The Impact of Short-term Thermal Stress on the Consequent Germination of Dill Seeds

A F Bukharov¹, A F Razin¹, D N Baleev¹ and M I Ivanova*¹

¹ All-Russian Scientific Research Institute of Vegetable Growing – a branch of the Federal State Budgetary Scientific Institution “Federal Scientific Center of Vegetable Growing,” Moscow region, 14 Selektsionnaya str., VNIISSOK Village143080 Russia
E-mail: ivanova_170@mail.ru

Abstract. The authors obtained new data on the germination of dill seeds, which were gathered from the first-order and second-order branches, after short-term thermal stress (40 °C). The seeds were germinated in a thermostat. The swollen seeds (4 replications with 100 seeds each) were incubated at 40 °C for 1 to 5 days. The seeds in the control group were not incubated. After the incubation, the seeds were transferred to the standard (t = 20 °C) conditions and germinated on filter paper in Petri dishes without light for 21 days. In this study, the authors employed the dynamic method of seed germination analysis. To plot the seed germination curve, the authors used a log-logistic regression with three parameters: b, d, and e. The statistical analysis was conducted in R 3.4.3. The authors determined the duration of heat stress that inhibits the germination speed and germination rate of seeds. Seeds gathered from the first-order branches (“first-order seeds” from now onward) after 1-day to 3-day incubation germinated similarly to the control group. Increasing the incubation period to 4–5 days sharply reduced the germination speed. Seeds gathered from the second-order branches (“second-order seeds” from now onward) were less resistant to high-temperature stress. Three-day incubation completely precluded germination. The germination time of 50% (T₁₀₀) of the first-order seeds slowed by 0.92±0.11–6.4±0.49 days because of the gradual increase in incubation time. The second-order seeds that incubated for 1–2 days germinated significantly slower than the control group seeds. After this point, further incubation precluded germination. Upon reaching the incubation threshold, the germination rate curves steepened and then continued falling up to the full germination stop. The maximum incubation time (at 40 °C), after which germination was possible in standard temperature conditions, was 3.69±0.06 days for the first-order seeds, and 2.00±0.19 days for the second-order roots. The differences in data were statistically significant at p-value < 0.001

Keywords: Dill (Anethum graveolens L.) · Thermal stress · Seed germination

1. Introduction
Seed heteromorphism is widespread in wild and cultivated plants [9, 19, 22, 30, 35]. The seeds may significantly vary in size, weight, color, morphology, germination characteristics, and other features [8, 27, 39]. In the Apiaceae family, the maternal environment directly affects seed quality since the seeds develop in umbels on different branch orders. [14, 16, 4]. The mother plant architecture affects the seed size, mass, physiology [1, 11, 27, 31], and dormancy [26, 34]. Seed heteromorphism is a mechanism of environmental adaptation [13, 15, 18, 37]. Therefore, current ecological instability makes researching the adaptive reactions of plants, including their germination period, relevant.
Temperature is one of the most crucial crop vitality factors [10, 20, 24, 38]. Some researchers believe that exceeding the optimal temperature by 10–15 °C causes physiological and metabolic changes in plants [33, 36]. The influence of maternal environment and stress factors on seed germination in the Apiaceae family is underexplored, especially in dill [6, 17]. Therefore, in this study, the authors researched the maternal environment heteromorphism of dill seeds and their reaction to thermal stress.

This study analyzes the effect of high (40 °C) temperature on embryo growth dynamics and germination of intact dill seeds harvested from different umbel orders.

2. Materials and Methods

This research was conducted in 2015–2017 at the All-Russian Scientific Research Institute of Vegetable Growing – a branch of the Federal State Budgetary Scientific Institution “Federal Scientific Center of Vegetable Growing.” The authors of this study researched first-order and second-order seeds of the “Centaur” variety of dill (Anethum graveolens L.). The seeds were harvested from plants grown in an unprotected area in the 45×10 cm. pattern, 50 days after the first-order umbels bloomed. The plants were sampled three times, with 30 plants in each replication. The seeds germinated in the TS 1/80 thermostat (SKTB SPU, Russia). The swollen seeds (4 replications with 100 seeds each) were incubated at 40 °C for 1 to 5 days. The control group seeds were not incubated. After incubation, the seeds were transferred to the standard (t = 20 °C) conditions and germinated on filter paper in Petri dishes without light for 21 days.

