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Effect of Temperature on Seed Germination in Spinach (*Spinacia oleracea*)

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***Abstract*. Breeding heat-tolerant spinach is an important project to meet the demand of increasing spinach production in heat conditions. Seed germination is the early stage to test, screen, and develop heat-tolerant spinach genotypes. The objective of this research was to determine temperature effect on the seed germination percentage and to select heat-tolerant spinach genotypes. A total of nine spinach genotypes were used in this research. The germination experiment was conducted using seven temperatures: 10, 15, 20, 25, 30, 32, and 35 °C under growth chambers. The temperature trials were conducted using completely randomized design (CRD) with three replicates. Spinach seed germination percentage varied among the nine spinach genotypes under the seven temperatures, indicating that genetic variation for heat tolerance existed in the nine spinach genotypes. 'Donkey', 'Marabu', and 'Raccoon' showed higher seed germination percentage with over 70% at 30 and 32 °C, indicating the three spinach genotypes had heat tolerance for germination. However, all spinach genotypes except 'Ozarka II' dropped their germination percentages sharply to less than 30%; 'Ozarka II' had 63% germination under 35 °C, indicating it is a good source of heat tolerance for seed germination. The higher germination percentages above 30 °C of 'Ozarka II', 'Donkey', 'Marabu', and 'Raccoon' may indicate their potential as donors of heat-tolerant traits in spinach breeding program.**

The economic value of spinach (*Spinacia oleracea* L.) continues to grow in the United States as well as globally due to its high nutritive content. Spinach production has been valued over $250 million in the United States in 2014 (USDA, 2015). Spinach is grown for fresh market, freezing, and canning; and 90% of the spinach grown in the United States is for fresh market (Naeve, 2015). California, Arizona, Texas, and New Jersey grow up to 98% of the commercial fresh market spinach (Naeve, 2015). Nearly half of California's spinach is grown in Monterey County, and although spinach can be grown there nearly year-round, production is limited to the regions and seasons that meet the temperature requirements of spinach (Koike et al., 2011). In Arizona and Texas, production mainly takes place in the winters (CFAITC, 2014).

Spinach is a cool-season vegetable and depending on climate, typically grown in the early spring or late fall when the danger of higher temperatures is not as high (Anderson, 2014). Prior experiments have shown that spinach seeds will germinate in soil temperatures from 5 to 30 °C with germination percentages highest at 20 °C and dropping abruptly between 25 and 30 °C (Atherton and Farooque, 1983). Spinach seed germination has been reported to cease entirely at 35 °C (Leskovar and Esensee, 1999). Substantial seedling root development requires temperatures above 18.9 °C, and top growth will be limited at temperatures below 12.3 °C and above 23.3 °C (Wilcox and Pfeiffer, 1990). Studies have been done on the heat-shock response of spinach, both with whole plants and detached leaf tissue. It has been reported that after being exposed to heat shock (35–50 °C) for 30 min, CO_2 assimilation decreases and pigment proteins in thylakoid membranes aggregate, slowing down the plant's ability to photosynthesize (Tang et al., 2007). In addition, the first heat-shock proteins in spinach leaf tissue are induced when the temperature reaches 28 °C and a full range of heat-shock proteins are produced at 36 °C (Somers et al., 1989). If a spinach genotype has a high germination percentage in high temperature such as 35 °C, it should be a heat-tolerant spinach in germination stage.

Rapid and uniform germination is also necessary for efficient crop production, both in field and greenhouse practices. Although it has been reported that seed treatments may be effective for increasing germination of spinach at higher temperatures (Katzman et al., 2001), managing this trait via selecting heat-tolerant genotypes is a more manageable practice for spinach producers.

