

Preservation of Old Potato Varieties

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What are old potato varieties?

A potato variety may be defined as a group of identical plants, sharing the distinctive characters of an original individual from whom they are derived by vegetative reproduction. A potato variety is considered distinct when it differs from all other known varieties by one or more recognizable characters whether they be of a morphological or of a physiological nature. Commercial potato cultivars are propagated through the tubers, since the propagation by seeds is hindered by a high degree of heterozygosity, self-incompatibility and male sterility, frequently accompanied by failures in fruit and seed set.

In Germany for the commercialization of seeds of agricultural species and vegetables the addition to the National List is necessary. The German Seed Act (Rutz, 1999) provides the legal frame for the National List which protects the consumer and ensures the provision of high quality seed and planting stock material of resistant and high performance varieties for farmers and horticulturists. When potato varieties are no longer on the official list they are considered as old potato varieties.

Why does anyone want to preserve old potato varieties?

As long as varieties are still on the market the breeders maintain the varieties. But what happens with those varieties not any longer on the National List? They find a refuge in collections of plant genetic resources also known as genebanks, otherwise they could be lost for ever due to their requirements for vegetative propagation.

Tab. 1: Options for the long-term preservation of old potato varieties

Field genebank	In vitro storage	Cryopreservation
Long-term experience with the technique		
yes	yes	no
Availability for distribution		
several months of the year	always	3 to 6 months after thawing
Easiness of delivery		
high, with restrictions	high	low in N ₂ , high as plants
Feasibility to verify the genetic integrity directly		
high	low	low
Chance to keep cured material pathogen free		
non	high	high
Risk to lose part of the collection through diseases, pests etc.		
yes	non	non
Risk to lose part of the collection through technical failures		
high	high	non
Risk for mislabeling		
high	high	only in the initial phase
Costs for initiating the collection		
depending on circumstances	medium	medium
Costs for maintaining the collection		
depending on circumstances	medium	low

Though originally conserved for plant breeding purposes they are hardly if at all requested for this purpose. Requests come mainly from research institutions. Since a few years there is also a small but growing interest in old potato varieties among the consumers. However, the market share is very low. Mass commercialization is prohibited through the German Seed Act.



Fig. 1a: Storage as in vitro plantlets

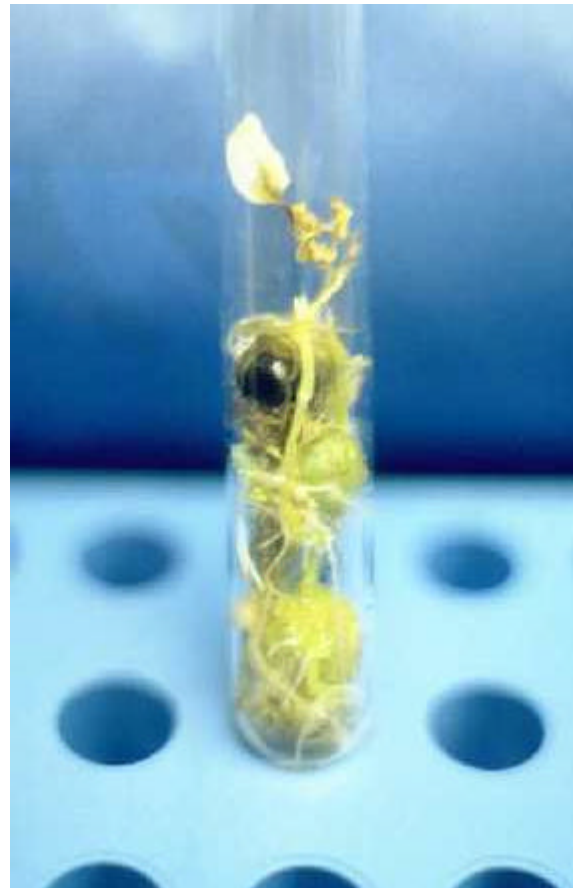


Fig. 1b: Storage as micro tubers

How can they be preserved?

