Original Research Article

In vitro Conservation of *Smallanthus sonchifolius* under Slow-Growth Conditions

Iva Skalova[1], Iva Viehmannova[1], Jan Vitamvas[2]

[1]*Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Czech Republic*
[2]*Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Czech Republic*

Abstract

The aim of this work was to optimize *in vitro* storage of yacon (*Smallanthus sonchifolius*) under slow-growth conditions. Medium-term conservation was tested on ½ MS supplemented with mannitol and sorbitol (1-3%), BAP (5 mg l^{-1}), or MS and ½ MS medium free of plant growth regulators. Within 60 days, height of plants, number of nodes, number of roots and their length were measured in all of these treatments. The results revealed that there was a decline in plant growth along with the increase in the concentrations of mannitol (1.88, 1.49 and 1.07 cm for 10, 20 and 30 g l^{-1} mannitol) and sorbitol (3.66, 3.19 and 1.74 cm for 10, 20 and 30 g l^{-1} sorbitol), and along with the decrease of nutrient concentration in the cultivation medium (4.74 and 2.24 cm for MS and ½ MS medium). The survival of explants was found to be minimal at the highest concentrations of osmotic regulators (70% in mannitol and 50% in sorbitol) and on the medium supplemented with BAP (60%), while maximal on MS medium, ½ MS medium and on ½ MS medium with addition of 10 g l^{-1} mannitol (in all cases 100%). On full-strength MS medium and medium with addition of BAP plant growth was rapid, suggesting that these media could be rather used for multiplication of yacon. On the basis of our results it can be concluded, that for medium-term conservation of yacon can be satisfactory used ½ MS medium and media supplemented by 10 or 20 g l^{-1} mannitol or sorbitol.

Keywords: Germplasm; medium-term preservation; yacon; cultivation medium; 6-benzylaminopurine; mannitol; sorbitol.

INTRODUCTION

Yacon (*Smallanthus sonchifolius* Poepp. & Endl.) H.Rob is a root crop native to the Andean region of South America, grown mainly in Peru and Bolivia in an altitude range of 1 000-3 770 m above sea level. It belongs to family *Asteraceae* and it represents a traditional crop of the original population of Peru used in folk medicine. Yacon contains fructooligosaccharides in its tuberous roots and polyphenolic antioxidants in the whole plant (Grau and Rea, 1997). Genetic material of yacon is not possible to preserve in the form of seeds, because of their high sterility (Chicata, 1998). Thus, it must be maintained and propagated via vegetative parts.

Conservation of plant germplasm by *in vitro* technology can be done using slow growth procedures or cryopreservation. Slow growth is usually achieved by reducing the culture temperature, by modifying culture media with supplements of osmotic agents, growth inhibitors, or by removing growth promoters (Charrier et al., 1991; Withers, 1991; Engelmann, 1991; Malaurie et al., 1998). *In vitro* conservation of germplasm was reported for many root and tuber crops, such as potato and cassava (Engelmann, 1998) or yam (Malaurie et al., 1998). However, as far as *in vitro* conservation is concerned, no report on preservation of yacon has been published yet.

The present study aims to develop a proper protocol for storage of yacon in slow growth conditions using osmotic agents (mannitol, sorbitol), cytokinin (6-benzylaminopurine) and lower concentration of nutrients in culture medium.

MATERIALS AND METHODS

Plant material

For *in vitro* experiments landrace ´NZL I´ was used. It is maintained at Institute of Tropics and Subtropics, CULS Prague since 1993 when it had been bought from Auckland in New Zealand. It is a high-yielding clone, which has tuberous roots with delicious sweet flesh. According to Viehmannová (2009) and Fernández and Kučera (1997) landrace „NZL I" is octoploid with chromosome number 2n=58.

Propagation of plant material for establishment of experiment

Plant material for mid-term conservation was obtained from continuous *in vitro* multiplication by axillary and apical meristems. The cultures had been multiplied every 4 weeks and cultivated on MS medium (Murashige and Skoog, 1962) supplemented with 30 g l^{-1} sucrose and 100 mg l^{-1} *myo*-inositol. Under photoperiod 16/8 h (light/dark), temperature 25/23 °C and the intensity of light 2000 lx (fluorescent lamp NARVA LT 36 W/010).

Slow growth treatments

The effects of osmotic agents mannitol (MAN) and sorbitol (SORB), cytokinin 6-benzylaminopurine (BAP) and reduced concentration of nutrients in medium on the survival and growth of *in vitro* yacon cultures were investigated. MS medium was used as a control variant.
List of treatments:

1) MS
2) ½ MS
3) ½ MS with 10 g l^{-1} MAN
4) ½ MS with 20 g l^{-1} MAN
5) ½ MS with 30 g l^{-1} MAN
6) ½ MS with 10 g l^{-1} SORB
7) ½ MS with 20 g l^{-1} SORB
8) ½ MS with 30 g l^{-1} SORB
9) ½ MS with 5 mg l^{-1} BAP

Twenty millilitres of media solidified with 0.8% (w/v) agar was dispensed in test tubes (25 × 150 mm) covered with plastic caps and autoclaved at 121 °C and 100 kPa. Stem segments ca. 2 cm in length containing axillary meristem were placed on cultivation media. For each treatment 10 repetitions were used. During the experiment, test tubes with plant material were placed in cultivation box and maintained under photoperiod 16/8 h (light/dark), temperature 25/23 °C and the intensity of light 2000 lx (fluorescent lamp NARVA LT 36 W/010).

