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## **PLANT GROWTH REGULATORS AFFECTING IN VITRO CALLUS, SHOOTS AND ROOTS INDUCTION OF *SILPHIUM PERFOLIATUM* L. – A NEW ALTERNATIVE MULTIFUNCTIONAL PLANT**

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### **ABSTRACT**

The aim of the study was to investigate the optimal factors needed for establishing an effective protocol for propagation of the cup plant. This species can be applied in medicine, animal feeding, and as a decorative, aparian energy-producing and fitoremediation plant. Currently, there is no sufficient sowing material in Poland that would do justice to the present attempts at propagating this species in *in vitro* growth cultures. As explants were used cotyledonary and leaf fragments with petioles, which were placed onto MS media supplemented with BAP in combination with IAA, ABA, NAA; and onto MS medium fortified with KIN and 2,4 – for callus and shoot bud initiation. The most efficient callus proliferation reported was on MS medium incorporated with various concentrations and combinations of auxins and cytokinins such as BAP (10 mg·dm<sup>-3</sup>) + NAA (1.0 mg·dm<sup>-3</sup>), BAP (0.5 mg·dm<sup>-3</sup>) + 2,4-D (1.0 mg·dm<sup>-3</sup>) + IAA (1.0 mg·dm<sup>-3</sup>) + NAA (1.0 mg·dm<sup>-3</sup>). The adventitious shoots formed from both the tested explants. It was found that the highest frequency of explants forming shoots were induced on MS medium containing BAP (5.0 mg·dm<sup>-3</sup>) + IAA (0.05 mg·dm<sup>-3</sup>) and KIN (0.2 mg·dm<sup>-3</sup>) + IAA (2.0 mg·dm<sup>-3</sup>), respectively.

**Key words:** Cup plant, micropropagation, callus, explants, cytokinins, auxins, *in vitro*.

### **INTRODUCTION**

In recent years, many scientific initiatives have been started in order to identify new valuable plants occurring in wild nature, which can be useful for the food and pharmaceutical industry, as well as for agronomic and environmental purposes. The cup plant (*Silphium perfoliatum* L.), a perennial belonging to the *Asteraceae* family, originates from the midwestern and southeastern United States, but now is also cultivated in other regions of the world.

What is species-characteristic here are the flowers in a form of yellow flower heads collected on shoot tips and achene-type fruits [28]. The opposite of the leaf of that perennial plant creates a bowl in which water gets accumulated, making the survival in drought periods possible. The cup plant, because of its features, is used for medical, feed, and energy purposes [19, 22, 23, 29].

*Silphium perfoliatum* has a wide range of practical merits. The plant is a good source of basic compounds such as carbohydrates (including inulin), proteins, amino acids, cellulose, minerals and ascorbic acid; and is a suitable raw material for extraction and production of functional food ingredients. The plant also produces bioactive secondary metabolites of the isoprenoid groups (essential oils, triterpenes and their glycosidic-saponin bonds), as well as phenolic and polyphenolic compounds (phenolic acids, tannins, flavonoids). Extracts from the herb show painkilling, anti-inflammatory, diaphoretic, strengthening, and expectorant properties [5, 11–15]. Moreover, a mixture of saponins isolated from the cup plant leaves exhibits blood cholesterol-lowering activity. Many triterpene saponins also possess anti-bacterial and anti-fungal activity related

to their function in plant defence against pathogens [8]. Additionally, flowers of the cup plant are an excellent source of pollen and nectar for bees owing to long flowering period and production of high amounts of pollen grains [29].

