MedCrave
Step into the World of Research

Horticulture International Journal

Short Communication

Open Access

CrossMark

Safeguard of plant germplasm through the in vitro culture

Volume 4 Issue 2 - 2020

Maurizio Micheli,[1] Francesco Prosperi,[1] Simona Facchin,[1] Daniel Fernandes da Silva[2]

[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy

[2]Universidade Estadual do Oeste do Paranà, Marechal Candido Rondon, Brasil

Correspondence: Maurizio Micheli, Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno74, Italy, Tel ++390755856260, Email maurizio.micheli@unipg.it

Received: April 16, 2020 | **Published:** April 24, 2020

Introduction

The last trends of the intensive cultivation systems need the employment of genotypes suitable to assure high productions; at the same time, large variations concerning the management of the most part of plant germplasm are required. All this involves a significant reduction of the native genetic resources consequently to the decreasing of cultivated species/varieties. Therefore, the biodiversity protection is considered the most important priority for the environment management and the development of a sustainable agriculture. Several recent activities are intended to preserve the genetic variability and to individuate effective and innovative tools for the plant germplasm safeguard. Increasing interest concerns the in vitro culture techniques for the ex situ conservation. For some years the application of the tissue cultures and the innovative encapsulation technology is carried out with that goal at the Department of Agricultural, Food and Environmental Sciences (University of Perugia). The former is applied through the axillary buds proliferation, by using uninodal vegetative explants or meristematic apexes (micropropagation). The encapsulation technology concentrates the easier management, handling, storability and transportability into reduced size products (capsules and/or synthetic seeds). Currently, both biotechnologies are employed to collect a considerable number of fruits, vegetables and ornamental genotypes.

Micropropagation

Plant tissue culture techniques, as micropropagation, are being widely used for large scale plant multiplication. By using a single explant several thousand plants can be proliferated in relatively short time period and space, under controlled environmental conditions, irrespective of the season and weather. Endangered and threatened plant genotypes have successfully been conserved by micropropagation in ex situ conditions.[1] The technique is based on the concept of totipotentiality of plant cells, that is the ability of a single cell to express the full genome by cell division and regeneration of tissues and whole organs.[2] Plant explants are usually cultured in aseptic conditions on artificial media containing all the nutrients required for the normal growth and development of plants: macroelements, microelements, vitamins, carbohydrates, growth regulators (auxins, cytokinins and gibberellins), other organic compounds and gelling agents. MS medium[3] is most extensively used for the micropropagation of a large part of plant species in vitro.[4] In vitro organ culture offers an alternative source for the conservation of endangered and rare genotypes. Tissue culture protocols can be used for preservation of vegetative tissues when the targets for conservation are clones instead of seeds, to keep the genetic background of a crop and to avoid the loss of the conserved patrimony.[5] Micropropagation is divided in five phases: selection and preparation of donor plants, decontamination and in vitro establishment, multiplication (or proliferation), rooting and acclimatization.

Several plant species have been studied and currently conserved at the Laboratory of Micropropagation and In Vitro Biotechnologies (LMIVB) of University of Perugia: olive, apple, mulberry, aquatic plants, kiwifruit, grape, pear, sequoia, lime tree, potato, holm oak, hop and myrtle. They are usually used for educational activities and experimental objectives, carried out with the aim to improve protocols and procedures of in vitro establishment (Figure 1), increase the multiplication rate (Figure 2) and maximize the rhizogenic ability (Figure 3) of genotypes.

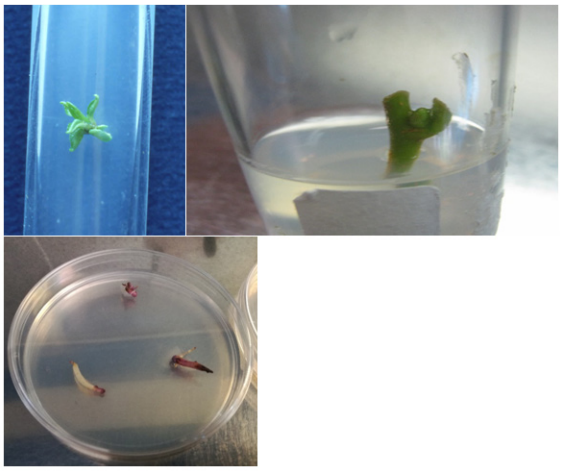

Figure 1 *In vitro* establishment of apple, hazel and potato (from left to right) (by M.Micheli).

