



Procedures for Generation of Potato Tuber Families from true (botanical) seed

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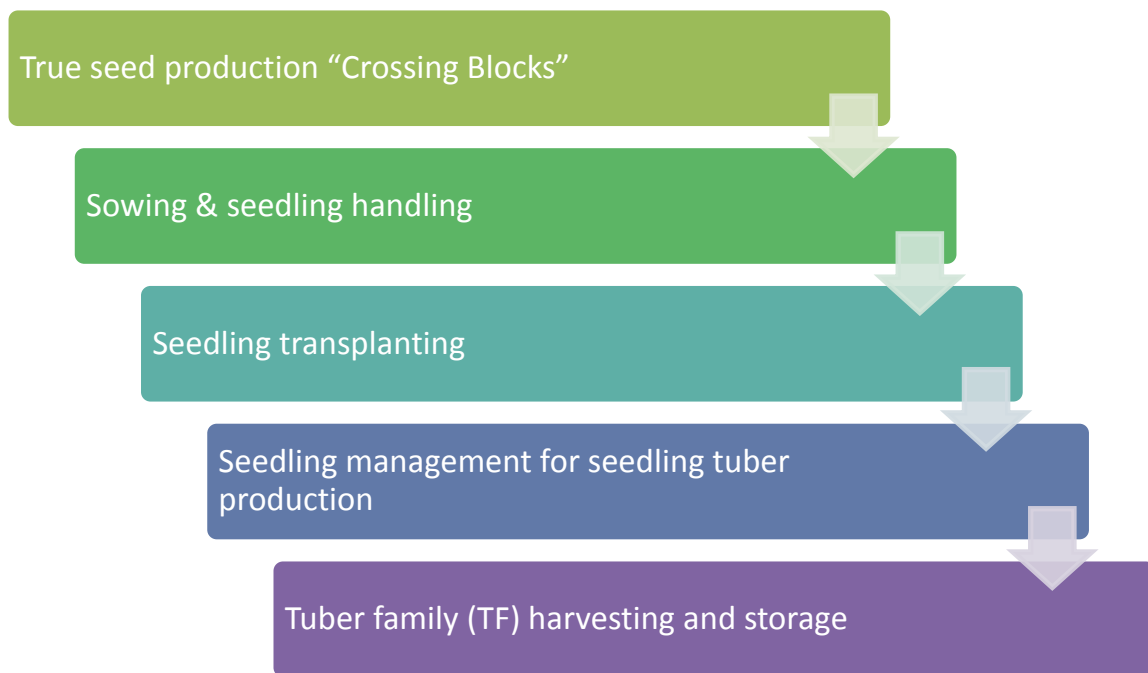


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[1]

Flowchart for the generation of Potato Tuber Families



Option 1

Option 2

TF for genetic studies and/or parental selection

- Requires 3 to 4 sets for multi-environment trials.
- Careful identification of families

TF for clonal selection

- Requires minimum 2 sets for keeping a clean copy
- Careful identification of individual genotypes



[2] Background

Potato breeding relies on the generation of new genetic combinations through sexual reproduction achieved by pollinating flowers to produce berries which contain botanical seeds. To distinguish botanical seed from vegetative tuber seed, the product of sexual reproduction is also called true seed (TS).

Potato breeders create new variation by generating true seed from natural open pollination or by performing directed crosses by transfer of pollen from one parental line to another.

Potato is highly heterozygous, therefore each cross results in a heterogeneous progeny (or true seed “family”) of which each seed is a unique genotype. Individual or sets of true seed families represent segregating populations.

CIP’s breeding program distributes segregating populations as TS families to collaborators and breeders of CIP’s Regional Programs and partners and clients from NARS and ARI. These TS families are then used in further genetic studies and parental selection and / or for clonal selection. In the first instance (Option 1- genetic studies), segregating populations from genetic mating designs aim primarily at drawing the required information to estimate genetic parameters, such as heritability, variance components, and parental value for the identification of superior or pairwise heterotic parents, while in the second case (Option 2- clonal selection) progenies originating from hybridization of selected parents are used for identifying clones for variety selection with appropriate combinations of genes for adaptation and consumers’ preference traits.

