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CULTIVATION OF SOAPWORT PLANTS (*SAPONARIA OFFICINALIS* L.) IN *IN VITRO* CULTURES

ABSTRACT

Saponaria officinalis L. is a medicinal plant from the family *Caryophyllaceae*. It occurs in most European countries, as well as in North Africa, America and Western Asia. The aim of the study was to find an optimal method of sterilization *S. officinalis* seeds in order to obtain sterile seedlings and to multiply axillary shoots from nodal explants isolated from them, on MS medium with the addition of growth regulators (RW). In this study, 4 variants of seed sterilization were compared using NaClO at the following concentrations: 1.5% (2), 2% (3) and 2.5% (4) for a period of 11 minutes. The control was a variant in which 70% C_2H_5OH was used for pre-sterilization for 1 minute (1). The highest percentage of sprouted, seeds and sterile seedlings and the lowest percentage of contamination were obtained in variant (4). As a result of micropropagation of soapwort in *in vitro* cultures, axillary shoots were formed from nodal explants from axillary buds. The highest percentage of explants with shoots (95%) and callus tissue (48%) and the highest number of shoots from one explant (5.95) were obtained in the second passage on MS medium with the addition of 4 $mg \cdot dm^{-3}$ BAP and 0.5 $mg \cdot dm^{-3}$ NAA. Due to the wide possibilities of using soapwort, it is advisable to continue research aimed at developing an optimal and efficient plant regeneration system of this species.

Keywords: medicinal plant, axillary shoots, micropropagation, plant growth regulators.

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INTRODUCTION

Soapwort (*Saponaria officinalis* L.) is a perennial plant from the family *Caryophyllaceae*. The plant occurs in the temperate climate zone in most European countries, including Spain, France, Italy, as well as in North Africa, America and Western Asia (Böttger and Melzig 2011, Saboohi and Mortazaeinezhad 2016, Slobodianiuk et al. 2021). *S. officinalis* is known as a weed in most countries of the world, and is sometimes grown in gardens as an ornamental plant. The soapwort inhabits open waste areas, roadsides or railway tracks, field balks, thickets and alluvial terraces (Saboohi and Mortazaeinezhad 2016, Grygierzec 2022). In Poland it a wild plant that can be found mainly in lowland areas (fig. 1). In addition to its decorative value, soapwort is cultivated and harvested mainly for its medicinal and cosmetic properties. In medicine, mainly the dried root and rhizome are used (Góral et al. 2018). Soapwort is a rich source of glycoside compounds - saponins, which foam when extracted with water. This species is also a source of fatty acids, flavonoids and other phenolic compounds and carbohydrates (D-glucose, D-galactose, D-fructose, D-sucrose) (Talluri et al. 2018, Budniak et al. 2021, Slobodianiuk at al. 2021). Due to the presence of biologically active substances, this species has antioxidant, anticancer, antibacterial, insecticidal and antifungal properties (Chandra et al. 2021). Research shows that soapwort root extract can also act as a substitute for acaricides, i.e. preparations used to combat mites. This property is very desirable in agriculture because it allows the use of pesticides to be reduced (Slobodianiuk et al. 2021). Soapwort root is used in the food industry as a raw material for the production of halva, turron and other sweets. (Korkmaz and Ozcelik 2011). Recently, there has been an intensive development of field crops of plants with medicinal properties, but many species are obtained from the wild. Therefore, a gradual decrease in natural resources of medicinal plants can be observed (Thiem and Kitkowska 2008). Due to the wide use of soapwort and obtaining it mainly from the natural state, it is justified to propagate this plant using the *in vitro* micropropagation technique, which involves growing plants in sterile conditions in most often glass containers on nutrient media (artificial substrates) under strictly controlled conditions. In order to initiate the culture, small plant fragments (shoots, leaves, cotyledons and seeds) called explants are used, which, after being isolated from the initial plant, are placed on sterile media (Figas et al. 2016).

Sterile seedlings obtained from seeds placed on media in *in vitro* cultures can also be used for micropropagation. Young seedlings are characterized by very good regenerative properties (Koziara 2002). Thanks to the micropropagation technique, it is possible to obtain certified seedlings that are genetically homogeneous and free from pathogens (Figas et al. 2016). Plants originating from *in vitro* cultures are characterized by high productivity and juvenile growth (Kucharska et

al. 2017). In the case of herbal plants, *in vitro* cultures provide a chance to obtain a valuable and homogeneous raw material. In the literature the information about this micropropagation *S. officinalis* plants is limited (Saboohi and Mortazaeinezhad 2016).

