High Maturation Temperature of Lettuce Seeds during Development Increased Ethylene Production and Germination at Elevated Temperatures

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ABSTRACT. Ethylene synthesis and sensitivity, and their relation to germination at supraoptimal temperatures, were investigated in lettuce (*Lactuca sativa* L.) seeds matured at 30/20 °C [12-h day/night, high temperature matured (HTM)] or 20/10 °C [12-h day/night, low temperature matured (LTM)]. HTM seeds of both thermosensitive ‘Dark Green Boston’ (DGB) and thermotolerant ‘Everglades’ (EVE) had greater germination at a supraoptimal temperature (36 °C), in both light or dark, than LTM seeds of DGB and EVE. HTM seeds of DGB and EVE produced more ethylene during germination than LTM seeds, regardless of imbibition conditions. The ethylene action inhibitor, silver thiosulfate, led to reduced germination in both cultivars. The ethylene precursor, 1-aminocyclopropane-1-carboxylic acid at 10 mM increased germination of both cultivars at supraoptimal temperatures, whereas germination of HTM seeds was greater than that of LTM seeds. No differences in ethylene perception were detected between HTM and LTM germinating seeds using a triple response bioassay. This study demonstrated that at least one method through which seed maturation temperature influences lettuce germination is by affecting ethylene production.

Optimum germination temperature for most lettuce cultivars is between 15 °C and 22 °C (Cantliffe et al., 2000). In many cultivars, temperatures above 25 °C reduce germination and above 27 °C, germination of many lettuce cultivars is totally inhibited (thermoinhibition). The ability of lettuce to germinate at supraoptimal temperatures (>28 °C) depends on both the genotype and the environmental conditions during seed maturation (Sung et al., 1998a). Lettuce cultivars can be divided in two temperature-related classes: thermosensitive and thermotolerant. Thermotolerant cultivars can germinate above 90% at ≤36 °C in light (Nascimento et al., 2000). Germination potential of some thermotolerant and thermosensitive cultivars can be influenced by temperature during seed maturation. Sung et al. (1998a) reported that germination at a range of supraoptimal temperatures of thermosensitive ‘Dark Green Boston’ and thermotolerant ‘Everglades’, ‘PI 251245’, and ‘Floricos 83’ seeds, matured at 30/20 °C (day/night) or 35/25 °C, was greater than that of seeds matured at 25/15 °C or 20/10 °C.

Researchers have attempted to identify the mechanism(s) of the promotive effect of supraoptimal maturation temperatures on subsequent lettuce seed germination. Weakening of the micro-pylar endosperm may be a prerequisite for lettuce germination, especially under supraoptimal temperatures (Cantliffe et al., 2000; Nascimento et al., 2000). Employing puncture force measurements, Sung et al. (1998b) observed that the resistance of the

endosperm was lower for both DGB and EVE seeds matured at 30/20 °C than at 20/10 °C. They also reported that both DGB and EVE seeds matured at 20/10 °C rather than 30/20 °C had more endosperm cells in front of the radicles. Nascimento et al. (2000) observed activity of the enzyme endo-beta-mannanase (EBM) in both DGB and EVE seeds prior to germination. The endosperm cells of lettuce are rich in galactomannans, a key substrate for this enzyme. Therefore, it was hypothesized that degradation of endosperm cell walls weakened the endosperm allowing the radicle to penetrate it (Cantliffe et al., 2000). Nascimento et al. (2000) reported that EBM activity was higher in lettuce seeds matured at 30/20 °C than at 20/10 °C.

The production of and sensitivity to ethylene also has been correlated with lettuce germination, especially at supraoptimal temperatures. Prusinski and Khan (1990) reported that ethylene production from nine lettuce genotypes correlated with their ability to germinate at both 32 °C and 35 °C as well as under salt and osmotic stresses. Abeles (1986) observed full reversion of thermoinhibition of ‘Grand Rapids’ seeds at 30 °C in dark by 10 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene.

