

# Carrot seed germination and ethylene production at high temperature in response to seed osmopriming

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## ABSTRACT

Carrot (*Daucus carota*) seed germination can be erratic or reduced under temperatures above 35°C. Priming circumvents seed thermoinhibition in several crops, including carrot. The involvement of ethylene in germination at supra-optimal temperatures has been reported in other species, but its role in carrot seed germination has not been examined. The objective of this study was to determine whether priming-mediated improvement in carrot seed germination at high temperature was associated with increased ethylene production. Seeds of two tropical carrot genotypes (Alvorada and Brasília) were incubated over a range of temperatures. 'Brasília' seeds were primed for 72 hours in aerated PEG solution at 15°C under light conditions and germination tested at 20 and 35°C. Seeds of a thermosensitive genotype 'Arrowhead' were primed for 0, 4, 8 and 12 days in aerated PEG 6000 solutions at 15°C under light conditions. Seeds were incubated at 20 and 35°C, and ethylene production was measured immediately before visible primary root protrusion. Temperatures above 30°C decreased seed germination percentage of 'Alvorada' and 'Brasília'. Priming increased 'Brasília' seed germination at 35°C. 'Arrowhead' germinated 96% at 20°C, and 13% at 35°C. Seed germination and ethylene production of 'Arrowhead', a thermosensitive genotype, increased in response to increased duration of priming. The results suggest that seed priming circumvents thermoinhibition of carrot seed germination by increasing ethylene production at high temperatures.

**Keywords:** *Daucus carota*, thermotolerance, stand establishment, osmoconditioning, cultivars.

## RESUMO

### Germinação de sementes de cenoura e produção de etileno em alta temperatura em resposta ao osmocondicionamento

A germinação de sementes de cenoura (*Daucus carota*) pode ser irregular ou reduzida em temperaturas superiores a 35°C. O condicionamento osmótico contorna a termo-inibição de sementes de diversas espécies, incluindo a cenoura. O envolvimento de etileno na germinação de sementes em temperaturas supra-ótimas tem sido relatado em outras espécies, mas o seu papel na germinação de sementes de cenoura não tem sido investigado. O objetivo deste estudo foi determinar se a tolerância de sementes osmocondicionadas de cenoura à germinação sob altas temperaturas está associada ao aumento da produção de etileno. Sementes de dois genótipos de cenoura (Alvorada e Brasília) foram incubadas em várias temperaturas. Sementes de 'Brasília' foram condicionadas por 72 h em soluções aeradas de PEG 6000 a 15°C sob luz e, em seguida, incubadas a 20 e 35°C. Sementes de 'Arrowhead', genótipo termo-sensível, foram condicionadas por 0, 4, 8 e 12 dias em solução aerada de PEG a 15°C sob luz. As sementes foram incubadas a 20 e 35°C e a produção de etileno foi determinada imediatamente antes da emissão visível da raiz primária. Temperaturas superiores a 30°C prejudicaram a germinação de sementes de 'Alvorada' e 'Brasília'. O condicionamento osmótico beneficiou a germinação das sementes de cenoura 'Brasília', a 35°C. Sementes de cenoura 'Arrowhead' germinaram 96% a 20°C, e 13% a 35°C. A germinação de sementes e produção de etileno no genótipo termo-sensível 'Arrowhead', aumentaram em resposta ao aumento da duração do condicionamento osmótico. Os resultados sugerem que o condicionamento osmótico contorna a termo-inibição das sementes de cenoura pelo aumento da produção de etileno em condições de altas temperaturas.

**Palavras-chave:** *Daucus carota*, termotolerância, estabelecimento de plântulas, condicionamento osmótico, cultivares.

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Carrot crops are established by direct seeding, and poor stands may occur when sowing is done during extremely high temperatures. Many publications relate the negative effects of high temperatures (35-40°C) on carrot stand establishment (Cantliffe & Elballa, 1994; Vieira *et al.*, 2005; Nascimento & Pereira, 2007; Pereira *et al.*, 2007; Nascimento *et al.*, 2008). In tropical areas, carrot production is vulnerable to

loss due to thermal stress during stand establishment (Vieira *et al.*, 2005), and most commercial carrot cultivars have reduced seed germination at high temperatures.