Traditionally, potato varieties have been and are still maintained in a field genebank. The major advantage of growing the varieties in the field is the potential to verify the genetic integrity of the variety directly. Distribution of material is possible for several months at the time of the year seed potatoes are traditionally requested. The major disadvantage of a field genebank, however, is the risk to lose part of the collection through diseases, pests, weather damage or other accidents. In Tab. 1 the second option for the long-term storage of potato varieties is the in vitro storage, either as plantlets (Fig. 1a) or as microtubers (Fig. 1b). This type of storage lowers the risk to lose material due to environmental stress. Once viruses or other pathogens have been eliminated cultures can be kept pathogen free. But the in vitro storage is not totally secure, potential risks are technical failures, secondary infection or mis-

labeling. The third option is cryoconservation, the storage in liquid nitrogen. Also this method has advantages and disadvantages. From the three options for long-term conservation of vegetatively propagated potato genotypes, field genebanks have the longest tradition, also in vitro storage techniques exhibit long-term experience whereas cryopreservation of potato as a routine storage method is fairly new.

Cryopreservation - a method for long-term storage?

First successful attempts to freeze meristems or shoot-tips of *Solanum tuberosum* genotypes were already published 1978 by Bajaj followed by a number of publications (see Schäfer-Menuhr et al., 1996 for references). Though the published methods were partially successful they have never been refined further for routine application in a collection of plant genetic resources. Thanks to the initiative of Lindsey L. Withers, a joint project between the Federal Agricultural Research Centre (FAL) and the German Collection of Microorganisms and Cell Cultures (DSMZ) supported by the Minister for Economic Cooperation through the International Plant Genetic Resources Institute (IPGRI) was originated to explore already existing freezing methods, select a promising one, refine it for a genebank routine and finally apply it. As scientist in charge I want to introduce the method and give an overview about my experiences using this method in the routine of both German collections of plant genetic resources, one of which still situated in Braunschweig and the other one in Gatersleben.

The published freezing protocols vary and are mostly the result of trial and error rather than a complete understanding of the freezing process. From the already existing freezing methods I selected ultra rapid freezing. With this freezing technique the intracellular ice crystallizes in microcrystals of a size which is supposed to be harmless to the integrity of the cell components. Then I had to decide on the plant material to use and a way enabling not only ultra rapid freezing, but also ultra rapid thawing which I think is also necessary and, thirdly, I needed a way to store the material in liquid nitrogen. The plant material supposed to be cryopreserved was stored under slow growth conditions. Comparative growth experiments led me to the decision that the plantlets should be grown in fairly high density with good aeration. Then I had to decide on the explants and their size. For ultra rapid freezing they had to be small, on the other hand small meristems of the size of those prepared for virus elimination were time consuming to prepare and had already a poor regrowth rate when not frozen. A good compromise were trimmed shoot tips.



Fig. 2a: Ultrarapid freezing



Fig. 2b: Storage in liquid nitrogen

The second problem was to find a kind of support for the shoot-tips that allows not only ultrarapid freezing but also ultrarapid thawing and that can be stored in liquid nitrogen containers in a way that they can be found again when needed. I am very much indebted to Erica Benson who introduced me to the idea to use small aluminum foils (described by Kartha et al., 1982) fitting into the standard 2 ml cryovials which can be labeled and stored in a documented place in the cryocontainer.

With these two fixed parameters (trimmed shoot-tips and aluminum foils as support) I started to optimize the method. Most experiments were performed with several varieties. The result was a simple, almost primitive, freezing method which is briefly described in the following (for details see Schäfer-Menuhr et al., 1996). The varieties to be frozen are propagated in high density (10-15 in vitro plantlets) in vessels allowing good air exchange. These are 12 cm high twist off jars with a hole in the lid into which a cotton plug had been inserted. Depending on the variety, it takes 3 to 7 weeks until the plantlets have reached a height of 10 cm. Then the shoot-tips are cut off, trimmed under a stereomicroscope in a way that they contain the apical meristem, leaf primordia and part of the covering leaves. The actual size varies depending on the in vitro growth habit of the variety. For one freezing experiment 100-150 shoot-tips are prepared and incubated in MsTo-medium (Towill, 1983) over night at 23 °C. The next day the shoot-tips are transferred into the cryoprotectant which is the same medium containing 10% dimethylsulfoxide. After incubation for 2 hrs (actually it is a broad plateau of 1-4 hrs) the shoot-tips are placed into small droplets (2.5 μ l) of cryoprotectant which had been distributed

on small rectangles of aluminum foil (Fig. 2a). The foils are inserted into labeled cryotubes which had been filled with liquid nitrogen. The tubes are closed and placed into the storage container (Fig. 2b). Directly after freezing one vial containing 10-12 shoot-tips is checked for its ability to regenerate plants. We froze every variety three times which means that about 20-30 cryovials are stored per variety.

