



Salicylates Promote Carrot Seed Germination At A Low Temperature



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Introduction

Sporadic and delayed seedling emergence is a major problem in carrot production that results in a reduced crop stand directly lowering root yield and quality. Seedlings emerge from 10 days to 30 days depending on field conditions. Seed size, viability, sowing depth, soil moisture and temperature are all known to contribute to poor and/or sporadic germination and emergence (Bewley and Black 1982, Rajasekaran et al. 1992, Hartman et al. 1997). Amongst all the factors that control germination, temperature is perhaps the most important environmental factor that regulates the timing of germination (Hartman et al. 1997). Temperature affects both germination percentage and rate (Bewley and Black 1982). Germination rate is invariably low at low temperatures but increases gradually as temperatures rise, similar to a chemical reaction (Koller 1972). There is little or no information on temperature requirements for carrot seed germination. Since carrot seeds are sown during April – May in the Maritime region of Canada, when soil temperature generally ranges from 2–5°C, it is possible that sporadic emergence and low crop stand could be due to such low soil temperatures.

Low soil temperature limits germination either by reducing imbibition, inhibiting key metabolic processes connected with germination, GA synthesis, or mobilization (Bewley and Black 1982), preventing inhibitor degradation or leaching, or by reducing enzyme activity and/or altering membrane potential (Bewley and Black 1982). Thermogenecity (heat production) in plants is now known to occur in the male reproductive structures of cycads and the flowers or inflorescences of some Angiosperm species (Meese and Raskin 1988). The heating is associated with a large increase in the cyanide-insensitive non-phosphorylating electron transport pathway, and activation of the alternative oxidase, glycolytic and Krebs cycles enzymes which provide substrate for thermogenesis. Calogen extracts from male flowers of voodoo lily has been found to contain salicylic acid. SA (Raskin et al. 1987) and application of SA to the sections of immature appendix lead to a 12°C increase in temperature. If this is so, treating carrot seeds with salicylates may promote germination at limiting low temperature.

This study examines the critical threshold temperature for optimal germination of carrot seedling and the possibility of whether salicylates hasten germination at a limiting low temperature. No information on salicylates in promoting seed germination at low temperature is available.

Materials and Methods

Seeds of carrot (*Daucus carota* var *sativus*) cv. Oranza, a high yielding slicer variety, were used for the entire study. Seeds of 1.6 – 1.8 mm from the same batch were obtained from Bejo (Bejo Seed Co, NY, USA). For the experiment to identify critical low temperature, seeds were visually sorted, counted and were placed on Munktel's No. 1F filterpaper in sterile petridishes (100 X 15mm) and incubated in temperature controlled germination chambers at 25 °C, 20 °C, 15 °C, 10 °C, 5 °C or 2 °C until the germination was completed. Seeds were submerged in distilled water (DW), equilibrated to respective temperatures for a period of 24h. After 24h, excess water was decanted and the seeds were moistened daily until the experiment was terminated on the 20th day. One hundred seeds were used for each treatment in each replication. Each treatment was replicated four times.

Separate experiments were conducted using various salicylates such as, salicylic acid (SA), acetyl salicylic acid (ASA) and 2,6-dihydroxybenzoic acid (DHBA) at 0, 1, 10, 100, 1000 mg l⁻¹. As solubility of DHBA in water was low it was dissolved in 1.6ml of ethanol and made up with DW. SA and ASA solutions were prepared using DW. One hundred seeds were used in each of the treatments and were replicated four times. The seeds were placed on filter papers in a sterile petri-dish and incubated at either 25°C or 5°C.

Germination was deemed to have occurred when the radicle was visibly extended beyond the surface of the seed, usually protruding through a fracture in the seed coat. Number of seeds germinated each day was counted and % of germination was calculated. Germination vigor was determined using the method described by Cabator (1982) modified to include the number of days of incubation at each temperature and each treatment. The vigor value was calculated as follows:

$$V = (a/1 + b/2 + c/3 + d/4 \dots x/n) / S^{1/100}$$

Where, a, b, c, d and x represent the number of seeds which had germinated after the first, second, third, fourth and nth day of incubation, respectively, and S represents the total number of seeds in the sample.

