PREVENTING THERMOINHIBITION IN A THERMOSENSITIVE LETTUCE GENOTYPE BY SEED IMBIBITION AT LOW TEMPERATURE

Warley Marcos Nascimento*

Embrapa Hortaliças, C.P. 218 - 70359-970 - Brasília, DF - Brasil. e-mail <wmn@cnph.embrapa.br>

ABSTRACT: Lettuce (*Lactuca sativa* L.) seed germination is strongly temperature dependent and under high temperatures, germination of most of genotypes can be erratic or completely inhibited. Lettuce seeds of 'Dark Green Boston' (DGB) were incubated at temperatures ranging from 15° to 35°C at light and dark conditions. Other seeds were imbibed in dark at 20°; 25°; 30°; and 35°C for 8 and 16 hours and then transferred to 20 or 35°C, in dark. Seeds were also incubated at constant temperature of 20° and 35 °C, in the dark, as control. In another treatment, seeds were primed for 3 days at 15°C with constant light. DGB lettuce seeds required light to germinate adequately at temperatures above 25°C. Seeds incubated at 20°C had 97% germination, whereas seeds incubated at 35°C did not germinate. Seeds imbibed at 20°C for 8 and 16 hours had germination. At 35°C, seeds imbibed initially at 20°C for 8 and 16 hours, had 89 and 97% germination, respectively. Seeds imbibed at 25°C for 16 hours, germinated satisfactory at 35°C. High temperatures of imbibition led to no germination. Primed and non-primed seeds had 100% germination at 20°C. Primed seeds had 100% germination at 35°C, whereas non-primed seeds germinate only 4%. The first hours of imbibition are very critical for lettuce seed germination at high temperatures.
Key words: germination, dormancy, seed priming, stand establishment

PREVENÇÃO DA TERMOINIBIÇÃO DE GENÓTIPOS TERMOSSENSÍVEIS DE ALFACE POR MEIO DA EMBEBIÇÃO DAS SEMENTES EM TEMPERATURAS BAIXAS

RESUMO: A germinação de sementes de alface (*Lactuca sativa* L.) é extremamente dependente da temperatura, e sob condições de altas temperaturas, a germinação da maioria dos genótipos pode ser errática ou completamente inibida. Sementes de alface 'Dark Green Boston' (DGB) foram incubadas a temperaturas variando de 15° a 35°C sob luz ou escuro. Outras sementes foram embebidas em escuro a 20°; 25°; 30°; and 35°C por 8 e 16 horas e então transferidas para 20 ou 35°C, no escuro. Sementes foram ainda incubadas sob temperatura constante de 20° e 35°C, no escuro, como testemunha. Em outro tratamento, sementes foram osmoticamente condicionadas por 3 dias a 15°C sob luz constante. Sementes de DGB requereram luz para germinar adequadamente sob temperaturas acima de 25°C. Sementes incubadas a 20°C germinaram 97%, enquanto sementes incubadas a 35°C não germinaram. Sementes embebidas a 20°C por 8 e 16 horas germinaram 100% a 20°C. A 35°C, sementes germinaram 89 e 97% quando embebidas inicialmente a 20°C por 8 e 16 horas, respectivamente. Sementes embebidas a 25°C germinaram satisfatoriamente a 35°C somente quando embebidas por 16 horas. Altas temperaturas de embebição levaram à ausência de germinação. Sementes osmoticamente condicionadas ou não germinaram 100% a 20°C; a 35°C, aquelas osmoticamente condicionadas germinaram 100%, enquanto às não condicionadas germinaram apenas 4%. As primeiras horas de embebição são bastante críticas para sementes de alface germinarem sob condições de altas temperaturas.
Palavras-chave: germinação, dormência, condicionamento osmótico, estabelecimento de plântulas

INTRODUCTION

Optimum germination temperature for most lettuce genotype seeds is around 20°C (AOSA, 1993), although some germinate at temperatures ranging from 5 to 33°C (Gray, 1975). Different cultivars vary in their response to germination at high temperatures (Thompson et al., 1979). In general, temperatures above 30°C adversely affect lettuce seed germination. High temperatures may decrease either germination rate or germination percentage (Cantliffe et al., 2000).