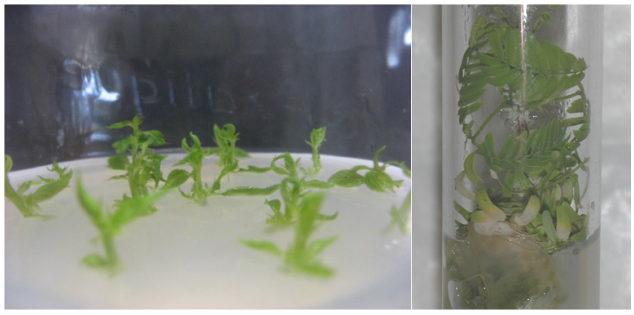

Figure 2 Proliferation of plum (left) and *Albiziajulibrissin* (right) (by M. Micheli).

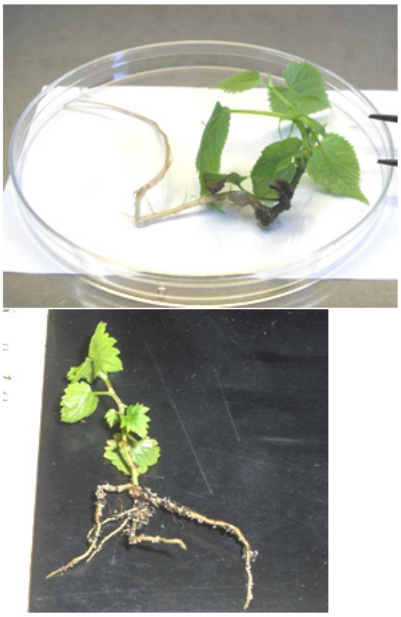

Figure 3 Rooted shoots of lime tree (left) and grape (right) (by M. Micheli).

Encapsulation technology

Recently different studies were carried out on the encapsulation, an innovative and promising technology for the germplasm conservation and the easy exchange of plants between nurseries, laboratories and Countries. The encapsulation technology concentrates the easier management, handling, storability and transportability of the reduced size products.

The protocols set up require the use of vitro-derived propagules (2-4 millimeters long) as unipolar explants (microcuttings) or bipolar propagules (as somatic embryos or microbulbs). They are covered by a gelled matrix with protective and nutritive functions.[6] The ionic complexation between the sodium alginate, solved into a nutritive matrix (artificial endosperm), and a solution of calcium chloride provides for the hardening of the explant coating, according to the 'egg box' model,[7] and gives the round shape to the final structure.

The products of the encapsulation are represented by capsules and synthetic seeds. The first ones can be defined as encapsulated portions of in vitro-derived plant tissues possessing the ability to evolve only in shoots (regrowth). They represent an effective tool for the exchange of elite and axenic plant material between laboratories due to its small size and relative easy handling, but also for the storage or the long-term conservation of plant germplasm. Synthetic seeds (or artificial seeds) instead are able to develop whole plantlets (conversion) under in vitro or ex vitro conditions. They can be the result of encapsulation of bipolar propagules or bipolarized microcuttings.[8] Synthetic seeds can be used like capsules for plant exchange, transport, storage and germplasm conservation, but also to grow plants in the nursery directly in ex vitro conditions reducing the time of acclimatization.[9]

Many experiences were conducted at our LMIVB of Perugia on encapsulation of several species/genotypes. Interesting results have been achieved optimizing the protocol of encapsulation (Figure 4), improving the regrowth of capsules (Figure 5) and increasing the conversion of synthetic seeds (Figure 6).

