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Slow growth storage and cryopreservation—tools to facilitate germplasm maintenance of vegetatively propagated crops in living plant collections

E.R. Joachim Keller*, Angelika Senula, Semuel Leunufna, Marion Grübe

Genebank Department, Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, D-06466 Gatersleben, Germany

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Abstract

In living plant collections, vegetatively propagated accessions are outstanding material with respect to vulnerability and labour amount. This is also true for the main vegetative material, held in the IPK, Gatersleben. A survey of the preservation of potato, garlic and other alliums, mint and yam is given. More than 630 accessions are in slow growth conditions. Amongst them, 99 clones of garlic and 35 of shallot have been tested to be virus-free. Cryopreservation is routinely applied for potato using the droplet method. The cryo-collection contains more than 1000 accessions, a part of which has been integrated from another collection, formerly established at Braunschweig. Cryopreservation of garlic has been used to store the accessions of the European core collection. Cryopreservation is successful in three *Dioscorea* species using a combination of the original vitrification with the droplet method. Investigations about morphogenesis and ultrastructural cell parameters before, during and after cryopreservation were included in these activities.

Keywords: Plants; Cryopreservation; Bank; Genetic resources

Entreposage sous des conditions de croissance lente et cryoconservation–des outils qui facilitent la conservation du plasma germinal des plantes utilisées afin d'obtenir des récoltes propagées des collections vivantes de plantes

Mots clés: Plantes; Cryoconservation; Espéces; Banque; Ressources génétiques

1. Introduction

Like all the other living organisms, plants are endangered by genetic erosion with an alarming extinction rate. This is true for all parts of plant germplasm likewise for wild species as well as for the genepool of cultivated plants.

* Corresponding author. Tel.: +49 39482 5267; fax: +49 39482 5741.
E-mail address: keller@ipk-gatersleben.de (E.R.J. Keller).

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The latter are especially endangered by the increasing use of a comparatively low number of modern highly productive varieties. Increasingly the farmers discard old landraces because of lower yield and lesser market chances. At the same time wild relatives of the crop species vanish due to habitat degradation and other human influences. Living crop plant collections, recently usually called genebanks have been established since the twenties of the last century. One of the most deserving historical personalities of the time of initiation of plant germplasm collections was Nikolai Ivanovitch Vavilov (1887–1943), who was the director of the Institute of Plant Industry (VIR) in St Petersburg from 1921 until 1940, performed worldwide collection missions and founded its well-known plant collection.

The majority of crop plant germplasm is stored in seed repositories at temperatures between −15 and −20 °C. Some seed genebanks, especially in developing countries, do not even use this low temperature and store seeds at 0–4 °C for economical reasons.

Much more problems are encountered with respect to such plant germplasm, which cannot be stored as seeds, because either it does not form seeds at all or their seeds are not storable (recalcitrant) or do not represent genetic identity with the parental material because of heterozygousity like in many clonal crops such as potato, fruit trees and many ornamentals. For this genetic material, in vitro slow growth storage and cryopreservation are the only ways to keep the material. Slow growth culture is a medium term method using in vitro culture of organs at low temperatures (around 4 °C for temperate crops and 10–15 °C for tropical germplasm). Cryopreservation is usually performed in liquid nitrogen at −196 °C. It is the only method for long-term conservation. Recently an increasing body of survey literature has been published, which summarized the progress in the various crops [1–3]. In contrast to the first phases of plant cryopreservation when slow freezing methods were prominent, the further development is more and more dominated by rapid freezing techniques either basing on encapsulation–dehydration [4], vitrification [5,6] or combination of both [7]. The Institute of Plant Genetics and Crop Plant Research at Gatersleben, Germany, is one of the largest European plant genebanks. It also keeps vegetative plant germplasm. In this publication, examples are given for the crop management and experimental results obtained with the main vegetative crops stored in this institute.

2. Potato

Potato is the largest vegetative collection of IPK. It consists of 2825 accessions of *Solanum tuberosum* L. and some material of related wild species (49 of 3063 accessions). The plant material is grown in the field. During the transfer into in vitro conditions, it passes a virus-cleaning phase using chemotherapy and thermotherapy for various viruses. These treatments are applied to in vitro shoot cultures. After establishment of virus-free material, it enters a cyclic slow growth maintenance consisting of a warm phase with long-day at 20 °C for 2–3 months, a microtuber-induction phase with short-day at 9 °C for 2–4 months and a cold storage period, in which microtubers are stored at 4 °C for 16–18 months [8]. The material is stored in vitro in the Groß Lüsewitz station in the North of Germany. For cryopreservation, it is sent to Gatersleben in the form of microtubers. After arrival, a pre-culture period is needed to multiply the material until enough shoot tips are ready for explant excision.

In IPK, the main cryopreservation method is basing on vitrification protocols, which are modified for the various crop species. For potato, the first reports concern slow freezing [9–12], rapid or ultra-rapid freezing [9,13–15], vitrification [16], and encapsulation/dehydration [4]. The main method for IPK has been elaborated by Schäfer-Menuhr et al. [17] in another institute at Braunschweig, Germany. The so-called 'droplet method' uses the high thermo-conductivity of aluminium foil to attain a very fast temperature drop during direct immersion into liquid nitrogen. It has then been introduced into the IPK genebank since 1997 [18,19].

The pre-culture conditions are very important for the cryopreservation success under standard conditions of the droplet method. As shown in Fig. 1 with two standard varieties, a clear difference has been found between the cultivations in culture rooms (variants A and B) and incubators (variants C and D) irrespective of the concrete temperature conditions, most of the differences being

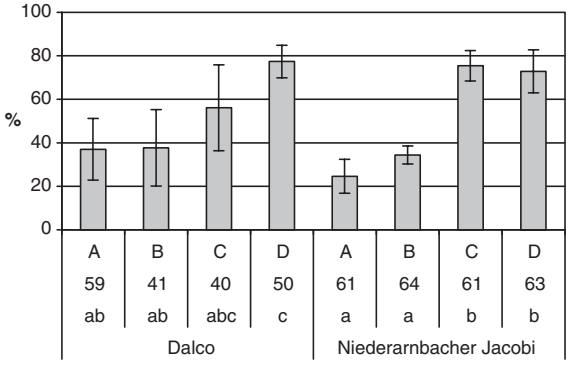

Fig. 1. Dependence of shoot regeneration rates two months after re-warming on 1 month pre-culture in different culture rooms (A–D) cultivated in vessel type V2. (A): cultivation room with 16 h light and permanent temperature of 25–26 °C; (B): cultivation room with 16 h light and permanent temperature of 18–20 °C; (C): a so-called '*Arabidopsis*-Incubator', type AR-75L3 with 16 h light at 25 °C and 8 h dark at 19 °C; (D): incubator CU-32L with 16 h light and permanent temperature of 20–21 °C, both incubators from Percival Scientific, Perry, IA, USA. Total number of explants per variant below the room type letters. Differences of values having one of the letters below these figures in common are statistically not significant in the χ^2-test (2×2 table). Significance level $p<5\%$, bar markers are the mean absolute differences MAD of two repetitions.