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Preliminary Evaluation of the Encapsulation Technology Used for Local Fruit Trees Germplasm Safeguarding

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Abstract: The encapsulation in a nutritive matrix is an effective technique which allows the conservation of *vitro*-derived explants and protects their viability. The encapsulated propagules are generated by submerging explants in a natural polysaccharide solution of sodium alginate and calcium chloride allowing to obtain uniform capsules. In this work, we present preliminary results of a study on the effect of an encapsulation protocol on any local fruit varieties, conserved in a Regional *in vitro* germplasm bank.

Key words: Encapsulation, *in vitro*, local variety, germplasm safeguarding.

1. Introduction

The encapsulation technology consists of the inclusion of some millimeters long *in vitro*-derived plant explants in a nutritive and protective matrix (*artificial endosperm*). It permits to maintain the viability and the ability to develop new shoots (*regrowth*) of the propagules even after storage [1]. Encapsulating matrix is composed by a natural polysaccharide, like alginate. Spherical capsule formation occurs through the polysaccharide solidification, in a process of "ionic exchange" (complexation) that is not detrimental to the explants. Actually, the ionic exchange is the most diffused method and sodium alginate is the most used encapsulating agent. Thanks to some sodium alginate properties, as moderate viscosity and speed of solidification, it possible to obtain uniform capsules of 4-6 mm diameter. The gelification is allowed replacing Na^{+} by Ca^{++} forming calcium alginate. The encapsulation is used also for plant germplasm safeguard through both cryopresevation [2, 3] and storage at low temperature (0-4 °C) [3, 4]. The aim of this study was to verify the possibility to use the encapsulation technology as a local fruit trees germplasm safeguarding tool.

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2. Materials and Methods

In this work three local fruit genotypes were studied: var. *A sonagli* (apple), var. *Marzaiola* (pear) and var. *Montebello* (plum). They are currently micropropagated and collected in the Laboratory of the Regional Germplasm Bank at the 3A Technology Park (3A-PTA) at Pantalla (Perugia, Central Italy).

At the end of the multiplication subculture of each genotype, uninodal microcuttings (3-4 mm long portions without leaves and with one or two axillary buds) were excised by the proliferated shoots and a part of them was immediately encapsulated, according to the procedure suggested by [1]. Thereby, each microcutting was dipped for few seconds into a sodium alginate (25 g/L) encapsulating solution, enriched with an artificial endosperm composed of half-strength MS basal medium, supplemented with 50 g/L of sucrose pH 5.5. In order to give hardness to the alginate coverage, the coated microcuttings were dropped into a calcium chloride solution (11 g/L) for 30 minutes. After complexation, the capsules were washed in a rinse solution, consisting of the artificial

endosperm without further additions, three times (15 minutes), in order to remove the toxic residual ions of chloride and sodium (Fig. 1).

Subsequently a control line (Th. A, not encapsulated microcuttings) was set up in comparison to four lines characterized by the different periods of storage (in liquid medium specific for each species and at 4 °C and in dark conditions) of capsules: 0 (Th. B), 30 (Th.1), 90 (Th.2) and 180 (Th.3) days. Then, all propagules were sown inside Magenta® vessels (7×7×10 cm) (4 vessels per thesis), each containing 5 propagules and 50 mL of the proliferation medium without growth regulators. All vessels were kept into the growth chamber at 23 ± 1 °C of temperature and photoperiod of 16 hours.

After 30 days and then additional 10 days, the viability (percentage of green colour microcuttings, without necrosis or yellowing appearance) and the regrowth level were monitored [5].

In addition, the clumps developed after 40 days from each microcutting (naked or encapsulated) were placed in Magenta® vessels containing a proliferation medium specific for each species. After further 40 days in growth chambers, the multiplication activity of shoots was monitored recording the following parameters: number of developed shoots, number of nodes and/or internodes, number and length of developed roots and callus production. Moreover other morphological aspects from explants were monitored (Fig. 2 and 3).

In all experiments data were analyzed by Student's t-test (NS, $P > 0.05$).

3. Results and Discussion

All propagules showed the highest level of viability (data not shown). The results of vegetative growth (regrowth) from three different varieties are reported in Table 1.

