Carrot Seed Germination at High Temperature: Effect of Genotype and Association with Ethylene Production

Warley M. Nascimento¹, Jairo V. Vieira¹, and Giovani O. Silva¹

Embrapa Vegetables, C.P. 218, 70359-970 Brasília-DF, Brazil

Kathleen R. Reitsma²

Iowa State University, North Central Regional Plant Introduction Station, Ames, IA 50011-1170

Daniel J. Cantliffe^{3,4}

Horticultural Sciences Department, Institute of Food and Agricultural Sciences, University of Florida, 1251 Fifield Hall, P.O. Box 110690, Gainesville, FL 32611-0690

Additional index words. *Daucus carota* L., thermotolerance, stand establishment, vigor, cultivars, germplasm

Abstract. Carrot (*Daucus carota* L.) seed germination may be erratic or reduced under high temperatures (above 35 °C). Even in tropical genotypes (tolerant to high temperatures during crop development), the negative effects of high temperatures on carrot stand establishment have been observed, especially during summer. The objectives of this study were to characterize commercial carrot cultivars and accession lines for their ability to germinate at high temperature and determine the ethylene production during imbibition at high temperature. Seeds from 34 commercial cultivars and 125 carrot accessions from the North Central Regional Plant Introduction Station were germinated at 25 °C (optimal) and 35 ± 0.5 °C (high) in constant light. Ethylene production during seed imbibition at high temperature was evaluated in some genotypes. Many of the commercial cultivars had reduced germination at 35 °C. 'XPC-3617', 'Alvorada', 'Brasilia', and 'Esplanada' had the greatest germination at 35 °C. A greater number of accessions germinated at 35 °C than did the commercial genotypes. The accession PI 319858 germinated 95% at both temperatures and was considered thermotolerant. Six accessions (Ames 7665, Ames 7698, Ames 25031, PI 167082, PI 294637, and PI 319858) germinated above 80% at 35 °C and were also identified as potential sources of thermotolerance. Fifteen other accessions (Ames 7694, Ames 25031, Ames 25036, Ames 25049, Ames 25705, PI 167082, PI 179687, PI 180834, PI 261782, PI 269486, PI 273658, PI 277710, PI 288242, PI 294637, and PI 319858) had thermotolerance ratios of $T35/T25$ 0.85 or greater (where $T35$ = germination at 35 °C and $T25$ = germination at 25 °C) and were identified for further testing. The identified thermotolerant genotypes might be useful for carrot seed germination mechanism studies as well as for breeding programs. Ethylene production during seed germination at high temperature was greater in thermotolerant genotypes than in thermosensitive genotypes. High correlations were observed between first germination count at 35 °C and ethylene production, total germination at 35 °C and ethylene production, and thermotolerance ratio and ethylene production.

Carrot crops are established by direct seeding, and poor stands may occur when sowing is done during extremely low or high temperatures. Many publications relate the negative effects of low temperature on carrot stand establishment (Cantliffe and Elballa, 1994; Corbineau et al., 1994; Nascimento and Pereira, 2007; Pereira et al., 2007; Vieira

et al., 2005). However, high temperatures (35 to 40 °C) may also delay or inhibit carrot seed germination in the field and reduce uniformity and total stand establishment (Cantliffe and Elballa, 1994; Nascimento and Pereira, 2007). In tropical areas, carrot production is vulnerable to loss from thermal stress (heat) during stand establishment (Vieira et al., 2005), and most commercial carrot cultivars have reduced seed germination at high temperatures (Pereira et al., 2007). Carrot seeds germinate over a range from 10 to 35 °C (Rubatzky et al., 1999) with an optimal range of 25 to 30 °C (Corbineau et al., 1994). The Association of Official Seed Analysts recommends an alternating day/night regimen of 30 to 20 °C (8/16 h) as the standard protocol for carrot seed germination tests (Association of Official Seed Analysts, 1993).

Superior genetic resources are required to reduce the risk of loss from high temperature stress. Within the carrot collection of the USDA-ARS, located in Ames, IA, there are 1129 accessions in the NPGS *Daucus* collection with 893 accessions available for distribution (K. Reitsma, personal communication). Germplasm characterization is an important function of a gene bank, and knowing the morphological and agronomic traits as well as their reaction to biotic and abiotic stresses in the individual accessions increases the potential usefulness of the germplasm collection (Day-Rubenstein et al., 2006).

Carrot cultivars differ in their sensitivity to high temperatures during seed germination (Pereira et al., 2007). One approach to improve thermotolerance (ability of seed to germinate at high temperature) is to transfer superior alleles from intrinsically thermotolerant wild relatives to less tolerant commercial cultivars (Senthil-Kumar et al., 2007). However, the genetic variation in thermotolerance among cultivated carrot gene bank accessions has not been determined (P. Simon, personal communication).

Thermotolerance in seeds is typically a multigenic trait (Senthil-Kumar et al., 2007), and there is no known thermotolerance mechanism in carrot. In lettuce, quantitative trait loci analysis may provide a new approach for elucidating the physiological factors controlling the imposition and release of seed thermoinhibition (Argyris et al., 2005). Tolerance to high temperatures during germination would seem to require constitutive genetic effects, although the mother-plant environment during seed development and maturation can also affect carrot seed quality (Gray et al., 1988) and influence thermotolerance as observed in other species (Sung et al., 1998). Also, tolerance to high temperatures during seed germination and early seedling growth involves acclimation effects such as synthesis of heat-shock proteins (Vierling, 1991). Oxygen requirements for seed germination may also be modulated by temperature (Bradford et al., 2007), and high temperatures during carrot seed imbibition may affect sensitivity to low oxygen tensions (Corbineau et al., 1994).

