cost

EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

FA

Cryopreservation of crop species in Europe

Proceedings of the final meeting

AGROCAMPUS OUEST INHP, Angers — FRANCE

February 08-11, 2011

Edited by:
Agnès Grapin
E. R. Joachim Keller
Paul T. Lynch
Bart Panis
Angeles Revilla Bahillo
Florent Engelmann

CryoPlanet
Cost Action 871

Food and Agriculture

COST Action 871

Cryopreservation of potato landraces using droplet-vitrification

Nataliia Shvachko and Tatjana Gavrilenko

N.I.Vavilov Institute of Plant Industry (VIR), Bolshaya Morskaya Street, 42-44, St. Petersburg, Russia.

1. Introduction

Presently, VIR holds the oldest and one of largest potato collections, consisting of approximately 8,700 accessions including cultivated and wild species. The VIR potato collection has a high practical importance, as it serves as the base for national breeding programs. One of the most important parts of the collection are landraces representing native cultivated species collected by VIR expeditions. The most important task today is to develop safety duplicates for the large field-maintained collection of cultivated potato species.

At present about 250 potato accessions of cultivated species are conserved *in vitro* (Gavrilenko *et al.* 2007). The *in vitro* collection was established with the following objectives: to conserve genetic diversity, to maintain a duplicate of the most important part of the field collections, to avoid losses of plant material maintained in the field collections and to conserve pathogene-free accessions under controlled conditions. Recently we have initiated a cryopreservation program for this *in vitro* material.

2. Materials and Methods

2.1. Plant material

The plant material used consisted of 15 accessions of Andean potato landraces (*Solanum tuberosum* ssp. *andigenum*) and of five accessions of Chilean potato landraces (*S. tuberosum* ssp. *tuberosum*). Each accession was represented by one clonal genotype. For cryopreservation we isolated both apical and axillary buds of 20 accessions (Table 1).

2.2. Cryopreservation techniques employed

In vitro plants were cultivated on MS (Murashige and Skoog 1962) medium at 22ºC, with a photoperiod of 16 h day/8 h night (Fig. 1A). For cryopreservation we used the droplet-vitrification method of Panis *et al.* (2005). Apical and axillary buds (from the upper part of microplants) were transferred in liquid LS medium for dehydration and after 20 min they were transferred in PVS2 solution (at 0°C). Buds were placed in small droplets of PVS2 medium on pieces of aluminum foil (Fig. 1B) and directly immersed in liquid nitrogen. Buds were rewarmed in unloading solution at room temperature.

In total 270 buds per accessions were isolated. Three repetitions were executed for control buds (cryoprotected, not cryopreserved) with 10 buds per repetition for each type of explant and 60 buds per accessions (30 - for control apical buds and 30 - for control axillary buds). 210 buds (both apical and axillary) per accession were cryoprotected and cryopreserved. Three repetitions for each type of explant were executed from the cryopreserved material with 20 buds per repetition to examine regeneration both for apical and for axillary buds – in total 120 buds per accession. Survival and regeneration percentages were determined for this material on week 3, 6 and 8 after rewarming (Fig. 1C). Besides that, 90 explants per accession were left in the cryotank for long-term conservation.

3. Results

As expected, a high correlation was observed between the survival and the regeneration percentages, both for apical and axillary buds (Table 1). Depending on the genotype, regeneration after cryopreservation (scored on week 8) varied from 15% to 86% with apical buds and from 15% to 77% with axillary buds. More than half (11 of 20) accessions had regeneration percentages higher than 50%. The percentage of regenerated plants using apical buds was significantly ($p \leq 0,05$) higher compared to axillary buds in six of 20 accessions: k-2084, k-3231, k-1751, k-1697, k-634, k-4499. The opposite situation was observed only in two accessions (k-3987, k-9002); however in this case differences in regeneration percentages were not significant (Table 1). No statistical differences in regeneration percentages were found between potato landraces belonging to different subspecies - *S. tuberosum* ssp. *andigenum* and *S. tuberosum* ssp. *tuberosum*. Cryopreservation of endangered potato landraces at VIR is in progress.

4. Discussion

The large field-maintained potato collections at VIR require the application of modern techniques for safety duplication. Cryopreservation could ensure the secure and reliable long-term conservation of the germplasm collection. In this study a droplet-vitrification procedure based on the work of Panis *et al.* (2005) was applied to 20 accessions of tetraploid potato landraces from the VIR *in vitro* collection. We showed that the application of this protocol is promising for cryopreservation of diverse tetraploid landraces of *S. tuberosum*, although survival and regeneration percentages were significantly affected by genotype. The regeneration of plants from apical buds was statistically higher than from axillary buds only in six of 20 accessions. In most cases, differences in regeneration between these two types of explants were not statistically significant. These results support the use of axillary buds of potato microplants in practical routine genebank cryopreservation.