Ethylene production and sensitivity to ethylene were measured in thermosensitive and thermotolerant lettuce seeds, matured at either 30°/20 °C or 20°/10 °C. The purpose of this study was to determine if maturation temperatures influenced in vivo ethylene synthesis during germination and whether the amount of ethylene produced was linked to the level of germination at supraoptimal temperatures.

Materials and Methods

PLANT MATERIALS. Lettuce seeds, from thermotolerant EVE and thermosensitive DGB cultivars, were used in this study. All seeds were produced at the same time at the Univ. of Florida in Conviron E15 (Controlled Environments, Pembina, N.Dak.) growth chambers. There were nine plants from each cultivar in each chamber. The plants were grown in 3.8-L pots with potting

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mix: 60% peat : 40% vermiculite as medium. The light intensity was 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. All plants were grown at 25/20 °C until uniform seed stalk development occurred, then they were transferred to different seed maturation temperatures. The seeds were produced under a 12-h photoperiod and two day/night temperature regimes, 30/20 °C (HTM) or 20/10 °C (LTM). All seeds were harvested manually at maturity, which was determined visually by the extent of flower drying and by seed color. The seeds from each plant were harvested several times, at the same time for all plants. The seeds from several harvests and several plants were combined for the experiments. All seeds were stored at 10 °C and 50% relative humidity for a minimum of 8 weeks prior to use.

SEED GERMINATION. For each treatment, three replications of 20 seeds were used. The seeds were placed on one layer Whatman #1 filter paper in 5.5-cm-diameter petri dishes. The filter paper was moistened with 1 mL deionized, ultra-pure water (Millipore, Cambridge, Canada). The paper was re-moistened as needed by adding 0.5 mL water. Germination was conducted at 20 or 36 °C in constant light (30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or constant dark in Precision Scientific (Winchester, Va.) incubators. Germination in dark was counted under a 25-V green safe light (General Electric, Hendersonville, N.C.). Radicle protrusion was used to indicate germination. The number of germinated seeds was counted at 10, 12, 16, 20, 24, 36, 48, 72, and 120 h after imbibition was initiated. The percent germination at 120 h was considered final and mean hours to germination (MHG) according to the formula proposed by Maguire (1962).

The ethylene action inhibitor, silver thiosulfate (STS), the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), or a combination of both were used to establish the effect of reduced ethylene perception or increased ethylene production, respectively, on germination of EVE and DGB seeds. STS was prepared according to the procedure of Abeles (1986). STS at 20 mM, used for all the experiments, was established as sufficient to affect lettuce germination (Nascimento et al., 2004). The ethylene precursor, ACC (Sigma, St. Louis), was prepared by dissolving in ultra-pure water; 10 mM was used for all experiments.

ETHYLENE DETERMINATION. Seeds of each cultivar and maturation temperature (30 per replication) were placed on one layer of 6 cm long $\times$ 0.5 cm wide Whatman #2 filter paper inside a horizontally-held plastic 1.2 $\times$ 7.5-cm culture tube (Fisher Scientific Co., Pittsburgh) sealed with a rubber stopper. The paper strip was moistened with 0.3 mL water at the start of the experiment and 0.15 mL water was added as necessary. The culture tubes were placed at 20 or 36 °C in continuous light (30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or continuous dark. Ethylene was measured at 9, 12, 15, 18, 21, 24, 36, and 48 h after imbibition was initiated. Germination was recorded immediately before the ethylene measurement. In dark, germination and gas sample withdrawals were conducted under the same green light as used for the germination experiments. A 1-mL gas sample was withdrawn using a gas-tight hypodermic syringe (Fisher Scientific Co.). The tubes were flushed with air and re-sealed for the next sampling. Each treatment was replicated three times. Ethylene was assayed using a gas chromatograph-Hewlett Packard Series II 5890 (Hewlett Packard, Agilent Technologies, Foster City, Calif.) equipped with a flame ionization detector. The carrier gas was nitrogen (N_2). The oven, injector, and detector temperatures were 130, 110, and 150 °C, respectively.