*Silphium perfoliatum* quite easily adapts to difficult environmental conditions, such as scarcity of mineral nutrients and water, soil contamination, and simultaneously shows high ecological plasticity [10]. Therefore, the cup plant can be utilized as a pioneer plant for reclamation areas, degraded by industry and municipal waste disposal [10, 28]. In addition, *Silphium perfoliatum* plantations produce large quantities of biomass considered to be a source of bioenergy [18, 19, 22, 23]. Despite the vast array of potential uses, only few studies have been carried out on the cup plant propagation [25], almost all the research focusing on its chemical composition only. The clonal propagation under aseptic conditions allows to produce a disease-free, superior quality planting material. It also helps in the development and rapid propagation of selected plants with desirable characters in shortest possible time.

The aim of the present research was to optimise the conditions of producing *Silphium perfoliatum* plants in *in vitro* cultures. The experiment investigated the effect of plant growth regulators on induction of the callus, shoot and root formation and can be essential for the development of an efficient and an effective method of micropropagation of that insufficiently spread species.

## MATERIALS AND METHODS

**Plant material and *in vitro* culture of explants:** The initial research material involved the seeds of cup plant (*Silphium perfoliatum* L.), which came from the Botanical Garden of the National Center of Plant Gene Resources of the Institute of Plant Breeding and Acclimatization (IHAR) in Bydgoszcz in Poland. Surface disinfection – sterilization of the isolated embryos was carried out by T by immersion in 70% ethyl alcohol (v/v) for 30 seconds, followed by treatment with 5% calcium hypochlorite (v/v) solution with 2–3 drops of Tween 20 for 5 minutes. The embryos were rinsed with autoclaved double – distilled water and placed by gentle pressing into test-tubes with a half-strength Murashige and Skoog medium (MS) [21] containing 30 g·dm<sup>-3</sup> sucrose and 0.8% (w/v) agar for germination. The pH of medium was adjusted to 5.8 with 1 N KOH or 1 N HCl and autoclaved at the temperature of 121°C for 20 min. Cotyledons and leaf fragments with petioles excised from 6-week-old seedlings were transferred on modified MS regeneration medium, fortified with different concentration of growth regulators: BAP (6-benzylaminopurine) (0.5, 2.0, 2.5, 5.0, 10 mg·dm<sup>-3</sup>), NAA (1-naphthaleneacetic acid) (1.0 mg·dm<sup>-3</sup>), IAA (3-indoleacetic acid) (0.05, 1.0, 2.0 mg·dm<sup>-3</sup>), KIN (kinetin) (0.2 mg·dm<sup>-3</sup>), ABA (abscisic acid) (0.45 mg·dm<sup>-3</sup>), 2,4 D (2,4-dichlorophenoxyacetic acid) (1.0 mg·dm<sup>-3</sup>). The medium was supplemented with 30 g·dm<sup>-3</sup> sucrose and solidified with 0.8% (w/v) agar. All cultures were incubated in growth room at a temperature of 25 ± 2°C, in 16 h photoperiod at a light intensity of about 40 mmol photons·m<sup>-2</sup>·s<sup>-1</sup>. After ten weeks of culture, the number of regenerating structures was determined.

**Statistical analysis:** The experiment was done in triplicate. Each replication consisted of 24 explants. The number of the cotyledon and young leaf explants forming callus, shoots and roots incubated on media with varying combination of growth regulators, were recorded. An analysis of variance was performed on all results using ANOVA. The significance of differences was evaluated by Tukey's test and LSDs were calculated at a significance level  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

The present paper describes the effect of different phytohormones on callus, shoot and root induction *in vitro* of *Silphium perfoliatum* from explants of various morphological potential. In the study, we have also shown that indirect organogenesis process is clearly stimulated by cytokinin application in combination with auxins.

