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MICROPROPAGATION AND ENCAPSULATION: USEFUL COMBINATION FOR NURSERIES

Maurizio MICHELI[1], Alvaro STANDARDI[2]

[1]University of Perugia, Department of Agricultural Food and Environmental Sciences,
Address: Borgo XX Giugno 74, 06121 Perugia (Italy), Tel. (++39) 075.5856260
[2]*Doctor Honoris Causa,* University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Address: Via G. Flamini 4, 06135 Perugia (Italy), Tel. (++39) 075.394522

Corresponding author e-mail: gastandardi@alice.it

Abstract

Encapsulation and micropropagation technologies were summarized and discussed as tools to exchange and to propagate valuable genotypes respectively. Each of these technologies shows advantages and problems for large and commercial diffusion whereas their integration represents a considerable innovation for the future nursery. In fact, uninodal microcuttings excised from in vitro proliferated axillary shoots or adventitious microshoots, both 3-4 mm long, can be encapsulated in a protective and nutritive covering after appropriates root induction treatments to obtain synthetic seeds. They are plant structures free from parasites, viruses included, and in several species able to convert in whole plantlets under in vitro *or* in vivo *conditions after storage and transport, like the zygotic seeds. In other words the combination of the micropropagation with the encapsulation permits to reach the advantages of both technologies in one tool (the synthetic seed) characterized by high cloning efficiency, perfect sanitary plantlet conditions, reduced space requirements and size, resistance to handling, storability, and transport. Some problems have to be solved before large scale diffusion of this innovative combination which can represents a promising tool for the future nurseries.*

Key words*: plant propagation, in vitro culture, synthetic seed, genotypes exchange.*

INTRODUCTION

It well know that the zygotic seeds are the natural organs for plant species conservation and dissemination on the territories and through time. Usually they are free from parasites, viruses included, and resistant to environmental stresses, manipulation and transport. Unfortunately they can't be employed for propagation and/or exchanging of cultivars, clones, and/or local woody ecotypes. In these situations the nurserymen have to employ one of cloning propagation methods like cutting, or grafting or layering to diffuse and to sure woody plant descent (Hartmann et al., 2002). But these methods require appropriate lot of mother plants which have to be cultivated adequately to maintain their genetic pureness and virus free status, as the national and international regulations impose. From some decades, in an increasing number of private nurseries the micropropagation techniques were introduced as innovative cloning methods because: (i) they are highly productive, (ii) requires a restricted amount of mother plants, and (iii) the production of new plantlets occurs in aseptic conditions. Moreover, the cloned plantlets are free from fungi, bacteria, parasites and viruses until they remain in the sterile conditions. In any case, the final products of cloning propagation methods consist in young plantlets, which are inadequate for conservation, commercialization and long distance transport because not resistant to sudden changes of temperature and water content. Moreover, the final stage of the *vitro*-derived plantlets occurs in pots or in soil where they can be infected by parasites and viruses (Standardi and Micheli, 2013).

Against these risks local and international regulations impose sanitary controls of the propagated plant material before their use in order to limit the diffusion of parasites. So, nurserymen are looking for a new procedure able to join the advantages of the micropropagation (high efficiency productive,

perfect sanitary plantlet conditions, reduced space requirements) with the characteristic of the zygotic seeds concerning size, resistance to handling, storability, and transportability (Micheli et al., 2003).

ENCAPSULATION TECHNOLOGY

From some years an increasing number of laboratories are involved in experiments concerning the encapsulation technology aimed to obtain the *synthetic seed* (or artificial seed or synseed or agamic seed), an innovative biotechnological product defined by Murashige (1978), as *an encapsulated somatic embryo*. The large number of studies aimed to find other suitable propagules allowed to extend the definition of the synthetic seed as an *artificially encapsulated* vitro-*derived somatic embryo, shoot bud or any* vivo-*derived plant tissues used as functionally mimic seeds for sowing and possessing the ability to evolve (to convert) into plantlet under* in vitro *or* ex vitro (in vivo) *conditions, which can retained even also after storage* (Bapat et al., 1987; Redembaugh, 1993; Rai et al., 2009).