ETHYLENE SENSITIVITY ASSAY. Sensitivity of HTM and LTM seeds of DGB and EVE to exogenously supplied ACC was measured using a triple response bioassay (Bleecker et al., 1988). Three replications of 25 seeds were placed on 1% Phytagar

(GibcoBRL, Gaithersburg, Md.) medium supplemented with 0, 3, 10, or 100 μM ACC. The media were sterilized by autoclaving for 25 min at 100 °C and 1.1 $\text{kg}\cdot\text{cm}^{-2}$ pressure, and 80 mL were dispensed per culture tray (Sigma). Seeds were sterilized with 20% commercial bleach solution (Publix, Lakeland, Fla.) for 20 min followed by three rinses with sterile water. Seedlings were grown at 20 °C in continuous dark for 10 d. On the 10th day, the hypocotyl length of each seedling was measured.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSES. Germination and ethylene experiments were conducted in a split-block experimental design with each treatment replicated three times. Germination temperature was considered the main block, and for percent germination (within either dark or light conditions) split-plots were: cultivar/maturation temperature, and solution; and for ethylene split-plots were: cultivar/maturation temperature, germination temperature, and light vs. dark conditions. Data were subjected to analysis of variance (ANOVA). For percent germination data, arcsin square root transformation was performed prior to conducting an ANOVA. The third order interaction (solution $\times$ germination temperature $\times$ cultivar/maturation temperature) was significant for each data set; therefore, actual nontransformed and transformed interaction means are presented. The third-order interaction transformed means were separated by least significant difference (LSD) at 5% level.

For the ethylene sensitivity assay, a randomized complete-block experimental design with treatments replicated three times was used. Hypocotyl length data were subjected to an ANOVA.

Results and Discussion

Germination of HTM and LTM seeds of DGB and EVE was 100% in water at 20 °C in either constant light or dark (Table 1). At 20 °C, all seeds with the exception of EVE-HTM, had lower germination percentages in 20 mM STS than in water. At 36 °C, in most cases, germination percentage of LTM seeds was lower than that of HTM seeds. Seeds were imbibed at 36 °C in 20 mM STS, generally, failed to germinate, regardless of maturation temperature or light conditions.

The promotive effect of ethylene on lettuce germination and reduced germination associated with reduced ethylene perception has been reported (Abeles, 1986; Kozareva et al., 2004; Nascimento et al., 2001). Nascimento et al. (2004) reported that while germination of both DGB and EVE was reduced at 35 °C by 20 mM STS, only DGB germination was reduced at 20 °C. Seed maturation at 30/20 °C may decrease sensitivity to STS. The inhibitory effect of STS on germination was more pronounced for both cultivars at 36 than at 20 °C (Table 1). Prusinski and Khan (1990) and Nascimento et al. (2000) hypothesized that at supraoptimal germination temperatures, lettuce seeds either had an increased requirement for ethylene or that ethylene production was reduced. Prusinski and Khan (1990) based their hypotheses on the observation that lettuce seeds and seedlings were more sensitive to ethylene under stressful conditions (including high temperature) than under optimal conditions, and that conversion of ACC to ethylene was reduced by increased imbibition temperature.

At 20 °C in light, HTM seeds produced more ethylene than LTM seeds (Table 2). Increasing the imbibition temperature to 36 °C in light ethylene production was greater in HTM seeds, while ethylene production in LTM seeds was similar at both 20 and 36 °C. Imbibition in dark as compared to light, at either 20 or 36 °C, reduced ethylene production in all seeds (Table 2). In dark at 20

Table 1. Germination percentage of 'Dark Green Boston' (DGB) and 'Everglades' (EVE) lettuce seeds, matured at 30/20 °C (HTM) or 20/10 °C (LTM), at 20 or 36 °C, in light or dark, in water or 20 mM silver thiosulfate (STS).^z

