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Proceeding Paper

Technology for the Production of Somatic Seeds Useful for the Storage of Valuable Genotypes of *Salvia officinalis* †

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Abstract: Current research has attempted to produce and convert somatic seeds that would enable the long-term preservation of valuable sage genotypes. The creation of artificial seeds consisted in placing explants capable to regenerating into plants in a protective casing. The method of obtaining somatic seeds used in this study allowed to obtain a high level of conversion of seeds into plants using apical buds (85%), and slightly lower in the case of axillary buds (62.5%). The results concerning the formation and conversion of somatic seeds allowed to obtain a high level of plant viability, which may prove the usefulness of the method of storing valuable *Salvia officinalis* genotypes.

Keywords: somatic seeds; *Salvia officinalis*; in vitro cultures

1. Introduction

Biotechnological methods are increasingly used to protect biodiversity. The protection of genetic resources is necessary not only for future generations, but also for obtaining new valuable varieties. Currently, gene banks and in vitro cultures enable medium- and long-term storage of plant material. Somatic seeds are also used to preserve genetic resources in gene banks. Artificial seeds contain any regenerative plant fragment in a protective bead, prepared for long-term storage or for commercial use. The definition of artificial seeds implies their use in the storage and mass multiplication of genetically identical and healthy plants. The aim of the research was to produce and convert somatic seeds that would enable the long- or medium-term storage of valuable genotypes of *Salvia officinalis* (sage). The development of a method to store valuable genotypes is very important for sage, which is an insect-pollinated plant and is usually blooms in the second year of cultivation. Moreover, somatic seeds can ensure the storage of plant line with a guarantee of genetic homogeneity and propagation of variable genotypes such as transgenic plants. In this context, research into the improvement of biotechnological methods for the production of somatic sage seeds is highly relevant.

2. Materials and Methods

2.1. Plant Material and Production of Somatic Seeds

The apical and axillary buds were used as explants for the production of somatic seeds. The explants (± 1.5 cm) were dissected from the 2-weeks-old multi-shoot cultures, then encapsulated with 1.2% sodium alginate solution, and then dripped into the 200 mM $CaCl_2$ solution. The occurring reaction of exchanging calcium ions with sodium ions leads to the formation of a beads with a hard polymer coat. As a result of mixing, the capsules take a spherical form containing a single explant. The somatic seeds were stored for 1 month at 4 °C.

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The somatic seeds were converted on MS medium containing 0.3 mg L^{-1} BAP. In the study we used 20 genotypes in 4 replicates. After 2 and 4 weeks, the following data were recorded: frequency of shoot formation, morphological data (color, vitrification, necrose etc.). The regenerated shoots were rooted on ½ MS medium containing 0.3 mg L^{-1} IAA in order to obtain the acclimatized plantlets. Conversion a and rooting proceeded at 25 °C ± 2 °C with a 16 h photoperiod under a photosynthetic flux of 120 μ mol m^2 s^{-1} (daylight fluorescent tubes). Experiments were performed for both types of explants in 4 replicates of 20 seeds.

2.2. Statistical Analysis

The results were expressed as percentage (%) of germinated seeds of three replicates each of 20 seeds. The data were statistically analyzed using one-way analysis of variance (ANOVA) and the statistical significance was determined applying. The data was compared by the least significant difference ($p \leq 0.05$) test using GenStat15 System.

3. Results

As the result of the conducted experiments the conversion ability of somatic seeds of valuable *S. officinalis* genotypes was verified. Somatic seeds were produced using both the apical and axillary buds (Figure 1).

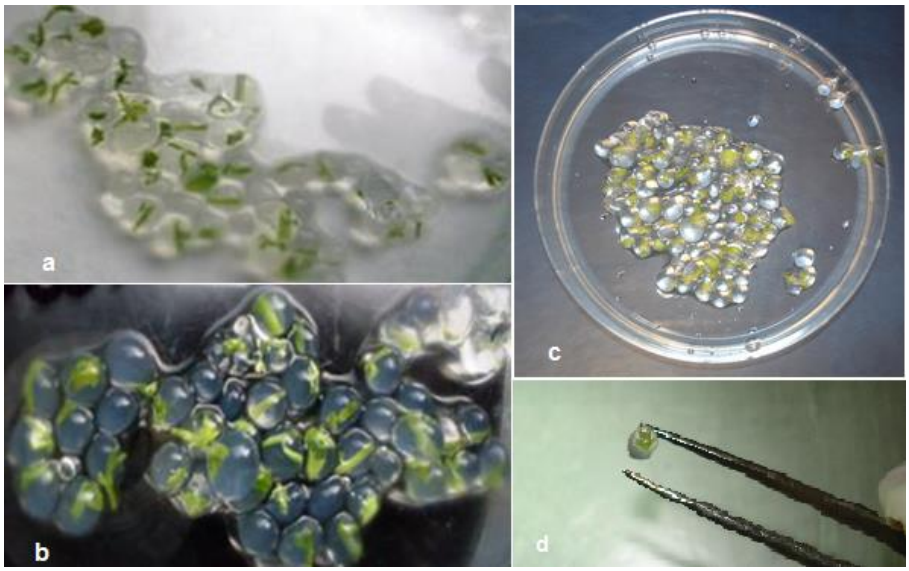

Figure 1. Somatic seeds of *Salvia officinalis*: (a) using apical buds; (b) using axillary buds; (c) after production in sterile conditions; (d) single somatic seed.