The GLM procedure (SAS Institute Inc. Cary NC, USA) was used for ANOVA with the Duncan Multiple Range Test for separation of differences between means for all the data.

Results

Exposure to various temperature regimes significantly ($p < 0.05$) influenced both germination percentage and vigor value (VV) of carrot seeds (Fig 1 and 2). Germination delayed as the temperature was lowered. At 25°C, the seeds germinated on day 2, continuing rapidly, reaching 96% on day 9. In contrast however, the seeds incubated at 5°C did not germinate until 7d, starting slowly thereafter, reaching 92% on day 13. At the lowest temperature of 2°C, there was a considerable lag in germination until 13d, registering the lowest germination percentage (26) on 19d. There was no improvement in germination thereafter.

As with germination percentage, VV also significantly and proportionately reduced as the incubation temperature lowered (Fig 2). More than 50% reduction in VV was observed at temperatures at 5°C. VV was the lowest at 2°C. The critical threshold temperature (GT_{50}) was observed to be 5°C.

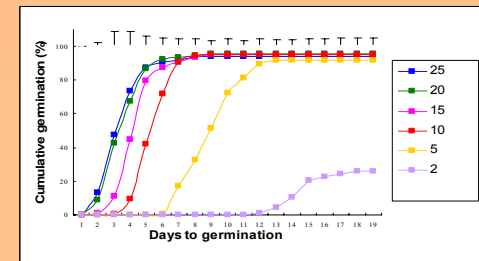


Figure 1: Cumulative Germination of Carrot Seedlings as Influenced by Various Temperature Regimes

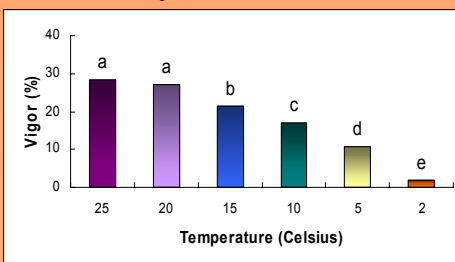


Figure 2: Vigor Value for Carrot Seedlings at Various Temperatures

DHBA hastened and promoted germination both at non-limiting temperature and limiting temperatures. The untreated seeds at 25°C germinated early (day 2) while ones incubated at 5°C germinated on day 10. Nearly, 95% of the seeds germinated on day 8 at 25°C as against 17 days with the ones at 5°C. DHBA at 1 mg l⁻¹ hastened and promoted germination significantly compared to the untreated control despite the seeds were incubated at a low temperature of 5°C. Increasing DHBA concentration however, delayed germination both at non-limiting and limiting temperature regimes. DHBA 1000 mg l⁻¹ was the most inhibitive of germination both at 25°C and 5°C.

Similar to DHBA, ASA also hastened and promoted germination at 5°C. ASA 100 mg l⁻¹ was the most effective in hastening and enhancing germination at 5°C. A significant enhancement in germination was observed in ASA 100 mg l⁻¹ treated seeds incubated at 5°C and day 5 and continued until day 9 remaining similar to that of untreated control, thereafter. There was no significant difference in germination on day 17 except with ASA 1000 mg l⁻¹. At 25°C, ASA showed no significant advantage at any concentration range. ASA 1000 mg l⁻¹ inhibited germination at both the temperature regimes.

SA had no significant influence at any concentration range on seeds incubated at 25°C. At 5°C however, SA at 1 mg l⁻¹ hastened and promoted germination significantly. Seeds treated with SA 1 mg l⁻¹ germinated early (day 7) and germination continued rapidly reaching the highest value on day 21. Germination percentage of SA 1 mg l⁻¹ treated seeds remained always higher than the untreated control until day 18, and no significant difference was observed thereafter. Germination percentage remained low with seeds treated with 10 mg l⁻¹ SA. SA 1000 mg l⁻¹ inhibited germination of seeds incubated at both temperatures completely.