Cultivar/ maturation temp	Light				Dark			
	20 °C		36 °C		20 °C		36 °C	
	Water	STS	Water	STS	Water	STS	Water	STS
	-----Germination (%)-----							
DGB-LTM	100 (90.0)	4.3 (7.1)	6.7 (12.2)	0.0 (0.0)	100 (90.0)	4.7 (10.2)	2.3 (5.2)	0.0 (0.0)
DGB-HTM	100 (90.0)	26.7 (30.1)	84.7 (71.3)	9.0 (17.3)	100 (90.0)	44.3 (41.7)	46.7 (43.1)	0.0 (0.0)
EVE-LTM	100 (90.0)	40.0 (39.2)	31.3 (33.7)	0.0 (0.0)	100 (90.0)	18.0 (24.4)	4.7 (10.2)	0.0 (0.0)
EVE-HTM	100 (90.0)	97.7 (84.9)	97.7 (84.9)	22.3 (28.1)	100 (90.0)	91.0 (76.0)	73.3 (59.2)	2.3 (5.2)
LS _{D0.05}	(12.3)				(11.3)			

^zData from light and dark incubation conditions were analyzed separately using ANOVA. Data was subjected to arcsin square root transformation prior to conducting an ANOVA. Transformed means are in parenthesis. Solution × germination temperature × cultivar/maturation temperature interaction was significant; therefore, second order interaction means (transformed) were separated using least significant difference (LSD) test.

Table 2. Ethylene production (nL·g⁻¹·h⁻¹) during germination and mean hours to germination (MHG) of 'Dark Green Boston' (DGB) and 'Everglades' (EVE) lettuce seeds matured at 20/10 °C (LTM) or 30/20 °C (HTM) at 20 or 36 °C in light or dark.

Cultivar/ maturation temp	20 °C				36 °C			
	Light		Dark		Light		Dark	
	(nL·g ⁻¹ ·h ⁻¹)	MHG	(nL·g ⁻¹ ·h ⁻¹)	MHG	(nL·g ⁻¹ ·h ⁻¹)	MHG	(nL·g ⁻¹ ·h ⁻¹)	MHG
DGB-LTM	30.5 ^z	18.6	13.6	17.7	36.4	15.8	4.5	25.4
DGB-HTM	43.8	14.2	19.5	13.5	71.5	12.4	35.4	12.6
EVE-LTM	30.2	19.1	11.5	18.8	25.0	20.6	4.0	24.4
EVE-HTM	46.6	13.8	17.7	13.3	82.4	15.8	29.4	9.5

^zEthylene values were calculated by averaging ethylene production at 9, 12, 15, 18, 21, 24, 36, and 48 h of imbibition. Cultivar/maturation temperature × germination temperature × light or dark interactions were significant; therefore, second order interaction means were separated using least significant difference (LSD) test. LSD_{0.05} for ethylene production is 7.9 and MHG is 4.6.

°C, ethylene production was similar for HTM and LTM seeds of DGB and EVE, while at 36 °C, ethylene production was greater for HTM than LTM seeds of both cultivars.

In light, at 20 or 36 °C, HTM seeds germinated more rapidly than LTM seeds (9 h vs. 12 h, respectively), regardless of cultivar, and associated with the rapid germination were earlier peaks in ethylene production for EVE seeds (Fig. 1A) and for DGB seeds (Fig. 1B) at 36 °C. In darkness, peaks of ethylene production were also earlier for HTM seeds of DGB than for LTM seeds at either 20 °C (radicle protrusion was 15 h LTM vs. 12 h HTM at 20 °C, 12 h LTM vs. 6 h HTM at 36 °C) or 36 °C (Fig. 2B). For EVE seeds (Fig. 2A), germinated at 20 °C, the peak of ethylene production was less for LTM seeds than for HTM seeds, as it was for DGB. Less ethylene was produced in dark than in light for all seeds (Table 2).

Ethylene production in all seeds, germinated at either 20 or 36 °C, decreased after germination was completed. Ethylene was only detected at or immediately after initial radicle protrusion; 6 to 15 h (data not presented). Ethylene was probably produced before radicle protrusion but not detectable until released from the seed coat. When radicle protrusion reached 90% of those seeds that germinated, peak ethylene evolution values were recorded (Fig. 1). Germination (MHG) was generally more rapid in HTM than LTM seeds (Table 2).

