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Pre-chill with gibberellic acid overcomes seed dormancy of Jerusalem artichoke

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Keywords
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Comments
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PRE-CHILL WITH GIBBERELLIC ACID OVERCOMES SEED DORMANCY IN JERUSALEM ARTICHOKE

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Abstract

Jerusalem artichoke is a valuable source of inulin, a polysaccharide. Inulin is a sustainable source of dietary fiber that enhances the immune system in humans. Although Jerusalem artichoke can be propagated vegetatively, breeders use cross-fertilization to produce novel varieties and hybrids with higher inulin yields. Seed dormancy can hinder the breeding progress because dormancy reduces the number of generations a breeder can obtain in one year. Current methods for breaking seed dormancy are time-consuming, and usually involve removing or pin-pricking the seed coat of these very small seeds, or using several weeks of seed vernalization or several months of storage. Breeders thus require better methods that are less time-consuming and achieve higher germination percentages. Here we studied germination, dormant seed, dead seed and the seedling growth rate of different Jerusalem artichoke genotypes and seed lots after seeds were treated with different methods to break seed dormancy. We evaluated fresh and stored seed lots using the following treatments: seed samples were planted on top of media moistened with either potassium nitrate, gibberellic acid or distilled water as control. Two identical sets of samples were planted: one set was germinated in a chamber at alternate 15−25 °C for 28 days, while the second set was pre-chilled at 5 °C for 14 days, before moving the samples to the alternate 15−25 °C chamber for 14 days. Our findings reveal that the highest germination percentage up to 85.3% was obtained when applying pre-chill with gibberellic acid. This novel dormancy-breaking treatment was thus effective in promoting fresh seed germination.

Keywords breaking dormancy · germination · scarify · stratification · seed vigor

1 Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is an herbaceous perennial plant native to North America. The genus Helianthus is in the Asteraceae family and comprises about 50 species (Kays and Nottingham 2008). Jerusalem artichoke has been cultivated widely across

the temperate and tropical climate zones. Jerusalem artichoke is a multipurpose crop that can be used to produce health food and products, animal feed additives and biofuel (Kerckhoffs and Renquist 2013). More recently, the crop was introduced into Thailand for commercial production as a functional food. Jerusalem artichoke tubers contain inulin, a non-digestible oligosaccharide that is beneficial to human health (Lingyun et al. 2007). The most important attributes of inulin are its nutritional caloric value and its water-soluble dietary fiber content (Stoop et al. 2007). Inulin is not metabolized by humans and animals and can act as a prebiotic, a non-digestible food ingredient that can be selectively fermented by beneficial organisms such as *Bifidobacteria* but limits growth of pathogenic or non-beneficial organisms such as *Escherichia coli* (Gibson and Roberfroid1995).

The most important production problems of Jerusalem artichoke under topical climate are low tuber yield, low inulin content and plant diseases caused by *Sclerotium rolfsii* and drought. Improvement of yield, inulin content, disease resistance and drought tolerance can be achieved through breeding. Hybridization of parents followed by selection of superior genotypes in segregating populations is a conventional breeding method in sexually-propagated crops and clonally-propagated crops such as sugarcane (Caieiro et al. 2010), cassava (Mezzalira et al. 2013) and Jerusalem artichoke. For Jerusalem artichoke, seed dormancy is a problem for development of segregating population for selection (Kays and Nottingham 2008). Although seed dormancy can be overcome perfectly through cold storage for three months (Lim and Lee 1989; 1990), this method is time consuming and can hinder breeding progress. In the tropics, Jerusalem artichoke can grow all year, and the crop can produce seeds three times per years. If the seeds are not dormant, hybridization and selection using fresh seeds can be carried out more than 2 times per year. Jerusalem artichoke also has poor seed set and, if seeds do not germinate due to dormancy, it is difficult to obtain large breeding populations for effective selection. In temperate environment, the progress of breeding programs will be slow because Jerusalem artichoke seeds require a period of storage to germinate. If the obstacle of seed dormancy is removed, multiple generations a year and selection of superior genotypes can be achieved by the use of greenhouse nurseries. The greatest benefit from this study is that these dormancy-breaking techniques could accelerate the breeding progress. Therefore, knowledge of seed dormancy in fresh and stored seed and breaking seed dormancy are important for Jerusalem artichoke breeding.