High seed vigor is necessary for tolerance to environmental stress (Heydecker, 1972), including high temperatures. For example, improved seedling emergence and uniformity of preconditioned lettuce seeds at high temperature was related to high vigor (Perkins-Veazie and Cantliffe, 1984). Examining two lettuce genotypes, these authors found that priming prevented thermodormancy in unaged but not in aged lettuce seeds. Seed aging can lead to some physiological and biochemical changes such as reduced ethylene and endo- β -mannanase activity and thus lead to thermoinhibition in germination, as observed in lettuce seeds (Nascimento et al., 2005).

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

Received for publication 17 Dec. 2007. Accepted for publication 2 Apr. 2008.

We thank the CNPq for financial support, process no. 470782/2004-8.

¹Research Scientist.

²Curator.

³Distinguished Professor and Chair.

⁴To whom reprint requests should be addressed; e-mail djcant@ufl.edu

been suggested that ethylene is necessary for germination at supraoptimal temperatures (Kozareva et al., 2004, 2006; Nascimento et al., 2000, 2004). The involvement of ethylene in carrot seed germination, especially under stress conditions, has not been studied.

The objectives of this study were to characterize commercial carrot genotypes and screen germplasm to identify lines with greater tolerance to germinate at high temperatures and then to determine if there is a possible correlation between ethylene production and seed germination at high temperatures.

Materials and Methods

Germination at high temperature of carrot cultivars from various commercial sources

Plant material. Seeds of 34 commercial carrot genotypes (hybrids and OP varieties) were used in this study (Table 1).

Germination test. 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 Co., St. Paul, MN) 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 ($30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in Precision Scientific (Winchester, VA) incubators at 25 °C (optimal) and 35 ± 0.5 °C (adverse). Germination was defined as visible radicle protrusion through the seedcoat and was evaluated after 5 (first count) and 10 (final count) d of incubation. A thermotolerance ratio ($T35/T25$, where $T35$ = germination at 35 °C, supraoptimal and $T25$ = germination at 25 °C, optimal) was created to minimize possible errors from using seeds with low viability overall.

Seed mass. Three replications of 100 seeds were weighed on an analytical scale to the nearest milligram.

Statistical analysis. Analysis of variance was performed with the Genes Program (Cruz, 2001), and the characters of first count, total germination, and the ratio of $T35/T25$ were transformed by $(x + 0.50)^{1/2}$ to attend

the normality presupposition of Lilliefors' (1967) test. The means were compared with the Scott and Knott (1974) test, and simple correlations between character pairs were calculated.

Screening carrot germplasm for seed germination at high temperature

Seeds from the Introduction Station (NCRPIS), located in Ames, IA, part of the U.S. National Plant Germplasm System, were used in this study. The accessions were selected according to seed quality (only the accessions that were regenerated in recent years and under like environmental conditions and protocols were used). Also included were a few accessions in which the donor (Korea) noted the accessions to have tolerance to high temperatures.

Germination test. Three replications of 20 seeds were incubated by using the same procedures as described previously for commercial seed samples.

Statistical analysis. The data from the 25 °C germination test were transformed by

Table 1. Analysis of variance and comparison of means for seed mass (SM), first germination count at 25 °C (FC25), total germination at 25 °C (TG25), first germination count at 35 °C (FC35), total germination at 35 °C (TG35), and thermotolerance ratio ($T35/T25$) from 34 commercial carrot cultivars.

