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Variation for Heat Tolerance During Seed Germination in Diverse Carrot [*Daucus carota* (L.)] Germplasm

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Abstract. **Carrot production is constrained by high levels of heat stress during the germination stage in many global regions. Few studies have been published evaluating the effect of heat stress on carrot seed germination or screening for genetic heat stress tolerance. The objectives of this study were to evaluate the response of diverse carrot germplasm to heat stress, identify heat-tolerant germplasm that may be used by plant breeders, and define the appropriate temperature for assessing heat tolerance in germinating carrot seed. To identify an appropriate screening temperature, three commercial hybrids and an open pollinated variety were evaluated at five temperatures (24, 32.5, 35, 37.5, and 40 °C). In preliminary studies, 35 °C was identified as the optimal temperature for screening heat tolerance of carrot seed. Cultivated and wild carrot plant introductions (PIs) (n = 270) from the U.S. Department of Agriculture (USDA) National Plant Germplasm System (NPGS) representing 41 countries, inbred lines from the USDA Agricultural Research Service (n = 15), and widely grown commercial hybrids (n = 8) were evaluated for heat tolerance under heat stress and nonstress conditions (35 °C and 24 °C, respectively) by calculating absolute decrease in percent germination (AD), inhibition index (II), relative heat tolerance (RHT), and heat tolerance index (HTI). All measurements of heat tolerance identified significant differences among accessions; AD ranged from −13.0% to 86.7%, II ranged from 35.7% to 100.0%, RHT ranged from 0 to 1.36, and HTI ranged from 0.0 to 1.45. The broad-sense heritability (H^2) calculations ranged from 0.64 to 0.86 for different traits, indicating a moderately strong genetic contribution to the phenotypic variation. Several wild carrot accessions and inbred lines displayed low levels of heat tolerance, whereas cultivated accessions PI 643114 (United States), PI 652400 and PI 652403 (Turkey), PI 652208 (China), and PI 652403 (Russia) were most heat tolerant. This is the first evaluation of heritability for heat stress tolerance during carrot seed germination, the first measure of HTI, and the first correlation calculation between heat and salt tolerance during germination in carrot.**

The ambient temperature of our planet has significantly increased over the past century (0.13 °C per decade in last 50 years) as part of current global climate change attributed to anthropogenic activities and natural factors (Rohde et al., 2013). Modern climate models have predicted that mean global temperature will rise by 1.1 °C by the end of this century and intense heat waves will also occur more frequently (IPCC, 2013). Heat stress, caused by elevated temperature, is a major threat for cereals, fruits, and vegetable production, especially in warm regions of the world (Farooq et al., 2011; Hasanuzzaman et al., 2013). Vegetables are a rich source of essential dietary micronutrients (Graham et al., 1999) but the effects of heat stress on the productivity of most vegetables have yet not been well-characterized (Fahad et al., 2017; Mahmood et al., 2017). It may be anticipated that cool-season vegetables, like carrot, are especially sensitive to heat stress.

Carrot (*Daucus carota* L., $2n = 2x = 18$) is a biennial, cool-season, Apiaceous root vegetable that is a rich dietary source of provitamin A carotenoids (α- and β-carotene), which is vital for human development and health (Tanumihardjo, 2012). The optimal germination temperature of carrot is 20 to 30 °C (Corbineau et al., 1994) and, like most crops, abiotic stress reduces carrot yield (Bano et al., 2014, Kahouli et al., 2014). Heat, drought, and/or salinity-induced stress contributes to the much lower carrot yields in countries like Pakistan and most of South Asia relative to North America or Europe (FAO, 2017).

Previous studies have reported that temperatures higher than 27 °C can cause significant reduction in root mass, which accounts for the yield of a carrot (Rosenfeld, 2004). High temperature causes heat stress and is considered to be one of the major abiotic factors that limits carrot production because of its adverse effects on seed germination, seedling emergence, and plant growth (Landjeva et al., 2008). Heat tolerance is a complex trait that varies with the severity of stress and plant growth stage. Therefore, there is a need to identify heat-tolerant carrot germplasm with stable growth and yield under high temperature at various stages of growth. Seedling establishment, vegetative growth, and the reproductive phase of the life cycle in carrot are all critical developmental stages, but without the ability to germinate under heat stress, tolerance at later stages of growth is irrelevant. Heat stress tolerance in seeds is typically a multicomplex and polygenic trait (Senthil-Kumar et al., 2007) that varies from species to species and environmental conditions (intensity and exposure period of stress). It has been demonstrated in previous studies that the seed germination of various crops, including wheat (Balla et al., 2012), maize (Iloh et al., 2014; Riley, 1981), rice (Shah et al., 2011), chick pea (Sleimi et al., 2013), and spinach (Hum-Musser et al., 1999), is adversely affected as temperature increases.

Several previous studies demonstrated the negative effects of low temperature on carrot seed germination and stand establishment (Nascimento and Pereira, 2007; Pereira et al., 2007; Vieira et al., 2005), but relatively little has been reported about the effects of high temperatures. Vieira et al. (2005) demonstrated that high temperature inhibits carrot seed germination. High temperature affects some physiological and biochemical processes like oxygen requirement during seed imbibitions (Nascimento et al., 2008). There is no known mechanism for thermotolerance in carrot and insufficient information is available to characterize the influence of high temperature on carrot seed germination and biomass production. The most expansive carrot germplasm evaluation, to date, for heat tolerance at the germination stage, was done by Nascimento et al. (2008), who evaluated 34 commercial cultivars and 63 diverse germplasm accessions. They evaluated heat stress traits including ethylene production, time to first germination, and RHT under 35 °C. The current lack of information limits our understanding of heat tolerance mechanisms in carrot. To expand the information needed to better understand those mechanisms, the objectives of this study were to identify the optimal temperature conditions for evaluating heat tolerance in carrot; to evaluate a large carrot germplasm collection for heat tolerance traits, including HTI, II, and AD at the germination stage; to evaluate the relationship between domestication status and root color on heat tolerance; and to calculate broad-sense heritability for carrot.

Materials and Methods

Germplasm. A total of 293 carrot genetic stocks consisting of 207 cultivated and 63 wild accessions from the USDA NPGS collection of PIs, 15 inbred lines from the USDA carrot breeding program, and eight commercial fresh market hybrids widely grown in the United States were included in this analysis. To better identify heat stress effects on germination, carrot accessions with more than 50% germination under nonstress conditions were included, as in recent evaluations of salinity tolerance (Bolton and Simon, 2019). The 293 carrot accessions originated from 41 countries and were classified into 14 regions based on their origin (Eastern Africa, Northern Africa, Southern Africa, North America, South America, Central Asia, Eastern Asia, Southern Asia, Western Asia, Eastern Europe, Northern Europe, Southern Europe, Western Europe, and Oceania) and they represent much of the global genetic diversity for carrot (Iorizzo et al., 2013). A subset of these accessions is also being evaluated in field trials in Bangladesh and Pakistan for heat tolerance at various stages of growth (A. Ali and M.A. Rahim, personal communication).

Determination of optimal heat tolerance evaluation temperature. A pilot experiment was conducted to determine the optimal temperature for evaluating heat tolerance in carrot. For this experiment, three widely grown commercial hybrids (coded as A, B, and C) and the cultivar Brasilia were selected to test seed germination under five temperatures (24.0, 32.5, 35.0, 37.5, and 40.0 °C). 'Brasilia' was included because it is widely grown in the warmer climate of Brazil and is known to be relatively heat tolerant, whereas the three hybrids represent cultivars bred for more temperate production areas. The temperature at which statistically most significant differences were recorded between accessions, that being the lowest *P* value, was chosen as the optimal temperature to evaluate heat tolerance at the seed germination stage.

Germination assay. This experiment was conducted using a randomized complete block design with six replications and two treatments, that is, control (24°) and heat stress (35°). Twenty seeds from each carrot accession were placed on P5 filter paper in 60 × 15 mm petri dishes (Fisher Scientific, Waltham, MA). Petri dishes were added with 7 mL of distilled water and were placed in complete darkness at 24 ± 1 °C for control in plastic bins and at 35 ± 1 °C in an incubator (Fisher Scientific) for heat stress.

Evaluation under 35 °C. Seed germination data were collected for a total of 10 d with measurements taken 2, 4, 6, 8, and 10 days after sowing. A seed was scored as germinated when the radicle had emerged and had a length of more than 1 mm. At each measurement time, any germinated seed was removed from the petri dish. Standard criteria for determining performance of carrot accessions under heat stress included final percent germination under nonstress conditions ($PG_{Control}$), final percent germination under heat stress (PG_{Heat}), AD, II, RHT, and HTI, as were used to evaluate salinity tolerance in carrot (Bolton and Simon, 2019). These measurements were calculated with the following equations: $AD = PG_{Control} - PG_{Heat}$; $II = 100 * (PG_{Control} - PG_{Heat})/(PG_{Control})$; $RHT = PG_{Heat}/ PG_{Control}$; $HTI = (PG_{Heat} * PG_{Control})/(PG_{Average})^2$, whereas $PG_{Average}$ is the average percent germination of all carrot accessions evaluated under no heat stress.

Data analysis. Statistical mixed linear models (Eq. [1]) were used for analysis of variance (ANOVA) for six measurements related to seed germination on the basis of carrot accessions, origin of carrot accessions, and heat treatments.

$$Y_{ij} = \mu + R_i + A_j + \varepsilon_{ij}, \qquad [1]$$

where Y_{ij} = the value of the measurements for the j^{th} carrot accession in the i^{th} replication where $i = 1, \ldots, 6$, and $j = 1, \ldots, 289$; μ = total mean (constant), R_i = effect of the i^{th} replication (random effect) on the response measurement, A_j = effect of the j^{th} accession (fixed effect) on the response measurement, and ε_{ij} = effect of the experimental error associated with ij^{th} observation. All analyses were performed in R. 3.4.4 (R Core Team, 2018). The lmer function in the lme4 package was used for ANOVA test (Bates et al., 2018). The mean separation analysis on the basis of region of origin of carrot accession was performed using least significant difference test function found in the agricolae package with alpha = 0.05 (De Mendiburu, 2014). Pearson rank correlations between measurements were calculated using the cor function found in the stats package (R Core Team, 2018).

The among-accession and within-accession variances were used for calculating broad-sense heritability (H^2) as derived from Falconer and Mackay (1996) by using Eq. [2] below,

$$H^2 = \left(\sigma^2 G/\sigma^2 P\right) = \left[\sigma^2 G/\left(\sigma^2 G + \left(\sigma^2 E/r\right) + \sigma^2 R/r\right)\right], \qquad [2]$$

where σ^2_G = genotypic (accessions) variance, σ^2_P = phenotypic variance, σ^2_E = variance due to experimental error, σ^2_R = variance due to replication, and r = the number of replications for each treatment. Variance components were derived using formulas: $\sigma^2_G = (MSA - MSE)/r$, $\sigma^2_E = MSE$, $\sigma^2_R = (MSR - MSE)/n$, with MSA = mean square accession, MSE = mean square error, MSR = mean square replication, r = number of replications, and n = number of accessions.

Results and Discussion

Optimal temperature for evaluation of heat tolerance at germination stage. Percent seed germination for the four cultivars evaluated in a preliminary study under nonstress conditions ranged from 44.0% to 84.0%, with a mean of 63.8%. Increasing temperature to 32.5 °C reduced the mean percent germination to 57.2% and lowered the range from 45.0% to 80.0%. At 35 °C, the mean percent germination was further reduced to 33.3% and lowered the range from 4.0% to 60.0%. At 37.5 °C, only 'Brasilia' germinated, whereas at 40 °C no germination occurred (Fig. 1). ANOVA for percent seed germination of the four carrot cultivars displayed a highly significant treatment effect (F = 154.63, $P < 0.0001$) and moderately significant accession effect (F = 4.78, P = 0.0044) on percent seed germination (Table 1). A significant difference between accessions was observed in the control temperature (24 °C), whereas the most significant difference between accessions was recorded at 35 °C (F = 13.18, P = 0.0002), indicating that 35 °C is an optimal temperature to screen carrot germplasm for heat tolerance at the germination stage. At 37.5 °C, only 'Brasilia' germinated, and at a low rate, whereas at 40 °C there was no seed germination (Table 2).