Under high temperature, lettuce seed is extremely sensitive and requires red light to germinate at its maximum (Blaauw-Jasen, 1981, Evenari et al., 1953; Georghiou & Thanos, 1983). Even for thermotolerant genotypes, such as 'Everglades' (Guzman et al., 1992), seed germination decreases when seeds are imbibed under high temperature in the dark (Sung, 1996; Nascimento & Cantliffe, 2000). Therefore, there is an important interaction between light and high temperature, and that may be mediated by the phytochrome system (Fielding et al., 1992; Taylerson & Hendriks, 1972).

Several methods to circunvent the effects of high temperature have been proposed including resistant germplasm, adjusting the seed production environment, and the use of growth hormones (Nascimento & Cantliffe, 1999). Seed priming has also been used to allow germination of lettuce seed at higher temperatures (Cantliffe et al., 1981; Guedes & Cantliffe, 1980; Khan et al., 1980/81; Valdes et al., 1985; Wurr & Fellows, 1984). The objectives of the present study were to determine the effects of different temperatures and light on seed germination for a thermosensitive lettuce genotype, and to evaluate the effects of low temperature imbibition for seed germination at high temperature.

MATERIAL AND METHODS

Lettuce seeds of 'Dark Green Boston' (DGB), a thermosensitive genotype (Nascimento, 1998; Sung, 1996), were used in this study. Three experiments were carried out:

Experiment 1: Seeds were placed on two layers of 5.0 cm diameter germination paper (Anchor Paper, Hudson, WI) moistened with 3 mL of distilled water. Water was added as needed to keep the filter paper moist. Blotters were covered with 5.5 cm Petri dish lids and incubated at 15°, 17.5°, 20°, 22.5°, 25°, 27.5°, 30°, 32.5° and 35°C under constant dark or light (~26 μmol m^{-2} s^{-1}) on a uni-dimensional thermogradient bar (Type DB 5000, Van Dok & De Boer, B.V., Holland). Total percent germination (normal seedlings) was evaluated after seven days (AOSA, 1993).

Experiment 2: Seeds were imbibed in the dark at 20°; 25°; 30°; and 35°C for 8 and 16 hours and then transferred to 20° or 35°C, in the dark. Other seeds incubated at constant temperature of 20° and 35°C in the dark were used as controls. Germination procedures were used as described for Experiment 1.

Experiment 3: Priming was used as a control procedure. Seeds were primed in 200 mm test tubes for 3 days at 15°C with constant light (~26 μmol m^{-2} s^{-1}) in an aerated solution of polyethylene glycol (PEG), at an osmotic potential of -1.2 MPa (30 mL of solution g^{-1} of seed). Aeration was provided by flow pump. The air was pre-hydrated by bubbling through water to minimize evaporation of the soaking solution. Afterwards, seeds were placed in a Buchner funnel, rinsed three times with 100 mL of distilled water and redried in an incubator at 15°C and 45% RH for two days. Radicle emergence was recorded daily for seven days. Total percent germination (normal seedlings) was evaluated at seven days (AOSA, 1993).

Four replications of 50 seeds were used in these experiments. Analysis of variance (ANOVA) using a randomized factorial design was performed. Treatments means were compared by the Tukey and F-tests (P = 0.05).

RESULTS AND DISCUSSION

DGB seeds germinated satisfactorily at temperatures below 22.5°C in the dark (Table 1). Early studies reported that light affects lettuce seed germination (Borthwick et al., 1952; Bewley & Black, 1994). For example, seed dormancy in lettuce may be induced when seeds are imbibed for a prolonged period in the dark (Toole et al., 1956). This effect may be alleviated if red light is applied to the imbibing seed early in the germination process (Scheibe & Lang, 1965). An interaction between imbibition conditions and temperature was observed in this study (Table 1).

