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SHORT COMMUNICATION

The effect of NAA and BAP on the multiplication of chamomile (*Matricaria chamomilla* L.) callus tissue *in vitro*

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ABSTRACT

The aim of the study was to determine the effect of the additive on NAA and BAP on the formation of callus tissue on leaf chamomile explants, cultivar, and to determine the optimal content of said growth regulators for the initiation of callus tissue *in vitro*. During the experiment fragments of chamomile from the collection of the Department of Genetics, Breeding and Biotechnology of Plant were laid on a medium with mineral composition according to Murashige and Skoog - MS (1962) enriched with auxin NAA in concentrations of 3 and 5 mg·dm^{-3} and cytokinin BAP in a concentration of 1, 2 , 5, 7 mg·dm^{-3}. Based on the conducted experiment it was found that the initiation of callus chamomile tissue is possible in the case of using leaf explants lined with MS in the combination of auxin NAA in concentration 3 or 5 mg·dm^{-3} and BAP from 1 to 7 mg·dm^{-3}. The propagation of chamomile callus tissue should be carried out on MS proliferating medium enriched with NAA at a rate of 3 mg·dm^{-3} and BAP in an amount of 7 mg·dm^{-3}. Explants laid on a medium with this composition develop a large mass of callus tissue.

***Keywords*:** Chamomile, callus tissue, *in vitro*, multiplication, growth regulators, *Matricaria chamomilla*, *Campyliaceae*

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1. INTRODUCTION

It is an annual perennial, long day of a light-sensitive nature. Present in almost the whole Eurasian region, and since the colonial period in both Americas. Taxonomically, it represents the family of *Campyliaceae* of a thin taproot with numerous ramifications. The empty, usually ramified stalk grows up to 15-40 cm in height. Alternate leaves, double sitting, with balanced flaky petals with sharp ends. main and secondary shoots have small single baskets with white lilac flowers. Flowering baskets with a semicircular bottom, narrow conical and empty during flowering ending. Marginal lilac, female, white flowers about 2-6 mm in length. Internal bisexual yellow tubes Five anthers fused in a tube covering the neck of the pistill. Dry-type fruit, longish, brown-green, a little bent in the apical tip. Has five ribs close to one side of the achenne, with no pappus. This plant is flowering from the beginning of May to the end of October (Gardiner, 1999).

Chamomile was already known in ancient times for its soothing properties. Used internally and externally. Written references on its influence on the human organism can be found in medieval Dominican and Benedictine books. Ointments, infusions, decoctions and fumes were treated by Pagans as a cure for burns, allegries, neuroses or ulcers (Rafieohossaini et al, 2010). Modern science has frequently proven the influence of active ingredients found in materials of chamomile on human health and mood. Young flowers are the most frequently used material from this plant. The gathered oil contains a large group of micro- and macroelements. The most important ingredient of the Chamomillae anthodium are etheric oils. They have a variety of applications like anti-imflammatory, antispasmatic, antianxiety, antibacterial, accelerating wound healing and antiulceric. According to some sources, a signifigant influence on people recovering from addictions was reported. These reports were stated not only in the Polish pharmacopeia, but also in the European edition, which is a sign on the impressive properties of the often neglected plant (Singh at al, 2011). These compounds consist of chemicelulosis, alpha-bisbolol and its oxides, spyroether, B-pharmesan, mircene and cadinene. Moreover, there is a large group of phlavonoids, such as apigenine, luteonine, or quartzetine (Kuznicka, 1984). One must not also forget about the cumarine derivatives such as umbeliphenol or herniarine. Spirocyclic polyacetylenes, mucus, choline and of course caretenoids, have less active properties, but are used as auxiliary substances to drugs ingredients based on chamomile (Singh at al, 2011)

The climate requirements are modest. The place should be well exposed to sunlight. The requirements of light soils, of good water-air ratio, medium, of good agricultural and environmental conditions. It's a calciophile. The most suitable crop rotation are root crops, papilionaceae, rapeseed. The cultivation is the best in the third year after the manure. Cultivating directly after the manure delays flowering, prolongs and worsens it, causes a too strong increase of leaf and sprout mass. The seed bed should be carefully prepared (medium plowing, harrowing), bearing in mind the size of the seeds. Chamomile is grown from seed to the ground from August to September, or in Spring, although the crops will be smaller then. Delaying the seeding is not recommended, because it causes worse hibernation of the plant, and as a result - worsening and delaying the crops (KeShun, 1997). Seeding 1.3-1.5 kg/ha. Soil rollling is recommended directly after seeding. The germination process occurs in full sunlight, therefore covering the plantation after seeding with a layer of soil is not advised. Fertilizing with potassium, nitrogen and calcium is essential for the cultivation success, while phosphorus has a minor influence. Fertilizer dose for chamomile: 40-50 N/ha, 50-60 P_2O_5/ha, 80-100

K_2O/ha. Nitrogen fertilizers are seeded in two periods: 1/3 of the dose before the seeding, 2/3 in the leaf rosetta phase and potasium and phosphor fertilizers entirely before the seeding. On acidulated soils it is necessary to lime before the seeding or for the forecrop in the amount of 1.5-3 t/ha $CaCO_3$ (KeShun, 1997).

