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Research Note

Stratification, scarification and gibberellic acid treatments of garden angelica (*Angelica arcangelica*) seeds

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Abstract

A study was made to examine methods to break seed dormancy in long-term stored seed lots of garden angelica (*Angelica arcangelica*), including cold stratification at 4°C for 60 or 85 days, scarification and gibberellic acid treatments. The results showed that stratification was needed but was not enough alone as 60 days gave almost no germination and 85 days 10%. Addition of gibberellic acid improved germination to up to 27% for the seeds stratified for 60 days and 41% for seeds stratified for 85 days, but with large variation among seed samples. Scarification + stratification gave a somewhat similar result, up to 12 and 22% germination, while the combined stratification + scarification + gibberellic acid treatment resulted in more than 60% germination. The results are useful for genebank managers and others involved in conservation and cultivation of garden angelica.

Keywords: *Angelica arcangelica*, garden angelica, gibberellic acid, scarification, stratification

Experimental and discussion

Plants have developed many survival strategies and seed dormancy is about spreading the time when seeds germinate (Galston, 1994). Inhibitors or physical barriers cause seed dormancy (Baskin and Baskin, 1998). Garden angelica (*Angelica arcangelica* L.), hereafter termed angelica, is adapted to cool and humid climates and is found in the Nordic countries, especially in the Arctic but also on high altitudes in Central Europe and Asia (Lid, 2005; Harkestad, 2017). The Vikings practiced cultivation of angelica, however, such cultivation more or less disappeared in the 18th century (Høeg, 1974; Dragland, 2000). Historically, the whole plant was used: seeds for flavouring drinks, leaves as

vegetables and young stems as candies. Today, angelica sees new interest, for example as part of the New Nordic Cuisine (Brandrud and Clausen, 2011), where chefs develop new recipes based on traditional plants or ingredients. For conservation purposes, there is also an interest and many museum gardens and botanical gardens maintain populations of angelica. The Nordic genebank (NordGen) has 38 accessions of angelica accepted for long-term conservation as part of their mission to conserve agricultural biodiversity (SESTO, 2019).

In the current study, we looked into dormancy breaking treatments of long-term stored angelica seeds maintained in genebanks. Newly harvested seeds are more able to germinate than one-year-stored seeds (Vashistha *et al*., 2009). Ojala (1985) found that a cold pre-treatment of seeds for six weeks in moist sand (cold stratification) improved germination, but the effect varied from genotype to genotype. That study also found that seeds kept in the dark did not germinate, whereas light promoted germination. Royal Botanic Gardens Kew (2019) report good results using agar with 8 hours light/16 hours dark and 25/10°C or with 12 hours light/12 hours dark and 21/11°C. They kept counting for 45 days but used no stratification. In practice, many experience that angelica seeds have serious dormancy, especially after some time in storage. Laufer (1984) showed that nearly all angelica seeds germinated after a very long stratification period (up to 1.5 years). Vashistha *et al*. (2009) demonstrated some effect of adding different chemicals and of scarification, but gibberellin (GA_3) was not included in that study. Ojala (1985) found no positive effect of adding GA_3 on angelica seed germination although it is known to stimulate germination for various plants (Stokes, 1965). The aim of our study was thus to develop germination protocols for stored angelica seeds, which we could use for viability monitoring of genebank accessions or could be used by others interested in cultivating angelica.

We hypothesised that a combined treatment would be useful. Thus, we run a multiple factor trial combining cold stratification, scarification and gibberellic acid (GA_3) application to various angelica seed samples (accessions). The accessions were obtained from NordGen, from a seed company (Impecta Seeds, Sweden = IMP) and from a local museum garden (Domkirkeodden, Hamar, Norway = DKO). The accessions were all from wild populations or with unknown origin. Prior to the start of the experiment, all NordGen seeds had been dried to 5-7% moisture content and had been packed in sealed bags and stored at −18°C. The samples used were of accession NGB20092 (collected in Iceland in 2008), NGB20093 (Iceland, 2008) and NGB24813 (Sweden, 2013). The IMP sample and the DKO sample were dried and kept under ambient conditions before the experiments started. No viability assessment was done prior to the experiment. Three replicates of 20 seeds per sample were stratified and/or scarified, put on filter paper (type 1731, Munktell, Falun, Sweden) in a 90 mm-diameter Petri dish. GA_3 (0.75 g L^{-1} water) was added to each Petri dish in the form of droplets at the beginning of the stratification period and approximately once a week during the stratification period and the germination test. Seeds were placed at 4°C (± 1°C) for 60 days with 12 hours light/12 hours dark for stratification or controls with no stratification (Experiment 1). We used four seed samples in this experiment. Scarification was done by removing the fruit covering structures by hand. Germination was examined by placing the Petri dishes on shelves under room

temperature, 22-23°C (± 2°C) with 12 hours light/12 hours dark. To minimise random errors, we rotated the Petri dishes during the trial. Germinated seeds were counted every third day as either geminated or not germinated, and to avoid double counting, seeds were removed when counted as germinated. We kept on counting germination for up to 60 days. Statistical analysis was carried out in R (R Core Team, 2019), using ANOVA and tree regression analysis. We analysed the counted numbers not the percentages and there was no need for transformation of the data. By ANOVA the main effects and all two ways interactions of the factors: accession, stratification, GA_3 and scarification were analysed. Several of the interactions were highly significant. Therefore, we combined the two-way interactions into new single factors for further analysis. Tree models are nonparametric, have no underlying assumptions and the method analyses both main effects and interactions simultaneously. We used a classification tree regression model to fit by binary recursive partitioning, whereby the data were successively split along coordinate axes of the explanatory variables so that, at any node, the split maximally distinguishes the response variable on the selected left and right branches. Each explanatory variable was assessed in turn, and the variable explaining the greatest amount of deviance in the response variable selected

