the LOW treatment (Fig. 6.5a). As a group, seeds that developed for at least the first 4 DAF in HT conditions had higher germination in 30°C-dark than the group of seeds that developed the first 5 DAF or more under LT; however, some minor differences within each group are observed (Fig. 6.5b). Similar results were recorded when germination was evaluated at -0.5 MPa, where seeds from the group of treatments with the first 4 or more DAF in HT had a higher GI than seeds that developed under LT during the first 5 or more DAF (Fig. 6.5c). Different results were observed from the AA test, where seeds from the HIGH, 3/4

LOW-1/4 HIGH, 2/4 LOW-2/4 HIGH, and 1/4 LOW-3/4 HIGH treatments produced a higher fraction of normal seedlings after AA than seeds from the LOW, 3/4 HIGH-1/4 LOW, 2/4 HIGH -2/4 LOW, and 1/4 HIGH -3/4 LOW (Fig. 6.5d).

DISCUSSION

In Exp. 1 I observed that lettuce seeds produced under the LT treatment were significantly heavier than seeds from the HT treatment (Table 6.1). Seeds from the LT treatment took ≈ 16 d to reach PM or maximum dry weight, compared with only ≈ 11 d for seeds under HT conditions (Fig. 6.1). This additional time of seed filling could explain the higher dry weight of seeds from the LT treatment. However, based on results from Exp. 2, the effects of temperature treatments on seed dry weight occurs early during lettuce seed development, during the first 4 and 5 d of seed development for the HT and LT treatments, respectively (Fig. 6.5a). According with the curves of lettuce seed dry weight accumulation shown in Fig. 6.1, this would be the phase of cell division and histo-differentiation that precedes the phase of cell expansion and reserve deposition (Bewley and Black, 1994). Thus, the effects of temperature on lettuce seed dry weight accumulation would not be fully explained by a longer period of reserve accumulation in seeds from the LT treatment. Gray et al. (1988) reported that lettuce plants at 30/20°C produced more flower heads and seeds per plant than plants at 20/10°C. During the performance of Exp. 1, more abundant flowering was observed in plants under the HT condition (no data available) and an alternative explanation to higher dry weight of seeds from the LT treatment would be that these seeds had less competition for photosynthates

than seeds from the HT treatment. An inverse relation between the number of flower heads per lettuce plant and individual seed dry weight has been previously reported (Izzeldin et al. 1980; Gray et al. 1988; Chapter 3).

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 (measured as radicle growth; Smith et al., 1973a) and seedling growth after emergence (Smith et al., 1973b). In Exp. 1, seed performance was evaluated by *i)* SG test or the ability to produce normal seedlings under optimal (20°C-light) conditions, *ii)* the AA test, *iii)* seedling growth and uniformity (vigor index and radicle length from SVIS), and *iv)* germinability (radicle emergence) at different optimal and sub-optimal conditions. Despite the significantly higher dry weight of seeds from the LT treatment, no differences in SG were observed (Table 6.1). When seed vigor was evaluated by the SVIS system, seeds from the LT treatment tended to have higher values of growth and uniformity, however these differences were significant ($p< 0.05$) only for the uniformity index and average hypocotyl length (Table 6.1). Higher vigor of seeds from the LT treatment would support the concept that heavier seeds are more vigorous; however, when the AA test was used for seed vigor evaluation, seeds from the HT treatment performed significantly better than seeds from the LT treatment (Table 6.1). Other authors (Pieta Filho and Ellis 1991, Sinniah et al. 1998) suggested that environmental conditions conducive for the production of heavier seeds do not necessarily provide higher seed quality. In Exp. 2, lettuce seed weight was not related with the production of normal seedlings after AA

(Figs. 6.5a, 6.5d). Based on these results, there is no clear relationship exists between lettuce seed size and vigor, and that the vigor classification of lettuce seeds may vary depending on the test used (e.g., seedling growth vs. AA test).