**Callus induction.** Considering both types of somatic explants, culture medium composition significantly influence callus induction in the cup plant (Tab. 1). Among the combinations used, BAP (10 mg·dm<sup>-3</sup>) + NAA (1.0 mg·dm<sup>-3</sup>), BAP (0.5 mg·dm<sup>-3</sup>) + 2,4-D (1.0 mg·dm<sup>-3</sup>) + IAA (1.0 mg·dm<sup>-3</sup>) + NAA (1.0 mg·dm<sup>-3</sup>), and BAP (5.0 mg·dm<sup>-3</sup>) + NAA (1.0 mg·dm<sup>-3</sup>) were the best for callus induction (Tab. 1) with 97, 95 and 88% from leaf explants and 74, 90 and 83% from cotyledonary explants, respectively (Fig. 1). Additionally, the leaf explants showed higher capacity for callus proliferation in comparison to cotyledonary explants on tested media (Tab. 1). It is noteworthy that both types of explants failed to form callus on MS medium supplemented with KIN and IAA. Our results showed that application of cytokinin in combination with low concentration of auxin was crucial for callus formation on the explants of the cup plant. The findings coincide with those reported in literature, which described *in vitro* culture of members of the *Asteraceae* family. For example, maximum callus induction and growth were recorded with BAP (3.0 mg·dm<sup>-3</sup>) and 2,4-D (2.0 mg·dm<sup>-3</sup>) in leaf explants of *Gerbera jamesonii* [16]. Similar trend was found for callus initiation from cotyledon and hypocotyl explants of dahlia *Dahlia cultivars*, which was also enhanced with increasing BA and NAA level in media [6]. Other authors reported that the most effective callus formation on leaf fragments of *Chrysanthemum morifolium* was observed on the MS media with lower concentration of BAP (0.065–0.65 mg·dm<sup>-3</sup>) and NAA (0.1 mg·dm<sup>-3</sup>) [30]. Aswath and Choudhary [3] also found a better rate of callogenesis on media with lower level of BAP for leaf fragments of gerbera. In our experiment callus mainly formed at the cut edges of the tested explants, directly in contact with the culture medium (Fig. 4A). The callus proliferated as greenish and yellow compact tissue.

Table. 1. Effect of growth regulators on the formation of callus, shoot and root on cup plant (*Silphium perfoliatum* L.) leaves and cotyledons explants

Growth regulators [mg·dm <sup>-3</sup> ]	Number of explants forming*		
	callus	shoots	roots

BAP	KIN	2,4 D	IAA	NAA	ABA	cotyledons	leaves	cotyledons	leaves	cotyledons	leaves
2.0			1.0			0.00b*	10.33b	0.00b*	0.67b	0.00b*	0.00b
5.0			0.05			0.00b	6.67b	0.00b	2.33a	0.00b	0.00b
2.5					0.45	0.00b	4.33b	0.00b	0.33b	0.00b	0.00b
2.0				1.0		0.00b	3.00b	0.00b	0.00b	0.00b	0.00b
5.0				1.0		20.00a	21.00a	0.00b	0.00b	0.00b	0.00b
10.0				1.0		17.67a	23.33a	0.00b	0.00b	0.00b	0.00b
	0.2		2.0			0.00b	0.00c	1.00a	0.00b	15.00a	2.33a
0.5		1.0	1.0	1.0		21.67a	22.67a	0.00b	0.00b	0.00b	0.00b
**Mean±						7.4	11.4	0.1	0.4	1.9	0.3
SD**						±10	±9.5	±0.4	±0.8	±5.1	±0.9
LSD[0.05]						4.863	8.978	1.000	1.000	2.999	1.527
* Values represent mean of three replicated experiments of 24 explants each. P<0.05											
** indicators computed for the explantats each; results are mean ± SD (standard deviation) of 24 replicates											