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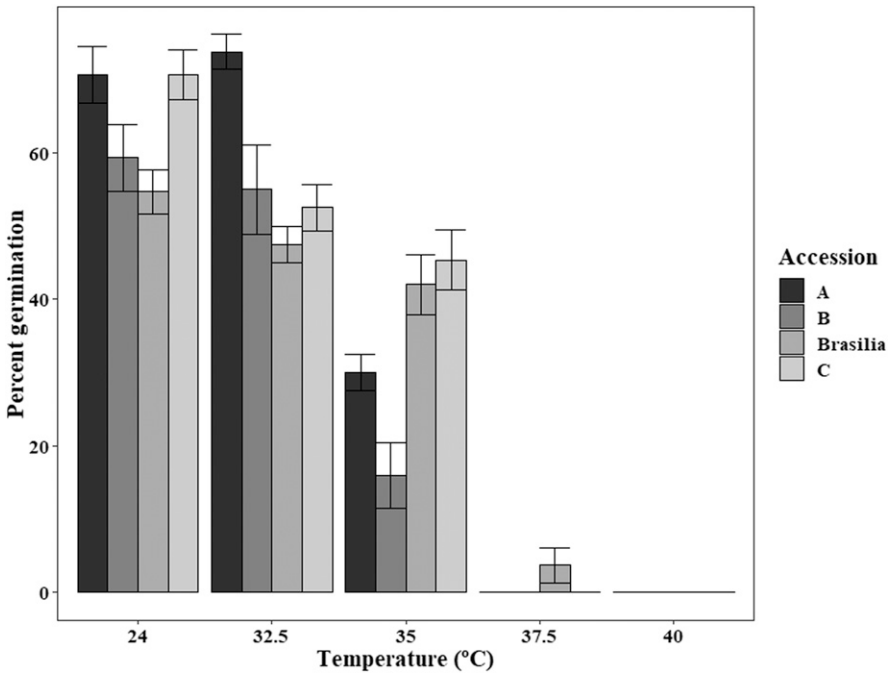

Fig. 1. Mean percent germination and standard error for three commercial hybrids (coded as A, B, and C) and the cultivar Brasilia under five different temperatures.

Table 1. Analysis of variance for pooled percent seed germination of four carrot accessions under five different temperatures.

Source	df	Sum of squares	Mean square	*F* ratio	Prob > *F*
Rep	16	1,050.00	65.60	0.70	0.7870
Treatment	4	55,618.00	13,904.50	147.29	<2.2E-16
Accession	3	1,523.00	1,523.00	5.38	0.0025
Error	57	5,383.00	94.40		

The results from this experiment indicate that a temperature of 35 °C is an optimal temperature to test for heat tolerance, which was the same temperature used by Nascimento et al. (2008).

Germination assay. The average percent germination for the 293 carrot accessions under nonstress conditions ranged from 50.0% to 100.00%, with a mean of 81.6% and a standard deviation of 12.6%. When seeds were tested under 35 °C, the average percent germination was reduced to 59.0% with a minimum value of 0.0%, a maximum of 97.5%, and a standard deviation of 19.5% (Fig. 2). These results indicate that heat stress significantly reduced percent seed germination in most carrot accessions. For all traits related to heat stress, there was a significant replication effect (*P* value < 0.0001) that may be attributed to variation within the incubator as reflected by the larger replication effect at 35 °C than at 24 °C (Table 3). Significant variation was recorded for the average percent germination among carrot accessions under control conditions ($F = 8.35$, $P < 0.0001$) (Table 3). PI 642756 (Netherlands), PI 643119 (France), and PI 652374 (Turkey) all had the maximum percent germination values (100.0%), under control conditions, whereas PI 652253 (India, 50.0%), PI 502914 (Germany, 51.1%), and PI 652154 (Netherlands, 51.2%) had the three lowest germination values (Supplemental Table 1). Percent germination also varied significantly under heat stress among carrot accessions ($F = 4.88$, $P < 0.0001$). PI 643114 (United States, 97.5%) had the highest percent germination value, whereas accessions PI 652208 (China), PI 652248 (Russia), PI 652400 (Turkey), and PI 652403 (Turkey) all had the second highest value (94.2%), indicating a high level of heat tolerance. Inbred B493B had the lowest percent germination value under heat stress (0.0%), followed by PI 652354 (Turkey, 4.2%), B7254B (7.5%), and PI 279764 (Syria, 10.8%), indicating that they were especially heat sensitive.

Several of the accessions with high percent germination under heat stress were cultivated PIs from diverse geographic regions. PI 643114 (United States), PI 652400 and PI 652403 (Turkey), PI 652208 (China), and PI 652403 (Russia) all had low AD and II values (–0.3% to 1.7%), high RHT values (0.95 to 1.0) and high HTI values (1.33 to 1.45). The most heat-sensitive carrot accessions were inbred lines and wild PIs. Inbred lines B493B and B7254B, and wild PIs from Syria (PI 279764) and Turkey (PI 652354) all had a percent germination less than 5.0% under heat stress, AD values from 50.8% to 86.7%, II values from 85.6% to 100.0%, and RHT and HTI values from 0.0 to 0.14. Surprisingly, the most tolerant accessions were not primarily from warmer regions of the world but were from very diverse geographic origins; and many tolerant accessions were cultivated varieties, suggesting that some degree of selection has occurred for heat tolerance in cultivated carrot in different parts of the world. Another interesting result was the low tolerance from several wild accessions native to regions with high levels of heat stress (Syria and Turkey), suggesting that adaptation to heat stress did not play a major role in carrot evolution and distribution based on these wild populations. In other species, sources of abiotic stress tolerance are often identified in crop wild relatives (Hajjar and Hodgkin, 2007). It appears that carrot differs from other species in this regard, as the least tolerant accessions are usually wild accessions whereas the most tolerant accessions are cultivated accessions. It should be noted that several wild carrot accessions were heat tolerant and several cultivated PIs were sensitive, contrary to general trends. That inbreds B493B and B7254B were highly sensitive was not surprising, as inbreeding depression may potentially make them more susceptible to the heat stress.

Interestingly, PI 652403 (Turkey) identified as heat tolerant in this study was also identified as one of the most salt tolerant accessions in a previous study, making this a potentially useful source of both salt and heat tolerance at the germination stage for carrot breeders (Bolton and Simon, 2019). Also, B493B had 0.0% germination under both salt stress in previous studies and heat stress in this study, indicating that this inbred line is very sensitive to two major forms of abiotic stress and can serve as a sensitive check in future studies.

AD in germination is a parameter used to measure heat stress ($PG_{Control} - PG_{Heat}$). AD is a useful trait to identify accessions with very low tolerance to heat stress because it measures the actual reduction in germination. In this experiment, mean AD for all accessions evaluated was 22.6% and ranged from –13.0% to 86.7% (Fig. 3A) with statistically significant variation observed among carrot accessions ($F = 3.02$, $P < 0.0001$) (Table 3). PI 652154 (Netherlands, –13.0%) and PI 478370 (China, –12.3%) had the lowest AD values, indicating heat tolerance. A total of 15 accessions had negative AD values, indicating that heat stress increased percent germination compared with the nonstress conditions. Accessions displaying a negative AD value often had a low percent germination under control temperatures and low percent germination values under heat stress close to the overall group average (≈60%). These low AD value accessions might not be useful sources of heat tolerance for breeders, but do provide interesting material to investigate the genetic control of increased germination under heat stress. PI 652354 (Turkey, 86.7%) and PI 515992 (Hungary, 84.2%) had the highest AD values, indicating that heat stress greatly reduced germination and thus they were very heat sensitive.

Table 2. Analysis of variance for seed germination of four carrot accessions under five different temperatures.

Temperature	Source	df	Sum of squares	Mean square	*F* ratio	Prob > *F*
24 °C	Rep	3	443.33	88.67	1.08	0.4114
	Accession	3	1,186.00	395.33	4.81	0.0154
	Error	9	1,234.00	82.27		
32.5 °C	Rep	3	317.19	105.73	2.37	0.1384
	Accession	3	1,579.69	526.56	11.80	0.0018
	Error	9	401.56	44.62		
35 °C	Rep	3	589.30	117.87	1.46	0.2593
	Accession	3	3,184.00	1,061.33	13.18	0.0002
	Error	9	1,208.00	80.53		
37.5 °C	Rep	3	17.19	5.73	1.00	0.4363
	Accession	3	42.19	14.06	2.46	0.1298
	Error	9	51.56	5.73		
40 °C	Rep	3	0.00	0.00		
	Accession	3	0.00	0.00		
	Error	9	0.00	0.00		

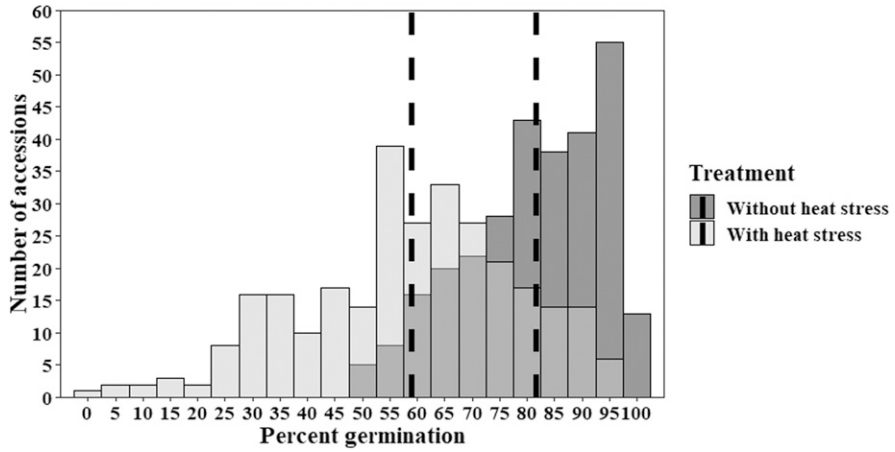

Fig. 2. Distribution and mean (dotted line) percent seed germination among 293 carrot accessions without heat stress (dark gray) and with heat stress (light gray).

II [II = 100*($PG_{Control} - PG_{Heat}$)/ $PG_{Control}$] values were significantly different among carrot accessions (F = 2.91, P < 0.0001) (Table 3) and had a range from 35.7% to 100.0% (Fig. 3B). PI 652154 (Netherlands, –35.7%) and PI 478370 (China, –22.3%) had the lowest II, agreeing with AD and indicating that heat stress increased germination for these accessions. B493B had a maximum II value of 100.0% followed by PI 652354 (Turkey, 95.6%), indicating that they are highly heat-sensitive accessions.

RHT (RHT = PG_{Heat}/ $PG_{Control}$) is a useful criterion for evaluating heat stress, as it gives a way to account for the percent germination under the control. RHT was also significantly different among carrot accessions (F = 8.34, P < 0.0001) (Table 3) with a population mean of 0.74 and a range from 0 to 1.36 (Fig. 3C). PI 652154 (Netherlands, 1.36) and PI 478370 (China, 1.22) had the highest RHT values, whereas B493B, 7254B, PI 279764 (Libya), PI 515992 (Hungary), and PI 652354 (Turkey) all had low values (0.0 to 0.14). Similar to AD, accessions with high RHT values displayed low percent germination values under control conditions and thus provide a more useful resource for understanding the genetics of performing better under heat stress rather than useful breeding material.