When germinated under light at temperatures between 30 and 40°C, 'Tango' lettuce seeds from the HT treatment had higher germination percentages and rates than seeds from the LT treatment (Figure 6.2a). Thermoinhibition of lettuce seed germination at high temperatures (≥ 30°C) is one of the most important problems affecting seedling establishment of lettuce (Wien, 1997). Levels of thermoinhibition vary among lettuce genotypes (Gray, 1975; Kozarewa et al., 2006) and seeds from cv. 'Tango' are known to be sensitive to high temperatures during germination (H.J. Hill, personal communication). Additionally, differences among seedlots within cultivars have also been observed (Wurr et al., 1986). My results are consistent with other reports documenting that producing lettuce seed at higher temperatures has a positive effect on reducing seed thermoinhibition (Koller, 1962; Gray et al., 1988; Drew and Brocklehurst, 1990; Sung et al., 1998; Kozarewa et al., 2006). Along with thermoinhibition, photodormancy is a common problem affecting lettuce seed germination and seedling emergence (Wien, 1997), and 'Tango" lettuce seeds are characterized by being highly photosensitive (Chapter 2,3; H.J. Hill, personal communication). However, seeds from the HT treatment had little or no photodormancy when germinated in dark at temperatures between 13 and 24°C (Fig. 6.2a). The lack of photodormancy on these lots of 'Tango' seeds may be explained by the light conditions during seed production, i.e. fluorescent light with high R:FR ratio (Chapters 4, 5). In addition, light requirements for germination of photosensitive lettuce genotypes have been shown to increase with

temperature (Ikuma and Thimann, 1964; van der Woude and Toole, 1980; Sung et al., 1998), which explains the reduced germination at 29°C (Fig. 6.2b). Independent of this relatively low photodormancy of the seeds, dark germination of seeds produced under LT was lower than for HT seeds at any of the evaluated temperatures, and differences increased along with the germination temperature (Fig. 6.2b). Lower photodormancy in seeds produced at 30/20°C vs. 20/10°C was also reported by Kozarewa et al. (2006) in two different lettuce cultivars.

Seeds produced under HT had lower sensitivity to increased exogenous ABA (Fig. 6.3a) and decreased water potentials (Fig. 6.3b) during germination. Seed dormancy has been positively related with ABA presence or sensitivity of seeds to this phytohormone (Benech-Arnold et al., 1991; Ni and Bradford, 1993; Yogeesha et al., 2006; Finch-Savage and Leubner-Metzger, 2006), and also sensitivity of germination to water potential (Ni and Bradford, 1993). Thus, seeds from plants grown under LT would be expected to be more dormant than those from the HT treatment. Additionally, it would be expected that the more dormant seeds from the LT treatment contain higher ABA concentrations, however, no significant differences ($p= 0.27$) in ABA concentration were found between mature lettuce seeds from LT and HT treatments (Table 6.1). Previously, it was shown that 'Tango' lettuce seeds produced under different light conditions exhibited different levels of dormancy, and in that case more dormant seeds had a higher ABA concentration at maturity than the less dormant seeds (Chapters 4, 5). These results could be explained by differences in the dormancy mechanisms between seeds from different experiments. When differences in lettuce seed dormancy were caused by different light conditions during production, the critical period for that effect was at the

end of seed development (after PM), during the phase of maturation and drying (Chapters 4, 5). In the case of temperature, the effects on dormancy occurred early during seed development (Figs. 6.5b, c), during the phase of cell division and histo-differentiation (Fig. 6.1; Chapter 2, Bewley and Black, 1994). In Arabidopsis (Karssen et al., 1983), sunflower (Le Page-Degivry et al., 1990), and maize (White et al. 2000), it has been observed that dormant seeds had a peak of ABA concentration early during seed development. When this ABA peak has been suppressed by using mutants (Karssen et al., 1993) or compounds that inhibit ABA synthesis (Le Page-Degivry et al., 1990), a lack of seed dormancy has been observed. A similar peak in seed ABA concentration was found in 'Tango' lettuce seeds (Chapter 2). It is known that ABA is synthesized from a carotenoid intermediate (Milborrow, 2001). It can then be deduced that conditions that affect carotenoid synthesis also should affect ABA synthesis. There are studies suggesting that carotenoid synthesis is affected by high (≥ 30°C) temperatures (Johima 1995, Haldimann 1996), therefore high temperature also should affect ABA synthesis. Based on this information and the results of Exp. 2, I hypothesize that the increase of germinability in lettuce seeds produced at higher temperatures would be explained, in part, by a reduction in the peak of ABA concentration in the seeds during their early development (first half of time to PM). Proving this hypothesis requires additional research.