		MS					
	df	SM	FC25	TG25	FC35	TG35	$T35/T25$
Block	2	0.001	57.56	4.86	3.37	18.98	0.02
Genotype	33	0.011*	403.31*	196.67*	9.70*	9.84*	0.01
Residue	66	0.001	45.28	38.80	2.42	2.60	0.01
CV		3.29	8.02	6.95	46.35	38.60	40.17
CVg/CVe		1.57	1.62	1.16	1.00	0.96	1.01
Genotype	Company	SM (g)	FC25 (%)	TG25 (%)	FC35 (%)	TG35 (%)	$T35/T25$
Arrowhead	Sakata	0.29 c ^z	99 a	100 a	4 c	7 b	0.07 b
Copperhead	Sakata	0.21 g	97 a	100 a	8 b	15 b	0.15 b
Trinity	Sakata	0.15 i	69 d	84 b	0 c	1 b	0.02 b
Columbia	Sakata	0.26 d	84 b	91 a	1 c	1 b	0.01 b
Spearhead	Sakata	0.14 j	93 a	96 a	13 b	20 a	0.21 b
XCR-3617	Sakata	0.22 f	97 a	97 a	57 a	68 a	0.70 a
Maverick	Siegers	0.12 k	93 a	100 a	3 c	11 b	0.11 b
Nantindo	Harris	0.17 h	85 b	89 b	9 b	23 a	0.26 b
R. Chantenay	Harris	0.14 j	79 c	83 b	7 b	9 b	0.12 b
Magnum	Harris	0.18 h	87 b	87 b	29 a	35 a	0.40 a
Scarlet Nantes	Harris	0.08 n	76 c	85 b	1 c	1 b	0.01 b
Topcut	Nunhems	0.28 c	93 a	93 a	13 b	20 a	0.22 b
Navajo	Nunhems	0.30 b	95 a	97 a	0 c	5 b	0.06 b
Primecut xx	Nunhems	0.13 k	96 a	96 a	13 b	28 a	0.29 b
Primecut xc	Nunhems	0.12 l	96 a	97 a	16 b	24 a	0.25 b
Topcut 93	Nunhems	0.25 e	95 a	97 a	17 a	35 a	0.35 a
Topcut xx	Nunhems	0.24 e	84 b	88 b	11 b	19 a	0.21 b
Sugarsnax xx	Nunhems	0.12 l	95 a	96 a	17 a	17 b	0.18 b
Sugarsnax xc	Nunhems	0.10 m	93 a	97 a	9 b	15 b	0.15 b
Esplanada	Embrapa	0.22 f	85 b	89 b	44 a	53 a	0.60 a
Brasilia EH	Embrapa	0.20 g	68 d	71 c	43 a	47 a	0.66 a
Alvorada EH	Embrapa	0.17 h	68 d	73 c	37 a	45 a	0.63 a
Alvorada IL	Isla	0.13 k	79 c	79 c	27 a	35 a	0.46 a
Brasilia IL	Isla	0.14 j	68 d	80 c	9 b	12 b	0.16 b
Kuronan	Isla	0.17 h	81 b	84 b	13 b	28 a	0.33 a
Tropical	Isla	0.16 i	81 b	88 b	36 a	43 a	0.48 a
Burpee A1	Burpee	0.18 h	84 b	88 b	1 c	15 b	0.16 b
Imperator 58	Ferry Morse	0.15 i	81 b	95 a	3 c	13 b	0.14 b
Danvers 126	Ferry Morse	0.15 i	76 c	84 b	23 b	25 b	0.31 b
Chantenay	Ferry Morse	0.10 m	52 e	79 c	5 c	9 b	0.12 b
Nantes C.	Ferry Morse	0.20 g	87 b	96 a	24 a	35 a	0.37 b
Senator	Clause	0.33 a	64 d	79 c	4 c	5 b	0.07 b
Major	Clause	0.13 k	96 a	99 a	19 b	21 b	0.22 b
Osiris	Clause	0.15 i	73 c	88 b	11 b	13 b	0.16 b

^zMeans within a column followed by different letters are significantly different at $P \leq 0.05$ according to Scott-Knott test.

*Significant at $P \leq 0.01$ by F test.

CV = phenotypic coefficient of variation; CVg/CVe = relation between genetic and environmental CV.

Table 2. Analysis of variance for first germination count at 25 °C (FC25), total germination at 25 °C (TG25), first germination count at 35 °C (FC35), total germination at 35 °C (TG35), and thermo-tolerance ratio ($T35/T25$) of 62 carrot accessions.

	df	MS				
		FC25	TG25	FC35	TG35	$T35/T25$
Block	2	1.02	0.40	2.00	0.10	0.005
Genotype	61	3.97*	0.89*	33.12*	32.90*	0.41*
Residue	122	0.44	0.21	0.64	0.55	0.009
CV		7.91	5.02	15.77	13.78	16.15
CVg/CVe		1.64	1.03	4.12	4.41	3.79
Means—group 1		79.88	83.49	56.83	61.50	0.75
Means—group 2		56.51	85.90	0.90	2.04	0.02

Significant at $P \leq 0.01$ by F test.

CV = phenotypic coefficient of variation; CVg/CVe = relation between genetic and environmental CV.

$\sqrt{x}$ and the data of 35 °C transformed by $(x + 0.50)^{1/2}$ to attend the normality presupposition. Analysis of variance and dissimilarity analysis based on Mahalanobis (D^2) Generalized Distances were performed with the Genes Program (Cruz, 2001), in which the dissimilarity matrix was transferred to the NTSYSpC Program (Rohlf, 2000) for the creation of a dendrogram based on the unweighted pair group method using the arithmetic averages clustering algorithm and determination of a cophenetic correlation coefficient between the matrix and the clus-

ters (Rohlf and Sokal, 1981). An estimation of hypothetical ideal (Ideotype) was performed, in which the values for each character were considered as the highest among the accessions. For the accessions of each group, a means analysis was performed following the procedures of Scott and Knott (1974). Accession means for each group were calculated for each character, and a simple correlation among the characters was also performed. Analysis of relative importance of the characters for the accession dissimilarity was done following Singh (1981). The

best genotypes for all characters for each group were chosen by using the selection index based on Pesek and Baker (1969).

Germination and ethylene production at high temperature

Plant material. From the first experiment, six genotypes were chosen according to their germination at high temperature and three classes were generated: thermosensitive ('Arrowhead' and 'Maverick'), intermediate ('Brasilia' and 'Magnum'), and thermotolerant ('XCR-3617' and PI 319858).

Germination test. Three replications of 25 seeds were incubated by using the same procedures as described previously.

