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Seed germination of calendula in response to temperature

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ABSTRACT

Calendula (*Calendula officinalis* L.) seeds contain high concentrations of calendic acid (C18:3) which can be used as tung and linseed oil substitutes. Calendula is adapted to temperate climate, but field studies in western Minnesota indicated that stand establishment was susceptible to high soil temperatures immediately after planting in spring. Consequently, understanding the temperature conditions that govern germination of calendula is necessary to incorporate the crop into crop rotations of the Upper Midwest, U.S. Temperature gradient bar and heat-shock experiments were used to characterize calendula (cv. 'Carola') sensitivity before and during germination. Seed germinated between 2 and 32 °C with the optimum germination temperature at 16–17 °C. Heat shock temperatures (35–40 °C) of less than 50 h duration reduced germination (at 16 °C) below 50%. At 45 °C, 100% seed lethality was induced within 24 h of heat treatment. Accordingly, calendula seed should be sown in the field only if forecasted soil conditions are expected to be below 30 °C during seed germination.

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1. Introduction

Calendula (*Calendula officinalis*), or pot marigold, is an annual plant that has been used historically for ornamental and medicinal purposes. Calendula typically grows 20–50 cm tall with yellow and orange flowers 4–7 cm in diameter (Tucker, 2007). Calendula is in the *Asteraceae*, and has composite-type flowers (i.e., arranged in capitula). The seed of calendula comes in three primary shapes: nuggets, winged, and hooked (Froment et al., 2003; Joly et al., 2013; De Clavijo, 2005). For agronomic production the nuggets are preferred because their shape is more compatible with seeding equipment. In the last 20 years calendula has been evaluated as a specialty oilseed crop due to its high calendic acid content (Meier zu Beerentrup and Röbbelen, 1987; Biermann et al., 2010; Cromack and Smith, 1998). Seed oil from calendula contains 59–65% calendic acid (C18:3), which has value in cosmetic, paint, and coating industries (Biermann et al., 2010). The cultivar 'Carola' was released in 2005 and is one of only a few commercial oilseed varieties (Gesch, 2013). The production and management practices for this oilseed crop have not been described fully for the Upper Midwest region of the U.S. Agronomic production of calendula has been evaluated primarily in Europe (Froment et al., 2003). Developing the best management practices for the production of calendula is necessary for optimized yield and profit for growers. Recent studies reported that calendula seed should be seeded at a depth of 1–2 cm by early May in central Minnesota fields (Joly et al., 2013; Gesch, 2013).

Observations during field trials suggested that high temperatures at planting time were associated with reduced calendula stand establishment. A germination study by Harrington (1921) concluded that at constant temperature calendula germination reached a maximum (65%) between 15 and 25 °C within 6 d. Germination of the medicinal cultivar 'Bonina Sortida' was rapid, reaching near maximum germination levels within one week in incubators (Koefender et al., 2009). For this cultivar, optimum temperature was 20 °C, at which over 80% germination was achieved, suggesting that calendula is adapted to germination during cool seasons. Lower levels of germination (52–76%) occurred at 15 and 25 °C, and only scant germination (11–28%) occurred at the higher temperatures of 30 and 35 °C. Seedling elongation and dry weight gain of this same cultivar were highest at 20 °C, in both light and dark conditions, whereas both variables were nil at 30 and 35 °C (Koefender et al., 2009). The related wild species, field marigold (*Calendula arvensis*), had similar germination responses to temperature as domesticated calendula: (a) a broad high-germination peak from 15 to 25 °C, (b) delayed germination at temperatures exceeding 30 °C, and (c) a near absence of germination at temperatures greater than 35 °C (De Clavijo, 2005). Similarly, an ornamental cultivar of calendula, 'Calypso Orange', produced only half as much dry matter and flower buds at 32 °C compared to 20 °C (Warner and Erwin, 2005). Moreover, high temperature hastened anthesis and decreased inflorescence size by one-third in this cultivar. Clearly, calendula appears intolerant of temperatures that easily could be reached in shallow soil layers after late spring planting or by air temperatures during summer in temperate zones. We selected the

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variety ‘Carola’ as the focus of this germination study because of its high seed oil content, commercial availability, and lack of reported germination requirements in the literature.

Here we determined the critical temperatures (T_b, T_o, and T_c) for calendula seed germination by examining germination rates over 9 d across temperatures ranging from 1 to 40 °C. Base temperature (T_b) is the temperature below which germination is 0%, optimum temperature (T_o) is the temperature where germination is maximal, and the ceiling temperature (T_c) is the temperature above which germination is 0%. Joly et al. (2013) reported the T_b for calendula to be 5.5 °C based on soil hydrothermal time to 50% field emergence. Hydrothermal time is a well documented measure where soil temperature and water potential are used to predict germination rates for different species. Using controlled temperature studies we sought to confirm the T_b for calendula and to determine the T_o and T_c, which can be used to facilitate seed sowing and agronomic production of calendula. Additionally, we determined the time period over which T_c is exerted by a series of heat-shock experiments.

2. Methods

2.1. Plant material

Nugget-type (annular) seeds of ‘Carola’ were used in all studies. Seeds were germinated on pre-moistened germination paper that was placed either on solid aluminum gradient bars or in glass Petri plates.