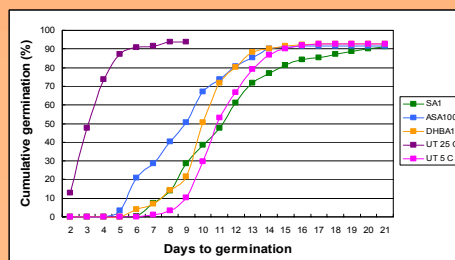


Figure 3: Cumulative Germination of Carrot Seedlings Influence by Various Treatments

Discussion

Lowering the incubation temperature significantly and proportionately reduced germination percentage and the vigor value of carrot seeds (Fig 1 and 2). The optimal germination temperature for carrot seed germination and vigor was 25°C (Fig 1). The critical temperature for carrot seed germination (GT_{50}) was found to be 5°C (Fig 1 and 2). This explains, at least to a certain extent, that sporadic emergence of field grown carrot seedlings may be due to the low soil temperature that prevail during sowing. A direct effect of low temperature on seed germination has been reported for many seeds (Bewley and Black 1982). Low temperature affects germination percentage by either limiting water flow and/or reducing the rate of thermochemical reactions by the seed (Bewley and Black 1982). A reduction in germination percentage and VV (Fig 1 and 2) indicates the possibility of limitation for water flow at low temperatures (Simon 1984), reduced water activity and/or a change in configuration of water molecules (Bewley and Black 1982). This would have reduced water availability, delaying imbibition thereby reducing germination at low temperature. Osmotic potential determines water uptake during the radicle emergence phase of seed germination (Hartman et al. 1997). A combination of increasing osmotic potential and/or changes in turgor potential can result in cell enlargement and initiate radicle emergence (Simon 1984, Hartman et al. 1997). Although no osmotic or turgor potential measurements were made in the present study, it is possible that low temperature would have caused a reduction in water flow affecting turgor during radicle emergence. It is also possible that low temperature may have reduced the synthesis or mobilization of cell wall loosening factors (Schopfer and Plachy 1985).

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Protein denaturation has also been reported at low temperatures (Bewley and Black 1982). Low temperature affects germination through certain protein conformational changes that are required to promote germination (Bewley and Black 1982). Although germination was delayed at 5°C, a completion of germination at this temperature on day 12 indicated that the delay may not be due to protein denaturation but possibly due to a lag in protein conformation (Fig 1). However, the lowest germination observed at 2°C (Fig 1) indicated the possibility of protein denaturation and/or changes in protein conformation. Since carrot seeds germinated and reached a maximal germination percentage as to that of 25°C (Fig 1 and 2), neither a failure of cell division nor a lack of protein synthesis or protein denaturation can be the factors delaying germination at 5°C. A similar response was observed by Simon (1984) with cucumber seeds that germinated readily after 350 h of imbibition at 5°C. Inhibitor experiments and incorporation of ¹⁴C-leucine into imbibing cucumber seed provided no support for a complete breakdown of protein synthesis in the cold, although the inability to synthesize certain proteins vital to the process of germination remains a possibility (McMenamin 1978).

Rate limiting diffusion may also be involved as the temperature approaches the minimum, and the temperature dependant transitions in cell membranes might be important (Simon 1984). The decline in rate of germination and vigor value also points to changes in membrane fluidity. It is also possible that a reduction in germination rate and vigor value may be due to low GA synthesis or availability as GA leaks out of storage compartments when membranes become much more permeable at low temperatures.