Seed dormancy is defined as the lack of germination in a specified period of time under otherwise favorable environmental conditions (Baskin and Baskin 2004) and the germination of dormant seeds is slow and not uniform (Majidi and Barati 2011). Many seed treatments that can promote germination have been reported in many crop species. Cold-moist stratification or pre-chill was successfully used in black mulberry to imitate overwintering in the field and provide the necessary stimulus required to overcome dormancy, increase

germination, and produce normal seedlings (Koyuncu 2005). Application of potassium nitrate (KNO_3) promotes germination and releases seed dormancy in sunflower (Maiti et al. 2006) and Ramonda serbica and R. nathaliae (Gashi et al. 2012). Gibberellic acid (GA_3) is a naturally occurring hormone associated with seed germination induction (Copeland and McDonald 2001) and plays an important role in the regulation of seed germination (Sarihan et al. 2005). GA_3 has been shown to increase seed germination of sunflower (Seiler 2010) and Gentiana rigescens (Zhang et al. 2012). Moreover, the stratification and chemical combination provides an attractive approach for breaking seed dormancy. Applying a solution of 500 ppm of GA_3 and 0.1% KNO_3 after stratification resulted in high germination of dormant water-lily tulip seeds (Rouhi et al. 2010).

Seed dormancy has hampered breeding efforts by limiting Jerusalem artichoke seed-propagation and multiplication (Lim and Lee 1989). Dormancy-breaking seed treatments are scantily available in the literature for Jerusalem artichoke. In previous investigations, germination of 60 to 70% could be obtained from storing Jerusalem artichoke seeds for 4 weeks at 2 °C followed by treating the seeds with 0.2% KNO_3 for one week at 10 °C (Mesken 1988). However, germination percentages were still low. Intact Jerusalem artichoke dormant seed subjected to low temperature stratification for 70 days germinated over 85% (Lim and Lee 1989) but this treatment requires a long time. The methods of removing and pin-pricking seed coat were very effective in breaking seed dormancy of Jerusalem artichoke, and the germination was 70 to 100% for seeds stored at room temperature for three months (Lim and Lee 1989; 1990). However, the long storage period hinders breeding progress because it is slow and the removal of seed coat is not practical for breeding programs which handle a large amount of hybrid seeds. Jerusalem artichoke seed is approximately 10-times smaller than sunflower which hinders seed coat removal. The average seed size in Jerusalem artichoke is 4.5 mg $seed^{-1}$ (Swanton et al., 1992) and in sunflower is 48 mg $seed^{-1}$(Alexander et al., 2001). Moreover, removal of the seed coat can cause high levels of disease infection. Cold stratification (pre-chill) and chemical treatments are very easy to apply and are time saving methods (Figure 1).

The objective of this study was to find a method to break seed dormancy in Jerusalem artichoke and reduce the number of dormant seed in a seed lot. This information will be useful for breeders and seed scientists interested in obtaining high seed germination of freshly harvested Jerusalem artichoke.

2 Materials and methods

The experiment was carried out in July 2012-March 2013 at the Seed Science Laboratory, Iowa State University, Ames, IA, USA. Jerusalem artichoke seed lots were kindly provided

by the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS), Ames, IA, USA (Table 1). The seed lots were chosen to represent seed lots of different age (freshly harvested or "fresh", and stored for different length of time or "stored"). The experiment consisted of 2 trials: trail 1 included 2 seed lots of fresh seed and 2 seed lots of stored seed; and trial 2 included 3 seed lots of fresh seed and 2 seed lots of stored seed (Table 1). These seed lots were either produced by open-pollination or inside cages to avoid cross-pollination. The stored seed lots were stored in a controlled temperature and humidity room at 4 °C and 25% relative humidity for 10 months for trial 1 and for 1 to 8 years for trial 2, while the fresh seed lot was used immediately for trial 1 and stored for two months under ambient conditions for trial 2. The seeds were not threshed from their bracts.

2.1 Trial 1: Assessing breaking dormancy treatment for promoting germination

Six seed treatments conditions were used (Table 2). The trial design was a randomized complete block design (RCBD) with 2 blocks or replications. Treatments and genotypes were analyzed as 2×6 factorial trial and the trial unit consisted of 40 seeds.

Forty Jerusalem artichoke seeds were arranged on the top of two 60 cm×30 cm paper towels (Anchor Paper, St. Paul, MN). Towels were moistened with either water, KNO_3 or GA_3 (Table 2). Then the seeds were covered with an additional paper towel, and the towels were then rolled loosely into a cylinder. The amount of solution or water in the towels was approximately 2.58 g of water per g of towel.