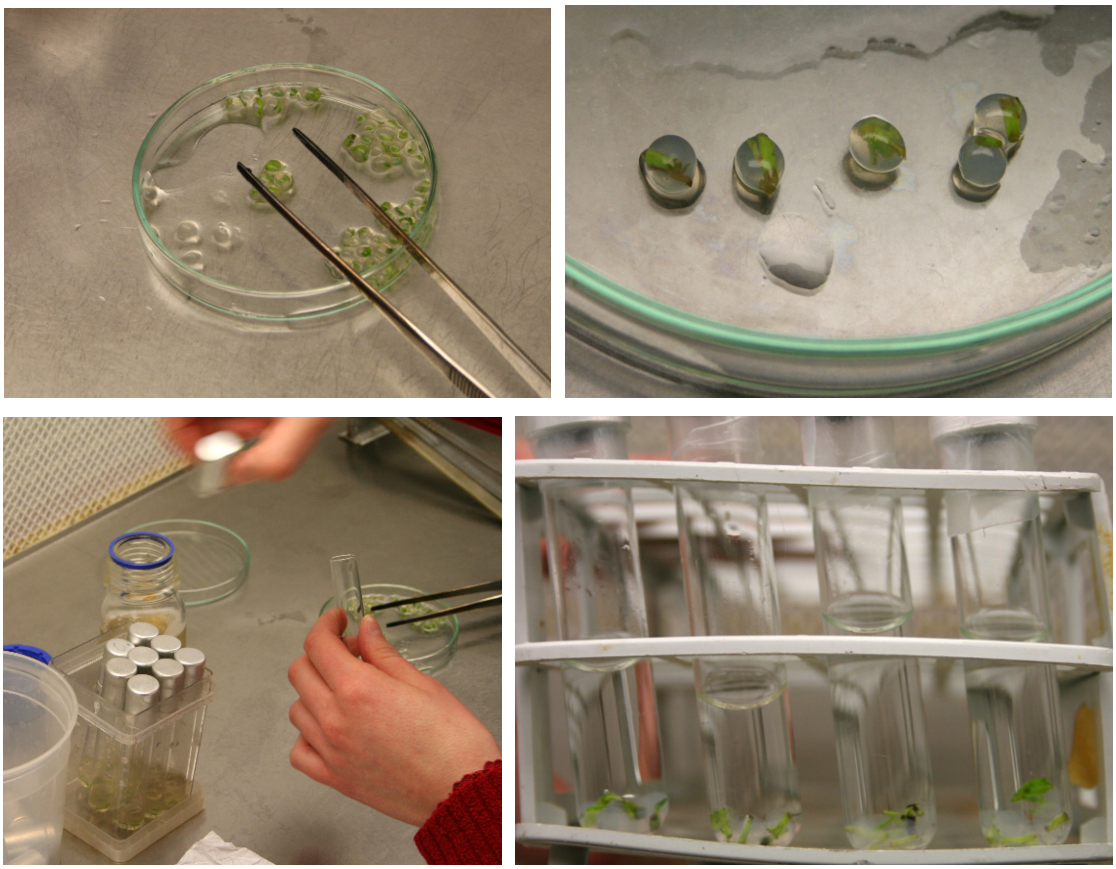

Fig. 1 Phases of capsules preparation.

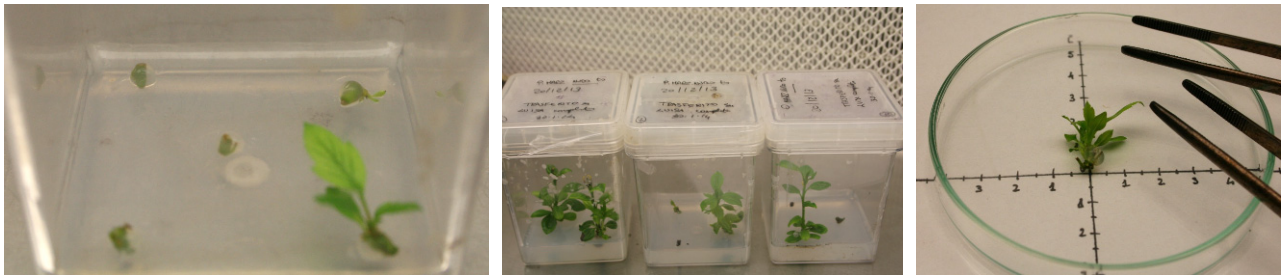

Fig. 2 Apple, Thesis 1: regrowth of capsules during the sowing on agarized medium (in the left); shoots during the multiplication phase (in the middle); clump proliferated after 40 days (in the right).