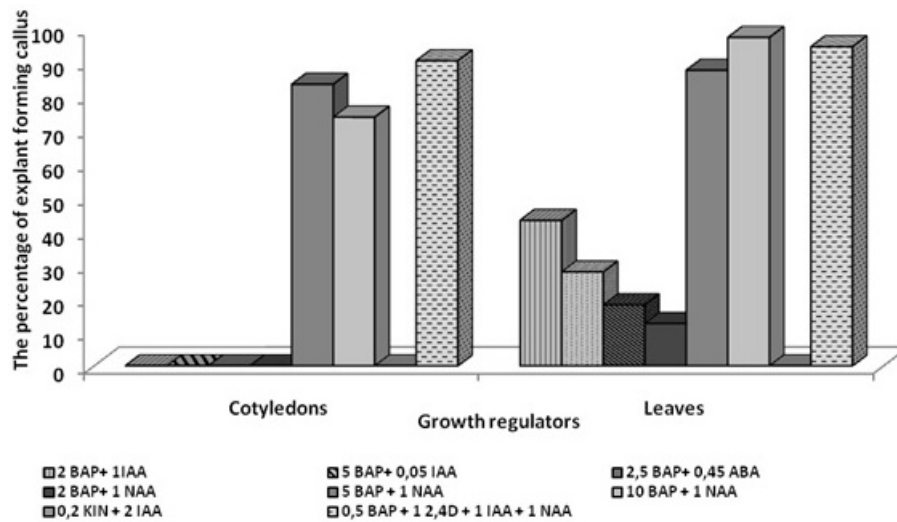


Fig. 1 . Effect of different hormonal composition in MS medium on the morphogenetic reactions of cotyledons and leaves explants of *Silphium perfoliatum*. The percentage of explants forming callus

**Shoot induction.** The results of the experiments showed that adventitious shoots were formed from explants (Fig. 4B). As shown in Table 1, the highest adventitious shoot initiation was obtained on young leaves explants cultured on MS medium with BAP (5.0 mg·dm<sup>-3</sup>) + IAA (0.05 mg·dm<sup>-3</sup>) added. As confirmed by the results, a lower percent response for cotyledons was noticed (Fig. 2). In that case, the shoots were only received on MS medium supplemented with KIN (0.2 mg·dm<sup>-3</sup>) and IAA (2.0 mg·dm<sup>-3</sup>) (Tab. 1). It is noteworthy that the absence of BAP in the medium led to shoot induction on this type of explants. Our results are in contrast with those reported by Aswath and Choudhary [3], who obtained most adventitious shoots of gerbera on MS media supplemented with NAA (2.0 mg·dm<sup>-3</sup>) and BAP (1.0 – 4.0 mg·dm<sup>-3</sup>). As the authors reported, with increasing concentration of NAA in the medium, BAP had a negative effect on shoot development. On the other hand, increasing the BAP concentration was superior for induction of shoots when combined with a high NAA concentration. In addition, these authors reported that the media containing only BAP induced lower frequency of shoots showing susceptibility to vitrification. The effect of BAP on shoot formation on explants of gerbera was observed by Jerzy and Lubomski [9]. In the experiment described by the authors this cytokinin at the concentration of 10 mg·dm<sup>-3</sup> stimulated the formation of the highest number of adventitious shoots but the shoots were breakable, short and demonstrated the symptoms of vitrification. On a medium with a lower level of cytokinins they observed less shoots but of normal habit.

Bhatia et al. [4], on the other hand, placed the leaf explants onto the MS medium, also containing 10 mg·dm<sup>-3</sup> BAP but supplemented with 2.0 mg·dm<sup>-3</sup> IAA. The adventitious buds were passaged onto the MS medium with 3.0 mg·dm<sup>-3</sup> BAP and 5.0 mg·dm<sup>-3</sup> NAA added. On those media callus was successfully induced. Overall, these results showed that the synergistic effect of BAP and IAA in the shoot formation process. The interaction of those phytohormones was described also in chicory *Cichorium intybus* L. by Manickam et al. [20] and confirmed in gerbera *in vitro* cultures by Thakur et al. [24] and Velayutham et al. [27]. Similarly, the synergistic effect of BAP and IAA for regeneration of shoots of another species, important for the commercial *Stevia rebaudiana* (Asteraceae), was reported by Jain et al. [7] and Laribi et al. [17]. In addition, the authors reported that an important factor in the process of regeneration of shoots are genotypic differences across cultivars and species.