Heat tolerance index [HTI = (PG_{Heat} *$PG_{Control}$)/($PG_{Average}$)^2] is an important trait to consider, as it takes into account both the percent germination under heat stress and under control conditions while comparing each accession to the population average, thus giving a ranking among all accessions evaluated. An accession with a high HTI will have higher percent germination under both conditions, making it a useful accession to be selected for use in a commercial growing setting. HTI was significantly different among carrot accessions evaluation (F = 7.28, P < 0.0001) (Table 3) with a population mean of 0.74 and a range from 0.0 to 1.45 (Fig. 3D). PI 643114 (United States, 1.45) and PI 652208 (China, 1.4) had the highest HTI, suggesting that they were highly heat tolerant and had high percent germination under nonstress conditions, whereas inbred lines B493B and B7254B, along with PI 652354 (Turkey), had the lowest HTI values (0.0–0.06).

The accessions used in this study demonstrate a wide range of phenotypic variation for each of the heat stress parameters measured. Exposure to 35 °C significantly reduced germination for most of the carrot diversity panel, agreeing with an earlier study (Nascimento et al., 2008). This evaluation reports a similar range of variation for heat tolerance as Nascimento et al. (2008), and evaluated nearly three times additional carrot accessions, including a broad range of cultivated open pollinated varieties. There were differences between these two evaluations for the four overlapping accessions ('Brasilia', PI 261782, PI 285613, and PI 537093) that may be attributed to differences in the seed lot and/or the number of replications that were used. These results suggest the value of evaluating a large number of diverse carrot accessions for heat stress, as has been suggested for salt stress in carrot (Bolton and Simon, 2019).

Heat tolerance according to geographic origin. Significant differences were observed for seed germination under control conditions and for all heat germination parameters among the 14 different geographic origins of carrot accessions (P values < 0.0001) (Table 4). When comparing cultivated, wild, inbred, and hybrid accessions, accessions from Eastern and Central Asia demonstrated an average higher heat tolerance at the germination stage. Accessions from Eastern Asia had a mean AD of 13.96% and a range from –12.3% to 36.8%, a mean II of 14.5, and a range from –22.3% to 39.1%, a mean RHT of 0.86 and a range from 0.61 to 1.22, and a mean HTI of 0.82, and a range from 0.26 to 1.40. Accessions from Central Asia had similar values and ranges to those from Eastern Asia with a mean AD of 13.7% and a range from –5.0% to 30.5%, a mean II of 15.4% and a range from –17.8% to 44.1%, a mean RHT of 0.85 and a range from 0.56 to 1.17, and a mean HTI of 0.83 and a range from 0.29 to 1.38. Accessions from Eastern Africa and Southern Europe only included one and six accessions, respectively, but were among the most heat sensitive, having RHT and HTI values of 0.52 or lower along with AD and II values (41.4% to 50.6%) (Table 5). Although accessions from Central and Eastern Asia were on average more heat tolerant, it is important to note the wide range of phenotypic variation within a single region and thus the need to evaluate multiple accessions from within a region when possible.

Heat tolerance according to domestication status and root color. No significant difference was observed for mean percent germination under nonstress conditions in comparing cultivated PIs (82.0%), wild PIs (80.2%), and inbred lines (78.4%) (Table 6). Under heat stress, cultivated PIs demonstrated a significantly higher tolerance than the wild PIs and inbred lines, with a mean percent germination under heat of 64.3% and a range from 12.5% to 97.5%, a mean AD of 17.7%, mean II of 20.0%, mean RHT of 0.80, and mean HTI of 0.81. An important observation to note is that most wild PIs originated from Western Asia (Table 7) and the inbred lines are exclusively from the USDA breeding program, potentially accounting for the trends we observed. No significant difference in heat tolerance was observed between the wild PIs and inbred lines for all traits, and most accessions from

Table 3. Analysis of variance for six measurements related to seed germination among 293 carrot accessions.

Measurement	Source	df	Sum of squares	Mean square	*F* ratio	Prob > *F*
Germination without heat stress	Rep	5	218.00	43.61	0.40	0.8565
	Accession	288	269,354.00	935.26	8.35	<2.0E-16
	Error	1,440	161,326.00	112.03		
Germination with heat stress	Rep	5	24,955.00	4,990.90	10.66	4.5E-10
	Accession	288	658,715.00	2,287.20	4.88	<2.2E-16
	Error	1,440	674,325.00	468.30		
Absolute decrease (AD)	Rep	5	22,563.00	4,512.70	7.71	3.6E-07
	Accession	288	509,434.00	1,768.90	3.02	<2.2E-16
	Error	1,440	842,581.00	585.10		
Inhibition index (II)	Rep	5	40,590.00	818.10	8.34	8.7E-08
	Accession	288	816,629.00	2,835.50	2.91	<2.2E-16
	Error	1,440	1,401,611.00	973.30		
Relative heat tolerance (RHT)	Rep	5	4.06	0.81	8.34	8.7E-08
	Accession	288	81.66	0.28	2.91	<2.2E-16
	Error	1,440	140.16	0.10		
Heat tolerance index (HTI)	Rep	5	4.38	0.88	10.78	3.4E-10
	Accession	288	170.32	0.59	7.28	<2.2E-16
	Error	1,440	116.94	0.08		

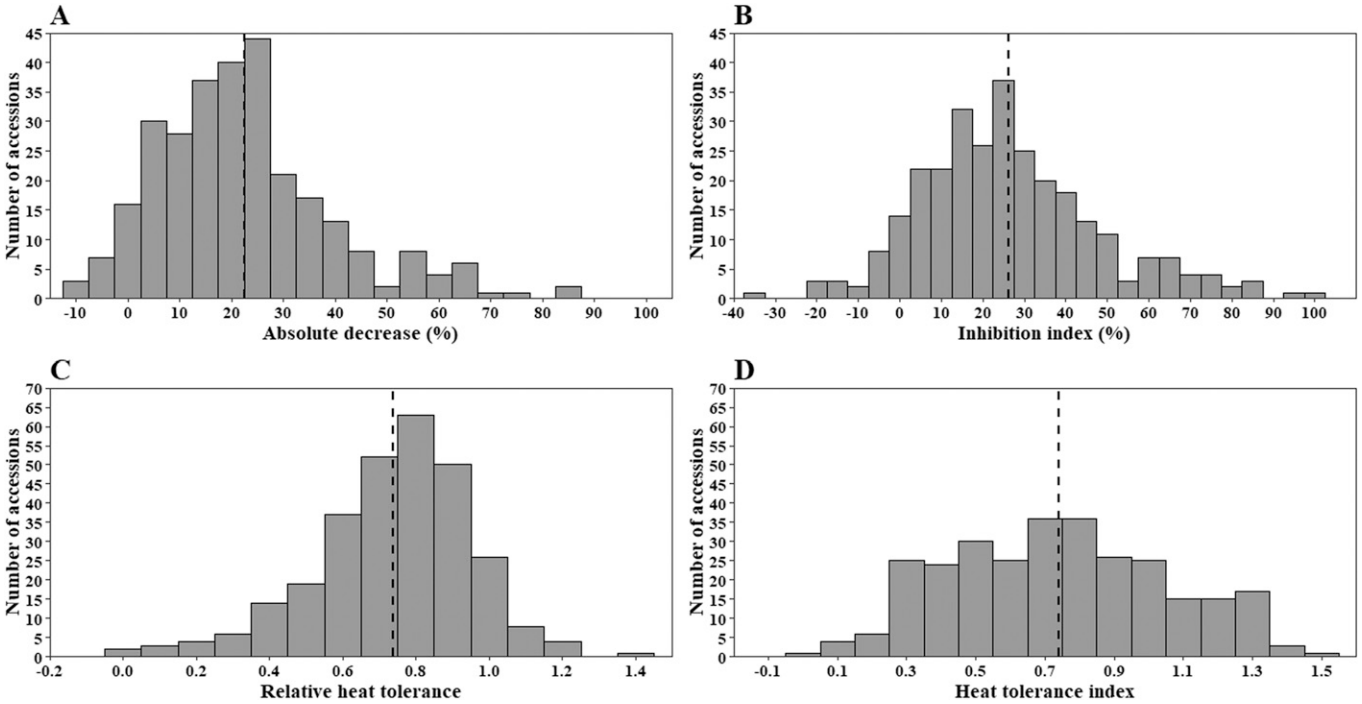

Fig. 3. Distribution and mean (dotted line) among 293 carrot accessions with four different measures of heat tolerance: (**A**) absolute decrease, (**B**) inhibition index, (**C**) relative heat tolerance, and (**D**) heat tolerance index.

these groups demonstrated higher sensitivity to heat stress than the cultivated accessions. There were some exceptions to the general trends based on domestication status. Inbred lines B5208B and Nb4002B both had moderately high HTI values of 0.95 and 0.93, respectively, whereas Ames 30259 (Tunisia) and PI 269487 (Pakistan), two wild PIs, have HTI values of 1.03 and 1.17, respectively. There were also many cultivated PIs with low levels of heat tolerance, such as PI 234621 (South Africa) and PI 515992 (Hungary), both of which have less than 25.0% germination under heat stress conditions.

Heat stress tolerance trait means of cultivated carrots did not vary according to root color under nonstress conditions. Under heat stress conditions, there was no significant difference among the white, orange, yellow, and red accessions with a range from 62.7% to 66.4% germination under heat stress and HTI range from 0.76 to 0.84. Purple rooted carrots were significantly more sensitive than the other colors, with a 55.1% germination under heat stress, down from 74.0% at 24 °C, and HTI of 0.62 (Table 6). It is important to note that the number of samples for each color category were not equal and were not equally distributed across geographic origins, thus the trends reported here should be confirmed with larger sample sizes.

Broad-sense heritability. A high broad-sense heritability (H^2) was observed for germination under nonstress conditions ($H^2 = 0.88$), for germination under heat stress ($H^2 = 0.79$), and for HTI ($H^2 = 0.86$), indicating a significant genetic basis for these traits. Heritability values for AD ($H^2 = 0.66$), II ($H^2 = 0.66$), and RHT ($H^2 = 0.64$) all were moderately high, suggesting a genetic basis for these traits. Interestingly, the heritability values for heat stress germination were lower than that observed in the same set of accessions for salt stress germination (Bolton and Simon, 2019). These differences in heritability suggest that either there is a smaller genetic component for heat tolerance germination in carrot, a larger environmental component that accounts for the phenotypic variation, or that it is a more genetically complex trait than salt tolerance germination with multiple genes responsible for the phenotype. These genetic factors warrant further investigation with the goal of identifying significant quantitative trait loci associated with heat tolerance at the germination stage.

Table 4. Analysis of variance for six measurements related to seed germination among carrot accessions from 14 geographic regions of origin.