A further product of the encapsulation technology is the *capsule* defined as *encapsulated portion of* vitro-*derived plant tissue possessing the ability to evolve (to re-growth) in shoots which can be reused only for micropropagation after storage and/or transport* (Standardi and Micheli, 2013).

The encapsulation procedure requires sterile conditions and includes three steps: coating, complexation, and rinsing. The single encapsulated propagules (few millimeters long) are enclosed in a spherical matrix covering them for 1-2 mm and containing nutritive compounds (*artificial endosperm*) suitable to maintain: (i) *viability* or green color, with no necrosis or yellowing along the period between encapsulation and use that included storage and commercialization; (ii) *re-growth* or growth of explants with consequent breakage of the encapsulating matrix and extrusion of at least one small shoot or root after sowing and (iii) *conversion* or contemporary growth and extrusion of epigeous and hypogeous organs with a vascular connection between shoot and root. This term correspond to germination used for gamic or zygotic seed (Carlson and Hartle, 1995; Gardi et al., 1999).

Besides the nutritive function, the encapsulating matrix protects the enclosed plant tissue against mechanical damages during manipulation, transport and storage because it resists to pressure of 1-2 Kg depending on the concentration of the encapsulating compound usually represented by sodium alginate (Barbotin et al., 1993). Moreover both capsule and synthetic seed can be stored for long period by cryopreservation or for short and medium time in refrigerator at 2-8 °C depending from the species (Engelmann, 1997; Micheli et al., 2007; Micheli et al., 2008; Lambardi et al., 2009). So, these characteristics make the encapsulated plant materials like to the zygotic embryos.

At present the encapsulation technology is enough expensive because requires intense manual labor but some machineries for preparation and encapsulation of the single propagules are under experimental evaluation. Essentially they have to perform the following three functions: (i) excision of the single and small propagule from the vegetative mass, (ii) dipping of each one into the encapsulation solutions, and (iii) complete covering of the plant material into the encapsulating matrix (Sakamoto et al., 1995; Brischia et al., 2001).

MICROPROPAGATION

It is well known that the micropropagation is a very productive technology which permits to obtain plantlets: (i) in aseptic conditions, (ii) in growth chamber with controlled light and temperature and, (iii) in appropriate nutritive formulations. In these conditions no risks of parasites and virus aggression during the plant regeneration process occur. Moreover, possible presence of virus in the donor plants can be eliminated if the size of the initial meristem is very small, about half millimeter or less (Micheli et al., 2008).

According to morphogenetic pathway used for *in vitro* plant propagation, the products of the micropropagation are: (i) somatic embryos (bi-polar organs) obtained by embryogenesis and potentially able to convert in plantlets, (ii) adventitious shoots (uni-polar organs) obtained by organogenesis from the initial plant tissue

and, (iii) axillary shoots (uni-polar organs) obtained by proliferation of the initial buds or meristematic tissues.
The somatic embryos possess both root and shoot apex and adequate size to be encapsulated for the synthetic seed production; nevertheless their use in the nursery activity is not suggested because of somaclonal variations can arise during the *in vitro* embryogenesis process (Donnelly and Vidaver, 1988; Gray et al., 1995).
Both adventitious or axillary shoots as unipolar explants have to be submitted to rooting inductive treatments to obtain whole plantlets which become autotrophic and marketable after acclimatization in soil or in pots and have to face possible virus infections (Standardi and Micheli, 2013). To avoid this risk and to sure the sanitary plant conditions required for local and international exchanges it is recommended to commercialize the micropropagated plantlets just at the end of the rooting step, inside the aseptic jars, on sterile media. But at this stage the plantlets are quite sensitive to water and temperature stresses and the risk of survival loss is very high, especially for long distance exchanges.