The use of the AA test has been proposed for evaluation of both seed vigor and seed storability (Copeland and McDonald, 2001). Previously, a significant correlation between results from the AA test and SG after 4 months of storage was observed for 'Tango' lettuce seeds (Chapter 4). Seeds produced under HT performed better after one

and two months of storage at 30°C and 74% RH (Fig. 6.4), and the effect of production temperature on seed storability (evaluated by AA test) occurs at the end of seed development, after PM (Fig. 6.5d). Thus, heavier lettuces seeds from the LT treatment not only had lower germinability than seeds from the HT treatment, but they also had poorer storability. In an early study, I observed a significant and negative correlation ($r= -0.98$; $p< 0.01$) between dark germination and storability of 'Tango' lettuce seeds produced under different day-length treatments (Chapter 4). In that study, the critical period of seed development for day-length effects was between PM and harvest (Chapter 4). Those results contradict data of the present study, where seeds from the HT treatment had higher germinability and higher storability. Additionally, these attributes were not correlated, because the critical period for production temperature effects on lettuce seed storability was different than that for the effects on germinability (Fig. 6.5). A possible explanation for these discrepancies is that the physiological mechanisms governing the responses of lettuce seed germinability to temperature and light treatments are different. In fact, effects of temperature on seed germinability occurred early in lettuce seed development (Fig. 6.5b, c), during the phase of cell division and histo-differentiation, and effects of the day-length treatments occurred after PM, during seed maturation and drying (Chapter 4).

I have already discussed a possible mechanism explaining the effects of temperature in seed germinability, however the question remains about differences in lettuce seed storability in response to the production temperature treatments. As shown in Fig. 6.1, the time between seed PM and the moment where seed water content (SWC) falls to $\leq 10\%$ varies depending on the temperature treatment: this period was ≈ 9 and 6 d

for ‘Tango’ lettuce seeds produced at LT and HT treatments, respectively. Vertucci et al. (1987) reported that photoconversion of Pr to Pfr on lettuce seeds required a minimal water content of 8%, over which photoconversion occurred at increased rates until reaching a maximum at 30% SWC, while photoconversion of Pfr to Pr required a minimal water content of 4%. Additionally, temperature has been suggested to affect the rate of Pfr degradation, with higher temperatures been associated to higher degradation rates (Saini et al., 1989). Seeds from the LT treatment spent ≈ 7 d after PM with a SWC over 30%, while this period was only ≈ 2 d for seeds in the HT treatment (Fig. 6.1). Therefore, it is reasonable to think that seeds from the LT treatment had a higher concentration of Pfr at harvest than seeds from the HT treatment. When dark germination was interrupted by FR-light, the percentage of germination was reduced significantly for seeds from the LT treatment, but not for seeds from the HT treatment (Table 6.2). These results support the hypothesis that relatively high germination in seeds from the LT treatment is explained by higher Pfr content in the seeds at harvest, and that another mechanism would be involved in promoting dark germination in seeds from the HT treatment. Based on previous experiments (Chapters 4, 5) it was suggested that the significant correlation between seed storability and photodormancy could be explained by the concentration of Pfr in the seeds at harvest, with lower storability being associated with higher Pfr concentrations. Thus, reduced storability of lettuce seeds produced at LT could be explained by a greater presence of Pfr at harvest; however, this is a hypothesis that requires further research.

In conclusion, my results confirm previous reports regarding the positive effects that producing lettuce seeds at higher temperatures (30/20°C) has in reducing seed thermoinhibition (Koller, 1962; Gray et al., 1988; Drew and Brocklehurst, 1990; Sung et al., 1998; Kozarewa et al., 2006). In addition, 'Tango' lettuce seeds produced at 30/20°C showed lower thermodormancy under a broader range of temperatures compared with seed produced at 20/10°C. Despite the reduction of seed dry weight associated with producing seeds at 30/20°C, there were no significant reductions in seed vigor, evaluated as seedling growth, and the AA test performance and seed storability were improved. Effects of production temperature in lettuce seed germinability occurred during the first phase of seed development (cell division and histo-differentiation) and therefore lower ABA production in the immature seeds may explains, in part, the observed effects. On the other hand, the effects of production temperature on seed storability occurred at the last phase of seed development, after PM, suggesting that this effect may be explained by a higher presence of Pfr in seeds from the LT treatment. Both hypotheses require further research.