Ethylene determination. Three replications of 0.1 g of dry seeds were placed on two layers of 3.0-cm diameter germination paper (Anchor Paper Co.), which were placed in the base of 50-mL volume vials sealed with rubber septa (Fisher Scientific, Pittsburgh, PA). The seeds in the vials were moistened with 4 mL of distilled water and then incubated under the same conditions as the standard germination procedures. After 24 h of imbibition (at radicle protrusion), ethylene production was determined. A 1-mL gas

Fig. 1. Dendrogram of 66 carrot accessions based on germination characters generated from a Mahalanobis Similarity Matrix. Ital. = Italy; Et. = Ethiopia; Ind. = India; Pol. = Poland; Fra. = France; Jor. = Jordan; Tur. = Turkey; Jap. = Japan; Russ. = Russia; Egy. = Egypt; NewZ. = New Zealand; SoA = South Africa; SoK. = South Korea; Germ. = Germany; Net. = Netherlands; Taj. = Tajikistan; Georg. = Georgia; Bulg. = Bulgaria; Gree. = Greece.

sample was withdrawn with a gas-tight hypodermic syringe (Fisher Scientific). Ethylene was assayed by 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 temperatures were 130, 110, and 150 °C, respectively.

Statistical analysis. The characters were transformed by $x^{1/2}$ to attain a normal distribution. A means analysis was performed following the procedures of Scott and Knott (1974), and simple correlations between character pairs were calculated.

Results and Discussion

Germination at high temperature of carrot seeds from different commercial cultivars. The commercial cultivars differed in most of the parameters examined (Table 1). No correlation between seed mass and seed germination at 25 and 35 °C was observed (data not shown). At 25 °C, seed germination of most cultivars was above 80% (Table 1); however, the subtropical cultivars (Alvorada, Brasília, Alvorada IL, and Chanetenay) were lower. Nevertheless, those three cultivars, along with 'XCR-3617', had the greatest germination under high temperature and also the highest thermotolerance ratio (Table 1). Most other cultivars had reduced germination at 35 °C. In another study, Carneiro and Guedes (1992) verified that 'Brasília' germinated 91% at 25 °C, whereas at 35 °C, germination decreased to 47%. Also, using the same cultivar, Nascimento and Pereira (2007) observed a higher reduction of germination at 35 °C. In addition, 'Brasília' carrot seeds that were previously incubated at 35 or 45 °C lost their ability to germinate when transferred to 20 to 25 °C, possibly as a result of an excessive increase in seed respiration and metabolic activities (Nascimento and Pereira, 2007) or a consequence of accelerated aging (Corbineau et al., 1994). Cultivars from the 'Brasília' group are cultivated largely in tropical regions (Simon et al., 2007). For example, in Brazil, ~80% of the carrot production area is sown with seeds from the 'Brasília' group (Nascimento et al., 2003). As described previously, seeds from these cultivars generally have lower germination at optimal temperatures compared with imported commercial cultivars. In open-pollinated cultivars such as 'Brasília', there is still high genetic diversity, which is one of the reasons 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 traits may compromise crop establishment, especially under conditions such as high temperatures at imbibition.

Screening carrot germplasm for seed germination at high temperature. In this study, 63 of the initial 125 accessions were eliminated as a result of low germination or low vigor (data not shown). This was done to improve confidence levels in the statistical

analysis. The analysis of variance from these results showed that all parameters were significantly different among the genotypes (Table 2) and the coefficients of variation were low, indicating good experimental precision, especially at 25 °C. The relation between the genetic and environmental coefficients of variation (CVg/CVe) were higher than 1, indicating predominance of genetic order effects, suggesting that satisfactory gains could be obtained from selection, especially at 35 °C.

According to the Mahalanobis Generalized Distance (D^2) (Mahalanobis, 1936), two large groups and a small group of two accessions near to the second group could be distinguished (Fig. 1). Our "Ideotype" (or ideal accession), a fictitious genotype originating from the higher values in each replication for each one of the characters, fit in the first group. The accession nearest to the "Ideotype" was PI 319858 from Japan. Thus, this PI was the most promising accession for good germination at both 25 and 35 °C. This accession germinated 95% under both temperature conditions and was considered ther-

motolerant. The accession grouping is important, because crosses between accessions from different groups (more distinct) might lead to heterotic gains (Cruz and Regazzi, 1997; Falconer, 1981) and populations with broader genetic variation (Mohammadi and Prasanna, 2003).

Based on the mean values for each group, it was observed that the accessions from the first group had the highest means, especially at 35 °C (Table 3). For the second group, the means did not have any significant differences at 25 °C, because only three clusters were formed (Table 4). At 35 °C, however, many accessions did not germinate or had low germination values. The accessions Ames 25915 from Turkey, PI 274298 from Pakistan, and Ames 23983 from Bulgaria had the highest germination at 35 °C, although their values were lower than those from the first group.

A high correlation was observed between the first count and total germination, especially at 35 °C (data not shown). For the same character under both temperature conditions, no significant correlations were observed,

Table 3. Mean comparison of first germination count at 25 °C (FC 25) and 35 °C (FC 35), total germination at 25 °C (TG 25) and 35 °C (TG 35), and thermotolerance ratio ($T35/T25$) from carrot germplasm accessions in group 1.