Measurement	Source	df	Sum of squares	Mean square	*F* ratio	Prob > *F*
Germination without heat stress	Rep	5	218.00	43.61	0.18	0.97
	Region	13	17,618.00	1,355.22	5.63	3.64E-10
	Error	1,715	413,062.00	240.85		
Germination with heat stress	Rep	5	24,955.00	4,990.90	6.79	2.83E-06
	Region	13	71,584.00	5,506.50	7.49	1.56E-14
	Error	1,715	1,261,455.00	735.50		
Absolute decrease (AD)	Rep	5	22,563.00	4,512.70	6.10	1.32E-05
	Region	13	83,612.00	6,431.77	8,370.00	<2.2E-16
	Error	1,715	1,268,404.00	739.60		
Inhibition index (II)	Rep	5	40,590.00	8,118.10	6.64	3.94E-06
	Region	13	120,634.00	9,279.50	7.59	8.99E-15
	Error	1,715	2,097,606.00	1,223.10		
Relative heat tolerance (RHT)	Rep	5	4.06	0.82	6.64	3.94E-06
	Region	13	12.06	0.93	7.59	8.99E-15
	Error	1,715	209.76	0.12		
Heat tolerance index (HTI)	Rep	5	4.38	0.88	5.51	4.92E-05
	Region	13	14.56	1.12	7.05	1.74E-13
	Error	1,715	272.70	0.16		

Table 5. Mean separation for relative heat tolerance (RHT), heat tolerance index (HTI), absolute decrease (AD), and inhibition index (II) across 14 geographic regions of origin for all 293 accessions [63 wild plant introductions (PIs), 207 cultivated PIs, 15 inbreds, and 8 hybrids].

Region of origin	Accessions	RHT		HTI		AD (%)		II (%)	
Eastern Asia	31	0.86	A[z]	0.82	AB	13.96	AC	14.52	AC
Central Asia	18	0.85	AB	0.83	AB	13.66	AC	15.36	ABC
Southern Asia	46	0.80	ABC	0.72	BC	16.18	AC	20.20	ABC
Eastern Europe	20	0.79	ABC	0.82	AB	20.06	ABC	21.55	ABC
Northern Europe	14	0.76	ABC	0.74	ABC	20.41	ABC	23.94	ABC
Western Europe	40	0.75	BC	0.76	ABC	21.84	ABC	25.32	ABC
North America	42	0.70	CD	0.86	A	25.19	ABC	29.65	ABC
North Africa	8	0.70	CDE	0.65	BC	26.14	ABC	30.14	ABC
Oceania	1	0.68	CDE	0.60	C	25.72	ABC	32.26	ABC
South America	2	0.64	CDE	0.80	ABC	33.29	ABC	35.93	ABC
Western Asia	61	0.63	DE	0.63	C	31.19	ABC	36.73	AB
South Africa	3	0.61	DE	0.63	BC	36.14	AB	39.41	AB
Southern Europe	6	0.52	E	0.48	C	41.17	A	48.45	A
Eastern Africa	1	0.49	E	0.46	C	44.17	A	50.61	A

[z]Means with the same letter are not significantly different using Fisher's least significant difference test at alpha = 0.05.

Table 6. Mean (± SE), for percent germination without heat stress (Nonstress), percent germination with 35 °C heat stress (Stress), absolute decrease (AD), inhibition index (II), relative heat tolerance (RHT), and heat tolerance index (HTI) separated by domestication status (DS) and primary root color (RC) [excluding wild plant introductions (PIs)] with number of accessions found in each category.

Factor	Category	Accessions	Nonstress		Stress		AD		II		RHT		HTI	
DS	Cultivated PI	206	82.0 ± 0.9	A[z]	64.3 ± 1.2	A	17.7 ± 1.0	A	20.0 ± 1.2	A	0.80 ± 0.01	A	0.81 ± 0.02	A
	Wild PI	63	80.2 ± 1.2	A	44.5 ± 2.3	B	35.8 ± 2.4	B	42.8 ± 2.9	B	0.57 ± 0.03	B	0.53 ± 0.03	B
	Inbred	15	78.4 ± 3.5	A	41.7 ± 5.3	B	36.6 ± 4.3	B	46.5 ± 6.6	B	0.54 ± 0.07	B	0.52 ± 0.07	B
RC	White	5	84.5 ± 6.7	A	62.7 ± 12.0	AB	21.8 ± 7.3	A	25.3 ± 8.2	A	0.75 ± 0.08	A	0.83 ± 0.21	AB
	Orange	153	83.0 ± 1.1	A	62.8 ± 1.5	AB	20.2 ± 1.2	A	23.4 ± 1.6	A	0.76 ± 0.02	A	0.81 ± 0.03	AB
	Yellow	38	82.2 ± 1.8	A	66.4 ± 2.3	A	15.8 ± 1.5	A	17.7 ± 2.0	A	0.82 ± 0.02	A	0.84 ± 0.04	A
	Red	9	76.8 ± 5.0	AB	64.6 ± 4.0	AB	12.2 ± 4.1	A	10.8 ± 4.9	A	0.89 ± 0.05	A	0.76 ± 0.09	AB
	Purple	19	74.0 ± 3.1	AB	55.1 ± 3.6	B	18.8 ± 3.9	A	22.3 ± 5.2	A	0.78 ± 0.05	A	0.62 ± 0.06	B

[z]Means with the same letter are not significantly different using Fisher's least significant difference test at alpha = 0.05.

Table 7. Correlation among seven heat stress parameters: absolute decrease (AD), hundred seed weight (HSW), inhibition index (II), under nonstressed condition (Nonstress), relative heat tolerance (RHT), heat tolerance index (HTI), and under heat stress (Stress).

Parameter	Nonstress	Stress	RHT	AD	II	HTI	HSW
Nonstress	1						
Stress	0.50	1					
RHT	–0.04	0.83	1				
AD	0.16	–0.78	–0.97	1			
II	0.04	–0.83	–1.00	0.97	1		
HTI	0.72	0.95	0.62	–0.56	–0.62	1	
HSW	–0.20	0.00	0.13	–0.15	–0.13	–0.17	1

Correlation among heat tolerance parameters, seed weight, and salt tolerance. Pearson correlation coefficients calculated for each of the heat tolerance parameters along with hundred seed weight (HSW) of each accession (Table 7) indicated that germination under nonstress conditions was highly correlated to the HTI (r = 0.74), but the correlation with germination under heat stress conditions (r = 0.50) was lower. Germination without stress was not strongly correlated to the other parameters evaluated (r = –0.20 to 0.16). These results indicate that percent germination under nonstress conditions does not predict heat tolerance germination in carrot. HSW had no correlation with percent germination under heat stress (r = 0.00) and a very weak negative correlation with germination under nonstress conditions (r = –0.20). These results were similar to those observed for salt tolerance germination where nonstress germination was also correlated with salt tolerance index (STI) (r = 0.54) and with stress salt stress (r = 0.40). Germination under salt stress was slightly

more correlated with HSW than germination under heat stress (Bolton and Simon, 2019). These data suggest that heat tolerance parameters and salt tolerance parameters follow similar trends, with heat tolerance having slightly stronger correlations among those parameters. Pearson correlation between HTI and the STI values calculated in Bolton and Simon (2019) display a strong correlation (0.69) between the two traits, suggesting that many of the accessions tolerant to one stress are also tolerant to the other. These results are not surprising, as heat stress and salt stress have similar effects on seed physiology and often cause similar stress responses. Although not evaluated in this study, it will be interesting to compare heat tolerance of these accessions in the field to heat tolerance at the seed germination stage and to determine if there is any correlation between the two studies.

Conclusions

This study identified a wide range of phenotypic variation for heat tolerance at the germination stage in a diverse collection of carrot germplasm. Five cultivated carrot accessions representing four countries (PI 643114, United States; PI 652208, China; PI 652248, Russia; and PIs 652400 and 654203, Turkey) were identified as the most heat-tolerant accessions, whereas inbred lines B493B and B7254B, along with wild accessions PI 279764 (Libya) and PI 652354 (Turkey) were identified as heat-sensitive accessions. The wide geographic range from which all the tolerant accessions originated was particularly surprising, as it suggests that heat tolerance has been under selection in multiple regions of the world where carrot is cultivated. This study adds to the current body of research for heat tolerance in carrot seed germination by identifying an optimal temperature for screening and measuring a high heritability of tolerance in a large collection of germplasm from diverse global regions. HTI was confirmed to be a particularly important measure of tolerance. This evaluation, coupled with recent evaluations of salt tolerance in the same diverse germplasm, provides valuable information for future studies of abiotic stress in carrot.

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Supplemental Table 1. Carrot accession, root color, country of origin, domestication status (DS), mean percent germination without heat stress (Nonstress) ± SE, mean percent germination with 35 °C heat stress (Stress) ± SE, mean absolute decrease (AD), mean inhibition index (II), relative heat tolerance (RHT), mean heat tolerance index (HTI), hundred seed weight (HSW), and rank based on HTI.