Parameter	Treatment		*p*-value[1]
	30/20°C	20/10°C	
Seed dry weight (mg·seed^{-1})	0.76	1.04	0.024
Normal seedlings at 20°C-light (%)	99.8	99.8	---
Normal seedlings after AA[2] (%)	59.5	1.8	0.001
Vigor index[3]	667	739	0.161
Growth index[3]	670	710	0.575
Uniformity index[3]	664	769	0.008
Radicle length[3] (pixels·seedling^{-1})	344	403	0.120
Hypocotyl length[3] (pixels·seedling^{-1})	90	103	0.015
Seed ABA content (pg·mg dry weight)	74.3	65.8	0.273

[1]: calculated from analysis of variance.
[2]: Accelerated aging for 72 h at 41°C and ~100%RH. Normal seedling percentages 11 days after planting are reported.
[3]: Values from SVIS© (Seed Vigor Image System)

Table 6.1: Parameters of quality for lettuce seed produced under high (30/20°C) and low (20/10°C) temperatures.

Germination condition	Temperature treatment		*p*-value[1]
	30/20°C	20/10°C	
Dark	89.5 ±8.1	80.5 ±9.1	0.573
Dark + FR[2]	74.9 ±6.3	11.5 ±3.2	0.009
***p*-value**	0.155	0.021	

[1]: calculated from analysis of variance
[2]: FR, far-red light breaks (four minutes) at 2, 4, 6, 8, and 24 h after start of imbibition

Table 6.2: Germination percentages of lettuce seeds at 20°C in continuous dark and dark plus FR breaks for seed produced under high (30/20°C) and low (20/10°C) temperatures. Data are means ± SE from four replications (50 seeds each).

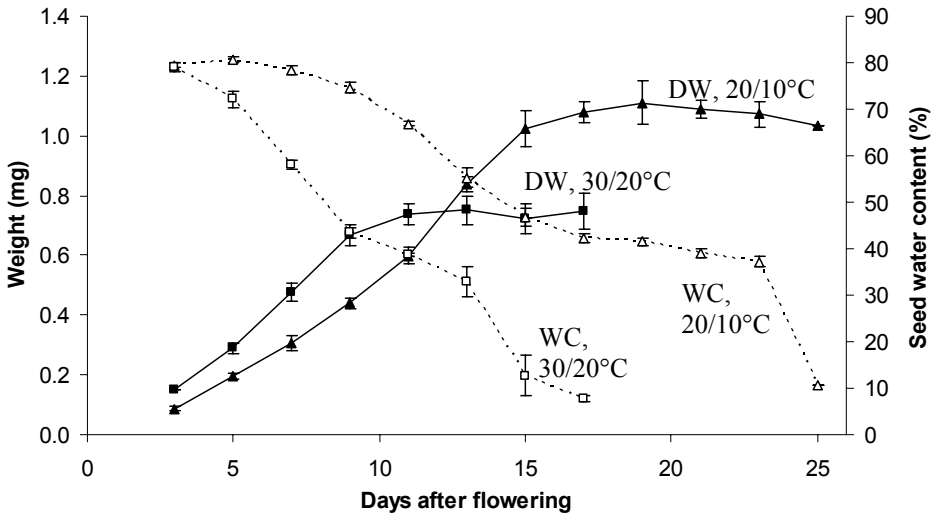


Figure 6.1: Seed dry weight (DW, solid line) and seed water content (WC, broken line) during development of lettuce seeds produced at 30/20°C (squares) and 20/10°C (triangles). Data are the average ±SE from four replications.

