The Role of Seed Analysis in Genetic Conservation[1]

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Abstract

Long term storage of seeds at freezing temperatures is one strategy for genetic conservation of tree species. It can be used to preserve species that produce seeds that remain viable after drying to a low seed moisture content. The U.S. Department of Agriculture Forest Service (USDA FS) National Seed Laboratory (NSL) began long term seed storage for genetic conservation in 2005. The program is mostly focused on five-needle pines (*Pinus*) and ash (*Fraxinus*) species. The genetic resources of both groups of species are eroding rapidly in the wild because of attack from invasive exotic pests for which there are no effective control measures. Seeds on the other hand, once placed in a freezer, are quite safe and require comparatively little maintenance. Part of the maintenance required for long term stored seed is to know the seeds are viable and sufficiently dry going into storage and that they remain alive and dry while in storage. To gain this information it is necessary to use seed analysis. The various seed analysis methods used at the National Seed Laboratory are described.

Introduction

Long term seed storage is a major tool for preserving the genetics of crop plants and perhaps the most famously reported example of this activity is the Svalbard Global Seed Vault in Norway for which the reader can find many references on the internet. But underpinning this magical arctic hideaway tunneled into a remote frozen mountain, and other collections of seeds held long term around the world, is seed analysis and seed technology. Before seeds are placed into storage they must be tested for viability and moisture. Viability must be assured because only living seeds are of any value in preserving the genetics, and moisture is tested because only seeds that can be and are dried to low moisture status can be kept long term in the freezer. These tests are conducted as the seed is first entered into the collection and then periodically after a specified number of years in storage. If viability is found to be declining then some seeds are planted to produce a fresh batch of seeds to replace the one that is declining in viability. Herbaceous plants produce seeds in 1 or 2 years generally, but trees typically have a comparatively long period of maturation before producing seeds. Because trees can take a number of years to mature and produce seeds and require large parcels of land to reach maturity, the practice of long term seed storage for trees was not practiced very extensively.

However, in 2005 the Chief of the U.S. Department of Agriculture Forest Service (USDA FS) expanded the role of the National Seed Laboratory (NSL) to include long term seed storage for genetic conservation. This was primarily in response to the loss of five-needle pines (*Pinus*) and ash (*Fraxinus*) species. Both groups of species are disappearing in nature because of attack from invasive exotic pests for which effective affordable control measures do not exist. Long term seed storage was an available cost effective method to preserve the genetic resources for both groups. This paper is a general overview of the testing conducted on seeds stored for genetic conservation and how those tests are used at the NSL. Extensive detail on seed testing can be obtained from the rules and handbooks published by the Association of Official Seed Analysts (www.aosaseed.com) and the International Seed Testing Association (ISTA, www.seedtest.org).

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The Testing Methods

Testing Seed Moisture

Seed moisture is the most important factor in preserving the viability of stored seeds (Justice and Bass 1978). Therefore, before a seed is placed in freezer storage, it is dried in air with a stable relative humidity in the range of 25 percent to 30 percent. Once the seed has reached moisture equilibrium with the air around it, it is ready to seal in a moisture proof container to maintain its dry condition. To be certain the seeds are dry, a seed moisture test is required. This moisture test can be conducted using the destructive method of oven drying at 103 ºC or the nondestructive equilibrium relative humidity (eRH) test (Baldet et al. 2009, Gold and Manger 2014, Karrfalt 2014). Because most of the seed lots in the NSL collection are irreplaceable and generally expensive to produce, the nondestructive eRH test is used to preserve as many seeds as possible. As an example, the cost of putting an ash sample into the collection is about $250. These samples on average weigh about 113.4 g (4 ounces). Therefore, the per-pound cost is $1,000, substantially more than commercially produced tree seed. The cost of the pine would be even higher as these trees are in more remote mountainous areas requiring more effort to visit, while the ash are generally reached by vehicle along well traveled roadways. An eRH test is done by placing the seeds in a sealed container along with the probe of a hygrometer (fig. 1). The air inside the container will equilibrate to the moisture status of the seeds. When the relative humidity in the container is in the range of 25 percent to 30 percent we know that the seeds are sufficiently dry and ready for storage. It is critically important that the seeds be at equilibrium internally. That means that inner and outer layers are at the same water potential. Otherwise the eRH test will be representative of the outer layers of the seed only and give a false reading. Sealing the seeds in the test container 16 to 24 hours before the test aids in ensuring the seeds have had time to reach equilibrium. The hygrometer probe does not need to be attached during this equilibration period. Once the probe is attached to the test container, the eRH can be taken after about 10 minutes. The reliability of this moisture testing method is seen in that samples tested with this method have lost no viability after 10 years of storage.