To plot the seed germination curve, the authors used a log-logistic regression with three parameters [6]:

- b – germination curve inclination;
- d – upper point of the seed germination curve (corresponds to the maximum germination rate);
- e – time at which 50% of maximum germination happened.

Statistical analysis was conducted in R 3.4.3 [21, 29, 25]. To determine the influence of temperature on embryo growth and seed germination, the authors used two-factor dispersion analysis. The correlation between the parameters was evaluated using Pearson correlation analysis. The differences in values were considered statistically significant at p-value ≤0.05.

3. Results

The previous three-week experiment has shown that the temperature of 40 °C is fatal to dill seeds [5]. In the control group grown at the optimal 20 °C, the germinability was 76% for the first-order seeds, and 57% for the second-order seeds.

The first-order seeds after 1-day to 3-day incubation germinated similarly to the control group. Increasing the incubation period to 4–5 days sharply reduced the germination speed (see figure 1). Second-order seeds were less resistant to high-temperature stress. Three-day incubation completely precluded germination.
Figure 1. Seed germination curves depending on the exposure to thermal stress (40 °C) and the place of seed formation on the mother plant: a – first-order; b – second-order. Source: Compiled by the authors.

Germination time of 50% (T50) of the first-order seeds after 1, 2, and 3 days of incubation increased by 0.92±0.11 days, 1.50±0.11 days, and 1.47±0.12 days, respectively, if compared to the control group (see table 1).

Table 1. The effect of thermal stress (40 °C) on T50 (time of germination of 50% of seeds) of the first-order (1) and second-order (2) seeds.

| Incubation time, days | Seed order | T50, days |
|-----------------------|------------|-----------|
| 0 – control           | 1          | 2.95      |
| 1                     | 2          | 4.23      |
|                       | 1          | 3.88      |
|                       | 2          | 4.38      |
| 2                     | 1          | 4.45      |
|                       | 2          | 6.45      |
| 3                     | 1          | 4.42      |
|                       | 2          | 0         |
| 4                     | 1          | 8.21      |
|                       | 2          | 0         |
| 5                     | 1          | 9.36      |
|                       | 2          | 0         |

Source: Compiled by the authors.

After 4 and 5 days of thermal stress, T50 increased by 5.30±0.41 days and 6.4±0.49 (at p<0.001) days, respectively. Compared to the control group, the germination time of the second-order seeds increased significantly (p<0.001) after 1 and 2 days of thermal stress. Further incubation (3–5 days) precluded germination.

The germination rate of first-order seeds varied significantly depending on the incubation time. Incubating for 1, 2, and 3 days decreased the germination rate by 4.4±1.1%, 6.3±1.1%, and 14.8±1.1% (at p<0.001) respectively (See Figure 2). Four and five incubation days considerably decreased the germination rate by 54.0±1.3% and 60.0±1.3%, respectively.

The germination rate of second-order seeds after 1 and 2 days of incubation decreased by 2.0±0.7% (p=0.02), and 19.2±0.7% (p<0.001) respectively. Further incubation at 40 °C precluded seed germination.
The curves, especially that of the second-order seeds, steepened upon reaching the incubation threshold. Then the curves declined steadily, up to the full germination stop.

The maximum exposure time, after which germination is possible in standard temperature conditions, was 3.69±0.06 days for the first-order seeds and 2.00±0.19 days for the second-order seeds. This difference of 1.61±0.20 days is statistically significant (p<0.001).

4. Discussion
Constant temperature stress of 40 °C negatively affects the seed swelling of lettuce, corn, and other plant species [3, 12, 32]. Thermal stress reduces the activity of nutritional enzymes, suppressing germination. Plants respond to thermal stress by producing heat-shock proteins. These proteins help maintain the proper assembly of oligomers; they unfold and recycle defective macromolecular complexes [23, 33, 36]. The thermal stress period, after which germination is possible in standard temperature conditions, was 3.69±0.06 days for the first-order seeds, and 2.00±0.19 days for the second-order seeds. Short-term heat stress suppresses seed metabolism. However, when returned to standard conditions, the affected seeds may restore their growth processes with a delay.

5. Conclusion
High-temperature stress negatively affects the germinability of dill seeds. The subsequent return to standard temperature conditions partially restores the seed metabolism, which is necessary for successful germination. These seeds may then germinate at standard temperatures but take longer to do so than the unaffected seeds. The maternal environment considerably affects seed germinability. Second-order seeds are more sensitive to thermal stresses and may not grow even with subsequent restoration of standard temperature conditions.