The aim of the study was to find an optimal method of sterilization soapwort seeds in order to obtain sterile seedlings and to multiply axillary shoots from nodal explants isolated from them, on MS medium (Murashige and Skoog 1962) with the addition of growth regulators (RW) from the group of auxins and cytokinins. In this work, 6-benzylaminopurine (BAP) and 1-naphthylacetic acid (NAA) were used in various modifications and concentrations.

Figure 1. Soapwort (*S. officinalis*) in the flowering phase in the Bydgoszcz area.

MATERIAL AND METHODS

The experiment was carried out at the Department of Agricultural Biotechnology of the Bydgoszcz University of Science and Technology. The starting material used to initiate the *in vitro* culture of soapwort were seeds that were disinfected using a chemical method. Plant seeds from natural conditions were used for the research. The experiment compared 4 variants of seed sterilization using sodium hypochlorite (NaClO). The first stage of sterilization included presterilization, in which only 70% ethyl alcohol (C_2H_5OH) was used to disinfect the seeds for 1 min. The second stage included proper sterilization, in which the following NaClO concentrations were compared: 1.5% (2), 2% (3) and 2.5% (4) with the addition of 2-3 drops of Tween 20 detergent. The action time of this active substance was in all variants 11 min. In the last stage, the seeds were rinsed three times in sterile bidistilled water. The control was the variant in which 70% C_2H_5OH was used for 1 minute (1) (tab. 1.).

Table 1. **Methods used to sterilize soapwort seeds.**

Variant	Pre-sterilization		Proper sterilization		Last stage of sterilization
	Substance disinfecting	Time exposure (min)	Active ingredient and concentration (%)	Time exposure (min)	
1. (control)	70% C_2H_5OH	1	-	-	bidistilled water, sterile (3x)
2.	70% C_2H_5OH	1	1.5% NaClO	11	bidistilled water, sterile (3x)
3.	70% C_2H_5OH	1	2% NaClO	11	bidistilled water, sterile (3x)
4.	70% C_2H_5OH	1	2.5% NaClO	11	bidistilled water, sterile (3x)

Sterilized soapwort seeds were placed individually in test tubes on ½ MS medium (Murashige and Skoog 1962) without the addition of RW growth regulators. The medium contained all the necessary macro and microelements. The medium contained a carbon source of 3% sucrose and was solidified with 0.8% agar. The medium had a pH of 5.7 and was autoclaved at a pressure of 0.1 MPa and a temperature of 121˚C for 25 minutes. The test tubes were placed in baskets with 24 compartments. Each sterilization variant included 24 samples (seeds). After inoculation on the medium, the seeds were stratified. For this purpose, the baskets with test tubes were moved to the refrigerator (temp. 4°C) for a period of 4 weeks. After this time, test tubes with germinated seeds were successively transferred to the phytotron, where strictly controlled conditions prevailed: 16 h photoperiod (16 h light/8 h darkness), white light intensity of approximately 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (daylight lamps of 40 W, PILA, Poland), temperature 25±2°C and air humidity 70%. The experiment was performed in 3 repetitions.

Under aseptic conditions, after 7 weeks of seedling growth, nodal sections were isolated and the first passage was performed. For this purpose, explants were placed on MS medium without growth regulators RW using sterile tweezers and a scalpel. After 4 weeks, the second passage was carried out; for this purpose, the developed side shoots were placed on MS media with the following composition: 0 RW (A) and with the addition of 1-naphthylacetic acid (NAA) and 6-benzylaminopurine (BAP) in the following modifications and concentrations (B, C, D, E):

- **A.** MS + 0 RW,
- **B.** MS + 4 $mg \cdot dm^{-3}$ BAP + 0.5 $mg \cdot dm^{-3}$ NAA,
- **C.** MS + 3 $mg \cdot dm^{-3}$ BAP + 0.5 $mg \cdot dm^{-3}$ NAA,
- **D.** MS + 4 $mg \cdot dm^{-3}$ BAP,
- **E.** MS + 3 $mg \cdot dm^{-3}$ BAP.