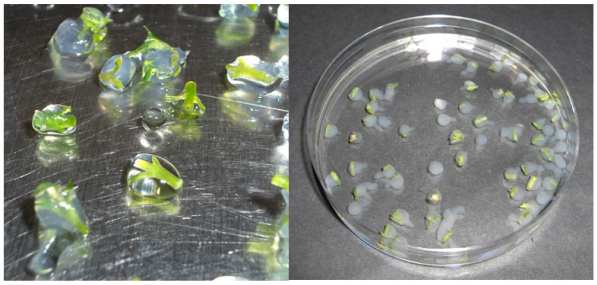

Figure 4 Encapsulated microcuttings of privet (left) and lavender (right) (by M. Micheli).

Figure 5 Regrowth of capsules of kiwifruit, peach and apple (from left to right) (by M. Micheli).

Figure 6 Conversion of synthetic seeds of *Rotalarotundifolia*, olive and *Narcissus* spp. (from left to right) (by M. Micheli).

The effort made on the development of encapsulation during these last three decades has made considerable progress. It has offered to researchers and nurseries an integrated package, including micropropagation for the production, commercialisation, exchange and conservation of plant germplasm. Now this technology cannot yet be used on a large scale, but its application can be suggested only for high value genotypes or plants easy-to-propagate. Many

Citation: Micheli M, Prosperi F, Facchin S, et al. Safeguard of plant germplasm through the in vitro culture. *Horticult Int J.* 2020;4(2):50–52.
DOI: 10.15406/hij.2020.04.00157

problems have to be overcome: high costs of manual labor, low rooting ability, control of contamination agents in no aseptic environment, low viability of encapsulated propagules during the long-term conservation. But a great number of the last studies on the encapsulation technology contributed to identify possible effective solutions, as the introduction of mechanical techniques for automating the processes, the use of specific Arbuscular Mycorrhyzal Fungi (AMF) for biotization of synthetic seeds, the application of Plant Growth Promoting Bacteria (PGPB) to increase vigour and growth of plantlets and consequent reduction of encapsulated propagules loss, the optimization of cryopreservation protocols.[6]

Acknowledgments

None.

Conflicts of interest

The authors declare have no conflict of interest about the publication of this paper.

References

1. Hussain A, Qarshi IA, Nazir H, et al. Plant tissue culture: current status and opportunities. In: Recent advances in plant in vitro culture. In: Leva A, Rinaldi LMR, editors InTech, Rijeka, Croatia. 2012.
2. Haberlandt G. Kulturversuche mit isolierten Pflanzenzellen. Sitzungsber. Akad. Wiss. Wien. Math.-Naturwiss. Kl Abt J.1902;111: 69–92.
3. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiol*. 1962;15: 473–497.
4. Bhojwani SS, Dantu PK. Plant tissue culture: an introductory text. Springer, New Delhi, India. 2013.
5. Tyagi RK, Agrawal A, Mahalakshmi C, et al. Low-cost media for *in vitro* conservation of turmeric (*Curcuma longa* L.) and genetic stability assessment using RAPD markers. In Vitro Cell. Develop. *Biol Plant*. 2007;43:51–58.
6. Standardi A, Micheli M, Encapsulation of *in vitro*-derived explants: an innovative tool for nurseries. In: Lambardi M, Ozudogru AE, et al. editors. Protocols for micropropagation of selected economically-important horticultural plants. *Series Methods in Molecular biology*. 2013;994:397–418.
7. Barbotin JN, Nava Saucedo JE, Bazinet C, et al. Immobilization of whole cells and somatic embryos: coating process and cell-matrix interaction. In: Synseeds: applications of synthetic seeds to crop improvement. In: Redenbaugh K. editor. CRC, Boca Raton, FL. 1993.
8. Micheli M, Standardi A, Fernandes da Silva D. Encapsulation and synthetic seeds of olive (*Olea europaea* L.): experiences and overview. In: Synthetic seeds: recent trends and future perspectives. In: Faisal M, Alatar AA. editors. Springer Nature, Switzerland. 2019.
9. Micheli M, Lambardi M. La tecnologia dell'incapsulamento e le sue applicazioni. *Italus Hortus*. 2019;26(3):3–13.