PERSPECTIVES FOR THE SYNTHETIC SEED

At the moment suitable *vitro*-derived explants for synthetic seeds production are considered both nodal portion (*microcutting*) excised from proliferated axillary shoots or adventitious ones at the *rosetta* stage, both 4-5 mm long. These propagules are able to re-growth in shoots spontaneously (uni-polar), whereas the synthetic seed is that if the encapsulated plant material develops a whole plantlet (*conversion*) after sowing (Bapat et al., 1987; Bapat, 1993; Redembaugh, 1993; Rai et al., 2009). So, they have to be submitted to appropriate inductive treatments before encapsulation. Several experiments were conducted to examine the effects of some treatments showing positive results in many woody fruit, medical and ornamental plants (Brischia et al., 2001; Sandoval-Yugar et al., 2009; Iklaq et al., 2010). Currently it is possible to suppose that the treatments to improve rooting and conversion of the synthetic seeds can be applied just before sown, like those to the zygotic seeds in the nursery (Harmann et al., 2002).
The most part of the experimentation concerning the conversion was carried out sowing the synthetic seeds on agar medium and in sterile conditions, appropriate only for exchanging of valuable genotypes between laboratories, even if it should be allowed also in *ex vitro* (or *in vivo*) conditions to satisfy completely the definition of synthetic seed. At this purpose promising results were obtained by addition of antimicrobials or fungicides to the sowing medium or to the encapsulating matrix (Germanà et al., 2007). Also the use of sowing substrates alternative to agar were essayed as jiffy, perlite, compo-cactea® and soil-mix with encouraging results (Saiprasad, 2001; Ray et al., 2009).
The combination of the *in vitro* plant regeneration with the encapsulation procedure seems to be a valuable perspective for propagation and exchange of high quality plant material but it is not immediately available for large diffusion in the nurseries, because some problems have to completely solved, as: (a) the poor conversion percentage both *in vitro* or *in vivo* in some species, (b) the full control of fungal and bacterial contamination in non aseptic environment, (c) the leaching of nutrient and dehydration risks from the coating during commercialization and storage, and (d) the reduction of manual labor for propagule preparation and encapsulation (Standardi and Micheli, 2013).

CONCLUSIONS

Research aimed to find solutions for these applicative problems is strongly recommended because the full value of the two technologies (encapsulation and micropropagation) increases when their integration will be reached. In fact, at moment the application of the encapsulation technology alone is limited to exchange plant material between laboratories involved on *in vitro* culture.
At the same time, micropropagation is very efficient as cloning method, but unable to assure the production of plantlets certainly virus free. Combining of micropropagation and encapsulation could produce an innovative technology, already detectable in nature from

time immemorial, when in autumn we can observe the spontaneous formation of plantlets on the falling leaves. During the winter, the leaf lamina protects and feeds the plantlet as our encapsulating matrix.

So, our innovative proposal is only one more example of "copy and paste" the nature!

REFERENCES

Bapat V.A., Mhatre M., Rao P.S., 1987. Propagation of *Morus indica* L. (mulberry) by encapsulated shoot buds. Plant Cell Report, 6: p. 393-395.

Bapat V.A., 1993. Studies on synthetic seeds of sandalwood (*Santalum album* L.) and mulberry (*Morus indica* L.). In: Redenbaugh K. (Ed.), Synseeds: Applications of Synthetic Seeds to Crop Improvement. CRC Press Inc, Boca Raton, California.

Barbotin J.N., Nava Saucedo J.E., Bazinet C., Kersulec A., Thomasset B., Thomas D., 1993. Immobilization of whole cells and somatic embryos: coating process and cell-matrix interaction. In: Redenbaugh K. (Ed.) Synseeds: Applications of Synthetic Seeds to Crop Improvement. CRC Press Inc, Boca Raton, California.

Brischia R., Piccioni E., Standardi A., 2001. Micropropagation and synthetic seed in M.26 apple rootstock (II): A new protocol for production of encapsulated differentiating propagules. Plant Cell Tissue Organ Culture, 68: p. 137-141.

Carlson W.C., Hartle J.E., 1995. Manufactured seeds of woody plants. In: Jain S.M., Gupta R.K., Newton R.J. (Eds.), Somatic Embryogenesis in Woody Plants, Vol. 1 History, molecular and biochemical aspects and applications. Kluwer Academic Publishers, Dordrecht.

Donnelly D.J. and Vidaver W.E., 1988. Somaclonal variation. In: Dudley T.R., (Ed.), Advances in Plant Science Series, Vol. 3, Glossary of Plant Tissue Culture. Dioscorides Press, Portland, Oregon.