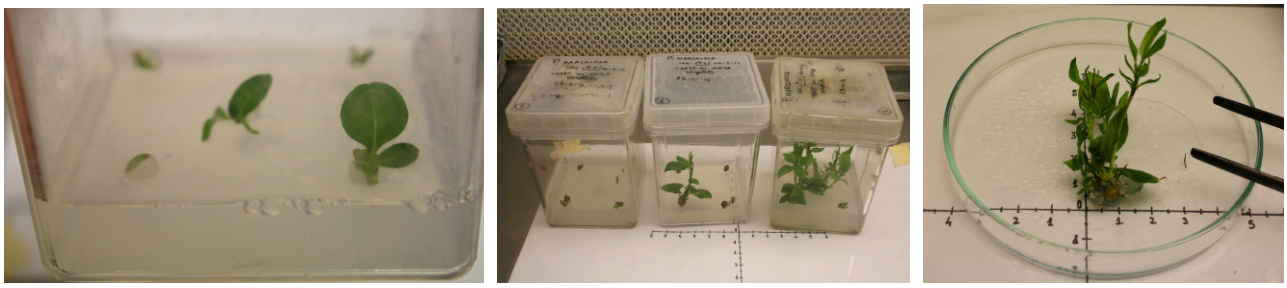

Fig. 3 Pear, Thesis 2: regrowth of capsules during the sowing on agarized medium (in the left); shoots during the multiplication phase (in the middle); clump proliferated after 40 days (in the right).

Table 1 Average values of regrowth (%) of the naked and encapsulated microcuttings.

Thesis	Apple cv. A sonagli	Pear cv. Marzaiola	Plum cv. Montebello
A (naked microcuttings)	58.3	50.0	41.6
B (not stored capsules)	55.0	75.0	100.0
1 (30 days of storage)	70.0	63.1	55.0
2 (90 days of storage)	73.6	100.0	15.0
3 (180 days of storage)	79.2	56.0	32.0

Apple cultivar benefited from the effect low temperature, which increased the regrowth of capsules from 55.0 % (B) to 79.2 % after 180 days of storage.

In pear, the maximum value of regrowth was monitored in thesis 2 (90 days of storage) while all encapsulated microcuttings showed higher vegetative activity than the control (thesis A).

Particular results were registered in relation to the plum cultivar (‘Montebello’) because the highest vegetative activity (100.0%) was recorded in thesis B, without storage, sowing the propagules immediately after the encapsulation. Moreover, the longer periods of storage at 4 °C (90 and 180 days) seem to reduce dramatically the regrowth.

Nevertheless it should be pointed that in plum the composition of the storage liquid medium, containing pectine, showed to be altered because of its anomalous density due to the cooling. It’s possible to suppose that a nutritive deficit could have conditioned the microcuttings viability in these two thesis.

In Table 2 the performances observed at the end of the subsequent multiplication phase are reported.

The average number of proliferated shoots was generally low in all species in comparison to the performances obtained during the multiplication subcultures [6, 7]. This could be attribute to the microcuttings miniaturization, required for the encapsulation protocol, which could have negative effects on the regrowth ability of propagules.

In apple genotype the length was higher in thesis 1 (9 mm), but it corresponds to the lower internode length (0.67 mm). As consequence, it indicates that this treatment allows to obtain the higher amount of usable nodes, considering the average number of proliferated shoots (1.13) (Fig. 2).

Table 2 Effect of the complete nutritive component on the shoots proliferation in the three genotypes.

Apple cv. A Sonagli	Shoots (n°)	Shoot lenght (mm)	Internode lenght (mm)
A (naked microcuttings)	1.00	5.67	1.08
B (not stored capsules)	0.67	7.33	1.32
1 (30 days of storage)	1.13	9.00	0.67
2 (90 days of storage)	0.59	6.50	0.76
Pear cv. Marzaiola			
A (naked microcuttings)	1.22	21.41	3.48
B (not stored capsules)	2.20	24.77	3.11
1 (30 days of storage)	1.65	28.29	2.74
2 (90 days of storage)	0.95	13.74	2.97
Plum cv. Montebello			
A (naked microcuttings)	0.75	9.33	1.47
B (not stored capsules)	1.20	11.07	1.20
1 (30 days of storage)	0.22	4.00	2.05
2 (90 days of storage)	0.15	7.50	0.70

In ‘Marzaiola’ (pear) the higher multiplication activity (2.2) is allowed if the shoots originate from not stored capsules. Good shoot elongation was monitored in all treatments (from 21.41 to 28.29 mm) except in thesis 2 (13.74). Moreover the lowest average internode length (2.74) corresponds to the highest shoot average length (thesis 1) also in this genotype (Fig. 3).

With respect to plum (‘Montebello’) the highest elogantion was achieved from shoots originated from not stored capsules (11.07 mm in thesis B). Any correlation seems to arise with average internode length.

In general the results reported in table 2 permit to hypothesize a clear effect of species and genotypes on the qualitative and quantitative aspects of the shoots proliferation as above described.

4. Conclusions

The results obtained in this preliminary study delineate opposing trends: the capsules of each genotype show satisfactory performances of regrowth ability after sowing, while the subsequent proliferation activity of shoots is not always corresponding. This is true especially for pear and apple varieties. Instead, the results registered in plum could be negatively influenced because some components (may be pectin) added to nutritive formulation did not perform their proper function. In consideration of the interesting potentialities of the encapsulation technology and conservation strategy diversification, further studies could allow decisive solutions to the emerged problems.

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