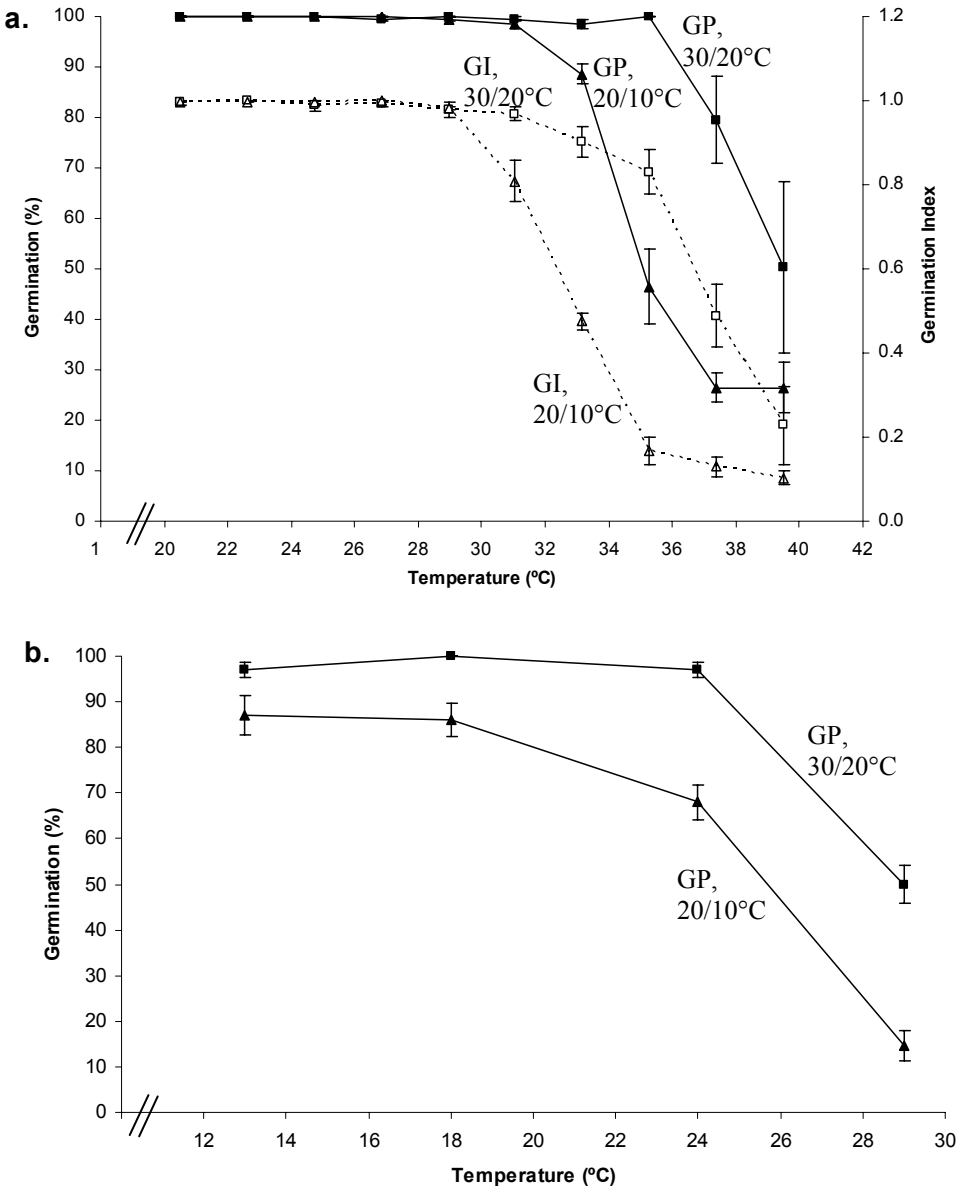


Figure 6.2: Germination percentage (GP, solid lines) and germination index (GI, broken lines) at different temperatures under light (**a**) and in dark (**b**; GP only) of lettuce seeds produced at 30/20°C (squares) and 20/10°C (triangles). Data are the average ±SE from four replications.