Genotypes	Origin	FC 25 (%)	TG 25 (%)	FC 35 (%)	TG 35 (%)	$T35/T25$
Ames 7661	Italy	85 h ^z	90 f	70 e	75 f	0.84 b
Ames 7664	Italy	92 d	93 d	67 g	67 k	0.72 c
Ames 7665	Italy	90 e	90 f	83 b	87 b	0.97 a
Ames 7680	Netherlands	72 n	72 o	42 s	48 u	0.69 c
Ames 7683	Soviet Union	78 k	80 k	45 r	50 t	0.63 d
Ames 7684	Georgia	70 o	73 n	60 k	60 n	0.85 b
Ames 7685	New Zealand	85 h	88 g	53 n	53 r	0.60 d
Ames 7694	Netherlands	68 p	70 p	62 j	62 m	0.89 b
Ames 7698	France	88 f	88 g	80 c	83 d	0.95 a
Ames 23894	Germany	70 o	87 h	8 B	18 D	0.21 e
Ames 25031	Turkey	82 i	83 i	83 b	85 c	1.03 a
Ames 25036	Russia	63 q	63 q	65 h	70 i	1.15 a
Ames 25049	India	73 m	77 m	62 j	65 l	0.85 b
Ames 25058	India	87 g	88 g	67 g	67 k	0.76 c
Ames 25082	Tajikistan	63 q	72 o	40 t	47 v	0.71 c
Ames 25378	Poland	80 j	90 f	15 A	22 C	0.25 e
Ames 25702	Syria	90 e	90 f	60 k	65 l	0.72 c
Ames 25705	Syria	62 r	62 r	48 q	52 s	0.88 b
Ames 25895	Turkey	68 p	88 g	23 x	33 z	0.38 e
Ames 25897	Turkey	72 n	78 l	20 z	30 A	0.40 e
Ames 25903	Turkey	73 m	87 h	33 u	45 x	0.53 d
Ames 27288	Poland	93 c	95 c	20 z	28 B	0.30 e
PI 167082	Turkey	92 d	95 c	80 c	80 e	0.84 b
PI 169487	Turkey	92 d	95 c	57 m	60 n	0.63 d
PI 169488	Turkey	73 m	73 n	52 o	55 q	0.75 c
PI 178900	Turkey	73 m	87 h	57 m	60 n	0.70 c
PI 179687	India	78 k	78 l	63 j	67 k	0.86 b
PI 180834	Turkey	68 p	70 p	63 j	67 k	0.96 a
PI 261782	France	78 k	78 l	70 e	73 g	0.94 a
PI 269486	Pakistan	75 l	78 l	58 l	68 j	0.89 b
PI 272258	South Africa	85 h	88 g	50 p	57 p	0.64 d
PI 273658	Ethiopia	87 g	88 g	67 q	75 f	0.85 b
PI 277710	Netherlands	60 s	60 s	50 p	58 o	0.98 a
PI 285613	Poland	92 d	92 e	63 j	73 g	0.80 c
PI 288242	Egypt	80 j	82 j	72 d	72 h	0.88 b
PI 288243	Egypt	82 i	83 i	53 n	57 p	0.69 c
PI 294637	Jordan	85 h	90 f	80 c	80 e	0.89 b
PI 319858	Japan	95 b	95 c	95 a	95 a	1.00 a
PI 319859	Japan	90 e	97 b	68 f	73 g	0.76 c
PI 537093	South Korea	85 h	88 g	30 v	45 x	0.51 d
Ideotype	—	100 a	100 a	95 a	95 a	0.95 a

^zMeans within a column followed by different letters are significantly different at $P \leq 0.05$ according to Scott-Knott test.

Table 4. Mean comparison of first germination count at 25 °C (FC25) and 35 °C (FC35), total germination at 25 °C (TG25) and 35 °C (TG35), and thermotolerance ratio (*T35/T25*) from carrot germplasm accessions in group 2.

Genotypes	Origin	FC25 (%)	TG25 (%)	FC35 (%)	TG35 (%)	<i>T35/T25</i>
Ames 23981	Bulgaria	68 a ^z	88 a	0 b	0 b	0.00 b
Ames 25562	Greece	23 c	62 c	2 a	5 b	0.07 a
Ames 25563	Greece	32 c	82 b	0 b	2 b	0.02 b
Ames 25564	Greece	52 b	90 a	0 b	2 b	0.02 b
Ames 25568	Greece	87 a	95 a	0 b	0 b	0.00 b
Ames 25572	Greece	73 a	90 a	3 a	3 b	0.04 b
Ames 25573	Greece	53 b	100 a	0 b	0 b	0.00 b
Ames 25574	Greece	73 a	92 a	0 b	0 b	0.00 b
Ames 25580	Greece	77 a	85 a	0 b	0b	0.00 b
Ames 25581	Greece	77 a	92 a	0 b	0 b	0.00 b
Ames 25583	Greece	52 b	77 b	0 b	0 b	0.00 b
Ames 25585	Greece	72 a	93 a	0 b	0 b	0.00 b
Ames 25587	Greece	35 c	85 a	0 b	0 b	0.00 b
Ames 25588	Greece	38 c	82 b	0 b	0 b	0.00 b
Ames 25589	Greece	60 b	85 a	0 b	0 b	0.00 b
Ames 25915	Turkey	83 a	95 a	3 a	7 a	0.07 a
PI 274298	Pakistan	53 b	97 a	5 a	12 a	0.12 a
Ames 23983	Bulgaria	45 c	78 b	3 a	8 a	0.11 a
Ames 25811	Turkey	43 c	87 a	0 b	3 b	0.04 b
Ames 25827	Turkey	58 b	88 a	2 a	2 b	0.02 b
Ames 25881	Turkey	62 b	80 b	2 a	2 b	0.02 b
Ames 25016	Germany	27 c	68 c	0 b	0 b	0.00 b

^zMeans within a column followed by different letters are significantly different at $P \leq 0.05$ according to Scott-Knott test.

indicating that the accessions with the highest germination in one temperature were not necessarily the highest for the other temperature. By analyzing the relative contribution of each character on dissimilarity, it was verified that the total germination at 35 °C had the highest contribution (58%) (Table 5). Thus, this character was considered the most important in the study; the characters of germination at 25 °C (FC25 and TG25) and the thermotolerance ratio had lower contributions to the distinction among accessions and thus were less useful during the evaluations.