The flasks with explants were moved to the phytotron (growth room). During the experiment, weekly observations were made and axillary shoots were systematically counted. After 6 weeks of culture, the multiplication coefficient was calculated and the following were finally determined: the percentage of explants forming side shoots, the average number of axillary shoots per explant, the average length of shoots, the percentage of explants forming callus tissue on the cut surface of the explant.

Data on the effect of RW on the number of shoots obtained from nodal explants of soapwort seedlings obtained in sterile conditions were subjected to analysis of variance in a completely random design. Single-factor analysis of variance (ANOVA) was performed. The arithmetic mean values are shown in tables ± standard deviation. Differences between mean values were assessed using the Tukey test, with a significance level of $\alpha = 0.05$. Statistical analyses were performed applying Statistica 10.0 software.

RESULTS AND DISCUSION

In this experiment, NaClO was used to disinfect soapwort seeds. At this stage of the experiment, the following NaClO concentrations were compared: 0% (1); 1.5% (2); 2% (3) and 2.5% (4). Among the tested variants, the most germinated seeds and sterile seedlings were obtained in the variant in which NaClO at a concentration of 2.5% was used for proper sterilization. The impact of the sterilization methods used in the experiment is shown in table 2. The percentage of germinated and sterile seedlings was 17%, and only 8% of the samples showed bacterial and fungal infections. In variant 1 (control), in which soapwort seeds were only subjected to initial sterilization, the contamination rate was 100% and the seeds also did not germinate.

Table 2. The influence of the applied sterilization variants of *S. officinalis* seeds on the number of sterile seedlings 4 weeks after the initiation of culture.

Variant	Variant sterilization	Number of germinated seeds (%)	Number of sterile seedlings (%)	Contamination (%)
1. (control)	70% C_2H_5OH,1 min.	0 (0)	0 (8)	24 (100)
2.	70% C_2H_5OH,1 min.; 1.5% NaClO,11 min.	2 (8)	2 (8)	4 (17)
3.	70% C_2H_5OH,1 min.; 2% NaClO,11 min.	2 (8)	1 (4)	3 (12)
4.	70% C_2H_5OH,1 min.; 2.5% NaClO,11 min.	4 (17)	4 (17)	2 (8)

In another study, Di Cola et al. (1997) obtained seedlings using the same compound for proper seed sterilization, but in a higher concentration of 10% for 20 minutes, and then the seeds were washed several times with sterile distilled water. According to Bhojwani and Dantu (2013), sodium hypochlorite, calcium hypochlorite, chloramine T, hydrogen peroxide, bromine water, silver nitrate, mercury chloride are the most often used to disinfect plant material.

The source of explants for initiating *in vitro* culture were sterile soapwort seedlings. The primary explants were nodal sections with buds in the leaf axils. As research by Saboohi and Mortazaeinezhad (2016) showed, this type of explant can be used to initiate *in vitro* cultures of soapwort. The authors used the internodes of this plant, which they isolated from plants under *ex vivo* conditions. According to Kanwar and Kumar (2008), this type of explant guarantees that the daughter plants are genetically identical to the mother plant. According to Assim (2008) and Hu and Wang (1983), the use of this type of explants allows obtaining genetically stable plants because they have the same histological structure and genetic structure as shoot apical meristems.

In the experiment, variants of media with the addition of BAP (cytokinin), and combinations of BAP and NAA (auxin), were used. The obtained results confirm the thesis that one of the most frequently observed effects of cytokinins in *in vitro* cultures is the stimulation of the formation of axillary shoots. As a result of changing the BAP concentration from 3 to 4 $mg \cdot dm^{-3}$, regardless of the presence of auxin in the medium, the percentage of explants with axillary shoots and callus tissue increased (tab. 3, fig. 2).

According to the observations, 3.70 and 3.96 shoots were obtained on MS medium with the addition of only BAP without the addition of auxin at a concentration of 3 and 4 $mg \cdot dm^{-3}$. The percentage of explants in which callus tissue developed on MS medium with the addition of cytokinin BAP alone was on average 13% and 17%, respectively on the medium with the addition of 3 $mg \cdot dm^{-3}$ BAP and 4 $mg \cdot dm^{-3}$ BAP.