The genotypes that grow as seedlings from true seeds produce tubers called “seedling tubers” that can be replanted as vegetative tuber seed, and hence clones can be established, maintained and propagated by asexual (vegetative) reproduction. A tuber family (TF) is the first generation (C1) of seed tubers from a TS family. Each true seed, seedling, seedling tuber, or clone is genetically unique, and may result in a new potato variety.

True seed produced at CIP is checked for quarantine diseases by laboratory testing of parental material and by following standard procedures for disease-free true seed and tuber family production.

Selection for traits with high heritability such as tuber color and skin texture, tuber number, shape and eye depth can be performed in tuber families thus reducing the size of the population to be tested in the field for the identification of clones for variety selection. (option 2)

This protocol attempts to provide a detailed description of the procedures to produce tuber families in order to orient and facilitate potato research toward more efficient breeding product achievements.

[3]

Methodology

Producing seedling generations

A. True seed sowing and seedling handling

- True seed of less than 6 months of harvest (average dormancy period) should be treated to overcome dormancy by soaking it for 24 h in 1500 ppm gibberellic acid (GA₃)
- True seed representing a progeny of individuals can be sown directly in a small tray (Fig.1) or clay pot or in nursery beds (Wiersema, 1986). Trays or pots must have some small holes in the base for proper drainage.
- *Substrate*: Potato seedlings require a substrate with high percentage of organic matter (e.g. plant compost or peat moss) and a very limited amount of clay to avoid a compacted substrate. A suitable substrate on volume basis may be 2 parts of sand, 1 part of soil and 2 parts of organic matter. If the soil already contains some organic matter, you may decide to use 1 part of organic matter only. The sand should be washed to eliminate salts. The components should be mixed thoroughly applying a certain amount of wetting for proper mixing. The mix is steam sterilized for half an hour at 80°C (180°F) and kept inside the sterilizer for 3 to 4 hours before using.
- *Planting in trays*: Trays do not need to be more than 2 inches deep since the rooting system of seedlings is rather shallow in early stages
 - Fill the trays with the substrate up to 1 inch from the top border. Make rows 0.4 inch deep spaced 2 inches apart (Fig. 1). A furrower as the one shown in fig.2 can be constructed. This consists of a wooden board with handles on one side and several lines of triangular strips one inch wide fixed to the opposite side.
 - True seed is sown at about 0.5 inch apart within each row and covered with the substrate from each side of the row (Fig. 3-4). A plastic stake with a waterproof sticker indelibly labeled with the cross or family name and planting date (Fig.4 and 5) must be put in the tray before starting sowing the next tray with another TS family to avoid labeling mistakes ending in a

wrong identification of TS families. If applicable use of barcode stickers are recommended to avoid mistakes and facilitate record-keeping.

- Immediately after sowing, moisten the substrate surface of the trays carefully using a watering can. A preventive treatment with a fungicide is recommended together with the first irrigation to prevent fungus diseases coming from incompletely disinfected substrates.
- Seedlings begin emerging approximately in two weeks after sowing and establish well in three weeks after germination (Fig. 6). Soil temperature of 15-25 °C is required for proper true seed germination. If required, shading with an agricultural black shade cloth can be used to lower the temperature. However shading during growth results in weak, elongated seedlings. Therefore, shade should be removed gradually after two or three weeks when the seedlings get established. If shading is still required to lower the temperature during the next weeks of growth prior to transplanting, a white shade cloth is recommended as this provides a good degree of sunlight
- Seedlings are watered lightly once a day or more depending upon the prevailing weather conditions. Keep the soil moist not wet. Use a fine sprinkling can or a hose pipe fitted to a weak pressure stainless steel sprayer to prevent removing the substrate.
- Once established, seedlings requires 2 weeks of growth prior to their transplanting into individual pots