Germination under heat stress may also play a significant role in selecting heat-tolerant genotypes. Historically, mass selection was the primary method for developing genotypes, with hybrid breeding becoming popular in recent years, but all are based on field testing (Morelock and Correll, 2008). Although field testing is necessary in many cases, there are numerous environmental effects that contribute to germination performance beyond that of temperature. Therefore, it may be useful to reduce the number of genotypes planted by preliminary testing, improving the statistical approach to reduce error and estimate genotype by environment interaction. Using germination as this pretest has been successful in other crops, such as sorghum (Tiryaki and Andrews, 2001), and would allow quicker and more efficient selections to be made.

The objectives of this study are to determine how temperature affects spinach seed germination and evaluate potential genetic variation for germinating under heat stress.

Materials and Methods

Nine spinach genotypes were used in this study. 'Donkey', 'Marabu', 'Tyee', 'Samish', and 'Raccoon' are commercially available genotypes (Snow Seed Company, 2013, http://snowseedcompany.com/). These genotypes were selected because of their popularity and heat-tolerant qualities. 'Donkey', 'Marabu', and 'Tyee' have been marketed as being heat tolerant or good for summer spinach production (Swallow Tail Garden Seeds, 2015). 'Samish' and 'Raccoon' are not described as heat tolerant and were chosen for comparison. Four genotypes from the University of Arkansas spinach breeding program, 'Ozarka II', 'Fallgreen', F88-310, and F88-354 (Brandenberger et al., 1991) were also used. 'Ozarka II' and 'Fallgreen' are genotypes that were released in 1984 and 1987, respectively, whereas F88-310 and

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F88-354 are breeding lines from the University of Arkansas spinach breeding program (Morelock and Correll, 2008). Most of the seeds from these genotypes were harvested in 2008 and stored at –20 °C. These genotypes were selected because of their frequent use in past spinach production in Arkansas, and also because these are advanced lines with very high white rust (Correll et al., 2010) and will likely be used as parents by the spinach breeding program.

The experiment was conducted in growth chambers at the Harry R. Rosen Alternative Pest Control Center of University of Arkansas. There were seven temperature treatments: 10, 15, 20, 25, 30, 32, and 35 °C. The previously reported optimum for spinach germination is 20 °C (Atherton and Farooque, 1983); therefore two temperatures below the optimum, 10 and 15 °C, were used. Previous studies have shown that germination is inhibited at 30 °C (Atherton and Farooque, 1983; Katzman et al., 2001) and suppressed altogether at 35 °C (Leskovar and Esensee, 1999). Therefore, 30, 32, and 35 °C were chosen as the heat-stress treatments.

Seeds were surface sterilized before the germination tests following methods of Sauer and Burroughs (1986). Seeds were first soaked for 1 h in distilled water, then rinsed for 30 s in 100% ethanol. Next, seeds were soaked in a 2% NaOCl solution for 10 min, and finally rinsed with autoclaved water three times.

The germination tests followed the procedures listed by the Association of Official Seed Analysts (AOSA, 1993). For each genotype, 50 seeds were placed on top of two sheets of blotter paper in 9-cm petri dishes. Dishes were premoistened with 2 mL autoclaved water (Heydecker and Orphanos, 1968) and placed into zip-sealing bags to prevent water loss. Seeds were allowed to germinate at the designated temperature for 21 d (AOSA, 1993). Germinated seeds were counted and removed on 7-d intervals beginning the 7th day after sowing. Seeds were considered germinated when 1 mm of the radicle had protruded through the seed coat. The experimental design was CRD with three replicates.

Table 1. Germination percentages of nine spinach genotypes at each of the seven temperatures.