Trejgell et al. (2007), conducting research on the micropropagation of *Carlina caulescens* - a plant belonging to the *Asteraceae* family, increasing the concentration of BAP in the medium from 1 to 3 $mg \cdot dm^{-3}$, obtained the effect of increased formation of callus tissue on the cutting surface of explants of the apical parts of seedlings. In their studies, the authors obtained a higher percentage of explants with callus tissue on the medium supplemented with 3 $mg \cdot dm^{-3}$ BAP (95%) than on the medium enriched with 1 $mg \cdot dm^{-3}$ (85%). In this experiment, the highest multiplication coefficient (5.9) was obtained using MS medium enriched with 4 $mg \cdot dm^{-3}$ BAP and 0.5 $mg \cdot dm^{-3}$ NAA and 95% of explants with developed axillary shoots (tab. 3, fig. 2, fig. 3). Based on observations, this phytohormone composition also influenced the height of individual shoots and the percentage of explants forming callus tissue. Enrichment of the medium with

auxin (NAA) stimulated the formation of callus tissue. The percentage of explants forming callus on media with the addition of NAA was 22% and 48%, respectively on media with 3 mg·dm^{-3} BAP + 0.5 mg·dm^{-3} NAA and 4 mg·dm^{-3} BAP + 0.5 mg·dm^{-3} NAA (tab. 3). Similar results were obtained by Saboohi and Mortazaeinezhad (2016), who recorded the maximum *S. officinalis* plant height (9 cm) on a medium containing 0.5 mg·dm^{-3} BA + 0.5 mg·dm^{-3} NAA, although they did not prove it statistically.

According to Beyl (2011) and Orlikowska (1997), a high content of growth regulators belonging to cytokinins in the medium and, at the same time, lower auxin influence the multiplication of plants in *in vitro* cultures. This phenomenon is explained by the fact that RW from the group of cytokinins, derived from adenine, which also includes BAP used in this experiment, abolish the dominance of the apical bud, stimulating the formation of axillary shoots from buds located in the leaf axils.

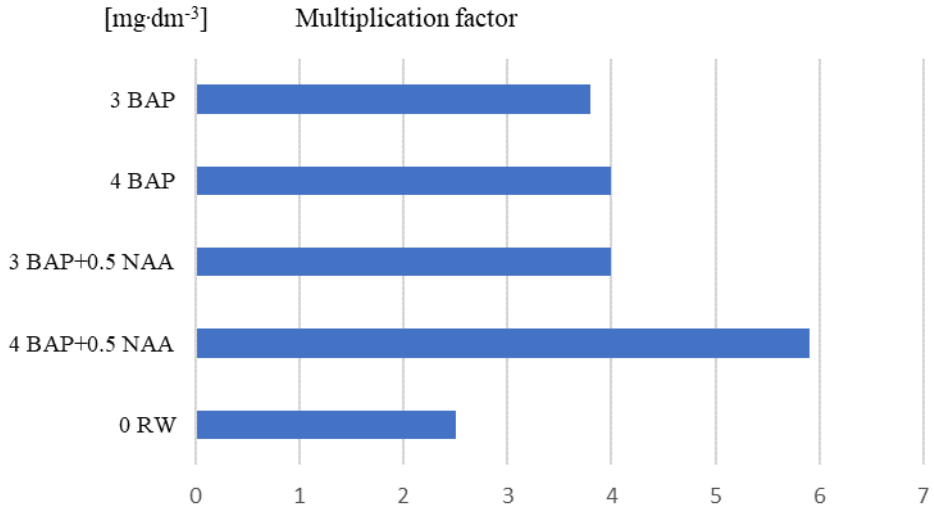

Figure 2. Multiplication factor of axillary shoots from the nodal sections of *S. officinalis* on MS and MS medium with the addition of BAP and NAA after 6 weeks of culture; NAA – 1-naphthaleneacetic acid (auxin), BAP – 6-benzyladenine (cytokinin).

Table 3. The effect of plant growth regulators on the effectiveness of multiplication of axillary shoots from nodal explants of *S. officinalis* in *in vitro* culture after 6 weeks of experiment.

