In Vitro Conservation of Potato and Sweetpotato Germplasm

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In vitro conservation of genetic resources has advanced considerably during this decade. Since 1975, CIP has contributed to developing tissue culture techniques for conserving germplasm of potato (*Solanum tuberosum*) (Roca, 1975), sweetpotato (*Ipomoea batatas*) (Sigueñas, 1987), and Andean root and tuber crops (Toledo et al., 1994). In vitro conservation is the most useful and efficient way to distribute clonal materials. It facilitates the availability of planting materials at any time, avoids the transfer of major pests and pathogens, and makes possible virus eradication through meristem culture (Roca et al., 1979; George, 1993). In addition, in vitro conservation is less expensive than cryopreservation of field-grown clonal materials (Florkowski and Jarret, 1990).

Short- and Medium-Term Conservation

In vitro plantlets grow in a Murashige-Skoog (MS) culture medium containing minerals, a carbon (C) source, vitamins, and a low concentration of growth regulators (Table 1). Plants exhaust the nutrients in this medium in 2-3 mo; therefore, in vitro plants have to be transferred frequently to fresh medium. The culture rooms generally have a temperature range similar to those needed to grow a given crop in the field. That is called short-term conservation. The interval between subcultures, however, can be extended through growth rate reduction by modifications to the environment or changes in some media components.

The addition of osmoticums or growth retardants to the medium has proved efficient for reducing growth rates of different plant species. Osmoticums such as mannitol or sorbitol reduce mineral uptake by cells through differences in osmotic pressures thereby retarding plant growth (Dodds and Roberts, 1985; Thompson et al., 1986). Growth retardants can produce some physiological changes or generate mutations, which can threaten the genetic stability of the materials conserved in vitro (Hughes, 1981; Lizarraga et al., 1989; Wescott, 1981). Reducing growth temperature close to 0°C for temperate plant species or several degrees below normal for tropical crops can also minimize the growth rate in many crops (Dodds and Roberts, 1985; George and Sherrington, 1984). In vitro plants growing in closed culture vessels have low concentrations of CO_2; C absorption is maintained by supplementing the medium with sugar. Reducing light intensity also affects growth rate by reducing photosynthetic requirements and therefore metabolism (Hughes, 1981).

A combination of osmoticums, low temperature, and low light intensity has been the most effective in lengthening periods between subcultures. At CIP, these procedures are applied to potato and sweetpotato with good results.

In vitro potato conservation

There are well-established protocols for micropropagation of potato (Espinoza et al., 1992), meristem culture (Lizarraga et al., 1991), in vitro tuber induction (Estrada et al., 1986), medium-term storage (Golmirzaie and Toledo, 1998), and cryopreservation (Golmirzaie and Panta, 1997).

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Table 1. Components of culture media of potato and sweetpotato.

Ingredients	Potato culture media			Sweetpotato culture media[a]		
	Propagation	Conservation	Tuber induction	Propagation I	Propagation II	Conservation
MS[b] (I L)	1	1	1	1	1	1
Stock solution	Stock 1	Stock 1		Stock 2	Stock 2	Stock 1
Sucrose (g/L)	25	25	80	30	30	30
Gibberelic acid (mg/L)	0.1			1		
Espermidine (mg/L)	-	-	-	-	-	20
Naphthalene acetic acid (mg/L)	-	-		-	0.5	-
Sorbitol g/L	-	40		-	-	20
Benzylaminopurine (mg/L)	-	-	5	-	-	-
Chlorocholine chloride (g/L)	-	-	0.5	-	-	-
Phytagel (g/L)	3.5	3.5		3.5	3.5	3.5
pH	5.6	5.6	5.6	5.8	5.8	5.8
Temperature range °C	18 – 20	6 - 8	18 - 20	23 - 25	23 - 25	18 - 21

a. Liquid media is also used in sweetpotato culture.
b. Murashige-Skoog salts.
Stock solution 1: 2 mg/L glycine, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine, 0.4 mg/L thyamine HCl.
Stock solution 2: 0.1 g/L arginine, 0.02 g/L putrescine, 0.2 g/L ascorbic acid, 0.002 g/L calcium d-panthotenic.

The protocol for medium-term in vitro conservation of the potato collection at CIP is as follows. Accessions are conserved in a conservation medium containing 4% sorbitol at a temperature of 6-8°C, and light intensity of 1,000 lux. That extends the in vitro conservation of the potato collection for 2-4 yr without subculture (Table 2). The use of sorbitol as an osmoticum is applied to many crops without any physiological changes such as callus formation or vitrification. But these undesirable reactions are produced when the media contains mannitol, which can affect potato genetic stability (Harding, 1994). After several years in in vitro culture, plantlets can recover normal growth after one to two subcultures in propagation media. This conservation method is one of the most efficient for managing a large number of potato accessions and the time interval between subcultures is longer than for other crops.

The production of microtubers in in vitro culture as an alternative method for long-term conservation of potato cultivars has also been evaluated at CIP. Microtubers are produced in 2 to 3 mo and can be stored at 10°C for up to 10 mo after harvest (Estrada, et al., 1986). The dormancy of these microtubers can be controlled by environmental changes (Estrada et al., 1986; Tovar et al., 1985). Alternatively, once the microtubers sprout, growth can be retarded, as with in vitro plants, for 2-4 yr by storing them embedded in a conservation medium.