Seed priming has been shown to be an effective treatment for the improvement of carrot stand establishment (Szafiroswska *et al.*, 1981; Brocklehurst & Dearman, 1983; Cantliffe & Elballa, 1994; Nascimento

& Pereira, 2007). Priming is based on seed hydration under conditions that allow the occurrence of metabolic activity but prevent cellular elongation and primary root emergence. The success of osmotic priming depends on several factors including duration of soaking.

The role of ethylene in seed germination has been extensively studied in several species (Abeles *et al.*, 1992),

and it was observed that ethylene may be involved in the removal of inhibitors and thus de-repress germination (Bewley *et al.*, 2013), especially under stress conditions. In lettuce, it has been suggested that ethylene is necessary for germination at supra-optimal temperatures (Nascimento *et al.*, 2000, 2004; Kozareva *et al.*, 2004, 2006). Nascimento *et al.* (2008) verified that the ability of carrot seeds to germinate at high temperatures is genotype-dependent, and the thermotolerant genotypes identified in that study might be useful for incorporating thermotolerance traits into breeding programs.

The involvement of ethylene in carrot seed germination, especially under stress conditions, has not been examined. The objective of this study was to determine whether priming-effects in carrot seed germination at high temperature was associated with increased ethylene production.

## MATERIAL AND METHODS

### Germination at range of temperatures using two sub-tropical cultivars

*Plant material* - Seeds of two lots from 'Brasília' and 'Alvorada' (Embrapa Vegetables) were used in this study. These cultivars are considered as intermediate in terms of thermotolerance trait (Nascimento *et al.*, 2008).

*Germination test* - Four replications of 25 seeds each were placed on two layers of 5.0 cm diameter germination paper (Anchor Paper, Hudson, WI) moistened with 3 mL of distilled water. Blotters were covered with 5.5 cm petri dish lids and incubated under constant light (fluorescent  $\sim 26 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) on a one-dimensional thermogradient bar (Type DB 5000, Van Dok & De Boer, B.V., Holland) at temperatures ranging from 20 to 40°C. Germination was defined as visible radicle protrusion through the seed coat and was evaluated daily during 10 days of incubation.

### Priming effect using a sub-tropical cultivar

*Plant material* - 'Brasília' carrot seeds from Embrapa Vegetables,

Brasília, Brazil, were used in this study.

*Seed priming* - Seeds were primed in 200 mm test tubes for 72 hours at 15°C with constant light ( $\sim 26 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in an aerated solution of polyethylene glycol (PEG 6000) at an osmotic potential of -1.2 MPa (30 mL g<sup>-1</sup> of seed). An aquarium pump provided aeration. The air was pre-hydrated by passing through water to minimize evaporation of the priming solution. Afterward, seeds were placed in a Buchner funnel, washed three times with 100 mL of distilled water and redried in an incubator at 15°C and 45% RH for two days.

*Seed germination* - Three replications of 25 seeds were placed in 5.5 cm petri dishes with two layers of 4.5 cm diameter #3 filter paper (Anchor Paper) moistened with 4 mL of distilled water. Additional distilled water was added as needed to keep the filter paper moist. Seeds were incubated under constant light (30  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in Precision Scientific incubators (Winchester, VA, USA) at 20°C (optimal) and 35±0.5°C (adverse), generating the total germination at 20°C (TG 20) and 35°C (TG 35), as well as the germination rate at 20°C (GR 20) and 35°C (GR 35). Germination was defined as visible primary root protrusion through the seed coat at 10 days of incubation. Germination rate was also evaluated daily during 10 days of incubation using the Maguire (1962) formula, where  $GR = N1/D1 + N2/D2 + \dots + Nn/Dn$ , N is the germinated seeds, and D is the number of days for germination.

### Priming duration and ethylene production at high temperature

*Plant material* - A thermosensitive genotype ('Arrowhead'), from Sakata Seed Co. was used in this study.