References
[1] Baleev D N, and Bukharov A F 2012 Biology of dill seeds formation and germination Russian Vegetables 2(14) pp 54-59
[2] Basra A S, Dhillon R, and Malik C P 1989 Influence of seed pre-treatments with plant growth regulators on metabolic alterations of germinating maize embryos under stressing temperature regimes Annals of Botany 64 pp 37-41 DOI:10.1093/oxfordjournals.aoa087805
[3] Bewley J D, and Black M 1982 Physiology and biochemistry of seeds about germination (Berlin, Heidelberg: Springer)
[4] Bianco V V, Damato G, and Defilippis R 1994 Umbel position on the mother plant: “Seed” yield and quality of seven cultivars of Florence fennel Acta Horticulturae 362 pp 51-58
[5] Bukharov A F, and Baleev D N 2013 Temperature stress and thermal rest of vegetable seeds of umbrella crops. Induction, manifestation and overcoming (Part I) *Vegetables of Russia* 2(19) pp 36-41

[6] Bukharov A F, Baleev D N, and Bukharaeva A R 2016 *Kinetics of seed germination. System of methods and parameters (training manual)* (Moscow, Russia: Russian State Agrarian Extramural University)

[7] Bukharov A F, Baleev D N, and Ivanova M I 2014 Morphometry of umbel crop seeds differences in the process of formation and germination *Bulletin of the Altai State Agrarian University* 7(117) pp 26-32

[8] Cao J, Lu X Y, Chen L, Xing J J, & Lan H Y 2015 Effects of salinity on the growth, physiology and relevant gene expression of an annual halophyte grown from heteromorphic seeds *AoB Plants* 7 plv 112 DOI:10.1093/aobpla/plv112

[9] Cheplick G P 1994 Life history evolution in amphicarpic plants *Plant Species Biology* 9 pp 119-131 DOI: 10.1111/j.1442-1984.1994.tb00092.x

[10] Chitwood J, Shi A, Evans M, Rom C, Gbur E E, Mote D … Hensley D 2016 Effect of temperature on seed germination in spinach (*Spinacia oleracea*) *Hort Science* 51 pp 1475-1478 DOI:10.21273/hortsci11414-16

[11] Corbineau F, Picard M A, Bonnet A, and Côme D 1995 Effects of production factors on germination responses of carrot seeds to temperature and oxygen *Seed Science Research* 5 pp 129-135 DOI:10.1017/s0960258500002749

[12] Drew R L K, and Brocklehurst P A 1990 Effects of the temperature of mother-plant environment on yield and germination of seeds of lettuce (*Lactuca sativa*) *Annals of Botany* 66 pp 63-71 DOI:10.1093/oxfordjournals.aoa.a088001

[13] Dyer A R, Brown C S, Espeland E K, McKay J K, Meimberg H, and Rice K J 2010 Synthesis: The role of adaptive trans-generational plasticity in biological invasions of plants *Evolutionary Applications* 3 pp 179-192 DOI:10.1111/j.1745-4710.2010.00118.x

[14] Eremenko L L 1975 *Morphological features of seed productivity in vegetable plants* (Novosibirsk, Russia: Nauka)

[15] Galloway J F, Etterson J R, and McGlothin J W 2009 Contribution of direct and maternal genetic effects to life-history evolution *New Phytopathology* 183 pp 826-838 DOI:10.1111/j.1469-8137.2009.02939.x

[16] Gray D, and Steckel J R A 1985 Parsnip (*Pastinaca sativa*) seed production: effects of seed crop plant density, seed position on the mother plant, harvest date, and method, and seed grading on embryo and seed size and seedling performance *Annals of Applied Biology* 107 pp 559-570 DOI:10.1111/j.1744-1748.1985.tb03172.x

[17] Holubowicz R, and Morozowska M 2011 Effect of umbel position on dill (*Anethum graveolens L.*) plants growing in the field stands on selected seed stalk features *Folia Horticulturae* 23 pp 157-163 DOI:10.2478/v10245-011-0024-3

[18] Lerner P D, Bai Y, and Morici E F A 2008 Does seed heteromorphism have different roles in the fitness of species with contrasting life-history strategies? *Botany* 86 pp 1404-1415 DOI:10.1139/b08-106