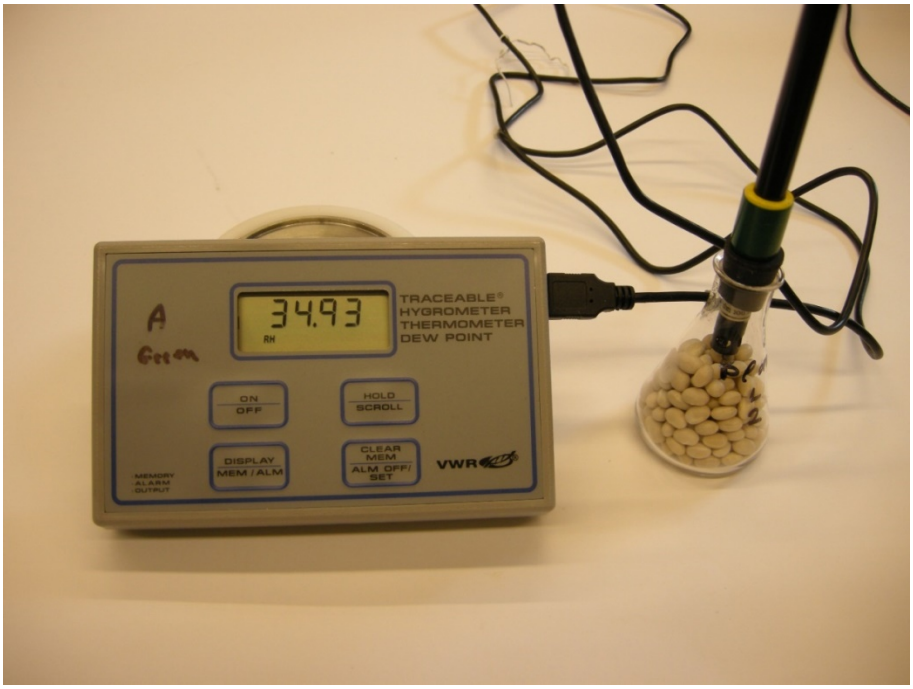

Figure 1—Testing the equilibrium relative humidity of seeds with a hygrometer.

Testing Viability

Storing dry seeds in a freezer is a good conservation strategy only if the seeds are alive. This means that seed viability must be tested before placing seeds into storage and periodically while they are in storage. Without this viability information, there is no way to know how many new plants can be produced from the seeds or if the seeds need to be replenished with a new collection.

Germination Testing

Germination is most simply defined as the emergence from the seed of a normal plant. A standard germination test for commercial purposes consumes 400 seeds, but that number of seeds is reduced for genetic conservation in order to minimize the number of seeds used for testing. This extends the time before the lot is reduced to the point where it needs to be replenished. 200 seeds or as few as 10 can be tested depending on lot size and the precision of estimate required. Germination is the gold standard for testing seeds as we actually see the full seedling produced.

Sometimes seeds do not germinate when placed in conditions favorable for germination. This result is usually referred to as dormancy. Various treatments are used to overcome dormancy, but moist chilling is most often used on trees. Some dormancy is easily overcome and only 30 to 60 days of chilling are needed to make most of the good seeds germinate. When dormancy is harder to overcome, then quick viability tests are often substituted for germination. These quick tests are described in the following sections. Even sometimes when the protocol to overcome a complex dormancy is known, a quick test is used in order to more rapidly complete the processing of seeds into the collection.