The results showed that 60 days of stratification alone was not enough to achieve germination (figure 1). Stratification + GA_3 or stratification + scarification significantly improved the germination ($P < 0.001$ for both). The tree regression showed that accession was the most important factor: seed lot DKO responded differently from the other samples. The next split was based on stratification treatment that showed to be a more important factor than both GA_3 and scarification in explaining the variance. Without stratification, only one single seed from one of the seed samples germinated. DKO was freshly harvested seeds and this may explain the better germination result in this sample compared with the stored samples.

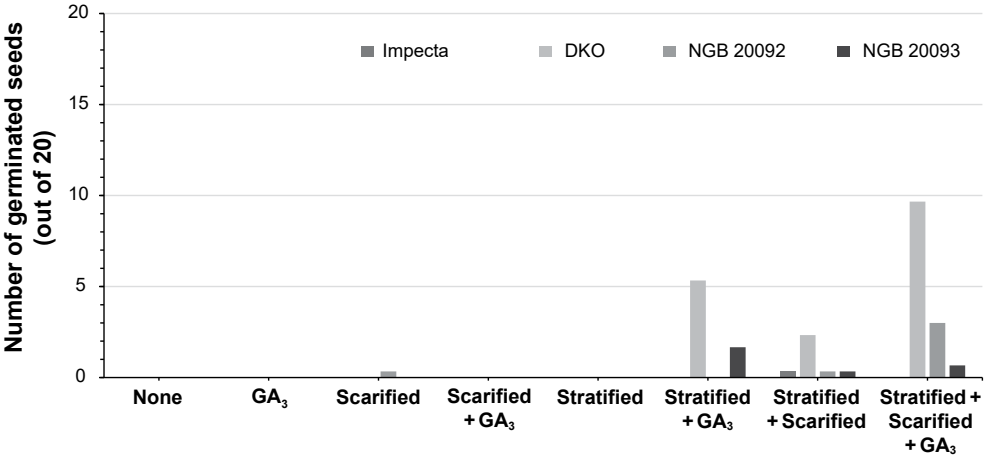

Figure 1. Germination of garden angelica (*Angelica arcangelica*) seeds with no treatment ("None"), gibberellic acid treatment (GA_3, 0.75 g L^{-1}), scarification (removal of fruit coat), 60 days stratification (cool and wet) and combinations of these treatments (Experiment 1). The results shown are the mean of three replicates of 20 seeds sown for each treatment combination and seed lot.

An additional trial (Experiment 2), with three replicates of 10 seeds per sample, was made with three long-term stored seed samples from NordGen. Now, we extended the stratification period to 85 days and had no controls with no-stratification (figure 2). Water and water + GA_3 was given as 4 ml per Petri dish at the beginning of the cold stratification period. This experiment gave an overall higher germination than experiment 1. The highest numbers were found with the combined treatment: stratification + scarification + GA_3 ($P < 0.001$). Germination exceeded 60% in two of the three seed samples and there was no significant difference between accessions.

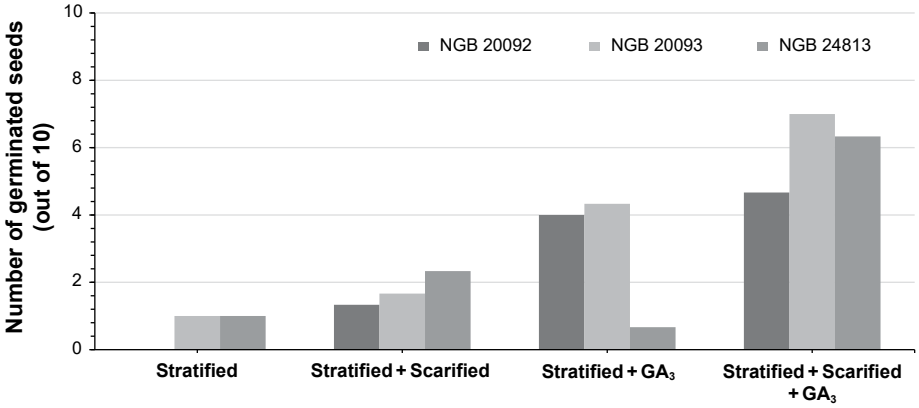

Figure 2. Germination of garden angelica (*Angelica arcangelica*) seeds after 85 days of stratification and with additional scarification treatment, gibberellic acid treatment (GA_3) and combinations of these (Experiment 2). The results shown are the mean of three replicates of 10 seeds sown for each treatment combination and seed lot.

To summarise, we have shown the importance of targeting both physical and physiological dormancy mechanisms in stored angelica seeds. By combining the treatments, we have shown that it is possible to shorten the stratification period from 1.5 years (Laufer, 1984) to two to three months, and the combined treatment has reduced the variation often found (Ojala, 1985; Vashistha *et al*., 2009). Although being fully aware of the low number of seeds applied in each germination test (much lower than the ISTA recommendation of 4 × 100), our research adds insight into the requirements for germination of stored angelica seeds. Such information is valuable for seed laboratories, farmers and conservationists and should be verified by additional trials using other genotypes and proper amounts of seeds.

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