[19] Makrushin N M 1987 *Basics of heterospermatology* (Moscow, USSR: Agropromizdat)

[20] Maraghni M, Gorai M, and Neffati M 2010 Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of Ziziphus lotus *Vegetables of Russia* (Saint Petersburg, Russia: Nauka) pp 36-41 DOI:10.17660/actahortic.1994.362.5

[21] Martin, A C 1946 The comparative internal morphology of seeds *American Midland Naturalist* 36 pp 513-660 DOI:10.2307/2421457

[22] Moles A T, and Westoby M 2006 Seed size and plant strategy across the whole life cycle *Oikos* 113 pp 91-105 DOI:10.1111/j.0030-1299.2006.14194.x

[23] Morimoto R I, Kline M P, Bimston D N, and Cotto J J 1997 The heat-shock response: regulation and function of heat-shock proteins and molecular chaperones *Essays Biochem* 32 pp 17-29 PMID: ncbi.nlm.nih.gov/pubmed/9493008

[24] Nascimento W M, Huber D J, and Cantliiffe D J 2013 Carrot seed germination and respiration at high temperature in response to seed maturity and priming *Seed Science and Technology* 41 pp 164-169 DOI:10.15258/stst.2013.41.1.19

[25] Necajeva J, and Ilevinsh G 2013 Seed dormancy and germination of an endangered coastal plant *Eryngium maritimum* (*Apiaceae*) *Estonian Journal of Ecology* 62 pp 150-161 doi.org/10.3176/eco.2013.2.06

[26] Nikolaeva M G, Lyanguzova I V, and Pozdnova L M 1999 *Seed Biology* (Saint Petersburg, Russia: Research Institute of Chemistry)

[27] Panayotov N 2009 Quality of pepper seed production depending on fruit position on the mother plant
Acta Horticulturae 830 pp 505-510 DOI:10.17660/actahortic.2009.830.72

[28] Panayotov N 2010 Heterogeneity of carrot seeds depending on their position on the mother plant Folia Horticulturae 22 pp 25–30 DOI:10.2478/fhort-2013-0147

[29] Stokes P 1952 A physiological study of embryo development in Heracleum sphondylium L. I. The effect of temperature on embryo development Annals of Botany 16 pp 441-447 DOI:10.1093/oxfordjournals.aob.a083326

[30] Sun H Z, Lu J J, Tan D Y, Baskin J M, and Baskin C C 2009 Dormancy and germination characteristics of the trimorphic achenes of Garhadiolus papposus (Asteraceae), an annual temporary from the Junggar Desert, China South African Journal of Botany 75 pp 537-545 DOI: 10.1016/j.sajb.2009.05.001

[31] Szafiroska A I 1994 The correlation between mother plant architecture, seed quality and field emergence of carrot Acta Horticulturae 354 pp 93-98 DOI:10.17660/actahortic.1994.354.10

[32] Takeba G 1980 Changes revealed by a tracer technique in the amino acid metabolism of thermodormant and non-dormant New York lettuce seeds Plant and Cell Physiology 21 pp 1627-1638 DOI: 10.1093/pcp/21.8.1627

[33] Tariq M, Waseem S, and Bilal H A 2010 An overview of the small heat shock proteins African Journal of Biotechnology 9 pp 927-949 DOI:10.5897/ajb09.006

[34] Thomas T H, Biddington N L, and Palevitch D 1978 Improving the performance of pelleted celery seeds with growth regulator treatments Acta Horticulturae 83 pp 235-244 DOI:10.17660/actahortic.1978.83.31

[35] Venable D L 1985 The evolutionary ecology of seed heteromorphism American Naturalist 126 pp 577-595 DOI:10.1086/284440

[36] Wang W, Vinocur B, Shoseyov O, and Altman A 2004 Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response Trends in Plant Science 9 pp 244-252 DOI:10.1016/j.tplants.2004.03.006

[37] Weber D J 2009 Adaptive mechanisms of halophytes in desert regions Tasks for Vegetation Sciences 44 pp 179-185

[38] Wen B 2015 Effects of high temperature and water stress on seed germination of the invasive species Mexican sunflower PLoS One 10 e0141567 DOI:10.1371/journal.pone.0141567

[39] Yao S, Lan H, and Zhang F 2010 Variation of seed heteromorphism in Chenopodium album and the effect of salinity stress on the descendant’s Annals of Botany 105 pp 1015-1025 DOI:10.1093/aob/mcq060