Seed imbibition under light increased the threshold for germination at higher temperatures; under light conditions, seeds germinated adequately at 27.5°C. These results agree with several reports which have shown that lettuce seeds imbibed at high temperatures are more dependent on red light for germination (Evenari et al., 1953; Georghiou & Thanos, 1983). Nascimento & Cantliffe (2000) reported that germination of 'Everglades', a thermotolerant lettuce genotype, exceeded 90% at temperatures up to 35°C under light conditions. However, germination of 'Everglades' decreased when seeds were imbibed under high temperature in the dark. However, DGB seeds did not germinate satisfactorily at temperatures above 27.5°C, even under light conditions (Table 1).

Table 1 - Percentage of germination of 'Dark Green Boston' lettuce seeds at different temperatures in light and dark conditions.

Condition	Temperature (°C)								
	15.0	17.5	20.0	22.5	25.0	27.5	30.0	32.5	35.0
					%				
Dark	100 Aa	100 Aa	96 Aa	83 Bab	40 Bc	3 Bd	0 Bd	0 Bd	0 Bd
Light	100 Aa	99 Aa	97 Aa	100 Aa	96 Aa	96 Aa	35 Ab	17 Ac	19 Ac

In each column, means with the same upper-case letter and, in each row, means with the same lower-case letter are not different, by Tukey Test ($P \leq 0.01$). There was interaction between conditions and temperature ($P \leq 0.01$).

In another study, seeds imbibed at constant temperature of 20°C in the dark, had 97% germination; at 35°C, seeds did not germinate (Table 2). However, when seeds were previously imbibed at low temperature (20°C) for 8 hours, germination at 35°C was 89%. Interactions among the treatments were observed in this study (Table 2). Lettuce seeds may germinate at high temperatures when the seeds are previously imbibed for a period of time at low temperatures. When seeds were imbibed for 16 hours at 20°C, germination at 35°C was 97%. The longer the seeds are imbibed at low temperatures, the higher their germination percentage after they were transferred to high temperatures. A prolonged period of imbibition at low temperatures might promote production of enzymes needed for lettuce endosperm loosening and consequent seed germination at high temperature (Nascimento, 1998). In addition, the length of phase II is temperature dependent. However, germination and endo-β-mannanase synthesis in lettuce at 15°C during the priming proceeded at adequate rates to circumvent thermodormancy later, at 35°C. At 25°C, seeds required 16 hours of imbibition to germinate adequately. Seeds imbibed at this temperature for 8 hours had only 43% germination (Table 2). Higher temperatures of imbibition affected seed germination at both temperatures of germination (Table 2).

Primed and non-primed seeds had 100% germination at 20°C (Table 3). At 35°C, non-primed seeds had only 4% germination, whereas primed seeds had 100%. An interaction between priming and germination temperature was observed for total germination but not for germination rate (Table 3). Primed seeds germinated faster than non-primed seeds, even at 20°C. Benefic effects of seed priming at high temperature were previously reported for lettuce (Cantliffe et al., 1981; Guedes & Cantliffe, 1980; Khan et al., 1980/81; Valdes et al., 1985; Wurr & Fellows, 1984, Eira, 1988), as confirmed herein. In general, lettuce seeds are primed in an osmotic solution under low temperatures, e.g., 15°C (Nascimento & Cantliffe, 1998). Seed priming extends the lag phase of water uptake by restricting radicle emergence, and in this phase major metabolic events occur preparing the seed for phase III and radicle protrusion (Bewley & Black, 1994). Therefore, a prolonged phase II during priming might promote a specific mechanism needed for seed germination at high temperatures (Nascimento et al., 2001).

The mechanism by which seed priming overcomes thermoinhibition or thermodormancy for lettuce is unknown. Guedes et al. (1981) observed a progressive loosening of the endosperm membrane during priming, a possible indicative of endosperm weakening, thus allowing lettuce seed germination to proceed at high temperatures. Nascimento et al. (2001) concluded that the activity of endo-β-mannanase, an enzyme which hydrolyses the mannan-polymers, and is produced and secreted by the lettuce endosperm, increased markedly in primed seeds before radicle protrusion, and this may be a crucial mechanism for endosperm weakening and consequently lettuce seed germination, especially under high temperatures.