Height of plants, number of nodes, number and length of roots were measured every 14 days for 60 days.

Statistical evaluation

The data from all experiments were analyzed using analysis of variance (ANOVA) and the least significant ($P < 0.05$) differences among mean values were estimated using Tukey´s HSD test [StatSoft STATISTICA 9.0]

RESULTS AND DISCUSSION

The important aspect for *in vitro* conservation is slow growth of plantlets and satisfactory survival after the conservation.

In our experiment, the osmotic regulators mannitol and sorbitol used at 1, 2 and 3% concentrations, significantly affected survival and growth of yacon plantlets. In general, a decrease in plant height and survival was observed with the increase of concentrations of mannitol and sorbitol, although the differences among particular concentrations were not statistically significant (Table 1). Similar responses of explants have been observed also by Tehrim and Sajid (2011) after sorbitol and mannitol treatments in *Vitis vinifera*, and by Lata et al. (2012) using the same osmotic agents in *Cannabis sativa*. Our results are also in accordance with study of da Silva and Scherwinski-Pereira (2011), where it had been reported that high concentrations may be harmful and cause plant death. Also in *Cedrus atlantica* and *C. libani* 6% mannitol in storage medium led to low survival and sprouting (Renau-Morata et al., 2006). On the contrary, Sarkar and Naik (1998) suggested that 2-4% mannitol could enhance survival of plant germplasm conserved *in vitro*.

Table 1: Characteristics of *Smallanthus sonchifolius* after *in vitro* conservation

Treatment	Survival (%)	Height of plants (cm)	Number of nodes	Number of roots	Length of roots (cm)
10 g l^{-1} MAN	100	1.88 ± 0.53abc	2.89 ± 0.42a	4.77 ± 0.69ab	4.26 ± 1.17ab
20 g l^{-1} MAN	90	1.49 ± 0.53ab	3.50 ± 0.45ab	4.33 ± 0.69ab	5.80 ± 1.17abc
30 g l^{-1} MAN	70	1.07 ± 0.53a	3.00 ± 0.42a	5.56 ± 0.69ab	2.13 ± 1.17a
10 g l^{-1} SORB	90	3.66 ± 0.53bcd	3.67 ± 0.42ab	2.38 ± 0.74a	5.66 ± 1.24abc
20 g l^{-1} SORB	80	3.19 ± 0.56abcd	4.63 ± 0.45ab	3.88 ± 0.74ab	6.21 ± 1.24abc
30 g l^{-1} SORB	50	1.74 ± 0.70abc	3.00 ± 0.57a	2.20 ± 0.93a	3.26 ± 1.57ab
5 g l^{-1} BAP	60	3.98 ± 0.53cd	5.33 ± 0.42b	2.57 ± 0.79a	9.21 ± 1.33bc
MS	100	4.74 ± 0.53d	4.67 ± 0.42ab	2.78 ± 0.69a	10.97 ± 1.17c
½ MS	100	2.24 ± 0.53abc	3.89 ± 0.42ab	6.11 ± 0.69b	3.93 ± 1.17ab

*Mean ± standard error

**Means in each column followed by the same letter (a, b, c, d) are not significantly different at $P < 0.05$ according the Tukey´s HSD test

*** Abbreviations: MAN - mannitol, SORB - sorbitol, BAP - 6-benzylaminopurine, MS –cultivation medium Murashige and Skoog (1962)

Comparing the influence of mannitol and sorbitol on plant growth, it could be suggested that mannitol decreases growth more than sorbitol, even thought this tendency is not statistically significant (Table 1). Likewise, in *Vitis vinifera* mannitol proved slightly better than sorbitol in iducing growth retardation (Tehrim and Sajid, 2011).

