



SLOW-GROWTH IN VITRO CONSERVATION OF ULLUCUS TUBEROSUS (LOZ.), AN ANDEAN TUBER CROP

Stacy Hammond¹, Iva Viehmannova¹, Jiri Zamecnik²

¹Czech University of Life Sciences Prague, Faculty of Tropical AgriSciences, Department of Crop Sciences and Agroforestry, Czech Republic ²Crop Research Institute, Plant Physiology and Cryobiology Laboratory, Czech Republic

ABSTRACT

Ullucus tuberosus (Loz.) of the Basellaceae family is a perennial tuberous crop, originating from the Andean region where it is cultivated for its edible tubers. The species is vegetatively propagated, which causes standard conservation of its genetic material difficult to perform using conventional methods. An efficient tool for conservation of vegetatively propagated plants represents slow-growth in vitro method that ensures the genetic resources secure maintenance and availability for farmers and plant breeders. This study focuses on optimizing the slow-growth in vitro protocol for medium-term conservation of the species using MS storage culture media supplemented with three osmotic agents at different concentrations: sorbitol (1, 2 and 3%, w/v), mannitol (1, 2 and 3% w/v), and sucrose (1, 3, 6, 9 and 12% w/v); or abscisic acid (ABA) (1, 2 and 3 mg/l). MS without osmotics and hormone was used as control. Forty plants per treatment including control was established, twenty were maintained at growing temperature of 5 °C and twenty at 17 °C. Shoot growth (cm), number of leaves, shoots and numbers of roots were evaluated for four consecutive months as a primary study within a longer period experiment. Results showed that the appropriate temperature for growth reduction while maintaining the vitality of plants for medium-term conservation of the species is 5 °C, while temperature of 17 °C is not appropriate as the plants grow at an exponentially higher rate. Mannitol 3%, sucrose 12 and 9%, sorbitol 3% and ABA 3 mg/l concentration yielded the best results in terms of growth reduction and maintenance of plant vitality during conservation when compared to the control and therefore appears to be the most effective for medium-term conservation of the species. However, further evaluation will be carried out over a 12 months period to determine what supplement is most effective in time and to have a fully optimised protocol. Conservation of this species is important, as it will ensure the constant availability of the species for both farmers and plant breeders.

METHODOLOGY

To carry out the experiment on medium term conservation of ulluco (Ullucus tuberosus), various osmotic agents at different concentrations such as sorbitol (1, 2 and 3%, w/v), mannitol (1, 2 and 3% w/v), and sucrose (1, 3, 6, 9 and 12% w/v) were used; abscisic acid (ABA) at concentrations (1, 2 and 3 mg/l) was tested as well. Half-strength MS (Murashige and Skoog, 1962) medium containing 30 g/l sucrose, 100 mg/l myo-inositol and pH adjusted to 5.7 was used as a basal culture medium for all treatments. MS without osmotics and hormone was used as control. Forty plants per treatment including control were established in culture tubes containing 15 ml of medium to carry out the experiment. Twenty plants were maintained at growing temperature of 5 °C and twenty at 17 °C to determine the influence of temperature on the growth of the plants. Growth and development of plants were evaluated each month for four consecutive months as a primary study within a longer period experiment. Shoot growth (cm), number of leaves, shoots and numbers of roots were evaluated. Statistical analysis of the collected data was performed using one-way ANOVA and Turkey's HSD test (P \leq 0.05) [StatSoft STATISTICA 12.0].





Figure 1. Root, leaf and shoot production after 4 months of in vitro storage under 17 °C



Figure 2. Root, leave and shoot production after 4 months of in vitro storage under 5 °C

CONCLUSION

Preliminary results showed that mannitol 3% followed by sucrose 12 and 9%, sorbitol 3%, and ABA at concentration 3 mg/l proved to be optimal treatments for growth reduction and maintenance of plant vitality at 5 °C suggesting that these conditions are suitable for conservation of the species. However, further evaluation will be carried out over a 12-month period to determine what supplement is the most effective in time and to have a fully optimized protocol. Optimization of slow growth of ulluco plays an important role in medium term conservation of this species. This technology of conservation will ensure the constant availability of the species for both farmers and plant breeders.

RESULTS

The most efficient osmotic agent for growth reduction while maintaining plant vitality proved to be mannitol (Fig. 7). The highest concentration tested, i.e., 3% when combined with a lower temperature of 5 °C inhibited the growth of plants at a significantly higher rate (Fig. 5) than those at lower concentrations and higher temperatures of 17 °C. Mannitol 3% also had a strong inhibitory effect on the production of roots, leaves and shoots at lower temperatures (Fig. 2). Lower concentrations of 1 and 2% also decreased the growth rate of plants but were not as effective. Sorbitol provided comparable results on the shoot growth (Fig. 4) and root reduction (Fig. 2) at 5 °C, however, plants were dwarfed with dense nodal segments. This effect was observed only at a concentration of 3%. Sucrose efficiently inhibited the shoot growth at concentrations of 9 and 12%, while low concentrations caused plants to grow at a higher rate than control (Fig. 3). Slow growth was the most effective when particular treatments of sucrose were combined with lower temperatures. ABA at 3 mg/l proved to have inhibitory effects on the height of plants cultivated at 5 °C (Fig.6). The mentioned concentration of ABA also proved to have a strong inhibitory effect on the root, leaf and shoot production at 17 °C (Fig. 1).



Figure 7. Plants of ulluco after 4-month cultivation on various media

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REFFERENCES

Murashige T, Skoog FA. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum 15: 473-497.

Cruz Lapa G de la, Gomez ZR. 1995. Conservacion de germoplasma in vitro: Experiencias en Oca, ulluco y mashua en Ayacucho. 1. Congreso Peruano de Cultivos Andinos. Ayacucho, Peru.

Corresponding author: Stacy Hammond · hammondstacy9@gmail.com