Temperatures during lettuce seed imbibition above the optimum for a specific cultivar may lead to two different phenomena: thermoinhibition, a reversible process, where the seeds do not germinate above a certain temperature, but will germinate after transfer to a lower temperature. Prolonged exposure to supraoptimal temperatures may induce a true dormancy, a phenomenon known as thermodormancy or secondary dormancy (Khan, 1980/81). In this case, the seeds will not germinate even when the temperature is reduced to a favorable range.

Table 2 - Percentage of germination of 'Dark Green Boston' lettuce seeds imbibed for 8 and 16 hours at different temperatures and incubated at 20 and 35°C in the dark.

Imbibition conditions		Germination at temperature	
Temperature	Period	20ºC	35ºC
ºC	hours	%	
20	8	100 Aa	89 Bab
	16	100 Aa	97 Aa
Mean		100 A	93 A
25	8	100 Aa	43 Bc
	16	73 Bb	81 Aab
Mean		87 A	62 B
30	8	41 Ac	01 Bd
	16	01 Ad	0 Ad
Mean		21 A	0 B
35	8	21 Acd	0 Bd
	16	05 Ad	0 Ad
Mean		13 A	0 B
Control (constant)		97 A	0 B

In each column, means with the same lower-case letter and, in each row, means with the same upper-case letter are not different by Tukey Test ($P \leq 0.01$). There were interactions among treatments ($P \leq 0.01$).

Table 3 - Total germination (TG) and time to first germination (FG) of 'Dark Green Boston' lettuce primed and non-primed seeds at 20 and 35°C in the light.

Treatment	Temperature(°C)			
	TG	FG	TG	FG
	%	hours	%	hours
Primed	100	5	100	4
Non-primed	100	17	4	18
Significance[1]	ns	**	**	**

[1] ns, Non-significant. ** Significant at $P \leq 0.01$ by F- test. There was interaction between conditions and temperature, at $P \leq 0.01$.

CONCLUSIONS

The first hours of imbibition are very critical for lettuce seed germination at high temperatures and thermoinhibition and/or thermodormancy can be by-passed prior to radicle protrusion. This is very important when using thermosensitive lettuce genotypes to obtain a good stand establishment either in a greenhouse (transplant industry) or on the field (direct seeding).

REFERENCES

ASSOCIATION OF OFFICIAL SEED ANALYSTS - AOSA. Rules for testing seeds. **Journal of Seed Technology**, v.16, p.1-113, 1993.

BLAAUW-JANSEN, G. Differences in the nature of thermodormancy and far-red dormancy in lettuce seeds. **Physiological Plantarum**, v.53, p.553-557, 1981.

BEWLEY, J.D. ; BLACK, M. **Seeds**: **Physiology of development and germination**. ed. New York: Plenum Press, 1994. 445p.

BORTHWICK, H.A.; HENDRICKS, S.B.; PARKER, M.W.; TOOLE, E.H.; TOOLE, V.K. A reversible photoreaction controlling seed germination. **Proceedings of the National Academy of Sciences**, v.38, p.662-666, 1952.

CANTLIFFE, D.J.; SHULER, K.D.; GUEDES, A.C. Overcoming seed thermodormancy in a heat sensitive romaine lettuce by seed priming. **HortScience**, v.16, p.196-198, 1981.

CANTLIFFE, D.J.; SUNG, Y.; NASCIMENTO, W. M. Lettuce seed germination. **Horticultural Reviews**, v.24, p.229-275, 2000.

EIRA, M.T. Condicionamento osmótico de alface: efeitos sobre a germinação e desempenho sob stress hídrico, salino e térmico. Piracicaba: USP/ESALQ, 1988. 90p. (Dissertação - Mestrado)

EVENARI, M.; NEUMANN, G.; STEIN, G. Factors modifying the influence of light on germination. **Nature**, v.172, p.452-453, 1953.