*Seed priming* - Seeds were primed in 200 mm test tubes for 0, 4, 8 and 12 days at 15°C with constant light ( $\sim 26 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in an aerated solution of polyethylene glycol (PEG 6000) at an osmotic potential of -1.2 MPa (30 mL g<sup>-1</sup> of seed). An aquarium pump provided aeration. The air was pre-hydrated by passing through water to minimize evaporation of the soaking solution.

Afterward, seeds were placed in a Buchner funnel, washed three times with 100 mL of distilled water and redried in an incubator at 15°C and 45% RH for two days.

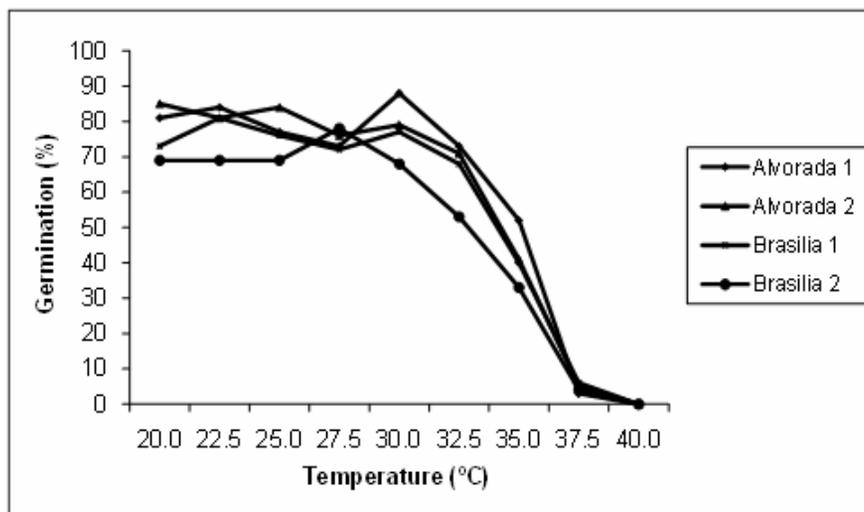
*Germination test* - Three replications of 25 seeds were incubated at 20 and 35°C using the procedures described above.

*Ethylene determination* - Three replications of 0.1 g of dry seeds were placed on two layers of 3.0 cm diameter germination paper in 50 mL vials. The filter paper was moistened with 4 mL of distilled water, the vials sealed with caps fitted with septa, and incubated under the same conditions as the standard germination procedures. After 12 hours (preliminary results indicated 12 hour-incubation, i.e, immediately before visible radicle protrusion as the ideal time), ethylene production was determined. A 1 mL gas sample was withdrawn with a gas-tight hypodermic syringe (Fisher Scientific). Ethylene was assayed using a Hewlett Packard Series II 5890 gas chromatograph (Hewlett Packard, Agilent Technologies, Foster City, CA) equipped with a flame ionization detector. The carrier gas was nitrogen. The oven, injector and detector were at 130°C, 110°C, and 150°C, respectively.

*Statistical analysis* - Analysis of variance was performed with the Genes Program (Cruz, 2006), and the characters of total germination and germination rate were transformed by  $(x + 0.50)^{1/2}$  in order to attend the normality presupposition of Lilliefors test. The means were compared through the Tukey test.

## RESULTS AND DISCUSSION

Temperatures above 30°C caused decreases in seed germination of both 'Brasília' and 'Alvorada' (Figure 1). The negative effects of high temperatures on carrot seed germination and consequently stand establishment have been previously observed in these tropical genotypes (Pereira *et al.*, 2007). However, in another study, 'Alvorada' and 'Brasília' along with XPC-3617 (Sakata) had the greatest germination



**Figure 1.** 'Alvorada' and 'Brasília' carrot seed germination at a range of temperatures (germinação de sementes de cenoura 'Alvorada' e 'Brasília' em diferentes temperaturas). Gainesville, University of Florida, 2007.