Plant growth regulator ($mg \cdot dm^{-3}$)		Percentage of explants forming axillary shoots (%)	Number of axillary shoots/explant (mean±SD)	Axillary shoot length (cm) (mean±SD)	Percentage of explants forming callus (%)
BAP	NAA				
4	0.5	95	5.95±3.29 a	6.95±3.60 a	48
3	0.5	60	3.89±3.79 b	6.01±3.01 b	22
4	-	73	3.96±3.21 b	4.66±3.59 c	17
3	-	85	3.70±2.23 b	4.37±4.41 c	13
-	-	72	2.44±1.79 c	3.94±2.70 c	0

Results are mean ± SD (standard deviation); within a column means followed by the same letter do not differ significantly at α=0.05 (Tukey test), NAA – 1-naphthaleneacetic acid (auxin), BAP – 6-benzyladenine (cytokinin)

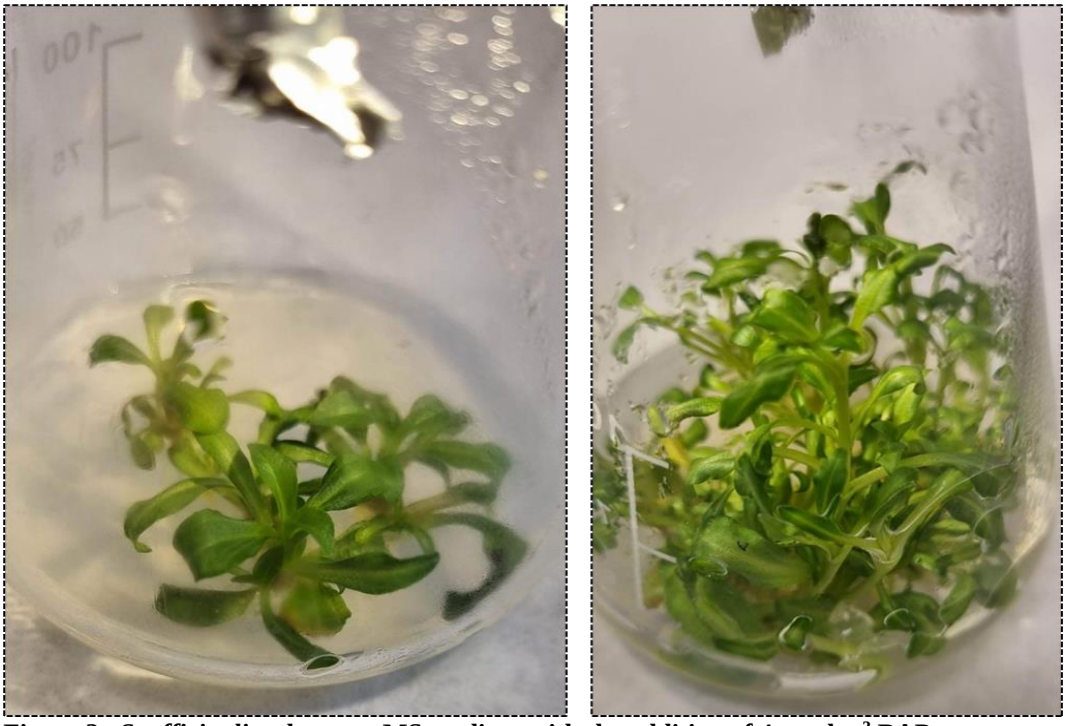

Figure 3. *S. officinalis* plants on MS medium with the addition of 4 $mg \cdot dm^{-3}$ BAP and 0.5 $mg \cdot dm^{-3}$ NAA (A) after 2 weeks and after 6 weeks (A) of observation.

CONCLUSIONS

1. The highest percentage of germinated seeds and sterile seedlings of soapwort (*S. officinalis*) and the lowest percentage of contamination were obtained in variant 4, in which 2.5% sodium hypochlorite was used for proper sterilization for 11 minutes.
2. A result of micropropagation of soapwort in *in vitro* cultures from nodal explants, axillary shoots were formed from axillary buds without adventitious organogenesis.
3. The highest percentage of explants with axillary shoots (95%) and callus tissue (48%) and the highest number of shoots from one explant (5.95) were obtained in the II passage on MS medium with the addition of 4 $mg \cdot dm^{-3}$ BAP and 0.5 $mg \cdot dm^{-3}$ NAA.
4. Due to the wide possibilities of using soapwort in medicine, industry and agriculture, it is advisable to continue research aimed at developing the optimal and efficient plant regeneration system of this species.

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