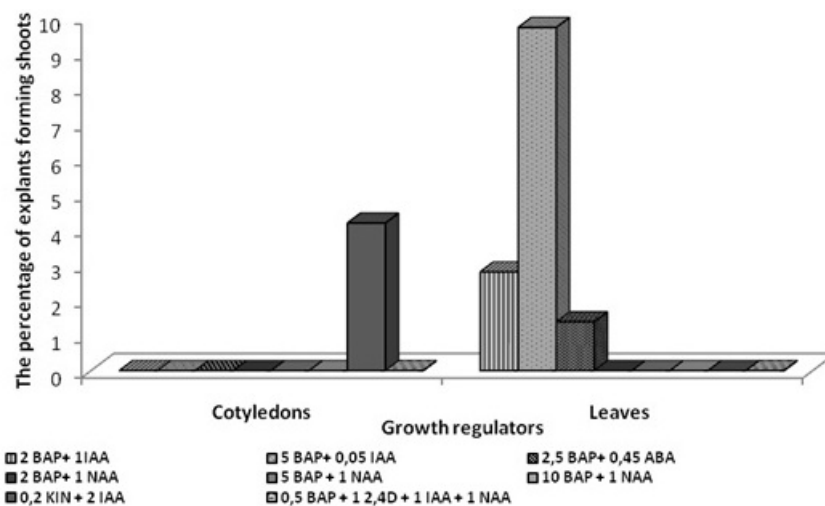


Fig. 2 . Effect of different hormonal composition in MS medium on the morphogenetic reactions of cotyledons and leaves explants of *Silphium perfoliatum*. The percentage of explants forming shoots

**Root induction.** In our experiment the root formation was observed on the explants of cotyledons and young leaves with petioles placed on the medium supplemented with KIN and IAA (Tab. 1) (Fig. 4C). In the presence of those phytohormones a 62% efficiency of rhizogenesis on cotyledon explants was reported. However, for young leaves with petioles, the response of the explants was poorer; a 9.7% rooting effectiveness was noted (Fig. 3). As reported in literature, the basic regulator for the induction of rhizogenesis is auxin. Most frequently IBA, NAA or IAA is added to the rooting medium, although sometimes roots get formed due to endogenous growth regulators [1, 2]. In the present study, the best rooting response was found only in the presence of the highest concentration of IAA in the MS medium.

Our results are consistent with those of Laribi et al. [17] who found that the highest rooting percentage and number of roots per shoot in *Stevia rebaudiana* were obtained on MS medium with IAA added. Indeed, in the case of many plants representing the *Asteraceae* family the process of induction of rhizogenesis occurred on MS medium fortified with IAA. In the course of micropropagation of chicory, also an intensified effect of IAA on the rooting process was noticed [27]. Phytohormone also stimulated the formation of single and adventitious roots in gerbera [3, 26] and dahlia [6]. On the other hand, Bhatia et al. [4] considered a half-strength MS with 1.0 mg·dm<sup>-3</sup> IBA and 45 mg·dm<sup>-3</sup> saccharose added to be the best gerbera rooting medium.