Fig 1. Preparing the trays for true seed planting

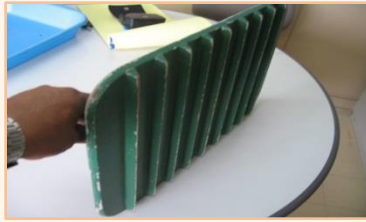


Fig 2. Furrower made of wood



Fig 3. True seed family planting



Fig 4. True seed families already planted and labeled with plastic stakes

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Fig 5. Plastic stake with waterproof sticker labeled with the family CIP number and planting date



Fig 6. Seedlings three weeks after planting

B. Seedling Transplanting

- Seedlings are ready for transplanting when they reach approximately 7.5 cm height (Fig. 7).
- Seedlings from each TS family are individually transplanted to 4" pots containing the same substrate used for true seed germination. However if a hydroponic system is available, use only washed sand.
- Label the first and last planted pot of each family with the cross or family name and transplanting date using a different stake color.
- Off-type and poorly growing seedlings should be removed during transplanting. Therefore care should be taken to plant an excess of TS in the initial planting. Our experience is to initially plant 200 seeds, transplant top 150 seedlings, and select at

least 120 individuals (siblings) per family when tuber families (TF) are intended for genetic studies or for estimation of the parental value of the progenitors (option 1). For TF intended for clonal selection (option 2), the final number of individuals can be more flexible, that is smaller and different per family, depending on the selection process during transplanting and harvesting, and according to the facilities and budget available for subsequent trials.

- Fill the pots halfway with the substrate, create a small hole or depression in the center of the soil, place the plant, cover it with the substrate and use your hands to pat the soil down firmly.
- If the substrate was not initially fertilized, fertilize each pot with 12-12-12 NPK fertilizer. Take the amount of fertilizer your fingers can pick and place it on the soil away from the plant stem, closer to the edge of the pot to prevent burning the roots. Water the plants using a watering can

C. Seedling management for seedling tuber production

- Hilling should be performed at 20 to 30 days after transplanting or when plants are 6 to 8 inches tall. Simply add substrate around the plants until 2/3 of the plant with leaves sticking out of the soil. You can take advantage while hilling to fertilize the plants by placing a small amount of urea (4 g) away from the plant before adding the substrate. Hilling gives tubers extra room to grow, encourage plants to produce more tubers and prevent them to come in contact with light until they are ready to harvest.
- A foliar fertilizer is recommended a week after hilling only if plants were not fertilized before. Do not exceed a 2% concentration to prevent leaf burn, and spray it on plant surface in the morning or late in the evening.
- Water the plants as needed. The soil should be moist to the touch but not soggy.
- Stop watering a week before harvest.

D. Tuber family (TF) harvesting and storage

- Cut off the vines when the potatoes are ready to harvest. This varies from two to three months after transplanting or when most plants turn yellow
- Dump the whole pot and dig to get the tubers (potatoes). Brush the tubers off with your hands to remove the substrate and place them back in the pot or just leave them carefully on a table (Fig.8).