Merged	Root Color	Country	DS	Nonstress (%)	Stress (%)	AD (%)	II (%)	RHT	HTI	HSW	Rank
Ames 25040	Red-Purple	India	Cultivated PI[z]	74.2 ± 2.4	49.2 ± 10.1	25.0	34.7	0.65	0.56	0.18	196
Ames 25732*	White	Syria	Wild PI	71.9 ± 7.4	35.8 ± 8.1	36.0	49.8	0.50	0.41	0.11	240
Ames 25773*	White	Syria	Wild PI	85.8 ± 3.3	60.6 ± 5.4	25.2	29.7	0.70	0.79	0.09	124
Ames 26382*	White	Portugal	Wild PI	63.3 ± 7.7	17.1 ± 6.7	46.2	60.8	0.39	0.13	0.13	285
Ames 26383*	White	Portugal	Wild PI	90.8 ± 2.4	25.8 ± 8.6	65.0	71.9	0.28	0.36	0.15	251
Ames 26384*	White	Portugal	Wild PI	88.3 ± 4.8	70.8 ± 9.7	17.5	19.0	0.81	0.95	0.15	77
Ames 27396*	White	Uzbekistan	Wild PI	75.2 ± 7.3	44.7 ± 13.9	30.5	44.1	0.56	0.55	0.08	197
Ames 27397*	White	Uzbekistan	Wild PI	72.8 ± 5.1	71.7 ± 5.3	1.1	0.1	1.00	0.79	0.08	125
Ames 27398	Orange	Uzbekistan	Cultivated PI	62.5 ± 4.0	55.8 ± 7.7	6.7	9.9	0.90	0.53	0.12	204
Ames 27399	Yellow	Uzbekistan	Cultivated PI	89.2 ± 3.0	88.3 ± 5.3	0.8	–0.2	1.00	1.18	0.11	30
Ames 27400*	Yellow	Uzbekistan	Cultivated PI	85.8 ± 3.3	90.0 ± 2.6	–4.2	–5.4	1.05	1.16	0.15	34
Ames 29084*	Orange	Tunisia	Cultivated PI	77.5 ± 5.6	64.2 ± 10.4	13.3	15.1	0.85	0.74	0.25	140
Ames 29087*	White	Tunisia	Wild PI	89.3 ± 3.9	43.5 ± 9.1	45.8	51.2	0.49	0.59	0.14	191
Ames 30198*	White	Tunisia	Wild PI	64.7 ± 5.0	63.3 ± 7.2	1.4	–1.9	1.02	0.61	0.20	183
Ames 30259*	White	Tunisia	Wild PI	90.8 ± 2.4	75.6 ± 6.0	15.3	16.4	0.84	1.03	0.08	55
Ames 30276	Orange	Tunisia	Cultivated PI	84.2 ± 3.8	46.7 ± 5.1	37.5	44.0	0.56	0.59	0.20	192
Ames 31193*	White	France	Wild PI	78.3 ± 4.4	55.8 ± 11.7	22.5	27.2	0.73	0.66	0.09	169
PI 163234	Yellow	India	Cultivated PI	58.0 ± 9.6	35.0 ± 9.9	23.0	29.4	0.71	0.31	0.21	267
PI 163235*	Yellow	Pakistan	Cultivated PI	82.5 ± 5.0	70.0 ± 6.2	12.5	12.7	0.87	0.86	0.25	101
PI 163238*	Orange/Yellow	India	Cultivated PI	80.8 ± 3.5	65.8 ± 4.4	15.0	18.9	0.81	0.81	0.14	115
PI 163239	Yellow	India	Cultivated PI	84.9 ± 3.9	64.2 ± 4.6	20.7	24.1	0.76	0.82	0.34	112
PI 163240	Yellow-Orange	India	Cultivated PI	83.3 ± 3.8	61.7 ± 12.2	21.7	22.5	0.77	0.74	0.19	141
PI 163241	Yellow/Orange	India	Cultivated PI	71.8 ± 4.5	74.3 ± 3.4	–2.5	–5.6	1.06	0.80	0.26	120
PI 164943	Yellow	Turkey	Cultivated PI	75.0 ± 5.9	65.8 ± 5.4	9.2	6.8	0.93	0.73	0.18	147
PI 165522	Orange	India	Cultivated PI	75.0 ± 6.1	45.0 ± 14.3	30.0	36.9	0.63	0.49	0.12	214
PI 167143	Purple-Yellow	Turkey	Cultivated PI	60.0 ± 5.6	53.8 ± 12.5	6.2	3.0	0.97	0.46	0.20	223
PI 167211	Purple	Turkey	Cultivated PI	60.0 ± 4.1	52.5 ± 5.9	7.5	13.9	0.86	0.49	0.21	215
PI 176563	Purple	Turkey	Cultivated PI	67.5 ± 5.0	29.2 ± 4.0	38.3	54.9	0.45	0.29	0.24	272
PI 177381*	White	Turkey	Wild PI	63.3 ± 3.8	39.2 ± 9.0	24.2	38.6	0.61	0.38	0.11	246
PI 181052*	Orange/Yellow	Pakistan	Cultivated PI	56.7 ± 5.4	30.0 ± 6.2	26.7	49.1	0.51	0.28	0.13	276
PI 181880	Orange/Yellow	Syria	Cultivated PI	76.3 ± 5.0	80.8 ± 8.1	–4.5	–5.9	1.06	0.95	0.17	78
PI 187235	Orange	Belgium	Cultivated PI	69.2 ± 3.0	39.2 ± 9.2	30.0	43.3	0.57	0.41	0.10	241
PI 187236	Orange	Belgium	Cultivated PI	63.3 ± 5.6	52.0 ± 5.2	11.3	17.1	0.83	0.51	0.09	207
PI 193504	Orange	Ethiopia	Cultivated PI	82.5 ± 5.3	38.3 ± 9.6	44.2	50.6	0.49	0.46	0.16	224
PI 205997*	Orange	Sweden	Cultivated PI	79.8 ± 5.3	59.2 ± 10.1	20.7	23.5	0.77	0.70	0.10	158
PI 211024	Yellow	Afghanistan	Cultivated PI	93.3 ± 4.0	84.2 ± 6.1	9.2	9.9	0.90	1.19	0.26	25
PI 211590	Yellow	Afghanistan	Cultivated PI	73.3 ± 1.7	54.2 ± 3.3	19.2	25.8	0.74	0.60	0.12	186
PI 218076*	Orange	Pakistan	Cultivated PI	96.7 ± 2.5	91.7 ± 1.7	5.0	4.9	0.95	1.33	0.27	8
PI 222250	Orange/White	Iran	Cultivated PI	85.8 ± 1.5	73.2 ± 4.4	12.6	14.5	0.86	0.94	0.15	81
PI 223504	Yellow-White	Afghanistan	Cultivated PI	76.2 ± 3.8	67.5 ± 6.7	8.7	9.7	0.90	0.77	0.21	131
PI 225867	Orange	Denmark	Cultivated PI	87.5 ± 4.2	30.8 ± 10.2	56.7	65.0	0.35	0.41	0.08	242
PI 225868	Orange	Denmark	Cultivated PI	95.8 ± 2.0	82.2 ± 5.0	13.6	14.5	0.86	1.19	0.11	26
PI 225869*	Orange	Denmark	Cultivated PI	90.6 ± 5.4	65.8 ± 11.9	24.7	26.6	0.73	0.90	0.13	90
PI 225870	Orange	Denmark	Cultivated PI	71.7 ± 5.6	33.3 ± 7.7	38.3	53.2	0.47	0.37	0.12	249
PI 225871	Orange	Denmark	Cultivated PI	75.8 ± 4.7	65.8 ± 12.6	10.0	13.2	0.87	0.77	0.10	132
PI 226043	Orange	Japan	Cultivated PI	83.3 ± 3.8	62.8 ± 5.3	20.6	23.8	0.76	0.78	0.14	129
PI 226309	Orange	Mexico	Cultivated PI	62.5 ± 5.9	55.8 ± 5.7	6.7	9.0	0.91	0.54	0.19	202
PI 226310	Orange	Mexico	Cultivated PI	96.7 ± 2.1	88.3 ± 5.3	8.3	8.9	0.91	1.29	0.20	13
PI 226464	Orange	Iran	Cultivated PI	74.4 ± 5.4	58.9 ± 7.1	15.5	17.6	0.82	0.65	0.18	173
PI 234620	Orange	South Africa	Cultivated PI	82.5 ± 2.5	60.0 ± 9.2	22.5	25.7	0.74	0.73	0.15	148
PI 234621	Orange	South Africa	Cultivated PI	94.3 ± 1.5	22.5 ± 6.0	71.8	76.3	0.24	0.32	0.16	262
PI 249535	Orange	Spain	Cultivated PI	87.5 ± 1.1	76.7 ± 6.3	10.8	12.4	0.88	1.01	0.15	59
PI 251228	Purple	Afghanistan	Cultivated PI	64.2 ± 4.7	47.5 ± 1.7	16.7	23.6	0.76	0.46	0.34	225
PI 256065	Orange/Yellow	Afghanistan	Cultivated PI	54.5 ± 3.6	30.0 ± 15.0	24.6	47.1	0.53	0.26	0.13	277
PI 256066*	Purple	Afghanistan	Cultivated PI	74.2 ± 4.2	28.3 ± 5.4	45.8	62.0	0.38	0.32	0.23	263
PI 261648	Orange	Netherlands	Cultivated PI	89.2 ± 4.0	56.7 ± 11.0	32.5	34.5	0.65	0.74	0.14	142
PI 261650*	Orange	Netherlands	Cultivated PI	96.7 ± 1.1	71.7 ± 5.4	25.0	25.9	0.74	1.04	0.13	52
PI 261781*	Orange	France	Cultivated PI	82.5 ± 3.4	35.0 ± 13.4	47.5	57.6	0.42	0.44	0.20	231
PI 261782	Orange	France	Cultivated PI	86.0 ± 6.0	58.6 ± 13.8	27.3	32.2	0.68	0.78	0.11	130
PI 261783	Orange	France	Cultivated PI	56.7 ± 6.0	44.7 ± 12.0	12.0	17.0	0.83	0.38	0.27	247
PI 263023	Orange	UK	Cultivated PI	90.0 ± 4.7	79.2 ± 5.5	10.8	10.7	0.89	1.07	0.13	50
PI 264232	Orange	France	Cultivated PI	62.5 ± 4.4	31.7 ± 8.0	30.8	50.1	0.50	0.31	0.10	268
PI 264234	Orange	France	Cultivated PI	97.5 ± 1.7	91.7 ± 4.0	5.8	6.2	0.94	1.35	0.13	4
PI 264235	Orange	France	Cultivated PI	90.0 ± 3.9	83.2 ± 5.2	6.8	7.6	0.92	1.13	0.12	38
PI 264238	Orange	France	Cultivated PI	99.2 ± 0.8	79.2 ± 9.4	20.0	20.3	0.80	1.18	0.13	31
PI 264543	Red/Orange	Japan	Cultivated PI	76.7 ± 4.8	70.8 ± 5.1	5.8	5.3	0.95	0.81	0.21	116
PI 264669	Orange	Germany	Cultivated PI	73.3 ± 3.3	65.8 ± 7.9	7.5	8.9	0.91	0.72	0.12	152
PI 267090	Orange	Tajikistan	Cultivated PI	78.3 ± 4.6	53.3 ± 4.8	24.9	31.5	0.69	0.63	0.13	178
PI 267091	Yellow	Soviet Union	Cultivated PI	94.2 ± 2.0	81.7 ± 4.6	12.5	13.1	0.87	1.16	0.17	35
PI 268382	Orange	Afghanistan	Cultivated PI	80.0 ± 2.6	78.3 ± 2.1	1.7	1.7	0.98	0.94	0.11	82
PI 269316	Orange	Sweden	Cultivated PI	81.7 ± 7.7	40.0 ± 6.8	41.7	50.0	0.50	0.51	0.14	208
PI 269319	Orange	Sweden	Cultivated PI	75.0 ± 5.3	82.5 ± 3.6	–7.5	–15.5	1.16	0.92	0.11	86

(*Continued on next page*)

Supplemental Table 1. (*Continued*) Carrot accession, root color, country of origin, domestication status (DS), mean percent germination without heat stress (Nonstress) ± SE, mean percent germination with 35 °C heat stress (Stress) ± SE, mean absolute decrease (AD), mean inhibition index (II), relative heat tolerance (RHT), mean heat tolerance index (HTI), hundred seed weight (HSW), and rank based on HTI.