Genotype	10 °C	15 °C	20 °C	25 °C	30 °C	32 °C	35 °C	LSM[y]
Ozarka II	68.7 B[z]	90.7 BC	87.3 ABC	81.7 BCD	78.0 A	75.0 AB	63.0 A	77.8 A
Fallgreen	56.7 C	68.7 E	74.3 BC	66.7 E	33.3 C	34.0 E	27.3 B	51.6 B
F88-310	91.0 A	94.7 ABC	68.0 C	76.0 CD	80.0 A	54.3 CD	23.3 BC	69.6 A
Donkey	96.0 A	92.7 ABC	86.7 ABC	93.3 A	73.3 A	81.3 A	20.7 BCD	77.7 A
Tyee	96.0 A	96.7 AB	89.3 AB	74.7 DE	44.0 C	34.7 E	19.3 BCDE	65.0 AB
Marabu	72.7 B	90.0 C	80.0 ABC	86.7 AB	70.7 AB	78.0 A	13.3 CDEF	70.2 A
F88-354	66.7 B	97.7 A	96.7 A	83.3 BC	70.7 AB	49.3 DE	9.7 DEF	67.7 A
Raccoon	94.0 A	98.0 A	84.0 ABC	88.7 AB	84.0 A	69.3 ABC	8.7 EF	75.2 A
Samish	91.3 A	80.0 D	85.3 ABC	82.0 BCD	58.7 B	58.7 BCD	6.7 F	66.1 AB

[z]Significant test of seed germination percentages of the nine spinach genotypes across each temperature. The capital letters represent the statistical significance at $P = 0.01$ level for each temperature listed in the column of the table.

[y]LSM signifies the least squares mean for each of nine spinach genotypes, estimated from JMP Genomics.