2.2. Gradient bar temperature incubations

Seeds were germinated on a temperature-gradient bar lined with filter paper as described by Berti and Johnson (2008) for 9 d with nine thermal couple probes evenly spread along the bar in the range of 1.1–39.0 °C. The thermocouples (copper-constantan) were attached flush to the aluminum bar with high temperature conductivity epoxy resin. The thermocouples were monitored with a CR-10X (Campbell Scientific, Logan, UT) data logger every 15 s and 1 h averages recorded. Seeds were placed in 3 rows of 10 at each thermal couple, each row served as an experimental replicate. Seeds were checked daily for germination and filter paper was remoistened as needed. Radical emergence of greater than 0.5 cm was considered germination, and the germinated seed was removed from the gradient bar. The entire experiment was performed three times.

Cumulative germination percent was calculated for each seed row after 9 d. Results from the experimental trials varied significantly and, consequently, different quadratic and cubic regression models were fit to each experimental trial. Critical temperatures were calculated from the different models.

Germination rate was calculated as the inverse of the number of days (d^{-1}) to reach 50% germination for each row of 10 seeds. If the seeds for a given treatment never reached 50% germination, the rate was entered as 0.0 d^{-1}. Germination rate was separated into sub- and supra-optimal ranges by visual inspection of the data. Linear regression models were fit to the sub- and supra-optimal data sets. The intersection of the sub- and supra-optimal regressions within each experimental trial denoted the T_o for germination rate. Both T_b and T_c were determined from the intercept of the sub- and supra-optimal regressions, respectively.

2.3. Heat shock incubations

Calendula seeds were incubated at three temperatures above the predicted T_c (35, 40, and 45 °C) expected to induce heat shock. Exposure times (treatments) were 0, 2, 8, 24, 48, 96, and 192 h, except trial 1 at 40 °C wherein seeds were incubated for 1, 2, 4, 8, 24, 48, and 192 h. All seeds were incubated at 16 °C following heat shock treatments for a total cumulative incubation period of 14 d. There were 20 seeds per plate and 3 plates per treatment. Petri plates were sealed with parafilm to prevent drying. Seeds were allowed to imbibe for 1 h at room temperature in standard Petri plates on germination paper wetted with 10 ml water before heat shock incubation. Dry seed treatments had 10 ml of water added to the Petri plate after heat shock incubation and before 16 °C incubation. Each experiment was performed at least twice. Cumulative germination percent was measured at incubation day 14 for each plate.

2.4. Tetrazolium chloride viability staining

Wet calendula seed was incubated at 45 °C for 0, 2, 8, or 24 h followed by incubation at 16 °C for a total cumulative incubation period of 24 h. Following modified procedures from the Tetrazolium Testing Handbook (Peters, 2000), seeds were bisected longitudinally, and the largest portion of the embryo was excised. There were 20 seeds per plate and 3 plates per treatment. The experiment was repeated twice. All 20 embryos from each plate were placed in 1 ml of 0.1% tetrazolium chloride solution and stored in the dark at room temperature for 4 h. Seeds were rinsed in 2 volumes of MiliQ water and scored for viability. Seeds only were considered viable if the entire embryo stained bright pink. All embryos with partial staining were recorded as dead. Percent viable seed was calculated for each treatment.

2.5. Statistical analysis

All statistical analyses were performed using SAS for Windows 9.3 (SAS Institute, Cary, NC). Gradient bar and heat-shock data were analyzed using nonlinear regression techniques. Based on general linear model (GLM) analyses, the percent viability for the two trials for tetrozolium staining did not differ significantly and the trials were pooled for each treatment. A Tukey’s HSD mean separation ($\alpha = 0.05$) was used to determine treatment differences.

3. Results and discussion

3.1. Germination of calendula at constant temperature

3.1.1. Cumulative germination percent

Calendula seeds that were germinated at different temperatures had a reduction in cumulative germination as gradient bar temperature increased and decreased around T_o (Fig. 1). Temperatures from 13.6 to 21.1 °C supported an average cumulative germination percent of 87 ± 8.3% SD after 9 d. The three experimental trials (1–3) were significantly different from each other, and quadratic and cubic regression models were fit to germination data within each experiment (Table 1). Critical temperatures (T_b, T_o, and T_c) were calculated from each model (Table 1). The predicted T_b was in the range of 0.0–3.2 °C. For five of the models the T_o was in the range of 15.6–16.8 °C, with the cubic model for experiment 1 giving a T_o of 11.7 °C, much lower than the others. This same pattern was observed for the T_c, with the cubic model for experiment 1 predicting the T_c as 23.7 °C, and the other five models ranging from 29.3 to 32.6 °C. Because of these discrepancies, the cubic model for experiment 1 was not used for critical temperature estimates. From these models we concluded that the T_o for calendula is 15.6–16.8 °C, T_b is 0–3.2 °C and T_c is 29.3–32.6 °C. Joly et al. (2013) reported a T_b for calendula of 5.5 °C for soil hydrothermal time based on field germination studies and simulated soil temperatures. Our models predict a lower T_b although the values are similar.