**Table 1.** Germination rate (GR) and total germination (TG) of 'Arrowhead' carrot seeds incubated at 20 and 35°C after different priming periods (velocidade de germinação (GR) e germinação total (TG) de sementes de cenoura 'Arrowhead' incubadas a 20 e 35°C após diferentes períodos de condicionamento osmótico). Gainesville, University of Florida, 2007.

Period (days)	GR20 (days)	TG20 (%)	GR35 (days)	TG35 (%)
0	2.9 a <sup>z</sup>	96 ab	6.8 a	13 c
4	2.5 b	92 b	4.4 ab	66 b
8	1.9 c	99 ab	2.4 b	96 a
12	1.7 c	100 a	1.9 b	97 a
CV(%)	4.87	3.13	25.54	8.28

<sup>z</sup> Means within a column followed by different letters are significantly different at  $p \leq 0.05$  according to Tukey test (médias seguidas por letras diferentes na coluna são significativamente diferentes a  $p < 0,05$  pelo teste de Tukey).

under high temperature; also they showed the highest thermotolerance ratio ( $Th/To$ , where  $Th$  = germination at 35°C, supra-optimal and  $To$  = germination at 25°C, optimal), (Nascimento *et al.*, 2008). The variation in percentage of germination at high temperature observed between the seed lots in the present study was possibly due to inherent differences in seed vigor. In another study (data not shown), carrot seeds from a thermotolerant genotype (XPC-3617) germinated 100% at both 20 and 35°C; however, when seeds were artificially aged (41°C/1 day), the germination remained 100% at 20°C, but declined to 82% at 35°C, showing that seeds of high vigor show higher tolerance to environmental stresses, including high temperatures.

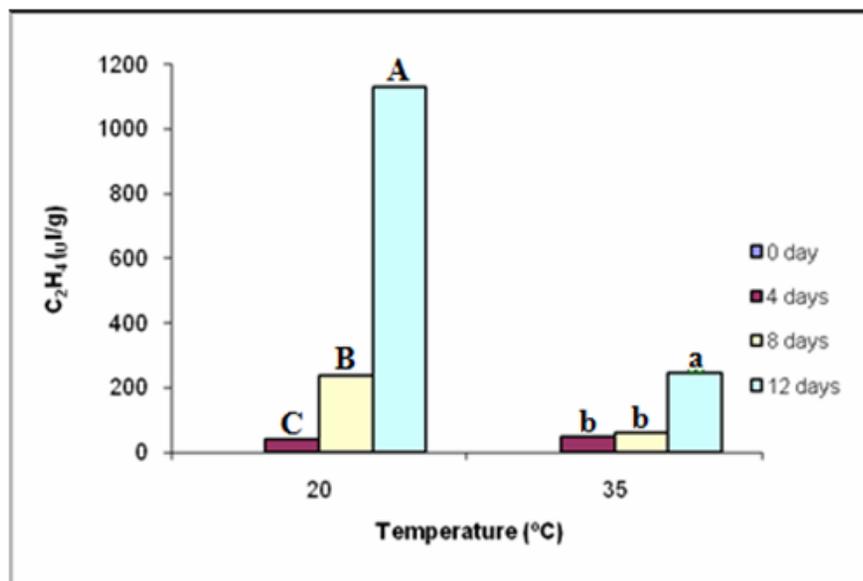
Nascimento *et al.* (2005) verified that aged lettuce seeds germinated poorly at 35°C compared with unaged seeds. Furthermore, seed aging can lead to physiological and biochemical changes including reduced ethylene production and endo- $\beta$ -mannanase activity, factors possibly contributing to thermoinhibited seed germination. Ethylene production during germination was also observed in aged tomato seeds compared with nonaged seeds (Siriwitayawan *et al.*, 2003). In addition, seeds from the sub-tropical cultivars 'Alvorada' and 'Brasília' generally have lower germination at optimal temperatures compared to imported commercial cultivars (Pereira *et al.*, 2007). In open pollinated cultivars such as 'Brasília', there is still high genetic

diversity, which is one reason why seed quality standards do not appear to be very well defined, leaving high variation in seed quality in terms of germination and vigor (Vieira *et al.*, 2005). These physiological seed quality traits may compromise crop establishment especially under conditions such as high temperatures at imbibition.