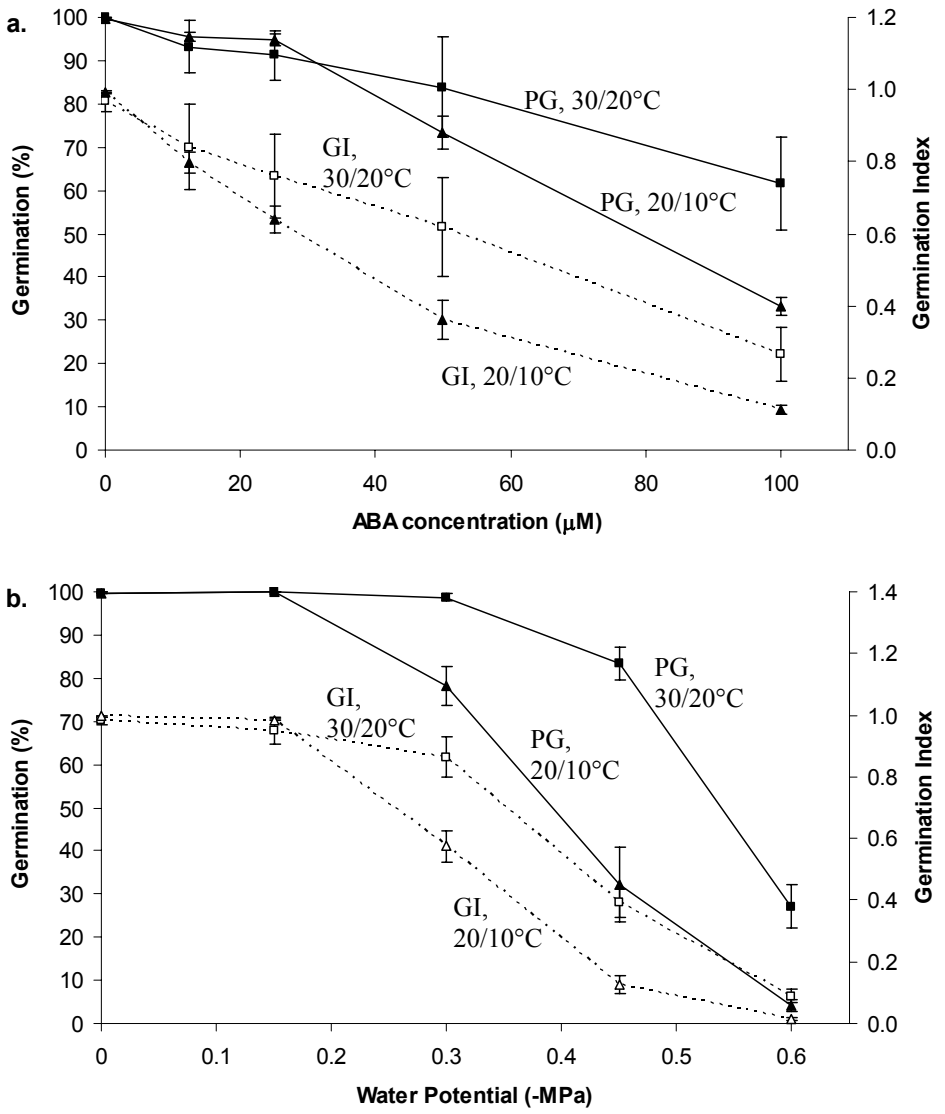


Figure 6.3: Germination percentage (PG, solid lines) and germination index (GI, broken lines) at different external abscisic acid (ABA) concentrations (**a**) and water potential (**b**) of lettuce seeds produced at 30/20°C (squares) and 20/10°C (triangles). Data are the average ±SE from four replications.

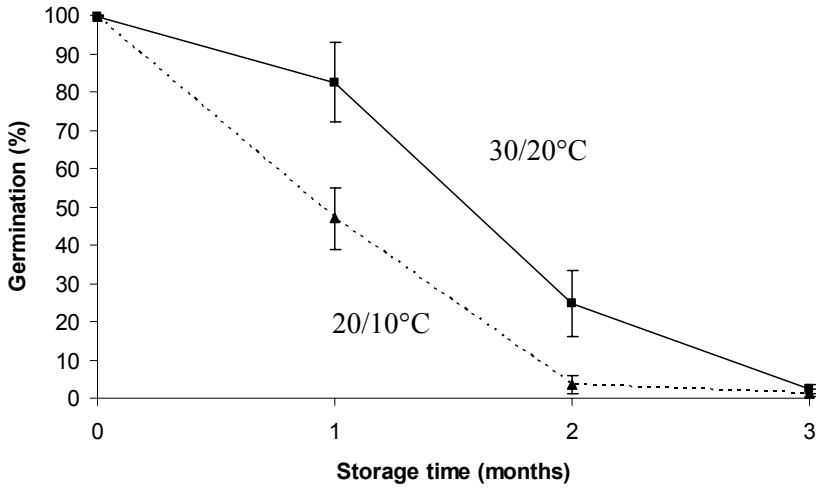
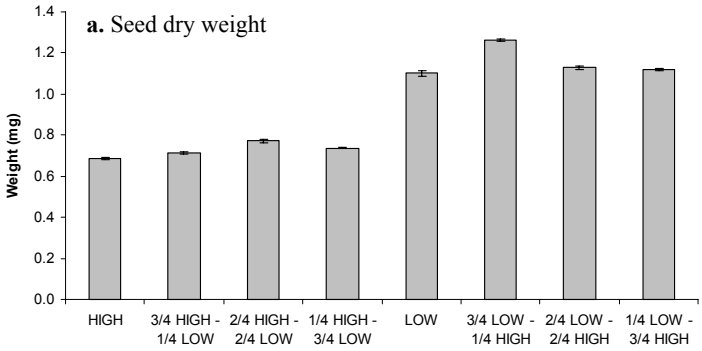
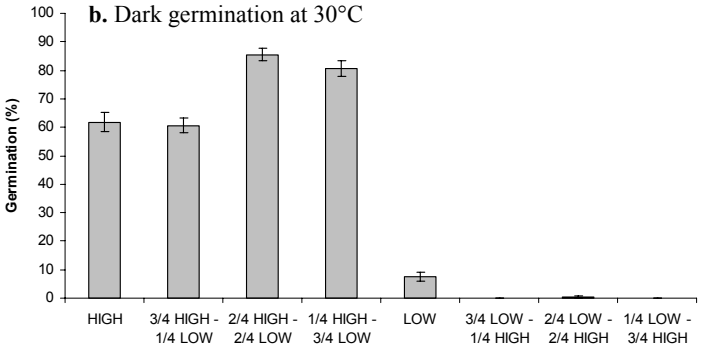
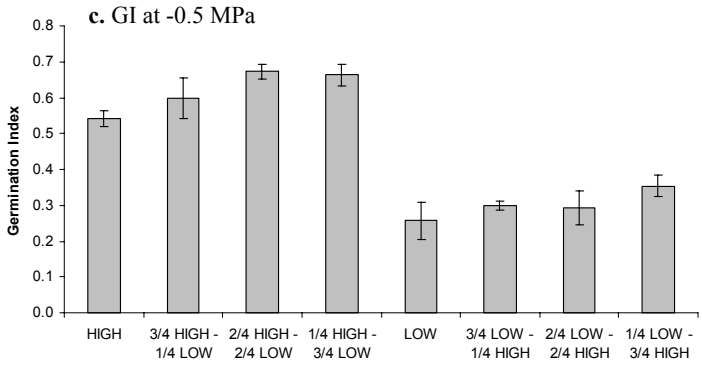