Merged	Root Color	Country	DS	Nonstress (%)	Stress (%)	AD (%)	II (%)	RHT	HTI	HSW	Rank
PI 269322	Orange	Sweden	Cultivated PI	82.5 ± 5.0	45.8 ± 11.4	36.7	39.4	0.61	0.54	0.08	203
PI 269485*	White	Pakistan	Wild PI	87.4 ± 3.8	74.2 ± 7.6	13.3	15.7	0.84	0.99	0.10	68
PI 269486*	Yellow	Pakistan	Cultivated PI	69.4 ± 6.0	54.2 ± 4.4	15.2	17.1	0.83	0.55	0.25	198
PI 269487*	White	Pakistan	Wild PI	88.3 ± 3.6	88.3 ± 3.1	0.0	–1.2	1.01	1.17	0.11	33
PI 269488*	Orange	Pakistan	Cultivated PI	95.0 ± 3.2	78.3 ± 7.0	16.7	18.3	0.82	1.13	0.12	39
PI 271044	Purple-Orange	India	Cultivated PI	52.5 ± 4.8	56.7 ± 7.3	–4.2	–14.3	1.14	0.44	0.28	232
PI 271470	White	India	Cultivated PI	69.8 ± 2.5	51.7 ± 9.8	18.1	26.8	0.73	0.55	0.29	199
PI 271471	NA	India	Cultivated PI	81.7 ± 3.8	74.2 ± 2.0	7.5	7.9	0.92	0.91	0.29	89
PI 274297*	White	Pakistan	Wild PI	62.5 ± 6.9	37.5 ± 9.0	25.0	40.7	0.59	0.38	0.12	248
PI 274298*	White	Pakistan	Wild PI	76.6 ± 5.8	55.0 ± 10.3	21.6	23.2	0.77	0.61	0.13	184
PI 277285	Orange	India	Cultivated PI	71.7 ± 5.4	54.2 ± 12.0	17.5	22.3	0.78	0.59	0.11	193
PI 277668	Orange	Netherlands	Cultivated PI	96.7 ± 2.1	51.0 ± 17.1	45.7	47.3	0.53	0.74	0.09	143
PI 279764	White	Libya	Wild PI	78.3 ± 3.6	10.8 ± 9.9	67.5	85.6	0.14	0.12	0.20	286
PI 279776	Purple	Egypt	Cultivated PI	75.0 ± 7.2	70.0 ± 8.4	5.0	3.4	0.97	0.80	0.30	121
PI 279777	Yellow	Egypt	Cultivated PI	80.0 ± 4.7	56.7 ± 6.7	23.3	27.4	0.73	0.67	0.22	166
PI 280706*	White	Chile	Wild PI	91.6 ± 2.1	58.3 ± 11.0	33.3	35.9	0.64	0.80	0.13	122
PI 285613	Orange	Poland	Cultivated PI	74.4 ± 4.4	61.7 ± 8.9	12.7	15.3	0.85	0.69	0.09	161
PI 294079	Orange	Japan	Cultivated PI	80.8 ± 3.0	50.0 ± 9.0	30.8	36.7	0.63	0.60	0.14	187
PI 294080	Orange	Japan	Cultivated PI	72.5 ± 5.9	65.0 ± 3.9	7.5	7.2	0.93	0.71	0.13	155
PI 294082	Orange	Japan	Cultivated PI	95.0 ± 2.6	88.3 ± 1.1	6.6	6.5	0.93	1.26	0.20	19
PI 294084	Orange	Japan	Cultivated PI	78.3 ± 10.5	65.0 ± 12.3	13.3	6.2	0.94	0.77	0.13	133
PI 294090	Orange	Japan	Cultivated PI	85.8 ± 2.7	64.2 ± 8.9	21.6	23.7	0.76	0.82	0.12	113
PI 295862	White	Spain	Cultivated PI	67.5 ± 8.5	25.8 ± 11.1	41.7	48.2	0.52	0.23	0.09	280
PI 306810	Orange	New Zealand	Cultivated PI	77.4 ± 4.4	51.7 ± 10.5	25.7	32.3	0.68	0.60	0.11	188
PI 319860	Red	Japan	Cultivated PI	78.3 ± 6.2	63.3 ± 13.6	15.0	14.1	0.86	0.74	0.17	144
PI 321688	Red	Japan	Cultivated PI	56.7 ± 3.8	56.7 ± 3.1	0.0	–2.7	1.03	0.48	0.17	218
PI 325993	Orange	Russia	Cultivated PI	97.5 ± 1.7	81.7 ± 12.5	15.8	15.8	0.84	1.19	0.11	27
PI 326009*	Orange	Uzbekistan	Cultivated PI	91.6 ± 2.8	79.2 ± 2.0	12.5	13.0	0.87	1.09	0.12	46
PI 326010	Orange	Tajikistan	Cultivated PI	80.8 ± 5.5	60.8 ± 3.0	20.0	21.8	0.78	0.73	0.16	149
PI 341204	Orange	France	Cultivated PI	74.2 ± 2.4	42.5 ± 5.1	31.7	43.0	0.57	0.48	0.15	219
PI 341207	Orange	France	Cultivated PI	56.9 ± 4.9	37.5 ± 9.9	19.4	24.2	0.76	0.29	0.19	273
PI 341208	Orange	France	Cultivated PI	67.5 ± 2.8	48.9 ± 9.6	18.6	27.3	0.73	0.50	0.17	209
PI 344447*	White	Iran	Wild PI	60.0 ± 1.8	50.0 ± 9.4	10.0	15.4	0.85	0.45	0.20	230
PI 419109	Yellow	China	Cultivated PI	70.3 ± 4.5	58.3 ± 7.2	12.0	16.1	0.84	0.62	0.16	181
PI 419110	Orange-Red	China	Cultivated PI	80.8 ± 3.3	67.5 ± 7.7	13.3	16.5	0.84	0.83	0.15	110
PI 419184	Orange	China	Cultivated PI	95.0 ± 3.2	58.2 ± 10.9	36.8	37.7	0.62	0.82	0.14	114
PI 430524	Yellow	Azerbaijan	Cultivated PI	59.6 ± 5.8	53.3 ± 5.6	6.3	2.8	0.97	0.46	0.15	226
PI 430527	Orange	Uzbekistan	Cultivated PI	94.2 ± 2.4	69.2 ± 3.5	25.0	26.4	0.74	0.98	0.13	70
PI 430528	Yellow	Uzbekistan	Cultivated PI	87.5 ± 2.8	64.3 ± 8.8	23.2	25.6	0.74	0.84	0.13	106
PI 430529	Yellow	Uzbekistan	Cultivated PI	87.5 ± 4.8	67.5 ± 8.0	20.0	20.1	0.80	0.88	0.16	98
PI 430531	Purple	Azerbaijan	Cultivated PI	85.0 ± 4.1	60.0 ± 13.8	25.0	29.0	0.71	0.77	0.16	134
PI 430532	Purple/White	Russia	Cultivated PI	97.5 ± 1.1	84.2 ± 8.6	13.3	13.6	0.86	1.23	0.11	23
PI 430533	Orange	Iraq	Cultivated PI	94.2 ± 2.4	89.2 ± 3.3	5.0	5.3	0.95	1.27	0.10	16
PI 430534	Yellow	Afghanistan	Cultivated PI	94.3 ± 2.0	72.3 ± 13.1	22.0	23.8	0.76	1.03	0.11	56
PI 432898*	Orange/Yellow	China	Cultivated PI	94.2 ± 5.8	71.7 ± 13.5	22.5	19.4	0.81	0.99	0.16	69
PI 432899	Purple/Yellow/Orange	China	Cultivated PI	70.0 ± 4.7	70.1 ± 11.3	–0.1	0.6	0.99	0.76	0.16	138
PI 432900	Orange	China	Cultivated PI	56.9 ± 5.4	55.8 ± 6.9	1.1	0.7	0.99	0.50	0.14	210
PI 432901	Orange	China	Cultivated PI	90.0 ± 2.6	66.7 ± 15.2	23.3	23.5	0.77	0.87	0.15	100
PI 451752*	Yellow-White	Netherlands	Cultivated PI	87.4 ± 4.3	67.5 ± 10.4	19.9	21.6	0.78	0.89	0.14	93
PI 451753*	Yellow	Netherlands	Cultivated PI	68.3 ± 5.3	35.8 ± 8.9	32.5	46.4	0.54	0.37	0.20	250
PI 451754*	Yellow	Netherlands	Cultivated PI	84.2 ± 3.5	55.8 ± 9.9	28.3	33.7	0.66	0.71	0.14	156
PI 451755	Yellow	Netherlands	Cultivated PI	85.8 ± 3.0	75.0 ± 7.4	10.8	12.4	0.88	0.97	0.09	73
PI 451756	Yellow-White	Netherlands	Cultivated PI	71.7 ± 2.1	46.4 ± 10.5	25.3	35.4	0.65	0.50	0.16	211
PI 451757*	White	Netherlands	Cultivated PI	88.3 ± 2.5	75.8 ± 8.0	12.5	13.8	0.86	1.01	0.13	60
PI 451758	Yellow	Netherlands	Cultivated PI	88.3 ± 5.1	82.5 ± 6.2	5.8	4.0	0.96	1.09	0.11	47
PI 451759	Yellow/White	Netherlands	Cultivated PI	88.3 ± 3.3	60.8 ± 6.1	27.5	31.1	0.69	0.81	0.11	117
PI 451761*	Yellow-White	Netherlands	Cultivated PI	94.2 ± 2.4	66.7 ± 4.9	27.5	29.4	0.71	0.95	0.14	79
PI 458857	Orange	Russia	Cultivated PI	83.3 ± 4.0	55.0 ± 10.2	28.3	32.9	0.67	0.68	0.13	164
PI 458858	Orange	Russia	Cultivated PI	96.7 ± 1.1	86.7 ± 4.6	10.0	10.1	0.90	1.26	0.11	20
PI 458859	Orange	Russia	Cultivated PI	58.9 ± 2.5	49.2 ± 7.2	9.7	14.2	0.86	0.43	0.15	234
PI 458860	Orange	Russia	Cultivated PI	95.8 ± 1.5	61.7 ± 16.8	34.2	35.1	0.65	0.88	0.10	99
PI 478369*	White	China	Wild PI	70.8 ± 5.5	75.8 ± 8.4	–5.0	–10.3	1.10	0.81	0.13	118
PI 478370	Orange	China	Cultivated PI	62.7 ± 3.7	75.0 ± 8.2	–12.3	–22.3	1.22	0.70	0.23	159
PI 478883*	White	France	Wild PI	83.4 ± 1.3	26.7 ± 6.2	56.7	68.0	0.32	0.33	0.16	258
PI 483348	Orange	Japan	Cultivated PI	79.8 ± 6.7	71.7 ± 6.5	8.1	3.0	0.97	0.83	0.18	111
PI 483352	Orange	Japan	Cultivated PI	88.3 ± 3.3	63.3 ± 10.7	25.0	28.3	0.72	0.85	0.17	103
PI 502239	Orange	South Africa	Cultivated PI	81.7 ± 5.4	67.5 ± 13.0	14.2	16.2	0.84	0.84	0.12	107
PI 502347	Orange	Uzbekistan	Cultivated PI	98.4 ± 1.0	93.3 ± 3.1	5.0	5.0	0.95	1.38	0.08	3
PI 502654*	Yellow/Purple	Pakistan	Cultivated PI	63.7 ± 5.1	42.5 ± 14.8	21.2	30.4	0.70	0.41	0.34	243
PI 502655*	White	Pakistan	Wild PI	82.5 ± 5.3	56.7 ± 7.2	25.8	28.8	0.71	0.69	0.18	162
PI 502656	Yellow-White	Pakistan	Cultivated PI	86.7 ± 3.3	69.2 ± 6.3	17.5	18.9	0.81	0.89	0.30	94
PI 502914	Orange	Germany	Cultivated PI	51.1 ± 6.3	47.5 ± 8.0	3.6	–1.5	1.01	0.36	0.12	252

(*Continued on next page*)

Supplemental Table 1. (*Continued*) Carrot accession, root color, country of origin, domestication status (DS), mean percent germination without heat stress (Nonstress) ± SE, mean percent germination with 35 °C heat stress (Stress) ± SE, mean absolute decrease (AD), mean inhibition index (II), relative heat tolerance (RHT), mean heat tolerance index (HTI), hundred seed weight (HSW), and rank based on HTI.