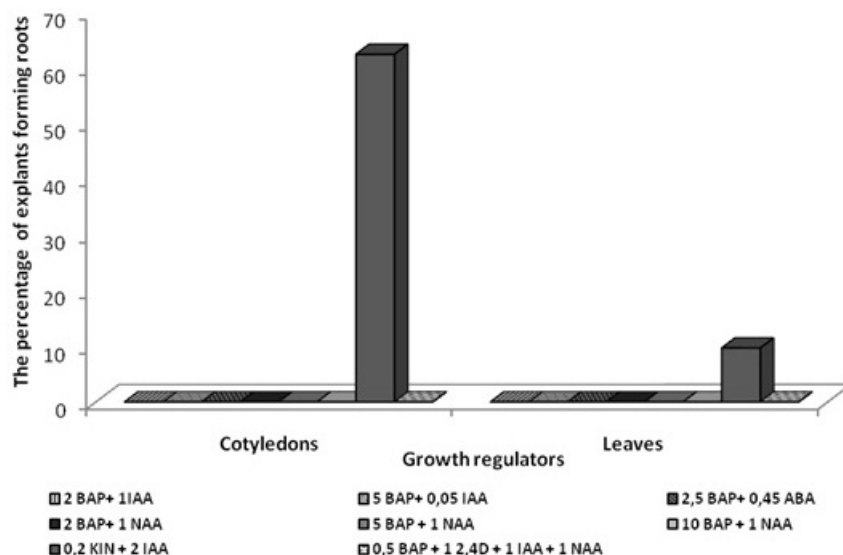


Fig. 3. Effect of different hormonal composition in MS medium on the morphogenetic reactions of cotyledons and leaves explants of *Silphium perfoliatum*. The percentage of explants forming roots

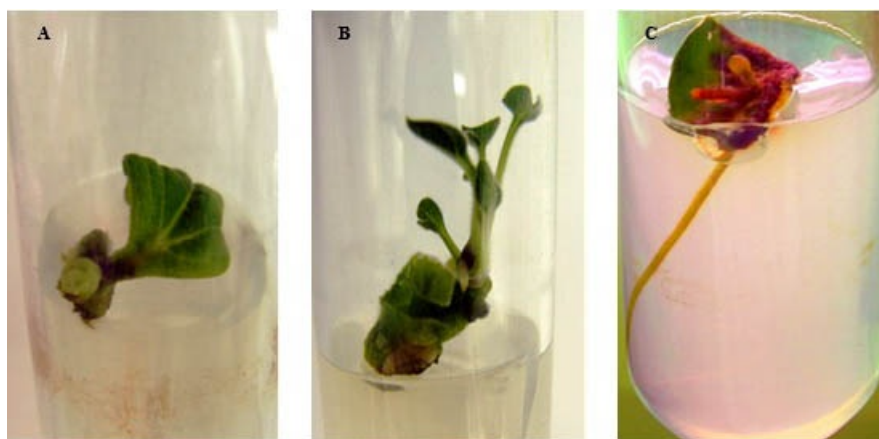


Fig. 4. The response of explants of *Silphium perfoliatum* to in vitro culture conditions. The callus tissue forming [A], the shoots regeneration [B] and roots induction [C]

## CONCLUSIONS

The in vitro regeneration protocol described in this report for induction organogenesis is a method to get plants from cotyledons and leaf explants. Results of the present work demonstrated that the callus and adventitious shoots were obtained on both types of explants. However, the parts of young leaves have a greater efficiency of regeneration. The present study reveals that the most effectiveness callus formations was observed for young leaf with petioles explants on the medium supplemented with 5 mg·dm<sup>-3</sup> BAP or 10 mg·dm<sup>-3</sup> BAP and 1.0 mg·dm<sup>-3</sup> NAA. Similar results were reported for explants on medium with an addition of 2,4 – D. The highest shoot initiation was obtained on MS medium with 5.0 mg·dm<sup>-3</sup> BAP and 0.05 mg·dm<sup>-3</sup> IAA. The root forming ability of the explants depended on growth regulators in a special way. In the presence of KIN and IAA on cotyledons explants the highest number of roots was observed. This study confirms that exogenous hormonal combination is specific for the type of explants for appropriate response. Advances in biotechnology, particularly methods for culturing plant cells and tissues, should provide new means for commercial processing of even rare plants and chemicals they produce. These new technologies will extend and enhance the usefulness of plants as renewable resources of valuable chemicals. In the future, biologically active plant-derived chemicals are expected to play an increasingly significant role in the commercial development of new products for regulating plant growth and for insect and weed control.

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