5. Acknowledgements

This study was supported by a collaborative project between the CRPGL, Luxembourg, the VIR, Russia and Bioversity International.

6. References

Gavrilenko T, Dunaeva S, Truskinov E, Antonova O, Pendinen G, Lupisheva J, Rogovaja V, Shvachko N (2007) Strategy of long-term conservation of germplasm of vegetatively propagated crops under controlled conditions. 2008. Research Bulletin of Applied Botany, Genetics and Plant Breeding. St.-Petersburg 164:273-285

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15:473-497

Panis B, Piette B, Swennen R (2005) Droplet-vitrification of apical meristems: a cryopreservation protocol applicable to all Musaceae. Plant Sci 168:45-55

Table 1 Results of application of droplet-vitrification method for 20 potato accessions and two types of explants (apical and axillary potato buds).

№	*S. tuberosum* subspecies	VIR catalog number	Number of apical/axillary buds[+]	Surviving,% apical / axillary buds	Regeneration % apical / axillary buds
1	*ssp. tuberosum*	k-2084*	60/60	31.7±1.4 / 21.0±2.5	21.7±2.0 / 15.1±2.5
2	*ssp. tuberosum*	k-3456	60/60	78.3 ±3.8 / 76.0 ±5.2	78.3 ±3.8 / 76.0 ±5.2
3	*ssp. tuberosum*	k-7573	60/60	22.5±1.4 / 23.0±1.4	15.0±1.4 / 17.9±1.4
4	*ssp. tuberosum*	k-7583	60/60	64.7± 7.1 / 79.3± 7.1	62.7±5.7 / 77.0±6.3
5	*ssp. tuberosum*	Juz-8969	60/60	23.3±1.9 / 21.3±1.8	18.3±1.9 / 20.0±5.8
6	*ssp. andigenum*	k-1688	60/60	21.6±2.0 / 20.0±2.5	16.7±1.4 / 15.0±2.5
7	*ssp. andigenum*	k-1697*	60/60	67.0 ±6.5 / 62.0±5.2	61.7 ±2.2 / 49.1±9.9
8	*ssp. andigenum*	k-1714	60/60	35.0±6.2 / 28.3± 5.1	28.3±5.1 / 23.3±2.7
9	*ssp. andigenum*	k-1751*	60/60	45.0±10.6 / 25.0±2.5	41.8±1.4 / 22.5±1.4
10	*ssp. andigenum*	k-1775	60/60	72.7±9.8 / 78.0±9.3	72.7±9.8 / 76.3±8.5
11	*ssp. andigenum*	k-3231*	60/60	86.3±2.5 / 48.3±1.8	86.3±2.5 / 48.3±1.8
12	*ssp. andigenum*	k-3987	60/60	66.3±3.8 / 75.7±5.2	66.3±3.8 / 75.7±5.2
13	*ssp. andigenum*	k-4617	60/60	27.5±8.6 / 52.5±10.1	17.5±2.8 / 36.2±13.7
14	*ssp. andigenum*	k-4634*	60/60	31.7±1.4 / 20.0±2.5	21.7±2.0 / 15.0±2.5
15	*ssp. andigenum*	k-4499*	60/60	76.0±2.1 / 47.0±2.1	76.0±2.1 / 47.0±2.1
16	*ssp. andigenum*	k-5588	60/60	72.0±2.2 / 67.0±5.3	72.0±2.2 / 67.0±5.3
17	*ssp. andigenum*	k-8201	60/60	62.0±3.5 / 54.0±2.8	62.0±3.5 / 54.0±2.8
18	*ssp. andigenum*	k-9002	60/60	59.3±3.6 / 63.0±1.4	59.3±3.6 / 63.0±1.4
19	*ssp. andigenum*	k-9571	60/60	20.0±5.8 / 22.5±1.4	20.0±5.8 / 17.5±1.4
20	*ssp. andigenum*	k-20579	60/60	68.0±5.0 / 51.0±7.7	68.0±5.0 / 51.0±7.7

*There were significant ($p \leq 0,05$) differences in regeneration between apical and axillary buds.
[+] In addition three repetitions for each type of explant with 10 buds per repetition were used as control for each accession.

Fig. 1 Steps of cryopreservation: *in vitro* plants (**A**), explants in the droplets of the PVS2 on pieces of aluminium foil (**B**) and plant regeneration (k- 1775) after cryopreservation (**C**).