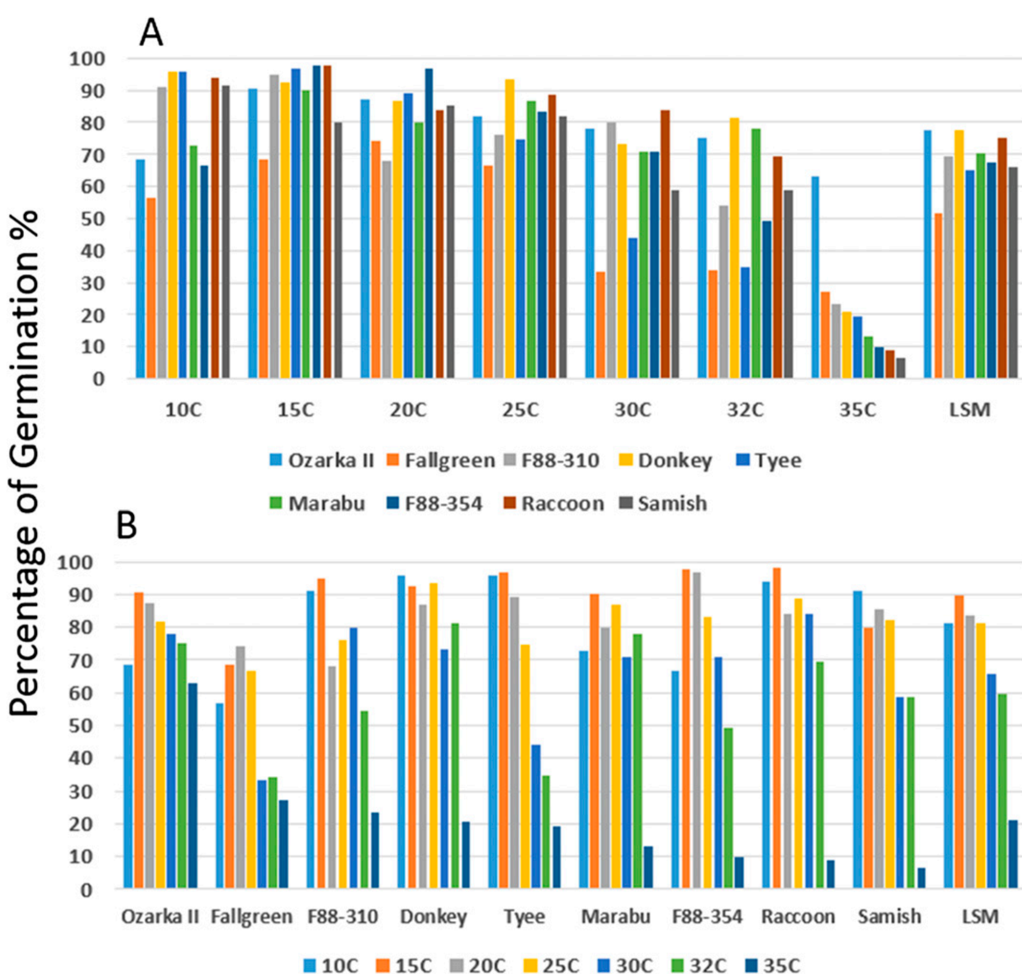

Fig. 1. Germination percentages of nine spinach genotypes at seven temperatures: (**A**) grouped by seven temperatures plus the LSM of each spinach genotype and (**B**) grouped by each of the nine spinach genotypes plus the LSM of each temperature. LSM = least squares mean of germination percentage calculating either from seven temperatures (**A**) or from nine spinach genotypes (**B**) by JMP Genomics and the figure was drawn using Microsoft Excel. In **A**, the spinach order is 'Ozark II', 'Fallgreen', F88-310, 'Donkey', 'Tyee', 'Marabu', F88-354, 'Raccoon', and 'Samish' and in **B**, the temperature order is 10, 15, 20, 25, 30, 32, and 35 °C.

Germination percentages were calculated and analyzed with Microsoft Excel and JMP Genomics (SAS, 2014). Microsoft Excel 2013 was used for data organization and drawing plots to display the germination percentage by temperature. Analysis of variance in JMP was used for statistical analysis. The least squares mean was calculated for each genotype by temperature as well as for each temperature by genotype. The student's *t* test is used to analyze the significant differences of the data.

Results and Discussion

Variation was observed among the nine genotypes for germination under the seven temperatures tested (Table 1; Fig. 1). 'Donkey', 'Tyee', F88-310, 'Raccoon', and 'Samish' had the highest germination percentages at 10 °C; 'Ozarka II', 'Marabu', and F88-354 had intermediate germination percentages; and 'Fallgreen' had the lowest germination percentage of the nine genotypes. At 15 °C, 'Raccoon' and F88-354 had the highest germination percentages (98% and 97.7%, respectively), although they were not significantly different from F88-310, 'Donkey', and 'Tyee' with 92% to 96%. Seven of the nine genotypes had similar germination percentages (80% to 89%) at 20 °C, which was expected because 20 °C is the optimal temperature for spinach seed germination (Atherton and Farooque, 1983). At 25 °C, 'Donkey', 'Marabu', and 'Raccoon' had the highest germination percentages between 86% and 93%. 'Ozarka II', F88-354, and 'Samish' made up the next group from 83% to 81%. F88-310 followed with 76%, then 'Tyee' with 74%, and finally 'Fallgreen' with the lowest of 66%.

Germination percentages remained unexpectedly high at 30 °C except for 'Fall Green', 'Tyee', and 'Samish'. Although seed priming has been reported to result in higher germination percentages for spinach seeds germinated at 30 °C (Katzman et al., 2001), studies have shown that germination inhibition begins at temperatures exceeding 20 °C, and germination is totally suppressed by 35 °C (Leskovar and Esensee, 1999). We observed that 'Raccoon' and F88-310 had above 80% germination, and 'Ozarka II', 'Donkey', 'Marabu', and F88-354 had above 70% germination at 25 °C. Further, at 32 °C, 'Donkey', 'Marabu', and 'Ozarka II' had germination percentages remaining above 75%. Finally, all genotypes except 'Ozarka II' had reduced germination percentage to less than 30% at 35 °C, while 'Ozarka II' had high germination with 63% (Table 1; Fig. 1).

Variation among genotypes may result from several factors including the production of heat-shock proteins (Hum-Musser et al., 1999). Organisms produce heat-shock proteins to respond to heat stress (Somers et al., 1989). These proteins function as molecular chaperones and may be crucial for cell survival under heat stress (Waters et al., 1996). Hum-Musser et al. (1999) evaluated heat-tolerant proteins in seeds germinated under heat stress for several genotypes, including the University of Arkansas genotype Fallgreen, and found a higher accumulation of heat-shock proteins in the genotypes with higher germination percentages under heat stress. 'Ozarka II' may have the ability to produce greater amounts of heat-shock proteins than the other genotypes we tested.