The best genotypes for all characters in each group, according to the selection index based on desirable gains of Pesek and Baker (1969), were for Group 1: Ideotype, PI 178900 from Turkey, PI 319859 and PI 319858 from Japan, and PI 294637 from Jordan; and for Group 2: Ames 25564, Ames 25573, and Ames 25587 from Greece, PI 274298 from Pakistan, and Ames 25811 from Turkey. Consequently, the crosses between the best accessions of each group might generate superior progenies for those characters.

Six accessions (Ames 7665, Ames 7698, Ames 25031, PI 167082, PI 294637, and PI 319858) germinated above 80% at 35 °C and were also identified as potential sources of thermotolerance (Table 3). Fifteen other accessions (Ames 7694, Ames 25031, Ames 25036, Ames 25049, Ames 25705, PI 167082, PI 179687, PI 180834, PI 261782, PI 269486, PI 273658, PI 277710, PI 288242, PI 294637, and PI 319858) had thermotolerance ratios *T35/T25* of 0.85 or greater and were identified for further testing (Table 3). These accessions had low germination at 25 °C; possibly they came from older seed stocks or had a higher incidence of dead seeds. It is difficult to separate genotypic differences in response to temperature from the effects of seed quality when seeds are not

all produced under the same ideal conditions or are stored for long periods (Ellis et al., 1987). Thus, the lack of ability/tolerance for germination at high temperature observed in several materials might be related to lower seed viability or vigor (Nascimento et al., 2005) rather than genotypic differences.

Germination and ethylene production at high temperature. Some of the commercial cultivars from the first experiment differed strongly in their upper limit for germination and were divided into three classes (within the thermotolerant types): thermosensitive, intermediate, and thermotolerant and used in the third experiment (Table 6). At 25 °C, seed germination differed statistically among the six chosen genotypes and was above 87% for all genotypes, except for 'Brasilia' (71%) (Table 6). However, seed germination was reduced at 35 °C and there were differences among the six genotypes generating three

Table 5. Relative contributions to the dissimilarity of 62 carrot accessions by the characters of first germination count at 25 °C (FC25), total germination at 25 °C (TG25), first germination count at 35 °C (FC35), total germination at 35 °C (TG35), and thermotolerance ratio (*T35/T25*).

Character	Percent of contribution to the dissimilarity
FC25	14.86
TG25	4.15
FC35	22.35
TG35	58.16
<i>T35/T25</i>	0.47

Accession dissimilarity calculated following Singh (1981).

different groups of temperature tolerance: the genotypes 'Arrowhead' and 'Maverick' germinated 32% and 43%, respectively, and were considered thermosensitive; these two genotypes also had the lowest thermotolerance ratio (Table 6). The genotypes 'Brasilia' and 'Magnum' germinated 52% and 60%, respectively, and were considered intermediate, whereas the genotypes PI 319858 and 'XPC-3617' germinated 85% and 93%, respectively, and were considered thermotolerant.

Ethylene production during germination at 35 °C was measured to determine possible differences among the thermosensitive and thermotolerant genotypes. In other species such as lettuce, the ability to produce ethylene during high temperature stress corresponded with their ability to germinate (Kozareva et al., 2006; Nascimento et al., 2000; Prusinski and Khan, 1990). In the present study, ethylene production during seed imbibition at high temperature was greater in thermotolerant genotypes than in thermosensitive genotypes (Table 6). In fact, high correlations were observed between first count at high temperature and ethylene production ($r = 0.91$), total germination at high temperature and ethylene production ($r = 0.84$), and thermotolerance ratio and ethylene production ($r = 0.97$) (Table 7). Prusinski and Khan (1990) suggested ethylene production during seed germination at high temperature

Table 6. Analysis of variance and results for first germination count at 25 °C (FC25), total germination at 25 °C (TG25), first germination count at 35 °C (FC35), total germination at 35 °C (TG35), thermotolerance ratio (*T35/T25*), and ethylene (ETH) production at 35 °C of six carrot genotypes.

	df	MS					
		FC25	TG25	FC35	TG35	<i>T35/T25</i>	Ethylene
Block	2	0.06	0.007	0.42	0.001	0.001	53.91
Genotype	5	1.22*	1.14*	14.35*	7.30*	0.09*	260.58*
Residue	10	0.04	0.03	0.41	0.45	0.007	35.70
CV		2.13	1.83	9.57	8.77	10.48	26.96
CVg/CVe		3.10	3.48	3.38	2.25	1.89	1.45
Genotypes		FC 25 (%)	TG 25 (%)	FC 35 (%)	TG 35 (%)	<i>T35/T25</i>	ETH (μL/g)
XPC-3617		97 a ^z	97 a	85 a	93 a	0.96 a	450.8 a
PI 319858		95 a	95 a	76 a	85 a	0.90 a	448.2 a
Magnum		87 b	87 b	49 b	60 b	0.70 a	334.5 a
Brasilia		68 c	71 c	47 b	52 b	0.74 a	420.5 a
Maverick		93 a	100 a	16 c	43 c	0.43 b	77.3 b
Arrowhead		99 a	100 a	19 c	32 c	0.32 b	36.5 b

^zMeans within a column followed by different letters are significantly different at $P \leq 0.05$ according to Scott-Knott test.

