Day-Length During Seed Development Affects Germinability and Storability of Lettuce Seeds

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Abstract

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), or ii) long day (LD). 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, or dark), seed germinability (% and rates) from 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, and 6 months of storage at 30°C, 74% RH. Stored seed presented a progressive and significant reduction of germinability for both treatments, however seeds from SD were more affected. The results indicated that day-length during seed development affected lettuce seed weight, germinability, and storability. In this case, germinability and storability were inversely related. The critical moment for day-length effects was also studied. Lettuce seed size was determined during early seed development. Conversely, lettuce seed germinability and storability were determined at the end of seed development, after physiological maturity.

Introduction

- Among the factors affecting seed quality are the environmental conditions under which the seeds are produced.
- Lettuce is one of the most important vegetables in the USA and the world. The establishment of this species requires high quality seed, which may germinate at sub-optimal conditions (e.g. high temperature).
- The relationship between seed germinability and storability remain poorly understood. This knowledge is important for management of seed stocks, germplasm conservation, and management of natural seed banks of weeds and wild species.
- The main objectives of this study were to determine how day-length of the mother plant affects lettuce seed quality and the relationship between seed germinability and storability.

Materials and Methods

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

Experiment 1, effects of day-length: 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 8 h of fluorescent light plus 8 h of incandescent light (~21 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 8 h of darkness daily. In both treatments the temperature was 23°C, constant. The experiment was replicated three times using plants from different sowing dates. Each replication was considered a block and consisted of 10 plants randomly assigned to each treatment. The harvest was performed manually extracting only fully matured flower heads of each plant.

Seed evaluation: For each replication, 3 groups of 100 seeds were used for dry weight determination. For the standard germination test, 100 seeds per replication (in groups of 50) were planted over 2 blotter layers saturated in dH_2O and germinated at 20°C with constant light. After 4 and 7 days just normal seedlings were considered as germinated.

Other germination tests were conducted using 2 groups of 50 seeds per replication, planted over 2 layers of blotters saturated with dH_2O or ABA solution. Germination (radicle emergence) was evaluated daily by 7 days. 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 in a thermogradient table at 14, 19, 24, or 29°C; germination was evaluated 4 days after sowing.

For the accelerated aging (AA) test, lettuce seeds were aged at 41°C and ~100%RH by 72 h, and then germinated following the standard germination protocol. Normal seedlings were evaluated 10 days after planted.

Seed storage: Seeds were stored at 30°C, 74% RH. Standard seed germination was evaluated after 2, 4 and 6 months of storage.

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 plants with ~25 flower heads each, labeled by flowering day, were assigned to each of the following treatments: **L**, LD during all seed development; **S**, SD during all 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. From 3 days after flowering, and each alternating day, 5 flower heads from plants in L and S treatments were sampled to determine seed wet and dry weight accumulation. For all treatments seed harvest was performed manually extracting only labeled and fully matured flower heads of each plant. The whole experiment was replicated two times.

Seed evaluation and data analysis. Seed dry weight, dark germination at 20°C, and germination of normal seedlings after accelerated aging were evaluated following the same methodology described for experiment 1. Differences among treatments were analyzed using ANOVA and the least significant difference (LSD, α=0.05) procedure. Specific groups of treatments were compared by contrast analysis.

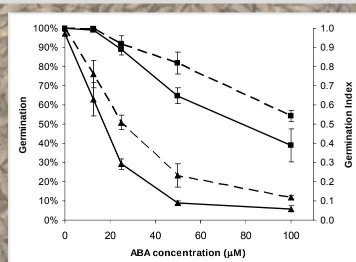

Figure 1. Lettuce seed germination percentage (square) and germination index (triangle) at different external abscisic acid concentrations of seeds produced under long (solid line) and short (broken line) days. Data are means ± SE of three replications.

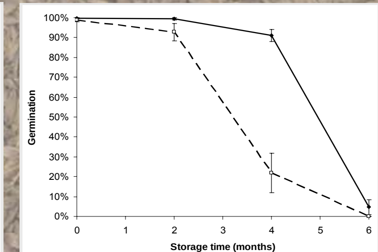

Figure 2. Lettuce seed germination percentage of normal seedlings after different storage periods at 30°C and 74%RH of seeds from long (solid line) and short (broken line) day treatments. Data are means ± SE of three replications.

Table 1. Parameters of quality for lettuce seed produced under long (LD, 16 h light) and short (SD, 8 h light) days.

Parameter	LD	SD	*p*-value[(1)]
Dry weight (mg/seed)	0.84	0.73	0.00
Germination % at 20°C	100	99	0.42
Germination Index at 20°C	0.98	1.00	0.02
Germination % at 30°C	21	60	0.03
Germination index at 30°C	0.05	0.35	0.06
Germination % after AA [(2)]	90	34	0.07
Dark germination % at 14°C	1	57	0.02
Dark germination % at 19°C	2	62	0.02
Dark germination % at 24°C	0	33	0.04

[(1)]: calculated from analysis of variance.
[(2)]: Accelerated aging for 72 h at 41°C and ~100%RH. Normal seedling percentages 10 days after planting are reported.