Engelmann F., 1997. In vitro conservation methods. In: Ford-Lloyd B.V., Newbury J.H., Callow J.A. (Eds.), Biotechnology and plant genetic resources: conservation and use. CAB International, Wallingford, UK.

Gardi T., Piccioni E., Standardi A., 1999. Effect of bead nutrient composition on regrowth ability of stored vitro-derived encapsulated microcuttings of different woody species. Journal of Microencapsulation, 16(1): p. 13-25.

Germanà M.A., Hafiz A.I., Micheli M., Standardi A., 2007. Preliminary research on conversion of encapsulated somatic embryos of *Citrus reticulata* Blanco, cv. Mandarino Tardivo di Ciaculli. Plant Cell Tissue Organ Culture, 88: p. 117-120.

Gray D.J., Compton M.E., Harreli M.C., Cantliffe D.J., 1995. Somatic embryogenesis and the technology of synthetic seed. In: Biotechnology in Agriculture and Forestry, 30: Somatic embryogenesis and Synthetic seed I. Springer-Verlag, Berlin Heidelberg.

Hartmann H.T., Kester D.E., Davies F.T., Geneve R., 2002. How plant propagation evolved in human society. In: Pearsons Education Inc. (Ed.), Hartmann and Kester's Plant Propagation: principles and practices. Prentice-Hall, Upper Saddle River, New Jersey.

Ikhlaq M., Hafiz I.A., Micheli M., Ahmad T., Abbasi N.A., Standardi A., 2010. *In vitro* storage of synthetic seeds: Effect of different storage conditions and intervals on their conversion ability. African Journal of Biotechnology, 9(35): p. 5712-5721.

Lambardi M., Benelli C., Caboni E., Engelmann F., 2009. Potenzialità dei semi sintetici per la crioconservazione di specie vegetali. Proceedings Giornata di Studio sulla Tecnologia dell'incapsulamento, Perugia 9 novembre.

Micheli M., Gardi T., Standardi A., 2003. La tecnologia dell'incapsulamento per la diffusione e/o la conservazione di materiale vivaistico. Italus Hortus, 10(4): p. 259-262.

Micheli M., Hafiz I.A., Standardi A., 2007. Encapsulation of *in vitro*-derived explants of olive (*Olea europaea* L. cv. Moraiolo). II. Effects of storage on capsule and derived shoots performance. Scientia Horticulturae, 113: p. 286-292.

Micheli M., Standardi A., Stanica F., 2008. The technology of *in vitro* encapsulation for propagation, exchange and storage of plant genotypes. Proceedings International Scientific Symposium on "Modern agriculture achievements and perspectives", Chisinau, Moldova, 21-23 October.

Murashige T., 1978. The impact of tissue culture in agriculture. In: Thorpe A. (Ed.), Frontiers of Plant Tissue Culture. International Association for Plant Tissue Culture, Calgary.

Rai M.K., Asthana P., Singh S.K., Jaiswal V.S., Jaiswal U., 2009. The encapsulation technology in fruit plants: A review. Biotechnology Advances, 27: p. 671-679.

Redenbaugh K., 1993. Introduction. In: Redenbaugh K. (Ed.), Synseeds: Applications of Synthetic Seeds to Crop Improvement. CRC Press Inc., Boca Raton, California.

Sakamoto Y., Onishi N., Hiroshima T., 1995. Delivery system for tissue culture by encapsulation. In: Aitken-Christie J., Kozai T., Smith M.A.L. (Eds.), Automation and Environmental Control in Plant Tissue Culture. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Saiprasad G.V.S., 2001. Artificial seeds and their applications. Resonance 6(5): p. 39-47.

Sandoval-Yugar E.W., Dal Vasco L.L., Steinmacher D.A., Stolf E.C., Guerra M.P., 2009. Microshoots encapsulation and plant conversion of *Musa sp*. cv. 'Grand Naine'. Ciencia Rural, 39(4): p. 998-1004.

Standardi A., Micheli M., 2013. Encapsulation of *in vitro*-derived explants: an innovative tool for nurseries. In: Lambardi M., Ozudogru E.A., Jain S.M. (Eds.), Protocols for micropropagation of selected economically-important horticultural plants. Springer, New York.