Alternatively, however, seeds require activation energy for germination. Activation energy is low at optimal temperature and high at low or sub-optimal temperatures (Simon 1984). Poor germination and low vigor value at low temperature may also be due to low activation energy, which is a result of enzyme catalysis. Enzyme catalysis becomes rate limiting at low temperatures. Delay or sporadic germination at low temperature implies that activation energy that is required for the seeds to germinate may not have been met. Energy for germination activation is derived from glycolysis and Krebs's cycle and the enzymes connected with both processes have temperature optima (Km) near 25°C for maximal activity (Bewley and Black 1982). This explains the highest germination rate and vigor value at 25°C in carrot (Fig 1 and 2). To activate seed germination at low temperature, either the genes connected with the glycolysis or Krebs's cycle have to be triggered or an alternate respiratory pathway to be turned on which would initiate the germination reaction by thermogenesis, increasing temperature inside the seed.

Salicylates, especially DHBA 1, ASA 100, SA 1 mg l⁻¹ significantly hastened and promoted germination at a low temperature of 5°C. Amongst all, ASA 100 mg l⁻¹ was the most effective in hastening germination at 5°C as seen on day 6 until day 10 compared to the untreated control (Fig 3).

Hastening germination at low temperature and germination inhibition at higher concentrations indicate that these compounds were absorbed by the seeds. Germination hastening by these salicylates at a low limiting temperature may be due to thermogenesis or calorigenic activity (Raskin et al. 1987). Measurement of temperature inside the seed was limited by the size of carrot seeds. However, the role of salicylates in thermogenesis and calorigenic activity in inflorescence of Arum lilies has already been demonstrated (Raskin et al. 1987). Application of SA led to an increase of as much as 12°C in an immature appendix (Raskin et al. 1987). Thermogenic activity of salicylates in several plants has been reported. Germination hastening at 5°C by salicylates may be triggering alternate – respiratory pathway, activating alternative oxidase (Raskin et al. 1987, 1989), and/or through activation of the glycolytic and Krebs's cycle enzymes which would have provided substrates for this metabolic activity. SA has also been reported to increase cyanide resistant respiration and associated heat production in young tobacco cell suspensions (Kapulnik et al. 1992). SA is also known to induce alternate oxidase protein, therefore, the enzymology of salicylates-induced germination promotion at a low temperature was not attempted in the present study, the germination response at low temperature due to salicylate treatment may perhaps be through their thermogenic activity (Raskin et al. 1987). Heat production inside the seed would have initiated a cascade of other thermochemical activities accelerating germination despite a low temperature. SA is also known to induce a rapid membrane depolarization (Raskin 1992) and it is possible that this would have facilitated a rapid leakage of germination inhibitors such as, ABA which otherwise will be limiting or delaying germination at low temperatures.

Although salicylates sustained high vigor value at 25°C, they failed to do so at a low temperature with any of the salicylates. If salicylates induces thermogenesis and increase respiratory activity this would be expected to deplete carbohydrate reserves thereby limit germination. However, maintenance of higher vigor value in salicylate treated seeds incubated at 25°C indicates that carbohydrate supply may not be a limiting factor and the low vigor value at 5°C in salicylate treated seeds may not be due to exhaustion. This indicates that some other factor may be limiting germination at low temperature, possibly, GA (Rajasekaran et al. 1992). Inhibition of germination by the salicylates at the highest concentration at 25°C or 5°C may be due to inhibition of ethylene biosynthesis (Rajasekaran and Blake 1999).

Amongst all the salicylates tested, ASA was the most effective in hastening germination at low temperature compared to DHBA or SA (Fig 3). However, DHBA has been shown to have a greater thermogenic activity than ASA or SA in Arum lilies (Raskin et al. 1989). It is possible that such a response could be ascribed to be due to tissue specificity (Kapulnik et al. 1992).

CONCLUSION

In conclusion, a temperature below 20°C has been found to be detrimental for carrot seed germination as it reduced germination percentage and vigor value. The critical temperature (GT_{50}) for carrot seed germination was 5°C. The salicylates such as, DHBA, ASA and SA hastened germination at 5°C. Among these compounds, ASA at 100 mg l⁻¹ was the most effective in hastening germination at 5°C compound to DHBA or SA. Germination hastening by ASA at low temperature may perhaps be due to its thermogenic activity triggering alternate oxidase genes, increasing energy for germination.

ACKNOWLEDGEMENT

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