Section 2.8

Sweetpotato sexual seed management

Zósimo Huamán and Humberto Asmat

One of the objectives of a genebank is to maintain the viability of the sexual seed stocks of all the accessions in the collection for a long time. The appropriate management of the sexual seed of the sweetpotato and related wild species includes the elimination of impurities, their disinfection, the seed number determination, their drying for storage, their packaging and storing, and their germinability tests.

Elimination of impurities

The seed stocks received by the genebank usually contain some impurities that must be eliminated. However, besides the remains of the capsules, pieces of inflorescences and other kinds of strange particles, there are also seed mixtures of other species, seed damaged by insects, and empty, deformed, or mummified seed. The

cleaning of small seed lots is usually done by hand. However, for larger seed lots, the use of seed blowers helps to discard these impurities and also the empty seeds.

Separation of viable and non-viable seed by the flotation method

The separation of viable (non-floating) seeds from those that may be immature or non-viable (floating), is easily made by pouring the seed into a glass or plastic container of 200 ml capacity containing a solution of water and any commercial detergent. The solution is then stirred in a circular way producing a whirlpool to submerge all seeds. After some minutes, all floating seeds are discarded by pouring off part of the solution. The seeds that stay at the bottom are then poured off into a plastic sieve and placed on a paper towel to dry.

In spite of the light weight of non-floating seeds, they can germinate in vitro. However, it is recommended that, for the maintenance of plant genetic resources, only the well-shaped and matured seed should be stored.

Seed disinfection

Before seed disinfection, all seeds damaged by insects should be eliminated. They are easily identified by the holes produced at the time the insect exits from the seed.

All seed apparently healthy is treated with an insecticide that has fumigant properties, like Vapona (DDVP) used in cereal seed stores. Vapona tablets, of 30 g approximately, gasify when put in the environment. For disinfecting sweetpotato seeds, these are placed in a container that can be hermetically sealed (petri dishes, for example) and Vapona is cut in pieces of 1 cm^2 approximately, using 1 piece per 100 seeds. Finally, the containers are sealed and stored for at least 30 days. After treatment with Vapona, the seeds are examined in order to throw away those seeds with insect emergence.

VAPONA

Common Name: DDPV (ANSI), Dichlorvos (ISO, BSI).

Chemical composition. 2,2-Dichlorovinyl dimethyl phosphate, or 2,2-Dichloroethenyl dimethyl phosphate.

Chemical properties. As a tablet, it gasifies in the environment. Steam pressure is 2.9 x 10^3 mbar at 20°C. Boiling point is 117°C. It works by inhalation as a fumigating insecticide. Controls weevils in grain storage.

The fungus *Fusarium lateritium* has been reported to be isolated in sweetpotato sexual seed germinated after being scarified with sulfuric acid and disinfected superficially with NaOCl (sodium hypochlorite). Fungus causes chlorotic distortion in the sweetpotato leaves and has been found in fresh sexual seed and in seed stored for many years.

Determining the number of seeds

In a genebank, it is important to determine the number of seeds in each accession in the collection in order to keep an updated record of the seeds in stock. Seed counting is usually done by hand, when there are few seeds. When the seed lots are large, there are several methods to estimate the number in a seed lot. Those most used are:

1. **Proportion of count to volume**, which consists in counting seed lots of 100, 500, and 1,000 seeds, depending on the number of available seeds of the same species or accession to be counted. These seeds are then placed in glass tubes big enough to hold the seed lot, and then a line is drawn with a waterproof marker at the level, which each seed lot reaches. These tubes are then used as tares to determine the seed number in seed lots that have the same seed size as the lot used to determine these volumes.

2. **Proportion of count to weight**, which is a relation between the weight of determined number of seeds (usually 100) from an accession with an abundant number of seeds, and then on the basis of the weight of this sample, the quantity of seeds in the whole seed lot is estimated. For this purpose, all the seeds of the accession are weighed, and then 100 seeds are counted and weighed. The formula to determine the number of seeds is:

   SN= (TW/WS)*100

   SN: Seed number

   TW: Total seed weight

   WS: Weight of sample (usually 100 seeds)

3. **Count using counting machines**, which is generally used for large seed numbers. The machines, usually digital counters, are previously calibrated to register precision counting for each size of seed. It is very important to clean the machine when a new accession is processed to avoid seed mixture from one seed lot to another.

Sweetpotato seed drying at 5% of moisture content

CIP has developed a simple drying method for the storage of sweetpotato sexual seeds under medium and long-term conditions. This method allows the moisture content of the seed to be reduced to about 5%. This is a seed moisture content that is recommended for long term storage of orthodox seeds.

The seed and silica gel, when it is completely dry, with a humidity colorimetric marker (blue when dry and red when humid), are weighed in an analytical balance with 3 decimals of approximation, considering a weight relationship of 1 g of seed to 2 g of silica gel. Then the seed and silica gel are placed in separate and uncovered containers (petri dishes, for example) inside a drying bell or a plastic tray completely covered with a transparent plastic bag, which is sealed once it is filled up with samples. If plastic trays are used, the plastic bag should be sealed with a masking tape. If the room temperature exceeds 20°C, or is lower than 15°C, the plastic trays are placed inside a refrigerator or incubator for 2 weeks, with temperature controlled at 17°C.

It is very important to keep the seeds separated from the silica gel to facilitate their removal when the silica gel changes color from dark blue to reddish, by absorbing moisture and becoming humid. During the 14 days of the treatment, humid silica is replaced by a dried batch of the same weight.