Excised Embryo (EE) and Tetrazolium (Tz) Tests

Excised embryo and tetrazolium tests along with x-ray are referred to as quick viability tests. In this way they are distinguished from germination and thought of as an estimate of what the germination value might be. For an excised embryo test the embryo is removed from the seed and placed in a controlled environment chamber where optimal temperatures, light levels, and moisture are provided (fig. 2.). Separating the embryo from the rest of the seed permits the embryo to initiate growth. Those embryos that initiate growth are said to have germinated. Seeds that contained embryos that germinate or remain sound in the excision tests are considered viable.

Figure 2—An excised embryo test of *Fraxinus* embryos. Greening and spreading of the cotyledons indicates these embryos come from live seeds.

The tetrazolium test uses a vital stain, tetrazolium chloride salt, which will stain living tissues a light pink and any dead seed tissue will not stain (fig. 3). The seeds are first imbibed with water, usually cut open slightly and then placed in a solution of the Tz salt. The whole test takes approximately 2 to 3 days. The EE test takes 7 to 10 days. The Tz test is evaluated usually under a microscope and each seed must be cut a second time to fully expose the embryo structures and food tissues. The excised embryos of some species do not germinate or do not germinate as well as the Tz test would indicate. The EE test therefore, is not used on those species. Combining germination with Tz is another strategy. Germination is first attempted and then any un-germinated seeds are tested with Tz. The sum of the Tz test and the germination test are added together for a single estimate of viability.

Figure 3—A viable *Viburnum* seed stained pink in a tetrazolium test. Note the embryo at the bottom of the seed.

X-ray Testing

X-ray testing is very fast and nondestructive. It clearly shows how many seeds are full and well-formed and how many are damaged (fig. 4). If seeds are properly handled during collection and cleaning it can be relied upon to give a good estimate of initial viability. However, x-ray has less reliability for seeds that have been stored because losses of viability in stored seeds are usually not visible morphologically. When the seeds are too rare to sacrifice any in a destructive test, initial viability is estimated exclusively with x-ray.

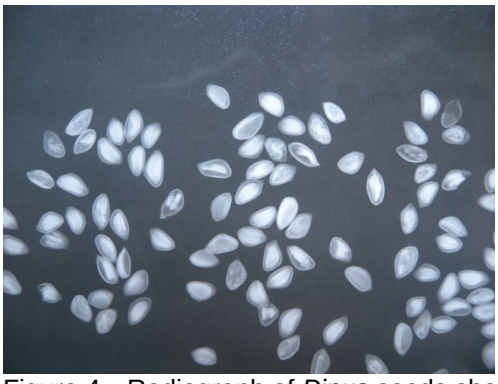

Figure 4—Radiograph of *Pinus* seeds showing many seeds that were likely damaged by insects. Seeds that appear completely dark are classified as empty and seeds with large portions that appear dark are damaged.

Purity

Purity tests indicate how much of a seed lot is pure seed and how much is trash. Genetic conservation seed samples are cleaned as close as possible to 99 or 100 percent pure to reduce the space used in the freezer. Therefore, testing for purity is not normally conducted on genetic conservation samples.

Seed Weight

The seed weight test tells the number of seeds per gram and ultimately the number of seeds in the collection. It is very important to know the number of seeds in a collection because this is part of knowing how many new trees can potentially be produced. In other words, this number says how large the genetic pool is in terms of numbers of individuals. For seed lots of the size used in the tree nursery trade, the International Seed Testing Rules require averaging the weight of eight 100-pure-seed subsamples to estimate the 1000 seed weight or number of seeds per gram. Because the genetic conservation samples are smaller and coming from one female tree they were believed to be more uniform than seed lots in the nursery trade. This potential greater uniformity suggested that possibly fewer seeds could be tested. A comparison between the standard ISTA procedure and a procedure using two subsamples of 100 seeds indicated the two procedures produce the same answer for our conservation seed lots. Therefore, seed weight determinations are made using only two subsamples of 100 seeds rather than the eight subsample ISTA method. Other aspects of the test are kept the same as the ISTA test. Reducing the number of seeds tested speeds up the process and reduces labor costs.

Summary

The seed analysis procedures described in this paper are indispensable to scientifically managing seeds stored for genetic conservation. They are needed to be sure the seeds are at a moisture level such that they remain viable during storage, to be sure the seeds are alive when entering storage, and to monitor seed viability over time.

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