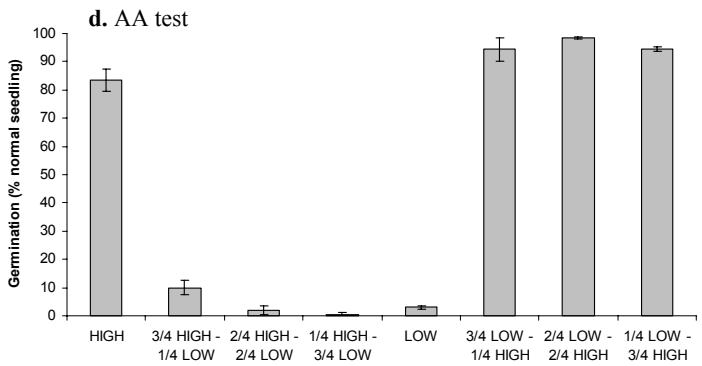
Figure 6.4: Germination (normal seedlings) after storage of lettuce seeds produced at 30/20°C (squares) and 20/10°C (triangles). Data are the average ±SE from four replications.

Figure 6.5: Seed dry weight (a), germination at 30°C (b), germination index (GI) at -0.5 MPa (c), and normal seedlings after accelerated aging (AA) (d) of lettuce seeds produced at **LOW** (all the time at LT= 20/10°C), **HIGH** (all the time at HT= 30/20°C), **3/4 LOW- 1/4 HIGH** (15 days at LT, then HT), **2/4 LOW- 2/4 HIGH** (10 days at LT, then HT), **1/4 LOW- 3/4 HIGH** (5 days at LT, then HT), **3/4 HIGH- 1/4 LOW** (12 days at HT, then LT), **2/4 HIGH- 2/4 LOW** (8 days at HT, then LT), and **1/4 HIGH- 3/4 LOW** (4 days at HT, then LT). Data are the average ±SE from four sub-samples of 50 seeds.


a. Seed dry weight
Weight (mg)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
HIGH
3/4 HIGH - 1/4 LOW
2/4 HIGH - 2/4 LOW
1/4 HIGH - 3/4 LOW
LOW
3/4 LOW - 1/4 HIGH
2/4 LOW - 2/4 HIGH
1/4 LOW - 3/4 HIGH


b. Dark germination at 30°C
Germination (%)
100
90
80
70
60
50
40
30
20
10
0
HIGH
3/4 HIGH - 1/4 LOW
2/4 HIGH - 2/4 LOW
1/4 HIGH - 3/4 LOW
LOW
3/4 LOW - 1/4 HIGH
2/4 LOW - 2/4 HIGH
1/4 LOW - 3/4 HIGH


c. GI at -0.5 MPa
Germination Index
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
HIGH
3/4 HIGH - 1/4 LOW
2/4 HIGH - 2/4 LOW
1/4 HIGH - 3/4 LOW
LOW
3/4 LOW - 1/4 HIGH
2/4 LOW - 2/4 HIGH
1/4 LOW - 3/4 HIGH


d. AA test
Germination (% normal seedling)
100
90
80
70
60
50
40
30
20
10
0
HIGH
3/4 HIGH - 1/4 LOW
2/4 HIGH - 2/4 LOW
1/4 HIGH - 3/4 LOW
LOW
3/4 LOW - 1/4 HIGH
2/4 LOW - 2/4 HIGH
1/4 LOW - 3/4 HIGH