Possibly, in HTM seeds, more ethylene production led to earlier and greater germination at 36 °C as compared to germination of LTM seeds. In dark at 36 °C, LTM seeds may not produce sufficient ethylene to trigger germination. The reason for the increased ability of HTM seeds to produce ethylene is unclear. Some of the biosynthetic steps leading to ethylene production may be enhanced during seed maturation at 30/20 °C. The activities of ACC synthase, the enzyme converting S-adenosylmethionine to ACC, and ACC oxidase, the enzyme converting ACC to ethyl-

ene, would be the most probable targets. These two enzymes are subject to complex regulation leading to strict control of ethylene production (Calvo et al., 2004; El-Sharkawy et al., 2004). A decrease in ACC oxidase activity during lettuce seed aging has been observed (Khan, 1994). This author reported that both naturally and artificially aged lettuce seeds had both a lower capability to germinate under stressful conditions and a reduced capability to convert ACC to ethylene. Siriwitayawan et al. (2003) reported that low-vigor tomato (*Lycopersicon esculentum* Mill.) and maize (*Zea mays* L.) seeds had fewer transcripts for ACC oxidase than high-vigor seeds. Perhaps imbibition at supraoptimal temperatures subjects lettuce seeds to similar stressful conditions as seed aging. Only seeds matured at 30/20 °C, capable of producing more ethylene, can fulfill the hypothesized increased requirement for ethylene and germination.

Imbibition in dark led to reduced ethylene production in seeds of all treatments. Nascimento et al. (2004) reported that EVE seeds produced less ethylene during germination in dark than in light. Saini et al. (1989) also observed higher ethylene production and greater germination in 'Grand Rapids' lettuce seeds in light than in dark. Even though the promotive effect of light on ethylene production during lettuce germination has been documented, the mechanism of this effect is unclear. Since most of the regulation of ethylene production was reported to be on the level of ACC synthase or oxidase (Calvo et al., 2004; El-Sharkawy et al., 2004), it may be speculated that during lettuce germination light promotes the expression and/or activity of one or both of these enzymes.

No information is available on the effect of light on expression of ACC oxidase genes or activity of ACC oxidase proteins in any plant species. There are several reports about stimulation of ACC synthesis by light. Sunohara et al. (2003) reported that ACC synthase activity was strongly enhanced in pesticide-treated

Fig. 1. Ethylene production ($\text{nL}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) by 'Everglades' (A) and 'Dark Green Boston' (B) seeds matured at 30/20 °C (HTM) or 20/10 °C (LTM) following imbibition in water at 20 or 36 °C in constant light. Arrows indicate the time when $\geq 90\%$ of final germination was achieved. Vertical bars indicate SE.

Fig. 2. Ethylene production ($\text{nL}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) by 'Everglades' (A) and 'Dark Green Boston' (B) seeds matured at $30/20^\circ\text{C}$ (HTM) or $20/10^\circ\text{C}$ (LTM) following imbibition in water at 20 or 36°C in constant dark. Arrows indicate the time when $\geq 90\%$ of final germination was achieved. Vertical bars indicate SE.

maize seedlings in light but not in dark. Bessler et al. (1998) hypothesized that light-stimulation of ethylene production in spanish moss (*Tillandsia usneoides* L.) was through an increase in the amount of available ACC. It is possible that light stimulates ethylene biosynthesis indirectly. Gallardo et al. (1992) reported that in vivo ACC oxidase activity was inhibited by abscisic acid (ABA) in embryonic axes of chick-pea (*Cicer arietum* L.) seeds. Light is known to inhibit ABA biosynthesis and stimulate ABA catabolism in seeds of many species, including lettuce (Roth-Bejerano et al., 1999). Therefore, light may enhance ACC conversion to ethylene in lettuce seeds by potentially counteracting the effect of ABA. Antagonism between ethylene and ABA during seed development and germination has been reported for several plant species, including thale-cress [*Arabidopsis thaliana* (L.) Heynh.] and hybrid petunia (*Petunia hybrida* hort. Vilm.) (Barry, 2004; Beaudoin et al., 2000; Ghassemian et al., 2000).