*Significant at $P \leq 0.01$ by F test.

CV = phenotypic coefficient of variation; CVg/CVe = relation between genetic and environmental CV.

Table 7. Correlation between first germination count at 25 °C (FC25) and 35 °C (FC35), total germination at 25 °C (TG25) and 35 °C (TG35), thermotolerance ratio ($T35/T25$), and ethylene production at 35 °C of six carrot genotypes.

	FC25	TG25	FC35	TG35	$T35/T25$	Ethylene
FC25						
TG25	0.97**					
FC35	0.05	-0.11				
TG35	0.18	0.05	0.97**			
$T35/T25$	-0.15	-0.28	0.97**	0.94**		
Ethylene	-0.36	-0.50	0.91*	0.84*	0.97**	

**Significant at $P \leq 0.01$; *significant at $P \leq 0.05$.

be used as a criterion to select thermotolerant lettuce cultivars. In another study, Nascimento et al. (2000) observed that ethylene production in thermotolerant lettuce genotypes was higher than in the thermosensitive genotypes; these authors also verified a high correlation between ethylene production and lettuce seed germination at high temperature. A relationship between carrot seed germination at high temperature and an increase in ethylene production during radicle protrusion was also established in this study. The amount of ethylene production might be developed as a screen for carrot thermotolerant genotypes. However, only seeds with high viability should be used.

Seed treatments (i.e., seed priming) (Cantliffe and Elballa, 1994) or cultural practices that improve seed viability and quality may also help circumvent carrot thermotolerance problems. Consequently, approaches optimizing seed quality during seed production in the field (Rubatzky et al., 1999) and the selection of superior, thermotolerant progenies during breeding programs (Vieira et al., 2005) should be used. Genotypic variability for germination at high temperatures has been detected in other species (Argyris et al., 2005; Pallais et al., 1987), and germination at sub- or supraoptimal temperatures may be a desirable trait for incorporating into carrot breeding programs. High genetic variability observed among progenies from the 'Brasilia' carrot group suggested that simple breeding methods may be applied to increase physiological seed quality (Vieira et al., 2005).

Conclusions

The ability of carrot seeds to germinate at high temperatures is genotype-dependent, and the thermotolerant genotypes we identified may be useful for incorporating thermotolerance traits into breeding programs. A relationship between thermotolerance and ethylene production during carrot seed germination at high temperature was also observed, and studies should be carried out to better understand the role of ethylene in carrot seed germination at supraoptimal temperatures.

Literature Cited

Abeles, F.B., P.W. Morgan, and M.E. Saltveit, Jr. 1992. Ethylene and plant biology. 2nd Ed. Academic Press, San Diego, CA.

Argyris, J., M.J. Truco, O. Uchoa, S.J. Knapp, D.W. Still, G.M. Lenssen, J.W. Schut, R.W. Michelmore, and K.J. Bradford. 2005. Quantitative trait loci associated with seed and seed-

ling traits in *Lactuca*. Theor. Appl. Gen. 111:1365–1376.

Association of Official Seed Analysts. 1993. Rules for testing seeds. J. Seed Tech. 16:1–113.

Bradford, K.J., D. Côme, and F. Corbineau. 2007. Quantifying the oxygen sensitivity of seed germination using a population-based threshold model. Seed Sci. Res. 17:33–43.

Cantliffe, D.J. and M. Elballa. 1994. Improved germination of carrot at stressful high temperature by seed priming. Proc. Flo. Sta. Hort. Soc. 107:121–128.

Carneiro, J.W.P. and T.A. Guedes. 1992. Influência da temperatura no desempenho germinativo de sementes de cenoura (*Daucus carota* L.), avaliada pela função de distribuição de Weibull. Rev. Bras. Sem. 14:207–213.

Corbineau, F., M.A. Picard, and D. Come. 1994. Effects of temperature, oxygen and osmotic pressure on germination of carrot seeds: Evaluation of seed quality. Acta Hort. 354:9–16.

Cruz, C.D. 2001. Programa Genes: Aplicativo computacional em genética e estatística. Viçosa, Editora UFV.

Cruz, C.D. and A.J. Regazzi. 1997. Divergência genética. In: Cruz, C.D. and A.J. Regazzi (eds.). Métodos biométricos aplicados ao melhoramento genético. Viçosa, UFV: Imprensa Universitária 6:287–324.

Day-Rubenstein, K., M. Smale, and M.P. Widrelechner. 2006. Demand for genetic resources and the U.S. National Plant Germplasm System. Crop Sci. 46:1021–1031.

Ellis, R.H., G. Simon, and S. Covell. 1987. The influence of temperature on seed germination rate in grain legumes, III. A comparison of five faba bean genotypes at constant temperature using a new screening method. J. Expt. Bot. 38:1033–1044.