Merged	Root Color	Country	DS	Nonstress (%)	Stress (%)	AD (%)	II (%)	RHT	HTI	HSW	Rank
PI 502915*	Orange/White	Germany	Cultivated PI	80.8 ± 4.0	45.0 ± 10.5	35.8	41.6	0.58	0.53	0.12	205
PI 502919	Orange	Germany	Cultivated PI	95.8 ± 2.4	69.2 ± 15.4	26.7	26.5	0.74	0.98	0.15	71
PI 503344	Orange	Lithuania	Cultivated PI	80.8 ± 3.5	83.2 ± 4.8	–2.4	–2.8	1.03	1.02	0.14	57
PI 503345*	Orange	Lithuania	Cultivated PI	93.3 ± 2.5	76.0 ± 8.7	17.4	18.8	0.81	1.07	0.09	51
PI 506444	Orange	Kazakhstan	Cultivated PI	97.5 ± 2.5	76.7 ± 7.7	20.8	20.8	0.79	1.12	0.12	43
PI 506445	Orange	Kazakhstan	Cultivated PI	59.7 ± 5.0	32.9 ± 8.1	26.8	42.3	0.58	0.29	0.15	274
PI 508473	Orange	South Korea	Cultivated PI	52.2 ± 2.9	32.5 ± 8.1	19.7	37.9	0.62	0.26	0.13	278
PI 509433	Purple/Yellow	Turkey	Cultivated PI	96.7 ± 1.7	71.7 ± 4.6	25.0	25.8	0.74	1.04	0.16	53
PI 509434	Red/White	Turkey	Cultivated PI	95.0 ± 2.2	85.0 ± 2.2	10.0	10.0	0.90	1.21	0.18	24
PI 515990	Orange	Hungary	Cultivated PI	99.2 ± 0.8	57.5 ± 8.1	41.7	41.9	0.58	0.86	0.11	102
PI 515992	Orange	Hungary	Cultivated PI	96.7 ± 1.7	12.5 ± 3.1	84.2	87.0	0.13	0.18	0.10	284
PI 531326	Orange	Hungary	Cultivated PI	70.8 ± 8.2	62.1 ± 8.8	8.7	2.2	0.98	0.66	0.09	170
PI 535887*	Orange	Poland	Cultivated PI	97.5 ± 1.1	69.2 ± 10.4	28.3	28.8	0.71	1.01	0.11	61
PI 537093	Orange	South Korea	Cultivated PI	90.8 ± 3.8	85.0 ± 3.2	5.8	5.3	0.95	1.16	0.20	36
PI 540419	Yellow	Uzbekistan	Cultivated PI	78.3 ± 8.6	83.3 ± 5.1	–5.0	–17.8	1.18	0.97	0.12	74
PI 540422	Yellow	Uzbekistan	Cultivated PI	71.4 ± 1.7	55.4 ± 8.6	16.0	22.3	0.78	0.60	0.10	189
PI 632381	Yellow	United States	Cultivated PI	99.2 ± 0.8	85.0 ± 12.1	14.2	14.2	0.86	1.27	0.14	17
PI 632382	Orange	United States	Cultivated PI	95.8 ± 2.4	78.8 ± 7.8	17.0	17.3	0.83	1.13	0.11	40
PI 632383*	Orange	United States	Cultivated PI	90.0 ± 9.0	59.2 ± 17.5	30.8	40.8	0.59	0.89	0.14	95
PI 632384*	Orange	United States	Cultivated PI	96.7 ± 1.7	69.2 ± 4.4	27.5	28.5	0.72	1.01	0.12	62
PI 632385	Orange	United States	Cultivated PI	99.2 ± 0.8	86.8 ± 4.7	12.3	12.3	0.88	1.29	0.15	14
PI 632386	Orange	United States	Cultivated PI	94.1 ± 2.4	70.8 ± 13.4	23.3	24.0	0.76	1.00	0.12	64
PI 632389	Orange	Netherlands	Cultivated PI	98.3 ± 1.7	80.8 ± 12.3	17.5	17.4	0.83	1.19	0.13	28
PI 632393	Orange	United States	Cultivated PI	95.0 ± 2.2	88.3 ± 4.2	6.7	6.9	0.93	1.26	0.12	21
PI 634651	Orange	United States	Cultivated PI	99.2 ± 0.8	90.0 ± 4.8	9.2	9.2	0.91	1.34	0.12	6
PI 634652	Yellow	United States	Cultivated PI	96.7 ± 2.1	85.8 ± 4.2	10.8	11.2	0.89	1.25	0.09	22
PI 634653	Orange	United States	Cultivated PI	97.5 ± 1.1	75.0 ± 10.2	22.5	22.6	0.77	1.09	0.12	48
PI 634654	Orange	United States	Cultivated PI	96.7 ± 1.7	71.7 ± 12.0	25.0	25.8	0.74	1.04	0.08	54
PI 634655	Orange	United States	Cultivated PI	96.7 ± 2.1	59.2 ± 12.8	37.5	37.9	0.62	0.85	0.13	104
PI 634657	Orange	Netherlands	Cultivated PI	95.0 ± 1.8	67.5 ± 17.2	27.5	29.4	0.71	0.97	0.14	75
PI 642755	Orange	United States	Cultivated PI	97.5 ± 1.1	55.0 ± 16.2	42.5	43.7	0.56	0.81	0.13	119
PI 642756	Orange	Netherlands	Cultivated PI	100.0 ± 0.0	88.3 ± 6.5	11.7	11.7	0.88	1.33	0.11	9
PI 642761	Orange	United States	Cultivated PI	96.7 ± 1.7	89.2 ± 4.0	7.5	7.8	0.92	1.30	0.14	12
PI 643114	White	United States	Cultivated PI	99.2 ± 0.8	97.5 ± 1.1	1.7	1.7	0.98	1.45	0.13	1
PI 643118	White	United States	Cultivated PI	97.5 ± 1.7	62.5 ± 5.9	35.0	36.1	0.64	0.92	0.09	87
PI 643119	Orange	France	Cultivated PI	100.0 ± 0.0	85.1 ± 8.2	14.9	14.9	0.85	1.28	0.09	15
PI 652121	Orange	Japan	Cultivated PI	94.2 ± 2.4	78.3 ± 5.9	15.9	16.7	0.83	1.11	0.14	44
PI 652136	Orange	Japan	Cultivated PI	85.8 ± 2.7	78.1 ± 5.8	7.8	8.0	0.92	1.00	0.14	65
PI 652137	Orange	Japan	Cultivated PI	85.8 ± 6.0	69.2 ± 5.7	16.7	18.6	0.81	0.90	0.12	91
PI 652138	Orange	Japan	Cultivated PI	90.7 ± 2.7	54.2 ± 6.3	36.5	39.1	0.61	0.73	0.15	150
PI 652152	Orange	UK	Cultivated PI	59.2 ± 8.0	32.5 ± 11.8	26.7	43.8	0.56	0.30	0.11	269
PI 652153*	Orange	Netherlands	Cultivated PI	90.0 ± 3.4	72.5 ± 6.6	17.5	18.6	0.81	0.98	0.12	72
PI 652154	Orange	Netherlands	Cultivated PI	51.2 ± 6.9	64.2 ± 4.4	–13.0	–35.7	1.36	0.49	0.10	216
PI 652155	Orange	Hungary	Cultivated PI	69.2 ± 3.8	46.7 ± 5.3	22.5	30.1	0.70	0.48	0.10	220
PI 652156*	Orange	Czech Republic	Cultivated PI	77.8 ± 2.6	50.8 ± 9.5	26.9	33.9	0.66	0.59	0.10	194
PI 652157	Orange	Soviet Union	Cultivated PI	75.8 ± 5.8	74.5 ± 9.6	1.3	–2.9	1.03	0.84	0.11	108
PI 652158	Orange	Georgia	Cultivated PI	74.2 ± 3.5	55.8 ± 10.1	18.3	25.4	0.75	0.63	0.11	179
PI 652163	Orange	Netherlands	Cultivated PI	74.2 ± 3.0	56.0 ± 10.2	18.2	24.9	0.75	0.63	0.10	180
PI 652171	Orange	Netherlands	Cultivated PI	78.3 ± 2.1	64.2 ± 5.7	14.2	16.9	0.83	0.75	0.14	139
PI 652173	Orange	UK	Cultivated PI	65.0 ± 4.3	66.7 ± 7.4	–1.7	–5.0	1.05	0.65	0.11	174
PI 652179	Orange/Yellow	United States	Cultivated PI	80.2 ± 4.3	70.8 ± 6.0	9.3	9.0	0.91	0.84	0.16	109
PI 652188	Purple	China	Cultivated PI	69.2 ± 5.1	63.3 ± 5.9	5.8	6.8	0.93	0.67	0.10	167
PI 652206*	Orange	Bulgaria	Cultivated PI	90.8 ± 3.0	87.5 ± 2.8	3.3	3.2	0.97	1.19	0.16	29
PI 652207	Orange	China	Cultivated PI	90.8 ± 4.9	56.7 ± 13.3	34.2	36.4	0.64	0.77	0.08	135
PI 652208	Orange/Yellow/Red	China	Cultivated PI	99.2 ± 0.8	94.2 ± 3.3	5.0	5.0	0.95	1.40	0.15	2
PI 652209	Yellow	China	Cultivated PI	89.2 ± 3.0	75.4 ± 6.7	13.8	15.0	0.85	1.01	0.18	63
PI 652242*	Yellow/White	India	Cultivated PI	83.3 ± 3.1	64.2 ± 7.6	19.2	21.3	0.79	0.79	0.17	126
PI 652243	Yellow	Turkey	Cultivated PI	78.1 ± 4.1	60.8 ± 13.2	17.2	19.7	0.80	0.70	0.28	160
PI 652245	White	India	Wild PI	75.0 ± 6.6	53.3 ± 4.8	21.7	25.8	0.74	0.61	0.23	185
PI 652246	Orange	Russia	Cultivated PI	66.7 ± 7.0	75.8 ± 4.2	–9.2	–19.9	1.20	0.77	0.24	136
PI 652247	Orange	Russia	Cultivated PI	65.0 ± 4.1	53.3 ± 12.2	11.7	21.6	0.78	0.55	0.18	200
PI 652248	Orange	Russia	Cultivated PI	95.0 ± 2.2	94.2 ± 3.3	0.8	0.5	1.00	1.34	0.21	7
PI 652249	Orange	Russia	Cultivated PI	90.0 ± 2.6	69.2 ± 4.6	20.8	23.2	0.77	0.94	0.15	83
PI 652252	Purple-Yellow	India	Cultivated PI	61.8 ± 5.0	40.0 ± 8.1	21.8	31.0	0.69	0.36	0.23	253
PI 652253	Red/Yellow	India	Cultivated PI	50.0 ± 3.7	56.2 ± 2.9	–6.2	–14.8	1.15	0.42	0.25	237
PI 652255	Orange/Purple	India	Cultivated PI	96.7 ± 1.7	74.2 ± 5.1	22.5	23.1	0.77	1.08	0.20	49
PI 652256	Purple/Yellow	India	Cultivated PI	79.8 ± 5.2	63.3 ± 9.6	16.5	16.7	0.83	0.74	0.20	145
PI 652257	Red	India	Cultivated PI	90.8 ± 2.0	68.4 ± 8.4	22.4	24.6	0.75	0.94	0.18	84
PI 652258	Orange	India	Cultivated PI	78.7 ± 2.7	55.1 ± 5.9	23.7	29.3	0.71	0.65	0.20	175
PI 652260	Orange/Yellow	India	Cultivated PI	85.0 ± 2.6	78.3 ± 7.7	6.7	7.4	0.93	1.00	0.15	66
PI 652261	Red	India	Cultivated PI	85.0 ± 4.7	78.3 ± 11.5	6.7	5.8	0.94	1.00	0.27	67
PI 652276	Orange/Yellow	Tajikistan	Cultivated PI	65.8 ± 7.2	42.5 ± 8.5	23.3	32.4	0.68	0.43	0.22	235

(*Continued on next page*)

Supplemental Table 1. (*Continued*) Carrot accession, root color, country of origin, domestication status (DS), mean percent germination without heat stress (Nonstress) ± SE, mean percent germination with 35 °C heat stress (Stress) ± SE, mean absolute decrease (AD), mean inhibition index (II), relative heat tolerance (RHT), mean heat tolerance index (HTI), hundred seed weight (HSW), and rank based on HTI.