At 20 °C, in both light and dark, all seeds germinated at 100%, regardless of their level of ethylene production (Tables 1 and 2). This demonstrated that even when ethylene production was reduced more than two times by the absence of light, enough ethylene was present to trigger germination of all seeds at 20 °C. Nascimento et al. (2000) and Kozareva et al. (2004) hypothesized that the requirements for ethylene increased at supraoptimal germination temperatures. This hypothesis was supported by the observation that germination of HTM seeds of DGB and EVE in both light and dark was accompanied by higher ethylene production at 36 °C than at 20 °C (Figs. 1 and 2).

The response of HTM and LTM seeds of both cultivars to exogenously supplied ACC and ACC/STS was examined in order to determine whether seeds matured at the two different temperature conditions responded differently to an ethylene precursor or to

an inhibitor of ethylene perception. At 20 °C in light, both HTM and LTM seeds of DGB and EVE germinated at 100% in water or 10 mM ACC (Table 3). When imbibition was conducted in 20 mM STS, germination of all seeds was significantly reduced. Germination in a combination of 10 mM ACC and 20 mM STS, was generally greater than in STS alone.

In darkness, all seeds germinated at or near 100% in water or 10 mM ACC at 20 °C (Table 4). Imbibition in 20 mM STS reduced germination of all seeds when compared to germination in water or ACC. When ACC and STS were combined, germination at 20 °C of all seeds was greater than germination in STS alone (Table 4). In darkness and water, germination of all seeds at 36 °C was lower than at 20 °C. Germination in 10 mM ACC was greater for HTM seeds than for LTM seeds (Table 4). Imbibition in 20 mM STS inhibited germination of all seeds at 36 °C.

The effects of ACC, STS, and combinations of both on germination of HTM and LTM seeds demonstrated the complexity of regulation of lettuce germination. Application of 10 mM ACC increased germination at 36 °C in light and dark (Tables 3 and 4). This indicated that the low amount of ethylene produced may have limited lettuce germination at supraoptimal temperatures. Nascimento et al. (2004) reported that EVE seeds produced more ethylene in ACC than in water.

At 36 °C in dark, germination of LTM and HTM seeds of both cultivars was increased when ACC was present. However, ACC had a stronger effect on germination of HTM seeds than of LTM seeds. It appears that the conversion of ACC to ethylene may be more efficient in HTM seeds than in LTM seeds. Possibly, HTM seeds accumulate more ACC or ACC oxidase, which facilitates germination at supraoptimal temperatures.

In order to test whether the enhanced germination of HTM

Table 3. Mean germination (%) in 10 mM 1-aminocyclopropane-1-carboxylic acid (ACC), 20 mM silver thiosulfate (STS), and 10 mM ACC + 20 mM STS at 20 and 36 °C in light of 'Dark Green Boston' (DGB) and 'Everglades' (EVE) lettuce seeds matured at 20/10 °C (LTM) or 30/20 °C (HTM).^z

Cultivar/ maturation temp	20 °C				36 °C			
	Water	STS	ACC	ACC + STS	Water	STS	ACC	ACC + STS
	Germination (%)							
DGB-LTM	100 (90.0)	0.0 (0.0)	100 (90.0)	0.0 (0.0)	26.7 (30.7)	0.0 (0.0)	73.3 (59.2)	1.7 (4.3)
DGB-HTM	100 (90.0)	35.0 (36.2)	100 (90.0)	9.0 (17.3)	80.0 (64.2)	10.0 (18.4)	98.3 (85.7)	15.0 (22.6)
EVE-LTM	100 (90.0)	28.3 (32.0)	100 (90.0)	0.0 (0.0)	33.3 (35.0)	0.0 (0.0)	86.7 (69.5)	3.3 (6.2)
EVE-HTM	100 (90.0)	88.3 (70.7)	100 (90.0)	22.3 (28.1)	100 (90.0)	23.3 (28.7)	100 (90.0)	76.7 (61.2)
LSD _{0.05}	(8.3)							

^zData was subjected to arcsin square root transformation prior to conducting an ANOVA. Transformed means are in parenthesis. Solution × germination temperature × cultivar/maturation temperature interaction was significant; therefore, second order interaction means (transformed) were separated using least significant difference (LSD) test.

