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In vitro preservation of somatic seeds and nonencapsulated hemp shoot tips

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Somatic seed technology and cold storage are used for rapid clonal propagation of plants and germplasm preservation. These methods provides genetic uniformity, pest and disease-free plants, easy to handle, and transport. The storage at low temperature reduces the metabolic rate, minimizes the risk of somaclonal variation and prolong storage time. The cold storage protocols for *Cannabis sativa* L. are scare and limited to drug type chemotypes. The aim of this study was to develop protocols for cold storage of nonencapsulated shoot tips and for alginate-capsulated somatic seeds.

Materials and Methods

In vitro growing hemp plants were used as source of explants (shoot tips). Hemp cultivars Epsilon 68, Globa and hybrid Carmagnola x K290 (marked as 1565) were tested. Cultures were maintained at 25 ±1°C, under 18/6 h light/dark photoperiod with 80-120 µmol m^2 s^{-1} fluorescent daylight. Shoot tips (0.5-1.0 cm, Fig. 1a) were excised from axillary shoots and placed vertically in Magenta vessels containing ½ MS medium and 8.5% agar. Explants (30 per vessel) were stored at 4 °C in the laboratory fridge in dark up to 9 months. Every 3 month, 12-15 ex-plants of each accession were set to regrow on the same medium (½ MS + IAA (0.5 mg L-1), 3% sucrose, 8.5% agar) and place in the growth chamber under the same conditions as hemp cultures. Regrowth of explants was recorded after 3 weeks of culture. For somatic seeds preparation shoot tips (Epsilon 68 and 1565) were excised and fully submerged in 3% alginic acid sodium salt with full-strength MS basal salt medium supplemented with 3% sucrose. Alginic acid coated buds were drop into 75 mM calcium chloride using automatic pipette for 30 min incu-bation to harden. Synthetic seeds were kept on ½ MS medium in Petri dishes sealed with parafilm. Dishes were stored in the laboratory fridge at 4°C in dark up to 3 months. The same regrowth conditions and medium was used for plant regeneration of somatic seeds.

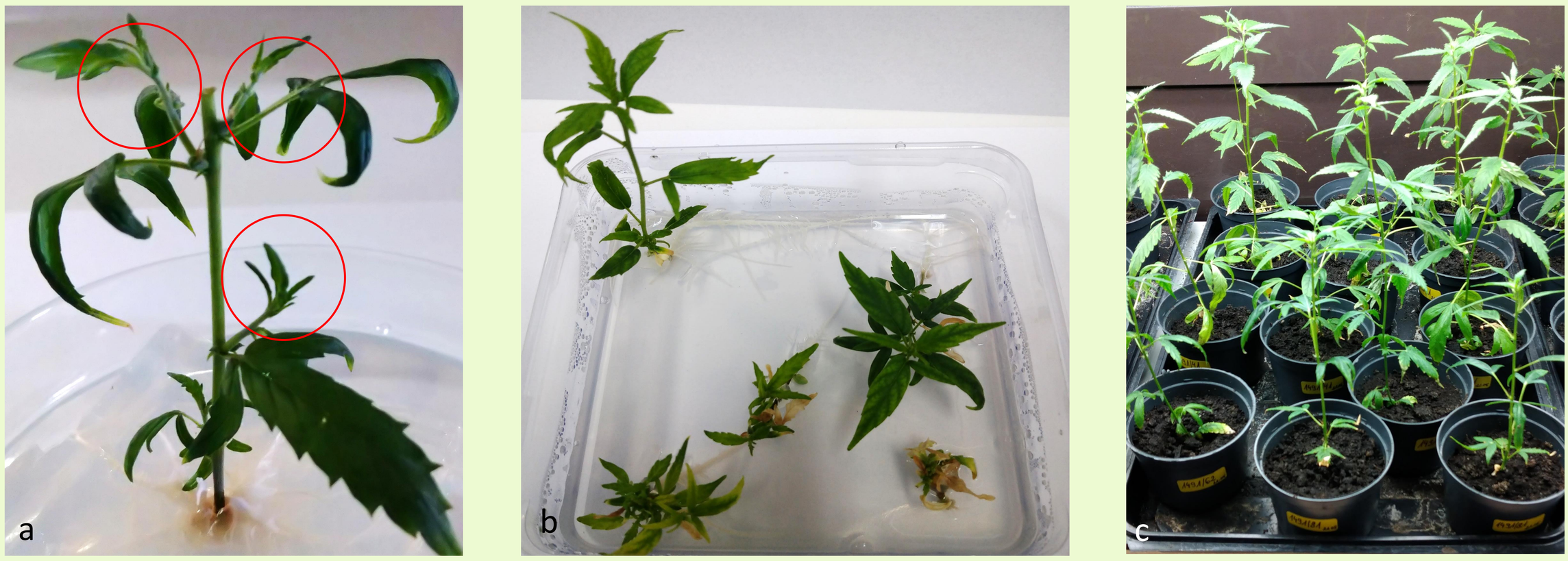

Figure 1. (a) Donor plant (1565) with red marked explants (shoot tips); (b). Rooted plants (Epsilon 68) originated from somatic seeds (3 months of storage), 5 weeks after germination; (c) acclimatization.

Results

Table 1. Effect of cold storage duration on rooting and shoot regeneration of unencapsulated explants of hemp

Accession	Storage in months	Survival (%)	Rooting Rate (%)	Mean no. of roots per explants (±SD)	Mean shoot length [cm]	Callusing explants (%)
Epsilon 68	3	100.0	85.7	5.00±3.61	3.08±1.72	31.0
	6	58.1	27.9	2.18±0.98	1.55±1.40	41.9
	9	16.7	4.8	0.92±0.17	0.56±0.24	2.4
Carmagnola x K290 (1565)	3	100.0	71.4	4.45±4.38	2.58±1.56	40.5
	6	81.8	45.5	3.48±1.95	1.92±1.59	72.7
	9	52.4	28.6	2.82±1.29	1.02±1.40	11.9
Globa	3	90.7	48.8	2.48±1.63	1.84±1,43	72.1
	6	86.0	44.2	2.30±1.90	1.50±0.76	69.8
	9	78.6	35.7	2.21±1.33	1.66±1.49	73.8

Table 2. Regrowth rates of somatic seeds after 3 months of cold storage recorded after 3 weeks after germination

Accession	Survival (%)	Rooting Rate (%)	Mean no. of roots per explants (±SD)	Mean shoot length [cm]	Callusing explants (%)
Epsilon 68	90	55.0	2.34±1.19	3.37±3.20	50.0
Carmagnola x K290 (1565)	100.0	90.0	2.98±2.35	1.50±1.12	70.0

After 6 months of storage 58-86% explants survived and 28%-45.5% of them fully re-generated root system and shoots (Table 1). After 9 months only 5%-36% of explants developed into vigorous plantlets. Moreover, the effect was genotype-dependent. After 3 months of somatic seeds storage relatively high regrowth rates (90% and 55%) was recorded for both hemp accessions. Well-developed, regenerated plants from encapsulated explants were successfully acclimatized (Fig. 1c).

In this preliminary study two forms of *in vitro* preservation were tested: nonencapsulated shoot tips and encapsulated explants in sodium alginate explants and stored at 4oC. Results of these preliminary studies show that both methods are potentially useful and suitable for hemp germplasm conservation. However, the protocol of cold storage should be optimized and adapted to the tested hemp genotype.

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