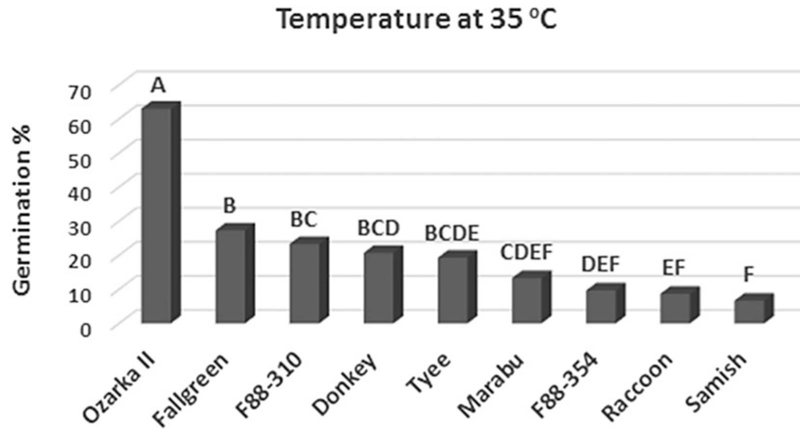

Fig. 2. Germination percentage of spinach genotypes at 35 °C. The letters A, B, BC, BCD, BCDE, CDEF, DEF, EF, and F represent the Student's *t* test at $P = 0.01$ level by JMP Genomics and the figure was drawn using Microsoft Excel.

Comparing 'Donkey', 'Marabu', and 'Tyee', genotypes marketed as heat tolerant, to 'Raccoon' and 'Samish', genotypes not marketed as heat tolerant, at 35 °C does not offer a clear illustration of accuracy in these heat tolerance claims (Table 1; Fig. 1). 'Donkey', 'Tyee', and 'Marabu' were expected to maintain higher germination percentages at higher temperatures, but 'Tyee' had the lowest germination percentage at 30 and 32 °C of the five genotypes. 'Raccoon' had the highest germination percentage of 84% at 30 °C. Only at 35 °C, 'Donkey', 'Marabu', and 'Tyee' as a group have higher germination percentages than 'Raccoon' and 'Samish' (Table 1; Fig. 1).

University of Arkansas germplasm has been incorporated into many genotypes over the years (Morelock and Correll, 2008), and several of these have been used in studies of spinach seed germination under heat stress (Hum-Musser et al., 1999; Leskovar and Esensee, 1999). Interestingly, the University of Arkansas genotypes seem to be among the more heat-tolerant genotypes. In this study, three of the four spinach genotypes from the University of Arkansas had higher germination percentages than the commercially available genotypes (Fig. 2). This may be the result of spinach selection under heat conditions in Arkansas, where spinach breeding lines are planted in the early fall and selected throughout the winter and spring in Alma, AR. Average high temperatures for Alma between August and October range from 24 to 34 °C (U.S. Climate Data, 2015), and recurrent selection of lines that are able to germinate and tolerate these or higher temperatures may have resulted in improved heat tolerance among University of Arkansas spinach germplasm. Recurrent selection has been shown to be a successful strategy in breeding for heat tolerance in wheat (Machado et al., 2010) and the unintended but nevertheless present selection pressure for heat tolerance in University of Arkansas spinach lines may result in future releases of heat-tolerant genotypes.

Conclusions

The optimum temperature for spinach seed germination was between 15 and 20 °C from this study. Above 20 °C, germination percentages decrease, with a sharp drop at 35 °C. Variation among spinach genotypes was observed, suggesting that breeding for heat tolerance is a possibility. The University of Arkansas genotype Ozarka II was more heat tolerant than the other genotypes tested and may be used as a donor of heat tolerance in the University of Arkansas spinach breeding program.

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