Falconer, D.S. 1981. Introdução à genética quantitativa. Tradução de Silva MA e Silva JC. Viçosa: UFV, Impr. Univ.

Gray, D., J.R.A. Steckel, J. Dearman, and P.A. Brocklehurst. 1988. Some effects of temperature during seed development on carrot (*Daucus carota* L.). Ann. Appl. Bio. 112:367–376.

Heydeker, W. 1972. Vigor, p. 209–252. In: Roberts, E.H. (ed.). Viability of seeds. Syracuse University Press, Syracuse, NY.

Kozareva, I., D.J. Cantliffe, R.T. Nagata, and H.J. Klee. 2004. New support for the involvement of ethylene in lettuce germination at supraoptimal temperature. Acta Hort. 631:31–37.

Kozareva, I., D.J. Cantliffe, R.T. Nagata, and P.J. Stoffella. 2006. High maturation temperature of lettuce seeds results in increased ethylene production and germination at elevated temperatures. J. Amer. Soc. Hort. Sci. 131:564–570.

Lilliefors, H.W. 1967. On the Kolmogorov-Smirnov test for normality with mean and variance unknown. J. Amer. Stat. Assoc. 62:399–402.

Mahalanobis, P.C. 1936. On the generalized distance in statistics. Proc. Nat. Inst. Sci. India 12:49–55.

Mohammadi, S.A. and B.M. Prasanna. 2003. Analysis of genetic diversity in crop plants. Salient

statistical tools and considerations. Crop Sci. 43:1235–1248.

Nascimento, W.M., D.J. Cantliffe, and D.J. Huber. 2000. Thermotolerance in lettuce seeds: Association with ethylene and endo- β -mannanase. J. Amer. Soc. Hort. Sci. 125:518–524.

Nascimento, W.M., D.J. Cantliffe, and D.J. Huber. 2004. Ethylene evolution and endo- β -mannanase activity during lettuce seed germination at high temperature. Sci. Agr. 61:156–163.

Nascimento, W.M., D.J. Cantliffe, and D.J. Huber. 2005. Seed aging affects ethylene production and endo- β -mannanase activity during lettuce seed germination at high temperature. Seed Sci. Technol. 33:11–17.

Nascimento, W.M. and R.S. Pereira. 2007. Preventing thermo-inhibition in carrot by seed priming. Seed Sci. Tech. 35:503–506.

Nascimento, W.M., J.V. Vieira, and M.C. Alvares. 2003. Physiological maturity of carrot seeds cv. Alvorada under tropical conditions. Acta Hort. 607:49–51.

Pallais, N., Z. Huaman, and S. Hopmans. 1987. Screening true potato seeds of native Andean cultivars for germinability under high temperatures. European Association of Potato Research, 10th Triennial Conference, Aalborg, Denmark, p. 292–293.

Pereira, R.S., W.M. Nascimento, and J.V. Vieira. 2007. Germinação e vigor de sementes de cenoura sob condições de altas temperaturas. Hort. Bras. 25:215–219.

Perkins-Veczic, P. and D.J. Cantliffe. 1984. Need for high-quality seed for effective priming to overcome thermotolerance in lettuce. J. Amer. Soc. Hort. Sci. 109:368–372.

Pesek, J. and R.J. Baker. 1969. Desired improvement in relation to selected indices. Can. J. Plant Sci. 49:803–804.

Prusinski, J. and A.A. Khan. 1990. Relationship of ethylene production to stress alleviation in seeds of lettuce cultivars. J. Amer. Soc. Hort. Sci. 115:294–298.

Rohlf, F.J. 2000. NTSYSpc numerical taxonomy and multivariate analysis system. Version 2.1. Exeter Software, Setauket, NY.

Rohlf, F.J. and R.R.N. Sokal. 1981. Comparing numerical taxonomic studies. Syst. Zool. 30:459–499.

Rubatzky, V.E., C.F. Quiros, and P.W. Simon. 1999. Carrot and related vegetable Umbelliferae. CABI Publishing, New York, NY.

Scott, A.J. and M.A. Knott. 1974. Cluster analysis methods for grouping means in the analysis of variance. Biometrics 30:507–512.

Senthil-Kumar, M., K. Ganesh, V. Srikanthbabu, and M. Udayakumar. 2007. Assessment of variability in acquired thermotolerance: Potential option to study genotypic response and the relevance of stress genes. J. Plant Physiol. 164:111–125.

Simon, P.W., R.E. Freeman, J.V. Vieira, L.S. Boiteux, M. Briard, T. Nothnagel, B. Michalik, and Y.C. Kwon. 2007. Carrot, p. 315–345. In: Prohens, J. (ed.). Handbook of plant breeding. Vol. 1. Springer, Berlin, Germany.

Singh, D. 1981. The relative importance of characters affecting genetic divergence. Indian J. Gen. Plant Breed. 41:237–245.

Sung, Y., D.J. Cantliffe, and R.T. Nagata. 1998. Seed developmental temperature regulation of thermotolerance in lettuce. J. Amer. Soc. Hort. Sci. 123:700–705.

Vieira, J.V., C.D. Cruz, W.M. Nascimento, and J.E.C. Miranda. 2005. Seleção de progênies de meio-irmãos de cenoura baseada em características de sementes. Hort. Bras. 23:44–47.

Vierling, E. 1991. The roles of heat shock proteins in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 42:579–620.