The obtained results of the observation during cultivation on MS medium containing 0.3 mg L^{-1} BAP are presented in Table 1.

Table 1. In vitro cultures of somatic seed of *Salvia officinalis.*

Somatic seed	Explant			Observation		
	W	B	L.S.D$_{0,05}$	1	2	L.S.D$_{0,05}$
	Mean values (%)			Mean values (%)		
Green	77.5^{a}	75.0^{a}	17.51	100.0^{a}	52.5^{b}	17.51
Brown - green	22.5^{a}	25.0^{a}	17.51	0.0^{b}	47.5^{a}	17.51
Brown	0.0	0.0	-	0.0	0.0	-
Growing	85.0^{a}	62.5^{b}	12.18	47.5^{b}	100.0^{a}	12.18
Non vitality	15.0^{b}	37.5^{a}	12.18	52.5^{a}	0b	12.18

Necrosed	0.0	0.0	-	0.0	0.0	-
Vittificated	30.0[a]	32.5[a]	13.71	0.0[b]	62.5[a]	13.71
Contaminated	0.0	0.0	-	0.0	0.0	-

*W - apical; **B – axillary. Values represent percentage (of germinated seeds) of three replicates each of 20 seeds. Diffrent lowercase letters in the same column indicate a significant difference at p≤0.05.

The method of obtaining somatic seeds used in this study allowed to obtain a high level of conversion of seeds into plants using apical buds (85%). The slightly lower percentage of regenerated plants was obtained for axillary buds (62.5%). However, the statistical significance of seed viability in the ANOVA analysis of variance was at the level of p <0.01 (Table 2). Therefore, it can be assumed that the usefulness of the developed method applies to both initial explants.

Table 2. ANOVA analysis of the traits observed in in vitro cultures of somatic seeds.

Source of variation	D.F.	Somatic seed					
		Green	Brown -green	Brown	Growing	Non vitality	Vittificated
Observation	1	9025.0 ***	9025.0 ***	0.0	11025.0 ***	11025.0 ***	15625.0 ***
Explant	1	25.0	25.0	0.0	2025.0 **	2025.0 **	25.0
Residual	12	258.3	258.3	0.0	125.0	125.0	158.3

** p<0.01; *** p<0.001.

For complete regeneration of plants, shoots were rooted in ½ MS medium containing 0.3 mg L^{-1} IAA. The results indicate that 4 explants out of 5 developed roots (4.067 ± 0.3914). Regenerated sage plants were characterized by proper growth and development at each stage of culture (Figure 2).

Figure 2. In vitro culture of *Salvia officinalis*: (a) somatic seeds placed on MS medium containing 0.3 mg L^{-1} BAP; (b) shoots regenerated from somatic seeds; (c) multi-shoot cultures; (d) regenerated plants on ½ MS medium containing 0.3 mg L^{-1} IAA.

4. Discussion

Since Kitto and Janick [1] began their first research on artificial seeds by working on carrots, there has been a continual interest in this topic. This led to a general definition an artificial seed also called a synthetic seed or synseed, seed analog, or manufactured seed with includes a range of plant structures, including somatic embryos, buds, shoots, or other meristematic tissues inside a coating, that can be sown in the same way as a conventional seed to produce a new plant [2]. The results obtained in this study have shown that somatic seeds of sage were capable of conversion and regeneration. The developed a

protocol was effective and enabled the complete regeneration of plants from apical and axillary buds. The use of this type of explants for the production of artificial seeds is increasingly an alternative to the use of somatic embryos [3]. There is only one report devoted to the production of artificial seeds of S. *officinalis*, that confirms the usefulness of this technology for the high medical and commercial value plants, such as sage [4]. Similar results regarding the formation of shoots from somatic seeds were obtained. However, in the present study MS medium, sucrose and gibberellic acid were not used as the components of the encapsulation gel, which significantly simplified the procedure. Therefore, our simplified procedure for producing artificial seeds and high conversion and regeneration efficiency of somatic seeds is a novelty in the study on the application of the technique of medium- and long-term storage genotypes of *Salvia officinalis*.

The potential benefits of using somatic seeds include rapid multiplication and long-term storage of a given plant line with a guarantee of genetic homogeneity. In addition, somatic seeds can provide for the delivery and propagation of variable genotypes such as transgenic plants with high genetic instability. Often, artificial seeds can be the only way to reproduce genetically modified plants [5]. In this context, the method obtained in own work could be develop and optimized in the future for the storage of valuable sage genotypes after genetic transformation [6].

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