FIELDING, A.; KRISTIE, D.N.; DEARMAN, P. The temperature of Pfr action governs the upper temperature limit for germination in lettuce. **Photochemistry and Photobiology**,v.56, p.623-627, 1992.

GEORGHIOU, K.; THANOS, C.A. Phytochrome control of skotodormancy release in Grand Rapids lettuce achenes. **Physiologia Plantarum**, v.57, p.352-356, 1983.

GRAY, D. Effects of temperature on the germination and emergence of lettuce (*Lactuca sativa* L.) varieties. **HortScience**, v.50, p.349-361, 1975.

GUEDES, A.C.; CANTLIFFE, D.J. Germination of lettuce seeds at high temperature after seed priming. **Journal of the American Society for Horticultural Science**, v.105, p.777-781, 1980.

GUEDES, A.C.; CANTLIFFE D.J.; NELL, T.A. Morphological changes during lettuce seed priming and subsequent radicle development. **Journal of the American Society for Horticultural Science**, v.106, p. 121-126, 1981.

GUZMAN, V.L.; NAGATA, R.T.; DATNOFF, L.E.; RAID, R.N. 'Florida 202' and 'Everglades': New butterhead lettuce cultivars adapted to Florida. **HortScience**, v.27, p.852-853, 1992.

KHAN, A.A. Hormonal regulation of primary and secondary seed dormancy. **Israel Journal of Botany**, v.29, p.207-224, 1980/81.

NASCIMENTO, W.M. Involvement of ethylene and endo-b-mannanase in lettuce seed germination at high temperature. Gainesville: University of Florida, 1998. 138p. (Thesis - Doctor)

NASCIMENTO, W.M.; CANTLIFFE, D.J. Germination of primed lettuce seeds after storage. **Proceedings of Florida State for Horticultural Society**, v.111, p.9699,1998.

NASCIMENTO, W.M.; CANTLIFFE, D.J. Circunventing thermodormancy in lettuce. **Acta Horticulturae**, v.504, p.147-152, 1999.

NASCIMENTO, W.M.; CANTLIFFE, D.J. Produção de etileno, atividade de endo-b-mananase e germinação de sementes de alface em resposta a luz e temperatura. **Revista Brasileira Sementes**, v.22, p.1-5, 2000.

NASCIMENTO, W.M.; CANTLIFFE, D.J.; HUBER, D.J. Endo-β-mannanase activity and seed germination of thermosensitive and thermotolerance lettuce genotypes in response to seed priming. **Seed Science Research**, v.11, p.255-264, 2001.

SCHEIBE, J.; LANG, A. Lettuce seed germination: Evidence for a reversible light induced in growth potential and for phytochrome mediation on the low temperature effect. **Plant Physiology**, v.40, p.485-492, 1965.

SUNG, Y. Identification and characterization of thermotolerance in lettuce seed germination. Gainesville: University of Florida, 1996. (Thesis - Doctor)

TAYLORSON, R.B.; HENDRICKS, S.B. Interactions of light and temperature shift on seed germination. **Plant Physiology**, v.49, p.127-130, 1972.

THOMPSON, P.A.; COX, S.A; SANDERSON, R.H. Characterization of the germination responses to temperature of lettuce (*Lactuca sativa* L.) achenes. **Annals of Botany**,v.43, p.319-334, 1979.

TOOLE, E.H.; HENDRICKS, S.B.; BORTHWICK, H.A.; TOOLE, V.K. Physiology of seed germination. **Annual Review of Plant Physiology**, v.7, p.299-324, 1956.

VALDES, V.M.; BRADFORD, K.J.; MAYBERRY, K.S. Alleviation of thermodormancy in coated lettuce seeds by seed priming. **HortScience**, v.20, p.1112-1114, 1985.

WURR, D.C.E. ; FELLOWS, J.R. The effects of grading and 'priming' seeds of crisp lettuce cv. Saladin, on germination at high temperature, seed 'vigour' and crop uniformity. **Annals of Applied Biology**, v.105, p.345-352, 1984.

Received October 10, 2001
Accepted May 22, 2003