In vitro sweetpotato conservation

In vitro techniques for micropropagation of sweetpotato cultivars are well established. Plantlets grow from 1 to 2 mo in

Table 2. Number of accessions per *Solanum* species conserved in vitro and interval between subcultures.

Solanum species	Interval between subcultures				
	2 yr	3 yr	4 yr	5 yr	Total
andigena	569	666	1,555	70	2,860
stenotomum	57	54	129	5	262
phureja	37	37	66	9	149
tuberosum	38	24	68	8	138
chaucha	23	23	61	1	108
goniocalyx	15	6	31	1	53
Juzepczukii	4	8	14	1	27
ajanhuiri	6	3	8	0	17
Other	184	125	280	18	607
Total	**933**	**946**	**2,212**	**113**	**4,204**

culture media containing minerals, a C source, polyamines, and growth regulators such as giberellic acid (propagation medium I, Table 1) in a culture room at 23-25°C and 3,000 lux (Lizarraga et al., 1990). Some plants show multiple shoot formation, phenolization, or shoot dormancy as a response to high stress. We have overcome this problem at CIP by using a culture medium containing naphthalene acetic acid (propagation medium II, Table 1).

A number of problems prevent the long-term conservation of sweetpotato. Many attempts to establish an efficient slow growth medium have failed due to a strong genotypic response to the modified culture media, low survival percentage under restrictive growth conditions, or the formation of callus and vitrification during storage.

Although plantlets cultured in growth retardants may have survival rates ranging from 70 to 90%, genotypic effects or toxic effects are seen. For example, plants grown in a medium containing abscicic acid at 5-20 mg/L had a survival rate of 70-85% after 8 mo, but showed strong genotypic effects (Desamero, 1990; Jarret et al., 1991). Plants grown with the retardant maleic hydrazide at 5 mg/L had a survival rate of 70-90% after 6 mo (Desamero, 1990). Plants grown with cycocel at 500 mg/L had the same survival rate after 1 yr (Guo et al., 1995). But toxic effects were observed with both maleic hydrazide and cycocel. Using kinetine as a growth regulator, Guo et al. (1995) achieved a 70% survival rate in 20 accessions after 1 yr in storage, but with some callus presented. Plants growing in MS medium diluted to 30% and 50% showed low survival (36-48%) after 4 mo in storage (Abreu et al., 1992; Aguilar and Lopez, 1993).

Sweetpotato plantlets can remain in 10-40 g/L sorbitol for 6-12 mo without subculture (Desamero, 1990; Acedo, 1993; Cubillas, 1997). They resume normal growth when placed in a culture medium without osmoticum. Sorbitol, however, can be metabolized by the plantlets after some months of storage and exhibit an incremental growth rate, effectively reducing storage time. Mannitol at 20-40 g/L has also been used in sweetpotato to extend the interval between subcultures to 1 yr (Aguilar and Lopez, 1993; Acedo, 1993; Mandal and Chandel, 1990; Guo et al., 1995; Desamero, 1990) but some callus formation and vitrification was reported.

Tropical crop species usually lose their viability at temperatures lower than those required for growth in the field.

Sweetpotato, however, can continue to grow at temperatures several degrees below normal field temperature. Sweetpotato plants grown at temperatures below 15°C for long periods have shown detrimental effects such as phenolization and necrosis (Desamero, 1990). CIP research has shown that temperatures of 16-18°C lengthen the storage period up to 1 yr in cultures containing 2% sorbitol. However, some detrimental effects were produced in 25% of accessions in the in vitro sweetpotato collection. Temperatures of 18-21°C are being tested. A culture medium containing 2% sorbitol, storage at 23-25°C, and 3,000 lux (Lizarraga et al., 1990) successfully maintained the sweetpotato collection at CIP 4-6 mo without subculturing. More recently, a new culture medium containing 2% sorbitol and 2% mannitol was tested using 30 accessions. Their survival rate was 82% after 16 mo of conservation (Table 3) (Cubillas, 1997). An evaluation of this method using several hundred accessions in the collection is under way.

Procedures for Optimum In Vitro Germplasm Conservation

In vitro conservation entails the risk of losing material due to cooling equipment failure, contamination of cultures, or mislabeling accessions. Therefore, the following recommendations will help to maintain an in vitro collection more efficiently.

- Frequently evaluate in vitro growth during the first month to detect plants with growth problems.
- Maintain aseptic conditions in the culture growth room to avoid sources of contamination (dust, dirt, mites, or contaminated material). Treat the room as a restricted area to prevent contamination.
- Equip the in vitro laboratory with an electronic alarm connected to a control panel that monitors environmental conditions.
- Develop a database with unique codes for each accession in the collection. Each accession should have at least two identification numbers for proper identification. Labels for the cultures should be generated directly from the database to prevent human errors while transcribing.
- Closely monitor the cultures with some frequency during in vitro storage. Isolate contaminated cultures as soon as they are detected to prevent spreading the problem to clean plantlets. Include specific antibiotics in culture media to

Table 3. Percentage of plant survival evaluated after 22 months of storage with three teatments of a modified culture medium for medium-term storage of sweetpotato.

Mo	2% mannitol 2% sorbitol	1.5% mannitol 2% sorbitol	2% sorbitol
6	100	100	100
8	98	96	94
10	92	88	84
12	87	82	79
14	85	77	70
16	82	70	61
18	76	63	53
20	71	57	43
22	66	51	37

eliminate endogenous bacteria in the cultures.

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