- Harvest provides an opportunity for selection. Individuals with off-type tubers can be discarded as well as those that did not yield enough tubers to build the number of TF sets desired for subsequent trials. The latter is particularly important when tuber families are intended for multi-environment trials to obtain genetic information or estimate the parental value of progenitors from a genetic mating design (Option 1). On the other hand, early selections of individuals for color and skin texture, tuber number, shape and eye depth can be performed when tuber families are intended for clonal selection (Option 2) thus reducing the size of the population for field testing.
- Packaging and labeling of tubers are performed based on the two aforementioned target options:
 - **Option 1: Genetic studies and/or parental selection.** Does not require to keep the identity of each genotype unless individual selections are planned. As many sets of TF as number of field evaluation trials planned should be formed. However this will also depend on the average number of seedling tubers produced. On average, up to three tubers per seedling with acceptable seed size are usually recovered at harvest. A tuber family is formed by bulking a seedling tuber from each sibling of a segregating TS family (Fig. 9). Tuber families are bagged in paper bags #25, labeled with the Family or CIP-Number, and a SET number assigned. Tuber families are recommended to have the same number of seedling tubers in order for all to be equally represented. An amount of 120 seedling tubers per family is a good minimum and allows their distribution in 40 seedling tubers per family and replication in field trials comprising three replications.
 - **Option 2: Clonal selection.** If facilities are available, it is ideal to keep the identity of each genotype in order to go back to a clean copy after selection trials. Therefore tubers from each sibling should be carefully identified with the family name followed by a decimal point and an identifier number that starts from 1 for the first harvested sibling, and continues with consecutive numbers up to the last harvested sibling. The largest tuber is retained and bagged in a small paper bag to be stored as a clean copy while the others are bagged and labeled separately to be used in one or more selection trials depending on the number of tubers available and locations desired.
- Harvested seedling tubers once bagged and labeled are stored at cold temperatures (3-5°C) to preserve tuber dormancy and quality until the planting season



Fig 7. Seedlings at the age of transplanting



Fig 8. Harvesting of seedling tubers

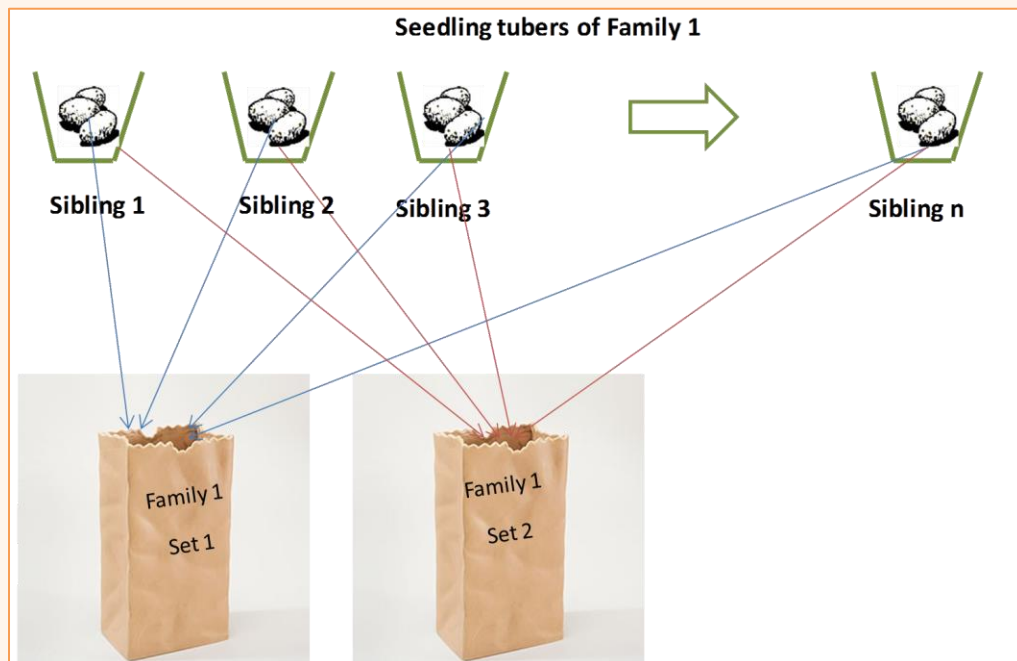


Fig 9. Procedure for building sets of tuber families.



[4]

Reference and Acknowledgement

Reference

- Wiersema SG. (1986) A method of producing seed tubers from true potato seed. Potato Research 29:225.

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Science for a food-secure future

The International Potato Center (known by its Spanish acronym CIP) is a research-for-development organization with a focus on potato, sweetpotato, and Andean roots and tubers. CIP is dedicated to delivering sustainable science-based solutions to the pressing world issues of hunger, poverty, gender equity, climate change and the preservation of our Earth's fragile biodiversity and natural resources.

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