Each roll of paper towels was placed inside a plastic bag to prevent excessive water loss and placed vertically inside a plastic cup. The plastic cups were placed inside environmentally controlled germinators under different temperature treatments (Table 2). Two different germinators (blocks) were used for this trial. Samples that required a pre-chill were placed inside a 5 °C chamber (HOBART) with no light prior to moving the samples to the germinators (HOFFMAN MANUFACYURING INC., International Agri-Supply, ALBANY, OREGON) at alternating 15−25 °C (15 °C and no light for 16 h, 25 °C and light for 8 h). The number of normal seedlings (AOSA 2012) was counted every 7 days. Data for final germination percentage, percentage of dormant seeds and percentage of dead seeds were recorded at 28 days.

Tetrazolium test

After final evaluation of seed germination (28 days), non-germinated seeds were tested for viability based on the tetrazolium test methodology for wild sunflower achenes (AOSA 2002;

Seiler 2010). The non-germinated achenes were cut at the distal end of the cotyledon region, the seed was removed from the achene and the seed coat or thin membrane was removed before seeds were placed in watch glasses containing a 1.0% 2, 3, 5-triphenyl tetrazolium chloride solution and incubated at 35 °C for 2h. The seeds were removed from tetrazolium solution, rinsed 2 or 3 times in water, and then evaluated according to the staining pattern. Seeds that stained uniformly red were considered viable. Percentages of dormant seeds and dead seeds were then calculated.

Statistical analysis

Data from fresh and stored seed lots were statistically analyzed separately. Analysis of variance for germination percentage, percentage of dormant seeds and percentage of dead seeds was performed according to a factorial trial in a randomized complete block design (RCBD) with two blocks (two chambers). Normality of the data for germination percentage, percentage of dormant seeds and percentage of dead seeds were tested by Kolmogorov-Smirnov test using SPSS 16.0 computer package (SPSS 2007). All data were distributed normally. The data were also tested for variance heterogeneity (Gomez and Gomez 1984). The variances within each dependent variable were homogeneous. Error variances of stored seeds were three-times larger than those of fresh seeds, so data sets were analyzed separately. When the genotypes × treatments interactions were significant, data were reanalyzed by genotype. Least significant difference (LSD) was used to compare mean differences. All calculations were done using computer software STATISTIX8 software program (Statistix8 2003). The seed germination percentage was presented as mean ± standard errors (SE) of the mean.

2.2 Trial 2: Assessing the effect of pre-chill with or without the use of chemicals

Trial 2 included stored seed lots from the same genotypes (Ames 22229 and Ames 22228) as trial 1 but from different seed lots. These seed lots were a bulk of seeds produced in 2005 and 2009 to 2011 (Table 1). The fresh seed included one seed lot from trial 1 (Beaula's) and 2 fresh seed lot (Ames 22227 and Ames 22228) from 2012 production (Table 1).

The treatments included in trial 2 were chosen among those from trial 1 where seed germination was highest (treatment 4−6) (Table 2). Three treatments from trial 1 were included in trial 2 (Table 2). The experimental design was an RCBD with 2 blocks and treatments and genotypes were arranged in a 2×3 factorial (stored seed) or 3×3 factorial (fresh seed). Data from stored and fresh seed were analyzed separately. The trial unit for each seed lot had 40 seeds.

Number of normal seedling (AOSA 2012), number of dormant and dead seed, were counted weekly for up to 35 days after samples were placed in the germinator. At 28 days after samples were placed in the germinator, the non-germinated seeds were scarified by pin-pricking of seed coat at the distal end of the cotyledon. Scarified samples were returned to the germinator and the number of normal seedling was evaluated again at 35 days. Seedling growth rate was evaluated in this trial only.

Seedling growth rate test for seed vigor evaluation

Seedling growth rate was evaluated by calculating the weight in mg per normal seedling. At each weekly count, the cotyledons of normal seedlings from the germination test were removed and the shoots and roots were separated by simply cutting at the juncture of the hypocotyl and the radicle. Shoots and roots were dried separately inside coin envelopes at 80 °C for 24 h and then weighed. The total dry weight of the normal seedling was divided by the total number of normal seedlings to obtain the seedling growth rate (SGR) in mg per seedling (AOSA 2009).

Statistical analysis

The data for all trials were based on evaluation at 28 days. After evaluation at 28 days, the non-germinated seeds were pricked and were re-evaluated at 35 days, but the results were not significantly different from 28 days. Therefore, the data at 28 days were reported. Statistical analyses for the data from fresh and stored seed lots at 28 days were carried out separately. Analysis procedures for all characters were those mentioned in the trial 1, and seedling growth rate was included in this trial.