CHAPTER 7

FINAL REMARKS AND FUTURE RESEARCH

Modifying the maternal plant environment has significant effects on lettuce seed quality. By controlling specific environmental conditions during seed production of 'Tango', the commercial lettuce cultivar used in this study, it was possible to modify seed size, germinability, thermoinhibition, photodormancy and storability. Changes in germinability include increasing germination percentage and rate under a wider range of conditions, which may be interpreted as an increase in seed vigor (AOSA, 1983). Storability is considered another component of seed vigor (Provert and Linington, 2006). In some cases (e.g. seed production at higher temperatures) both aspects of seed vigor were improved at the same time, thus an overall improvement of seed vigor was achieved. However, alterations in the maternal light environment caused opposite responses in seed germinability and storability (they were inversely related), so in this case the effects over seed vigor were dependent on the vigor test selected.

During lettuce seed development, a peak of ABA concentration was observed at approximately 65% of the time to physiological maturity. This ABA peak seems to be involved with the onset of desiccation tolerance and accumulation of reserves in the embryo. I hypothesized that changes in the magnitude of this ABA peak could be part of the mechanism by which higher temperatures during seed development increase germinability. Testing this hypothesis would contribute to a better understanding of how maternal environment affects seed dormancy and germinability.

Restricted water availability during seed production had little effect on many aspects of lettuce seed quality, although a significant increase in seed weight and the production of fewer seeds per plant were observed. Of particular interest for seed producers is the significant gain in water productivity (seed yield per volume of water consumed) attained with restricted irrigation, especially because of the arid regions and conditions in which most lettuce seeds are produced. Experiments at a commercial scale would permit the evaluation of the best practices to attain higher yields and seed quality with efficient use of irrigation water. Management of plant populations to compensate for reduced growth and seed production per lettuce plant is another aspect that should be evaluated in these experiments.

Producing 'Tango' lettuce seeds under environments enriched in red light has a significant effect on reducing thermoinhibition and photodormancy. This is a novel and promising approach to the production of lettuce seeds with improved germinability, however there are some aspects that should be further investigated, such as: *i)* undesired

reductions in seed storability and possible effects on seed yield, *ii)* effects on other lettuce cultivars of commercial importance, and *iii)* the feasibility of modifying maternal light environment at commercial scale.

Similarly, the possibility of producing seeds with improved storability by reducing the R:FR ratio of the maternal environment may be of interest for germplasm centers and seed companies (e.g. to facilitate stock management of genotypes or species without germinability problems), and should be further studied. In these studies, one of the first questions that should be addressed is if the effects of light quality on storability are observed in other lettuce genotypes and, more importantly, in other species. Additionally, finding a significant correlation between light spectrum quality of the maternal environment and storability in a wide range of species would be important for understanding the seed bank dynamics of native and weed plants. Basically, it would mean that seeds produced under canopy shadow (i.e. lower R:FR ratio) have higher storability and are better prepared to remain viable for longer periods of time.

For temperature and light, the critical period during lettuce seed development at which storability is affected was the maturation desiccation phase, after physiological maturity. Improvement of germinability of seeds from maternal plant environments rich in red light also occurred during this period. I hypothesized that the accumulation of Pfr in the seed during this phase would be involved in both responses. Because the importance of this hypothesis for different areas of seed biology it should be further investigated. Some questions that future research should address are: *i)* is the higher ABA concentration observed in seeds produced under red-rich environments a response to higher Pfr accumulation?, *ii)* is there a cause-effect relationship between seed storability

and Pfr accumulation?, *iii)* are the changes in ABA concentration and sensitivity involved in the storability effects?, *iv)* are gibberellins concentration and/or sensitivity affected by the light environment during this phase?, if so, are these changes mediated by Pfr?.

In my experiments I described the significant effects that temperature and light quality of the maternal plant environment have on diverse aspects of lettuce seed quality. Still remaining is the question of the interaction that these two factors may have on each aspect of seed quality. This is an important matter to be studied because of the possibility of synergism or antagonism between these factors in their impact on seed development and quality, and their role in the physiological mechanisms governing effects of maternal plant environment on seeds.

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