Merged	Root Color	Country	DS	Nonstress (%)	Stress (%)	AD (%)	II (%)	RHT	HTI	HSW	Rank
PI 652277	Orange	Mongolia	Cultivated PI	91.6 ± 2.5	65.8 ± 15.0	25.8	27.5	0.72	0.90	0.11	92
PI 652288	Orange	Kazakhstan	Cultivated PI	60.0 ± 7.0	54.2 ± 11.4	5.8	–8.4	1.08	0.44	0.11	233
PI 652290*	White	Poland	Wild PI	82.5 ± 4.2	46.6 ± 8.0	36.0	44.3	0.56	0.59	0.12	195
PI 652291*	White	Portugal	Wild PI	83.3 ± 3.1	17.5 ± 11.2	65.8	78.4	0.22	0.21	0.15	283
PI 652334	Purple	Syria	Cultivated PI	85.8 ± 2.4	65.8 ± 5.7	20.0	22.7	0.77	0.85	0.15	105
PI 652335	Purple	Syria	Cultivated PI	75.3 ± 4.5	36.7 ± 3.8	38.6	51.3	0.49	0.42	0.19	238
PI 652336	Purple	Syria	Cultivated PI	57.5 ± 5.1	58.3 ± 6.4	–0.8	–6.3	1.06	0.50	0.17	212
PI 652338*	White	Syria	Wild PI	82.5 ± 4.6	59.2 ± 9.1	23.3	26.0	0.74	0.72	0.23	153
PI 652341*	White	Syria	Wild PI	89.2 ± 2.4	25.0 ± 13.7	64.2	72.6	0.27	0.34	0.15	255
PI 652344*	White	Syria	Wild PI	81.7 ± 2.1	27.5 ± 16.0	54.2	64.7	0.35	0.32	0.21	264
PI 652346*	White	Syria	Wild PI	89.2 ± 3.5	34.2 ± 16.0	55.0	61.3	0.39	0.46	0.14	227
PI 652347	White	Syria	Wild PI	82.2 ± 3.9	28.3 ± 9.7	53.9	61.7	0.38	0.32	0.13	265
PI 652348*	White	Turkey	Wild PI	58.3 ± 6.5	31.7 ± 9.6	26.7	44.4	0.56	0.29	0.18	275
PI 652349*	White	Turkey	Wild PI	83.3 ± 7.5	27.5 ± 8.6	55.8	63.9	0.36	0.33	0.10	259
PI 652351	White	Turkey	Wild PI	85.8 ± 4.4	28.3 ± 12.8	57.5	64.8	0.35	0.34	0.14	256
PI 652352*	White	Turkey	Wild PI	87.5 ± 3.4	54.2 ± 7.7	33.3	36.1	0.64	0.69	0.16	163
PI 652353*	White	Turkey	Wild PI	88.3 ± 2.8	60.8 ± 11.4	27.5	30.2	0.70	0.80	0.11	123
PI 652354*	White	Turkey	Wild PI	90.8 ± 4.4	4.2 ± 0.8	86.7	95.6	0.04	0.06	0.16	288
PI 652356*	White	Turkey	Wild PI	90.8 ± 3.0	45.8 ± 13.2	45.0	48.9	0.51	0.62	0.15	182
PI 652358*	White	Turkey	Wild PI	66.7 ± 5.1	60.0 ± 10.8	6.7	7.5	0.92	0.60	0.10	190
PI 652359*	White	Turkey	Wild PI	91.7 ± 3.1	16.7 ± 4.9	75.0	81.7	0.18	0.23	0.11	281
PI 652362*	White	Turkey	Wild PI	86.7 ± 2.8	41.3 ± 8.0	45.4	51.6	0.48	0.53	0.12	206
PI 652364*	White	Turkey	Wild PI	83.3 ± 1.7	53.3 ± 7.8	30.0	35.4	0.65	0.66	0.14	171
PI 652368*	White	Turkey	Wild PI	94.2 ± 4.0	55.8 ± 10.4	38.3	39.9	0.60	0.79	0.16	127
PI 652369*	White	Turkey	Wild PI	94.7 ± 2.0	67.2 ± 4.9	27.6	28.9	0.71	0.95	0.08	80
PI 652370*	White	Turkey	Wild PI	86.7 ± 5.4	55.0 ± 7.9	31.7	34.4	0.66	0.71	0.16	157
PI 652372*	White	Turkey	Wild PI	75.8 ± 4.0	44.2 ± 6.4	31.7	39.0	0.61	0.49	0.09	217
PI 652373*	White	Turkey	Wild PI	69.2 ± 6.6	33.2 ± 11.0	35.9	45.1	0.55	0.33	0.10	260
PI 652374	Yellow	Turkey	Cultivated PI	100.0 ± 0.0	75.8 ± 16.5	24.2	24.2	0.76	1.14	0.17	37
PI 652379*	White	Turkey	Wild PI	89.2 ± 3.5	31.7 ± 13.0	57.5	63.9	0.36	0.42	0.06	239
PI 652380*	White	Turkey	Wild PI	72.5 ± 4.2	31.7 ± 12.2	40.8	54.2	0.46	0.33	0.08	261
PI 652382*	White	Turkey	Wild PI	82.4 ± 4.9	18.3 ± 4.9	64.1	76.2	0.24	0.22	0.16	282
PI 652384*	White	Turkey	Wild PI	82.5 ± 4.2	53.8 ± 11.3	28.7	31.0	0.69	0.64	0.14	176
PI 652387*	White	Turkey	Wild PI	62.8 ± 4.6	35.0 ± 15.0	27.8	37.3	0.63	0.30	0.12	270
PI 652388*	White	Turkey	Wild PI	80.7 ± 6.3	28.3 ± 14.6	52.4	63.7	0.36	0.34	0.11	257
PI 652391*	White	Turkey	Wild PI	82.5 ± 2.8	24.2 ± 15.6	58.3	70.9	0.29	0.30	0.18	271
PI 652392*	White	Turkey	Wild PI	65.8 ± 4.7	25.0 ± 15.9	40.8	61.5	0.38	0.24	0.15	279
PI 652393*	White	Turkey	Wild PI	77.4 ± 3.5	37.5 ± 8.6	39.9	50.4	0.50	0.43	0.17	236
PI 652394*	White	Turkey	Wild PI	85.0 ± 3.4	69.2 ± 6.5	15.9	18.5	0.82	0.89	0.12	96
PI 652396*	White	Turkey	Wild PI	71.5 ± 6.0	44.6 ± 6.7	26.8	33.2	0.67	0.46	0.20	228
PI 652398*	White	Turkey	Wild PI	80.6 ± 4.4	53.3 ± 10.3	27.3	34.0	0.66	0.66	0.18	172
PI 652399*	White	Turkey	Wild PI	70.7 ± 3.4	45.0 ± 11.3	25.7	38.7	0.61	0.50	0.10	213
PI 652400	Orange	Turkey	Cultivated PI	95.8 ± 2.4	94.2 ± 4.9	1.6	1.1	0.99	1.35	0.18	5
PI 652401	Orange	Turkey	Cultivated PI	90.0 ± 7.2	85.8 ± 3.5	4.2	2.3	0.98	1.18	0.15	32
PI 652402	Orange	Turkey	Cultivated PI	96.7 ± 2.1	77.5 ± 4.4	19.2	19.8	0.80	1.13	0.15	41
PI 652403	Orange	Turkey	Cultivated PI	94.2 ± 1.5	94.2 ± 2.7	0.0	–0.3	1.00	1.33	0.22	10
PI 652404	Orange	Turkey	Cultivated PI	93.3 ± 1.1	56.7 ± 7.9	36.7	39.2	0.61	0.79	0.14	128
PI 652405	Orange	Turkey	Cultivated PI	97.5 ± 1.1	76.7 ± 15.4	20.8	21.7	0.78	1.13	0.15	42
PI 652406*	White	Turkey	Wild PI	82.5 ± 2.5	58.3 ± 7.9	24.2	30.0	0.70	0.73	0.14	151
PI 652407*	White	Turkey	Wild PI	77.5 ± 7.9	35.8 ± 10.9	41.7	58.0	0.42	0.47	0.09	221
PI 652409*	White	Turkey	Wild PI	88.3 ± 2.1	51.8 ± 9.9	36.5	39.9	0.60	0.67	0.11	168
PI 652410*	NA	India	Cultivated PI	95.8 ± 3.3	88.3 ± 2.1	7.5	7.1	0.93	1.27	0.26	18
PI 652411*	White	France	Wild PI	88.3 ± 2.8	51.0 ± 11.4	37.4	42.4	0.58	0.68	0.09	165
Brasilia	Orange	Brazil	Cultivated PI	54.7 ± 3.0	42.0 ± 4.1	12.7	23.2	0.77	0.35	0.11	264
B0493B	Orange	United States	Inbred	55.8 ± 6.8	0.0 ± 0.0	55.8	100.0	0.00	0.00	0.15	289
B2327B	Orange	United States	Inbred	67.5 ± 8.2	46.7 ± 6.9	20.8	21.6	0.78	0.45	0.19	229
B2566B	Orange	United States	Inbred	90.0 ± 3.7	28.3 ± 9.6	61.7	67.5	0.32	0.38	0.13	245
B5208B	Orange	United States	Inbred	90.8 ± 3.3	68.3 ± 13.5	22.5	25.6	0.74	0.95	0.12	76
B5238B	Orange	United States	Inbred	69.2 ± 5.8	49.2 ± 11.5	20.0	29.7	0.70	0.54	0.20	201
B6279B	Orange	United States	Inbred	63.3 ± 7.0	38.3 ± 9.5	25.0	42.3	0.58	0.40	0.12	244
B7254B	Orange	United States	Inbred	58.3 ± 6.4	7.5 ± 4.8	50.8	85.9	0.14	0.06	0.18	287
DH1[y]	Orange	Netherlands	Inbred	87.5 ± 2.8	23.3 ± 3.3	64.2	73.0	0.27	0.31	0.20	266
L1408B	Orange	United States	Inbred	65.0 ± 1.8	35.0 ± 3.9	30.0	45.6	0.54	0.34	0.18	254
Nb4001B	Orange	United States	Inbred	83.2 ± 2.2	56.2 ± 8.2	26.9	32.8	0.67	0.71	0.15	154
Nb4002B	Orange	United States	Inbred	88.3 ± 8.8	65.8 ± 9.3	22.5	28.0	0.72	0.93	0.15	85
Nb6526B	Orange	United States	Inbred	82.5 ± 5.1	59.8 ± 6.5	22.7	25.0	0.75	0.73	0.16	146
Ns5154	Orange	United States	Inbred	96.7 ± 1.7	60.8 ± 8.8	35.8	36.4	0.64	0.88	0.17	97
P1129B	Purple	United States	Inbred	93.3 ± 2.1	33.3 ± 7.2	60.0	63.8	0.36	0.46	0.18	222
R6636B	Red	United States	Inbred	84.2 ± 10.1	53.3 ± 17.1	30.8	19.8	0.80	0.64	0.20	177
A	Orange	United States	Hybrid	70.7 ± 3.8	30.0 ± 2.5	40.7	57.6	0.42	0.32	0.20	262
B	Orange	United States	Hybrid	59.3 ± 4.6	16.0 ± 4.5	43.3	73.0	0.27	0.14	0.19	284
C	Orange	United States	Hybrid	70.7 ± 3.4	45.3 ± 4.1	25.4	35.9	0.64	0.48	0.16	218

(*Continued on next page*)

Supplemental Table 1. (*Continued*) Carrot accession, root color, country of origin, domestication status (DS), mean percent germination without heat stress (Nonstress) ± SE, mean percent germination with 35 °C heat stress (Stress) ± SE, mean absolute decrease (AD), mean inhibition index (II), relative heat tolerance (RHT), mean heat tolerance index (HTI), hundred seed weight (HSW), and rank based on HTI.

Merged	Root Color	Country	DS	Nonstress (%)	Stress (%)	AD (%)	II (%)	RHT	HTI	HSW	Rank
D	Orange	United States	Hybrid	98.3 ± 1.1	89.2 ± 8.2	9.2	9.1	0.91	1.31	0.12	11
E	Orange	United States	Hybrid	93.3 ± 2.8	77.5 ± 6.9	15.8	16.5	0.84	1.09	0.24	45
F	Purple	United States	Hybrid	80.0 ± 6.6	62.5 ± 9.2	17.5	21.4	0.79	0.77	0.19	137
G	Orange	United States	Hybrid	89.0 ± 1.6	68.3 ± 9.0	20.7	23.4	0.77	0.92	0.20	88
H	Yellow	United States	Hybrid	94.2 ± 2.4	72.5 ± 10.1	21.7	22.0	0.78	1.02	0.10	58

[z]PI = plant introduction.
[y]Developed and donated by Rijk Zwaan.