Though the method is comparably fast, there are, without doubt, limitations concerning the number of samples that can be frozen. A limiting factor is the overnight incubation which is absolutely necessary for the success of freezing. This means freezing is reduced to 4 days of the week or even reduced to two days per week if there is a legal holiday in the middle of the week. If one technician does everything she/he can freeze 50 varieties per year which means 10 years for 500 varieties. The freezing efficiency can be raised considerably if two persons are available for the freezing work. It's quite realistic to assume that they can freeze 150 varieties per year. This would reduce the time for freezing 500 varieties to 3-4 years.

During the five year period of the project Ellruth Müller and I froze 219 varieties and genotypes. Theoretically, the two of us could have frozen 500 varieties or more, if we would not have had to deal with other tasks like finding a suitable method, optimizing the technique, verifying the genetic stability and last but not least writing reports and publications. Our inheritance to Gunda Mix-Wagner who continued the work at the genebank in Braunschweig later on, was a simple freezing method, a post freezing regeneration system which certainly works at a suboptimal level for several genotypes, but has the advantage that plants have been obtained from all varieties frozen so far, and two liquid nitrogen containers with 63 852 shoot-tips in 5 321 cryovials.

Of every batch frozen 10-12 shoot-tips have been thawed to check the ability for plant regeneration after freezing. These regrowth controls were used to calculate the survival and plant regeneration rates. Since only 10-12 shoot-tips were thawed for the regrowth controls (for the first varieties frozen it were only 5-6), the calculations are more approximations than statistically secured data. In addition, neither the regeneration medium nor the culture conditions are optimal for all genotypes. The survival rates for most varieties are high - approximately 80 % on the average. More important for a genebank, however, are the plant regeneration rates. They are, on the average, 40 % (40 % means in practice 4 plantlets per 10 frozen shoot-tips) and depend on the genotype. This genotypic effect is well known to anyone working with in vitro cultures of potato. As an example, the regeneration ability of the first 200 varieties frozen are shown in Fig. 3. The columns represent percentage groups. I think it is safe to assume that a plant regeneration rate of 30 % is not only sufficient for the maintenance of the variety but also for a potential distribution. Fig. 3 shows clearly that this is the case for 129 varieties. It is now a question of time and confidence in the method what to do with the 71 varieties having plant regeneration rates below 30 %. Since they have also proved that they regenerate plants, enough vials have been stored. Thus, shoot-tips of several vials can be thawed in case the variety should be requested. The fact that plants have been obtained from all 219 varieties which had not been especially selected for the freezing procedure presented in this paper makes this method attractive for collections of plant genetic resources.