Table 4. Mean germination (%) in 10 mM 1-aminocyclopropane-1-carboxylic acid (ACC), 20 mM silver thiosulfate (STS), and 10 mM ACC + 20 mM STS at 20 and 36 °C in dark of 'Dark Green Boston' (DGB) and 'Everglades' (EVE) seeds matured at 20/10 °C (LTM) or 30/20 °C (HTM).^z

Cultivar/ maturation temp	20 °C				36 °C			
	Water	STS	ACC	ACC + STS	Water	STS	ACC	ACC + STS
	Germination (%)							
DGB-LTM	98.3 (85.7)	5.0 (12.9)	100 (90.0)	35.0 (35.7)	3.3 (8.6)	0.0 (0.0)	15.0 (22.6)	0.0 (0.0)
DGB-HTM	100 (90.0)	48.3 (44.0)	100 (90.0)	90.0 (71.6)	63.3 (52.9)	0.0 (0.0)	93.3 (75.2)	3.3 (8.6)
EVE-LTM	100 (90.0)	18.3 (25.3)	100 (90.0)	85.0 (67.4)	0.0 (0.0)	0.0 (0.0)	20.0 (26.3)	0.0 (0.0)
EVE-HTM	100 (90.0)	63.3 (52.8)	100 (90.0)	100 (90.0)	66.7 (54.8)	5.0 (12.9)	96.7 (83.9)	33.3 (35.3)
LSD _{0.05}	(7.6)							

^zData was subjected to arcsin square root transformation prior to conducting an ANOVA. Transformed means are in parenthesis. Solution × germination temperature × cultivar/maturation temperature interaction was significant; therefore, second order interaction means (transformed) were separated using least significant difference (LSD) test.

seeds was not due to enhanced sensitivity to ethylene, the ability of seeds matured at different temperatures to perceive exogenously supplied ACC was tested using a triple response bioassay. During this bioassay, a reduction of hypocotyl length with increasing ACC concentrations evaluates sensitivity to ethylene. Using this bioassay, no differences between the hypocotyl length of HTM and LTM seedlings were detected (data not shown). Even though differences in the perception of ethylene between germinating seeds can not be entirely excluded, it appears more probable that differences in the amount of ethylene produced is the “ethylene-related” factor determining the ability of lettuce seeds to germinate at high temperature.

Ethylene has been implicated to be one of the factors regulating lettuce germination, especially under stressful conditions (Abeles, 1986; Kozareva et al., 2004; Nascimento et al., 2000; Saini et al., 1989). Seed maturation at supraoptimal temperatures also has been reported to improve lettuce germination at high temperatures (Sung et al., 1998a). Our results demonstrated that the promotive effect of high environmental temperatures during seed maturation on subsequent germination was associated with increased ethylene production. Therefore, thermosensitive lettuce seeds can be made thermotolerant, when they are produced under higher than optimal temperatures (e.g., 30/20 °C), while thermotolerant seeds can be made thermosensitive, when they are matured under low temperatures (e.g., 20/10 °C). The latter finding indicates that the sensitivity to, and tolerance of high imbibition temperature of different lettuce genotypes are relative, and depend on the balance of the seeds’ genetic predisposition to thermoinhibition and the environmental conditions during seed maturation. The different capability of seeds matured at low and high temperatures to convert ACC to ethylene suggests that the ethylene biosynthesis may be enhanced in seeds matured at supraoptimal temperatures. However, more studies are required to elucidate the relationship between ethylene biosynthesis and seed maturation temperature.

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