3 Results and discussion

Jerusalem artichoke breeding is hindered by seed dormancy. This dormancy is lost over time through a natural process called seed after-ripening (de Casas et al. 2012). However, breeders do not have the time necessary for this natural process to occur. To advance selection of improved germplasm breeders need good seed germination (Qu and Widrlechner 2012). This study evaluated known seed germination enhancing methods used in seed analysis to improve seed germination and break dormancy of fresh and stored Jerusalem artichoke seed.

3.1 Trial 1: Assessing breaking dormancy treatment for promoting germination

The germination percentage and percentage of dormant seeds of stored seed lots were significantly different among genotypes and seed treatments (Table 3). However, differences among genotypes and seed treatments were not significant for percentage of dead seeds. The interactions between genotypes and seed treatments were significant for germination percentage and percentage of dormant seeds but not significant for percentage of dead seeds. In fresh seed lots, significant differences were observed among seed treatments for germination percentage only. The differences among genotypes and the interactions between genotypes and seed treatments were not significant for germination percentage, percentage of dormant seeds and percentage of dead seeds.

In stored seed lots, the interaction between genotypes and seed treatments was significant (Table 3). Consequently, data were reanalyzed by genotypes. The germination percentages in genotype Ames 22229 ranged from 37.5 to 97.5%, and the differences among treatments were significant (Figure 2a). Pre-chill with KNO_3 and pre-chill with GA_3 had the highest germination percentages (93.8 and 97.5%, respectively) which were higher than the control (37.5%) and KNO_3 (55.0%), but these treatments were not statistically different from GA_3 (70.0%) and pre-chill (81.3%). Pre-chill was significantly different from control but it was not statistically different from KNO_3 and GA_3. Germination percentages of Ames 22228 were not significantly different at $p \leq 0.05$; and the germination percentage ranged from 83.8 to 91.3%. Previous findings in Jerusalem artichoke showed that seed coat removal and seed coat pin-pricking promoted germination percentage of dormant seed from 0 to 100% but did not significantly promote seed germination if the initial germination percentage was higher than 88.4% (Lim and Lee 1990). However, the removal of seed coat is laborious and not practical for breeding programs which handle large amounts of hybrid seed. Our results showed that pre-chill in combination with growth promoting chemicals can provide a good alternative for removing seed dormancy in Jerusalem artichoke.

The percentage of dormant seeds in control treatments of Ames 22229 was high (51.3%) but low for Ames 22228 (10.0%) (Figure 1b), even though both seed lots were produced in 2011 (Table 1). The use of pre-chill with KNO_3 and pre-chill with GA_3 significantly decreased the percentage of dormant seed in Ames 22229 compared to the control. None of the treatments significantly decreased the number of dormant seeds in genotype Ames 22228. The percentage of dead seeds was also not significantly different for both genotypes, ranging from 2.5 to 13.8% (Figure 2c).

In fresh seed lots, the interaction between the genotypes and seed treatments was not significant (Table 3). The germination percentage was highest for seed lots treated with pre-chill with GA_3 (62.5%) (Figure 2a). The germination percentage of all treatments ranged from 10.6 to 62.5%, and $KNO_3$was the lowest (10.6%) but not different from the control (17.5%). The percentages of dormant and dead seeds were not significantly different among treatments.

Jerusalem artichoke dormant seeds incubated for 70 days at low temperature and wet conditions (3.5 °C on wet cotton wool) germinated more than 85% (Lim and Lee 1989). However, the incubation time is long. Our study showed that this time can be shortened by the addition of pre-chill. The effect of pre-chill and chemical treatments on seed germination was greater in fresh (freshly harvested) seed lots than in stored seed lots, depending on their degree of dormancy. In some cases, the percentage of dormant seeds decreased from over 50% to close to 0% (Figure 2b). We expected to find greater levels of seed dormancy in fresh seed lots, since seed dormancy is naturally lost in storage through a physiological process called "after ripening" (Copeland and McDonald 2001).