Seed priming may be effective to favor carrot seed germination at high temperatures. Priming of 'Brasília' seed, however, was of limited success in improving germination at high temperature. At 35°C unprimed and primed seeds germinated at 35% and 65%, respectively (data not shown). Germination at 20°C in both primed and unprimed seeds was 82%. As cited above, the physiological quality of those genotypes such as 'Brasília' group is lower when compared to other commercial carrot cultivars and this may have affected the better response to seed priming.

The low response to seed priming in this study was possibly due to the short period of soaking during priming. In another study, Nascimento *et al.* (2013) observed that extended priming duration may benefit results at high temperature from carrot seed priming. Accordingly, priming duration was examined for its influence on germination of thermosensitive carrot seed at high temperatures. The results are shown in Table 1. Germination of unprimed seeds was 96% at 20°C and 13% at 35°C. Priming increased germination rate and total germination at both temperatures, and improved germination responses were observed in response to longer priming duration. For example, priming periods of 4, 8, and 12 days resulted in total germination at 35°C of 66%, 96%, and 97%, respectively. At 25°C germination rate was 2.9 days in unprimed seeds and 1.7 days in seeds primed for 12 days; at 35°C germination rate was 6.8 days in unprimed seeds and 1.9 days in seeds primed for 12 days (Table 1). Thus, extending the duration of seed priming proved highly effective for improving seed germination at high temperature.

No ethylene production was observed in unprimed seeds at 20 and 35°C



**Figure 2.** Ethylene production of 'Arrowhead' carrot seeds incubated at 20 and 35°C after different priming periods (0, 4, 8 and 12 days). Means followed by the same uppercase letter and the same lowercase letter were not significantly different by Tukey test at  $p < 0.05$  [produção de etileno de sementes de cenoura 'Arrowhead' incubadas a 20 e 35°C após diferentes períodos de condicionamento osmótico (0, 4, 8 e 12 dias). Médias seguidas pela mesma letra maiúscula e mesma letra minúscula não são significativamente diferentes ( $p < 0,05$ ) pelo teste de Tukey]. Gainesville, University of Florida, 2007.

(Figure 2). For primed seeds, ethylene production was lower at 35°C than at 20°C. High temperatures have been shown to inhibit ethylene production in lettuce seeds (Nascimento *et al.*, 2000). Ethylene production increased markedly in response to priming and priming duration. It is not clear, however, whether the increased ethylene production in response to priming was simply due to increased seed vigor. Nascimento *et al.* (2005) verified that aged lettuce seeds germinated poorly at 35°C compared with unaged seeds and that seed aging reduced ethylene production during subsequent seed germination. In tomato, priming was accompanied by a significant increase in ethylene production during radicle protrusion compared with non-primed seeds (Siriwitayawan *et al.*, 2003). The authors suggested that priming reduced the time required to initiate synthesis and/or activity of both ACC-synthase and ACC-oxidase. In our study, ethylene production was measured after 12 hours of imbibition (immediately before visible radicle protrusion). Thus, a possible mechanism of seed priming in circumventing thermoinhibition of carrot seeds would be by increasing

ethylene production during germination at high temperatures. In lettuce, seed priming or adding aminocyclopropane-1-carboxylic acid (ACC) during incubation increased germination at 35°C (Nascimento *et al.*, 2004). A relationship between thermotolerance and ethylene production during carrot seed germination at high temperature was also observed by Nascimento *et al.* (2008). The authors showed that ethylene production during carrot seed germination at high temperature was higher in thermotolerant than in thermosensitive genotypes. These authors also observed a high correlation between ethylene production and carrot seed germination at high temperatures. Similar results were observed with lettuce seeds (Nascimento *et al.*, 2000).

The results suggest that a possible mechanism circumventing thermoinhibition of carrot seeds by seed priming is due to increased ethylene production during germination at high temperatures.

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