When silica gel is not available, rice dried at high temperature can be used as a desiccator. Good results can be obtained by using at least 3 changes of dried rice in the two weeks of treatment.

Package sealing and storing

Once the seeds have been counted and dried, they are placed inside aluminum foil pouches which contain aluminum foil between two polyethylene sheets (PET 12µ, AL, 9µ, PE 70µ) to make it water proof. Then, these seed packages are sealed by heating. Metal or glass containers that can be hermetically sealed can also be used.

Once seeds are in packages, they can be stored in refrigerated chambers with temperatures under 4°C for short or medium storage conditions. For long term conservation, seed packages are stored in freezers at -10 to -20°C.

Seed germination tests

The sweetpotato sexual seed has a thick, very hard and impermeable testa, which makes seed germination difficult and requires scarification. There are several methods, both chemical and physical -

mechanical, that make the hard testa seed permeable and facilitate its germination.

1. **Chemical scarification with sulfuric acid**, which consists in placing the seeds in plastic sieves, and immersing them in a suitable glass containing concentrated sulfuric acid (90% approximately) for 20 minutes. The treated seeds are then rinsed in running water during the whole night to completely eliminate acid residues.

   This method has the advantage of treating large numbers of seeds, and those that do not germinate can be scarified again. The disadvantage is that there is not 100% of seed germination after this treatment. This scarification method must be executed with a lot of caution by qualified staff, because of the sulfuric acid toxicity.

2. **Scarification by wasting the seed testa with sandpaper**, which is carried out after disinfecting the seeds for five minutes in three solutions against fungi and bacteria. The first solution is hydrochloric acid (HCI) 1 N; the second is soapy water and the third is an ethanol solution at 96%. Sweetpotato seeds have a groove called hilium and the plantlet emerges from one of its extremes. The scarification of the testa consists in rubbing the opposite side to the hilium with sandpaper for wood of medium thickness in order to wear that side of the testa until it is very thin.

   If these treated seeds are going to be put to germinate, they should be placed on filter paper which is then soaked with sterile water, or placed in a plastic net in a container with sterile water for 6 to 8 hours. After this time, the seeds will be swollen and the radicle will emerge.

3. **Breaking the opposite side to the hilium of the seed using a scalpel**, which is the physical-mechanical method most used and involves making a little cut with a sterile scalpel in the opposite side to the hilium to avoid damaging the embryo. This method allows the scarification of 100% of the seeds and it is also non-toxic. However, it demands a lot of man/hours because it implies treating each seed individually.

It has been reported that sweetpotato seeds are viable for more than 20 years when stored at 18°C and 45-50% of relative humidity. The viability of sweetpotato seeds stored in genebanks is determined by in vitro seed germination tests. This is done by placing 100 seeds, depending on the number of seeds in stock of recently scarified seed in petri dishes with filter paper moistened with distilled water. The petri dishes are then placed in a germinator, refrigerator, or incubator at a constant temperature of 18°C. The evaluation is made by daily

counts of germinated seeds after 10 days, and then at 14 and 21 days from when the germination test began. The number of germinated seeds will be the percentage of germination in that seed lot. The seeds that do not germinate after 21 days are probably non-viable or dead.

Seed multiplication

Seed multiplication is done when the seed number or their germinability is under the minimun levels. In the case of *Ipomoea batatas* accessions, seed is usually obtained from the clonal material maintained in the genebank. For wild *Ipomoea* accessions, a seed sample of about 30 seeds is used for multiplication.

Since the main objective of a genebank is to maintain, insofar as possible, the original genetic variability of the samples, this is accomplished by:

1. Incrementing seed as little as possible.

2. Using appropriate techniques for long term storage, to ensure that seed viability decreases as little as possible.

3. Using 30 plants at least per accession for seed multiplication.

4. Pollinating all plants of each accession with a pollen mixture from several cultivars of the same geographical area. If seed of wild species is being multiplied, intercrossing is made between different pairs of genotypes of the same accession.

5. Preparing seed lots for the multiplication of each wild *Ipomoea* accession, in such a way that each female-plant contributes with approximately the same seed number. In the case of sweetpotato cultivars, seeds produced by all the plants in each accession are collected.

The number of seeds produced will depend on the flowering intensity, which in turn depends on the ecological niche where seed multiplication takes place, its latitude and its photoperiod. Besides, it will also depend on the sexual compatibility of the genotypes used in the multiplication. This sexual compatibility also depends on biotic factors such as the sporophytic incompatibility system at the level of the stigma, through the stile, and at the ovary level.

Bibliography

Clark, C.A.; Hoy, M.W.; Stine, B.S. 1992. Isolation of *Fusarium lateritium* from sweetpotato true seed from different countries. Phytopathology 82(10):1073.

International Potato Center (CIP). 1981. El arroz tostado: un agente desecante de semillas. CIP Circular 9(7):1-3

Jones, A. and Dukes, P.D. 1982. Longevity of stored seed of sweet potato. HortScience 17(5):756-757.

Martin Jr., J.A. 1946. Germination of sweet potato seed as affected by different methods of scarification. Proc. Ame. Soc. Hort. Sci. 47:387-390.

Santos, R.C. dos;Melo Filho, P. de A.; Jurubeba, A.C. 1989. Teste de germinacao em sementes da batata-doce. En: 29. Congresso Brasileiro de Olericultura. Horticultura Brasileira, 7(1):76.

Steinbauer, C.E. 1937. Methods of scarifying sweetpotato seed. Proc. Ame. Soc. Hort. Sci. 35:606-608