Dormancy of Jerusalem artichoke seeds is affected by the seed coat and cotyledons that restrict water and oxygen absorption (Lim and Lee 1990). Our study also showed that the effects of pre-chill and chemical treatments combined enhanced seed germination of Jerusalem artichoke. In some case, this germination percentage was higher than the germination percentage of either pre-chill or chemical treatment alone (Figure 2a). Pre-chill facilitates diffusion of oxygen through the seed coat into the seeds, breaking seed dormancy (Balouchi and Sanavy 2006). A pre-chill applied at the appropriate time and for the proper duration did promote seed germination (Nkomo and Kambizi 2009). Other authors have reported that GA_3 treatment alone did not enhance seed germination in all species (Rouhi et al. 2010). Gibberellins increase cell wall plasticity and promote the hydrolysis of starch to sugar which reduces the water potential inside the cell; this result in water diffusion into the cell which causes cell elongation. There is evidence that natural gibberellins-like substances appear during successive stages of after-ripening and germination (Sarihan et al. 2005). Seed treatments of 1 mM GA_3 for one hour increased germination of wild H. annuus achenes from 13 to 88%, and H. petiolaris from 1.5 to 85% (Seiler 2010), but seed treatments of 500 ppm GA_3 was not significantly different from water-treated control in Heliopsis helianthoides (L.) Sweet (Zlesak 2007).The use of KNO_3 has been an important seed treatment in seed-testing laboratories for many years without a good explanation for its mechanism of action (Çetinbaş and Koyuncu 2006).

A combination of pre-chill and GA_3 could be used to reduce the stratification time in many species (Pipinis et al. 2012) and was used to improve seed germination percentage in black mulberry seeds (Koyuncu 2005) and Carpinus betulus and C. orientalis (Pipinis et al. 2012). Our study showed that this dormancy-breaking treatment also was effective in promoting seed germination in Jerusalem artichoke.

Trial 2: Assessing the effect of pre-chill with or without the use of chemical

Similar to trial 1, the results from stored seed lots and fresh seed lots were evaluated separately. The number of treatments was reduced from six to three treatments which all included pre-chill; and seedling growth rate also was evaluated. In stored Jerusalem artichoke seed lots, genotypes and seed treatments and their interactions were not significantly different for germination percentage, percentage of dormant seeds, percentage of dead seeds and seedling growth rate (Table 3 and Figure 3).

In fresh Jerusalem artichoke seed lots, genotypes were significantly different for germination percentages, percentage of dead seeds and seedling growth rate but not significantly different for percentage of dormant seeds (Table 3). Treatments were significantly different for germination percentages and seedling growth rate but not significantly different for percentage of dormant and dead seed. The highest germination percentage was recorded for seeds treated with pre-chill and GA_3 (85.3%) (Figure 3a). Dormancy in Jerusalem artichoke seeds is very deep and it takes several months under ambient conditions to break dormancy (Kays and Nottingham 2008). Jerusalem artichoke is characterized by the type of dormancy known as 'physiological dormancy' and non-deep sub-type, and cold-stratification or GA3 may promote germination (Baskin and Baskin 2004). Lim and Lee (1989) showed that freshly harvested seeds stored for 3 months at room temperature and treated by temperature, light and GA_3 had almost 0% germination, while seeds stored for 27 months at room temperature had germination percentage of 47.5%. Mesken (1988) improved the germination of freshly harvested Jerusalem artichoke seeds using cold stratification with a 0.2% solution of KNO_3. However, germination percentages obtained in the previous studies were lower than in our study (62.5 to 96.5%). Seed treated with pre-chill with KNO_3 had the highest seedling growth rate (1.34 mg per seedling). Our study showed that the combinations of pre-chill and KNO_3 had the highest seedling growth rate for fresh seed lot, but the differences were not significant for stored seed lots. Seedling growth rate is a measure of seed vigor (AOSA 2009). Heavier seedlings have greater dry matter accumulation and likely will have better chances of emergence and survival in the field. Seed vigor can be utilized to predict successful field establishment under different environments (Grey et al. 2011). Previous research also confirmed that KNO_3 promoted seed vigor in several species. Jerusalem artichoke seedlings that derived from seeds typically are less vigorous in contrast to other Helianthus species in which seeds are used in commercial production (Kay and Nottingham 2008). Mamidi and Pirasteh-Anosheh (2013) revealed that KNO_3 had a greater increasing effect on radicle and shoot length and levels of seedling growth in sunflower. To our knowledge, this is the first report that KNO_3 also improves seedling vigor in Jerusalem artichoke.

4 Conclusions

An effective and practical method in breaking Jerusalem artichoke seed dormancy and to obtain greater seed germination percentage is highly desirable for Jerusalem artichoke breeding programs. Our results showed that a combination of pre-chill at 5 °C for 14 days and followed by germination at 15−25 °C for 14 days with 500 ppm GA_3 was the best method for promoting germination of fresh seed lots. However, this dormancy breaking treatment was ineffective in stored seed lots probably due to low dormancy levels in the seed. Future research should explore the use of these seed dormancy-breaking treatments in Jerusalem artichoke prior to planting the crop in the field.

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