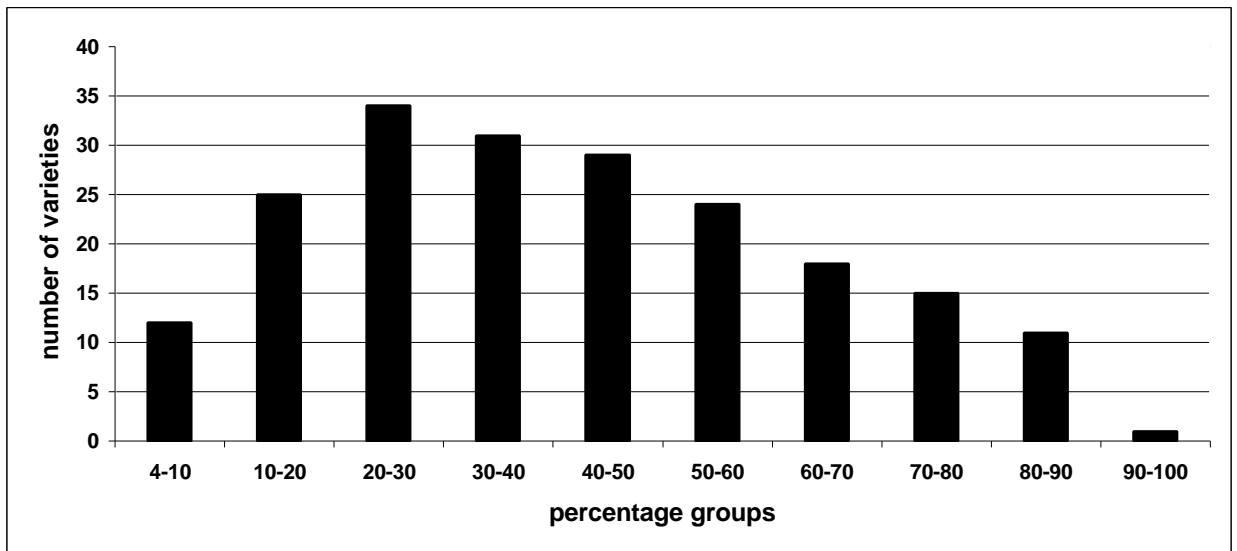


Fig. 3: Plant regeneration of the first 200 varieties frozen

An important criterion whether or not this freezing method is useful for the long-term storage of potato varieties is the question whether the genetic characteristics of the varieties or genotypes are maintained. It is a matter of definition how narrow or how wide the term "genetic identity" is defined. A 100% congruence is difficult to prove and is also not reasonable. For a practical application it should be sufficient to show that the characteristic attributes of a variety are conserved for a potential use in future breeding programs.

The most effective method to show that the freezing and regeneration process did not change the properties of a variety was the evaluation of the phenotype of regrown plants. Among the regrown plants of 98 varieties only one abnormal plant was found which was most likely polyploid. There were also a few weak or tiny plants with poor or no tuber production. I do not think that the reduced vitality had anything to do with the freezing process, because we observed it also among the control plants. Similar phenomena have been described in the literature (Lommen 1996a, 1996b). Besides these few exceptions the regrown plants grew vigorously and could not be distinguished from the control plants.

The other two methods, flow cytometric measurements and DNA-fingerprinting, respectively, were less effective tools to prove the genetic identity. Though flow cytometry is an easy method for the determination of ploidy levels, the results are difficult to interpret, if the analyzed material is not in the same physiological state. However, the results showed clearly that no polyploid specimen was among the analyzed plants.

Employing DNA-fingerprinting we did not find unusual banding patterns, but this does not mean anything. Variants can only be detected, if the mutation is within the cleavage site of the respective restriction enzyme. In addition to that, it is very cumbersome to distinguish between real mutations and artifacts which occur when impurities in the extract disguise the cleavage site. In my opinion variation or somaclonal variation are no problem when using cryopreservation for long-term storage. The frequency is certainly not higher than in a field genebank or in in vitro storage.

Almost five years of using this simple freezing technique in the routine of a collection of plant genetic resources has shown that this method allows the secure storage of potato varieties and genotypes. The most important factors for the regeneration of plants after freezing are the preculture of donor plants and the postculture after freezing.

After termination of the initial project freezing was gradually continued in the FAL, however, initially at a reduced rate due to a reduction of staff. Activities increased considerably later on within the frame of the EU project “Genetic resources of potato including conservation, characterization and utilization of secondary potato varieties for ecological production systems in Europe”. In 1997 cryopreservation of potato was also started at the Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben using the same freezing protocol but micro tubers (Fig. 1b) as starting material. The growth controls in both institutes show that survival and plant regeneration is in about the same order of magnitude as was previously found by us.

Perspectives

When reflecting about the years, cryopreservation of potato is carried out and in the discussions pro and con cryopreservation one question that seems to be completely unimportant to those making final decisions in research policies, is the fate of the cryopreserved potatoes in the far future. We are very confident that the samples will still have the same regeneration behavior after many years and theoretically the samples can be stored forever and will not lose their vitality as long as liquid nitrogen is refilled. The problem is that the know-how is lost very fast when the freezing activities are interrupted for a longer time in spite of the simplicity of the method and an easy to follow manual. The danger increases when all samples are put into storage and when they are no longer integrated in an in vitro collection. Without in vitro culture techniques the samples cannot be thawed and regenerated. Therefore it is quite clear that cryopreservation as sole long-term storage technique cannot substitute in vitro culture collections or field genebanks, if old potato varieties are to be conserved for future generations.

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