Results and Discussion

Experiment 1. Seeds produced under LD were significantly heavier than those produced in SD conditions (Table 1). Heavier seeds are commonly believed to perform better. However, the seeds from this experiment presented similar results for the standard germination test, which was close to 100% normal seedlings in both treatments. Seeds from SD showed significantly better germination (% and GI) at 30°C (Table 1). Thermoinhibition of lettuce seed germination at high temperature (≥ 30°C) is a problem affecting establishment of lettuce crop. Previous published reports (1, 2, 6) indicated that seed produced at higher temperatures (~30/20°C) presented an improved germination at high temperature, however the effect of day-length over lettuce seeds thermoinhibition has not been reported.

Day-length during seed production also affected the ability of the seed to germinate at increased external ABA levels (Fig.1). In both treatments, germination percentage and GI were reduced by higher ABA concentrations, although seeds from LD were more sensitive to this phytohormone (Fig.1). ABA has been traditionally associated with seed dormancy, and our results suggest that lettuce seed produced under SD presented a higher germinability, or less dormancy, than seeds produced under LD.

Dark germination was significantly higher in seeds from SD at 14, 19, and 24°C, but at 29°C seeds from both treatments had germinations close to 0% (Table 1). The light requirement for lettuce seed germination has been extensively studied (3, 4, 5, 8), and variations depending on cultivar (5) and germination temperature (3,7) have been reported (3, 7). However, differences due to day-length during seed development have not been reported previously.

Lettuce seeds from LD performed better after AA, producing a higher fraction of normal seedlings than seed from SD conditions (Table 1). These results suggest that seeds from LD, despite their lower germinability at sub-optimal conditions, would be more vigorous and longer lived than seeds from SD. The evaluation of germination after 2, 4 and 6 months of storage at 74%RH (Fig. 2) corroborated this assumption.

A negative correlation between lettuce germinability and storability parameters was observed (Table 2). The most negative (-0.98) and significant (p< 0.001) correlation was between dark germination at 19°C and standard germination after 4 months of storage. These results suggest that physiological dormancy and storability would be inversely related, however a causal relationship remains unclear.

Table 2. Correlation coefficient between parameters of lettuce seed germinability and storability.

	Dark germination at 19°C	Germination in ABA, 50 µM	Germination at 30°C	Germination after AA
Germination after 4 months at 30°C and 74%RH	-0.983 (0.000)[(1)]	-0.586 (0.221)	-0.687 (0.132)	0.940 (0.005)
Germination Index after 4 months at 30°C and 74%RH	-0.865 (0.026)	-0.262 (0.616)	-0.401 (0.431)	0.821 (0.045)
Germination after accelerated aging (AA)	-0.984 (0.000)	-0.375 (0.463)	-0.469 (0.348)	___

[(1)]: *p*-value for the correlation.

Experiment 2. As may be observed in Fig. 3 and Table 3 the effect of day-length on lettuce seed dry weight was produced early during seed development (first 6 days after anthesis), while the effect over dark germinability and storability was determined at the end of the seed development (≥ 12 days from anthesis). Physiological maturity under both day-length conditions was estimated to happen ~11 days after anthesis (data not shown). According to these results, the day-length conditions during lettuce seed desiccation would be critical in determining physiological seed quality. Possible mechanisms involved in these responses are currently being studied.

In summary, these results indicated that day-length during seed development affected lettuce seed size, germinability, and storability. In lettuce cv. 'Tango', the critical moment for the effect of day-length on germinability and storability was at the end of seed development, after physiological maturity, and both attributes were inversely related. The Asteraceae family includes numerous other crop, weed and native species in addition to lettuce, and significant effects of day-length over seed quality in the related species may be expected. The information presented here is relevant for seed production of high quality lettuce seeds, and may support the development of rational weed control strategies and a better understanding of natural seed bank dynamics.

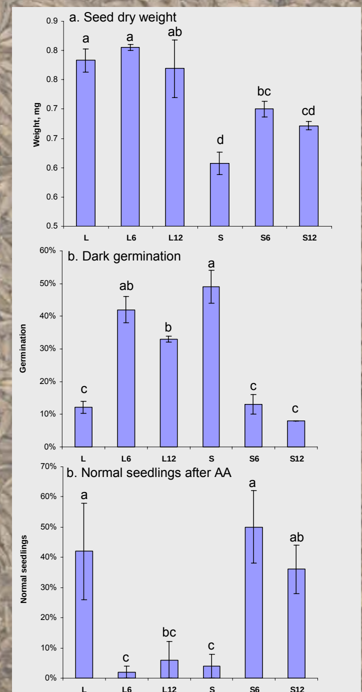

Figure 3. Seed dry weight (a), dark germination at 20°C (b), and normal seedling after accelerated aging (c) from seeds produced under different combinations of long (LD) and short (SD) days: **L**, LD during all seed development; **S**, SD during all seed development; **L6**, 6 days in LD, then SD; **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 a same graphic, treatments with different letters are significantly different (LSD, α= 0.05).

Table 3. Contrast analysis of specific group of treatments[1] to determine critical moment for long (LD) and short (SD) day effects.

Contrast	*p*-value
Dry weight, average (L, L6, L12) vs average (S, S6, S12)	0.0007
Dark germination, average (L, S6, S12) vs average (S, L6, L12)	<0.0001
Germination after AA, average (L, S6, S12) vs average (S, L6, L12)	0.0022

[1]: Treatments:
L, LD during all seed development; **S**, SD during all 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.

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