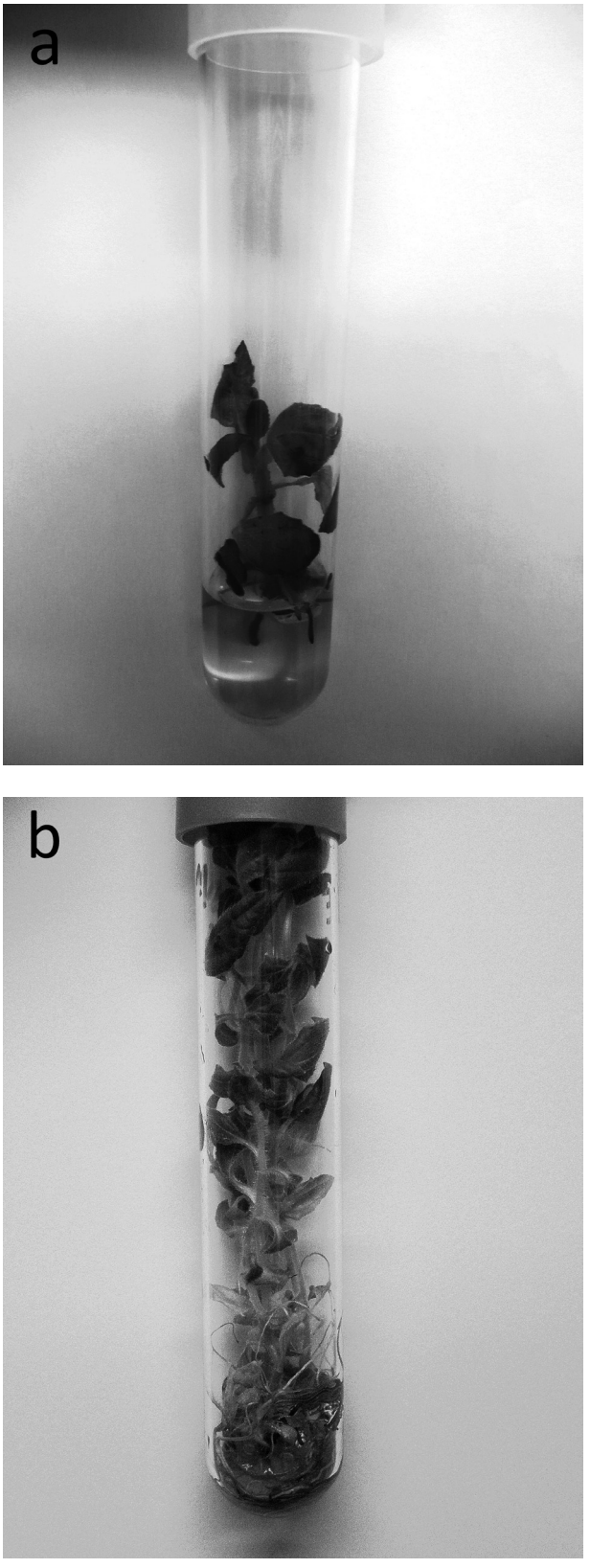

Figure 1: Comparison of yacon plantlets after medium-term conservation: a) on ½ MS medium; b) on MS medium

Media MS and ½ MS provided absolutely vital plantlets at the end of experiment (Table 1). A reduction of nutrient concentration significantly decreased height of plantlets cultivated on ½ MS when compared with plants on full strength MS medium (Fig. 1a, b).

The addition of 5 mg l^{-1} BAP in the ½ MS medium resulted in fast growth of plantlets and undesirable rapid development of plants for conservation purposes. Moreover, plantlets filled the culture vessel within 6 weeks and this overgrowth led to drying up of the cultures. On this medium, plants could be stored for a maximum of 40 days with 100% survival. Therefore, this medium should be rather used for multiplication of plant material. By contrast, for *in vitro* conservation of *Elettaria cardamomum* BAP was successfully used (Tyagi et al., 2009). Da Silva and Scherwinski-Pereira (2011) in *Piper aduncum* and *P. hispidinervum* used other plant growth regulator, abscisic acid, and achieved depressed shoot growth. The plant survival was however negatively affected.

Number of nodes was significantly influenced neither by osmotic agents, nor nutrient concentration. Rather higher number of nodes was produced only by plants on medium with 5 mg l^{-1} BAP, the difference compared to most other treatments, however, was not statistically significant.

Plantlets growing on ½ MS medium, media with all concentrations of mannitol, and 20 g l^{-1} sorbitol produced more roots per plant compared to the other treatments, while mean length of roots on MS medium and ½ MS medium supplemented by 5 mg l^{-1} BAP was higher in comparison with above mentioned media.

On the basis of stem, callus was frequently produced. Nevertheless, it did not have a negative effect for conservation of germplasm. During the experiment, no morphological changes were observed in plants of all treatments. Similar results were reported also in *Cedrus* spp. (Renau-Morata et al., 2006), where slow-growth storage did not cause phenotypical changes, and the genetic stability was confirmed by molecular markers.

In conclusion, medium with decreased nutrient concentration (½ MS) or media supplemented with lower concentrations of osmotic agents (10 or 20 g l^{-1} mannitol or sorbitol) can be successfully used for slow growth of yacon plantlets, maintaining high survival rate and low plant height after the 60 days conservation. For rather longer preservation of plant material, ½ MS medium could be recommended, not only for already mentioned advances but also from the economical point of view (lower nutrient concentration, absence of expensive osmotic agents).

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Corresponding author:

Iva Skálová
Czech University of Life Sciences Prague
Institute of Tropics and Subtropics
Kamýcká 129, 165 21 Prague 6
Czech Republic
E-mail: iva.skalova@seznam.cz