Plantation maintenance involves weed control, hoeing, pest and disease control. Weed control and hoeing of the soils surface layer should be started in Autumn up to when the cultivated plant covers the inter-row to avoid competition between the cultivated and intrusive plants. Chamomile is usually resistant to diseases. It is only vulnerable to mildew. It is often attacked by pests: aphids, --- They are treated by applying appropriate insecticides (KeShun, 1997).

The tissue of the callus in *in vitro* conditions has a remarkably big growth ratetherefore its breeding in properly applied conditions is not a problem. The only aspect of signifigant importance is the proper choice of the type and ammount of given growth regulators. In order to initiate the production of the callus tissues the plant should be mechanically injured, for example by cutting one of the living parts of the plant and next putting it on a balanced nutrition solution. Applying callus cultures taken from *in vitro* explants can be taken into account in the industrial as well as scientific field. Cells taken from tissue cultures, under appropriate stimulants, behave similarly to bacteria cells. Their growth and proliferation matches the logarithmic growth of some microorganisms. Using this feature, in absolutely sterile conditions any number of undifferentiated tissue for initiating the next cultures, cuttings or scientific research purposes can be collected. The conditions gained in solar bioreactors using the selection chamber and mutagenic compounds let us create plant tissues of properties impossible to achieve outside the laboratory. The amount of acquired secondary metabolites let us reduce production costs or easier acquiring of the metabolite of a certain type (Bhatia S. et al, 2015).

The goal of the research was to define the most appropriate level of plant height regulators to produce the highest mass of callus tissue.

2. MATERIAL AND METHODS

The starting material applied to initiate callus cultures were leaves gathered from chamomile "Złoty łan" seeds in 2013. The explants were gathered from plants cultivated *in vitro* in the Department of Genetics, Breeding and Biotechnology of the West Pomeranian University of Technology in Szczecin. It is a tetraploid vareity and even stable yielding (about 1000 baskets). It has a high amount of oil, azulene and guaiazulene. Bizabol occurs in amount typical to other vareities. It is a variety suitable for automated crops. Explants for initiating callus cultures in sterile *in vitro* cultures were taken from Chamomile growing on MS nutrients (Murashige and Skog, 1962), enriched in plant growth regulators. In order to initiate the proliferation of the callus, the cut leaves have been injured by one cut along the main conductive nerve. Next the injured leaves were moved to 330 ml jars containing 30 ml of nutrition solution. This was carried out in sterile conditions under the camera with laminar flow of air. These samples were stored in a growth chamber with a stable temperature of 24 ºC +1 ºC and light intensity of 40 PAR ($\mu E\ m^{-2}\ s^{-1}$). After three weeks of initiating the culture, the mass obtained by every explant was specified. The obtained results were processed statistically by variety analysis in a complete randomization system. The averages obtained during the measurement were compared with each other with the Turkey test with the signifigance level $\alpha = 0.05$.

The calculations collected from the Turkey test confidence half-ranges let us determine homogenious groups, which varied from each other in a statistically signifigant degree. The individual homogenious groups were assigned to letters of the alphabet.

3. RESULTS

Picture 1. Chamomile explants with cultured callus tissue on MS medium supplemented with 1 mg·dm^{-3} BAP and 3 mg·dm^{-3} NAA

Picture 2. Chamomile explants with cultured callus tissue on MS medium supplemented with 7 mg·dm^{-3} BAP and 3 mg·dm^{-3} NAA

The callus tissue has developed on all explants lain onto each kind of nutrition solution applied in the experiment. The most important increase of callus tissue weight were shown in the explants lain onto the nutrition solution enriched in BAP in the concentration 7 mg·dm^{-3} (1.04 g) and NAA in the concentration of 3 mg·dm^{-3}. The experiment has shown, that higher BAP concentration acted positively on the proliferation of the callus tissues. Yet with identical values of BAP and various concentration of NAA to themselves, the auxin of lower presence value acted more efficiently on its increase.

Table 1. The mass of callus tissue developed on fragments of leaf blades of chamomile lined with nutrient solutions with varying concentrations of plant growth regulators.

No.	Media		Mass of callus tissue [g]	
	Growth regulator [mg·dm^{-3}]			
	BAP	NAA	Mean	Results
1	1	3	0.893	ab
2	2	3	0.825	abc
3	5	3	0.893	ab
4	7	3	1.042	ab
5	1	5	0.788	bcd
6	2	5	0.686	cd
7	5	5	0.609	d
8	7	5	0.928	ab

4. CONCLUSIONS

On the basis of the experiment we can say that the explants lain onto the nutrition solution enriched in NAA in the concentration of 3 mg·dm^{-3} and BAP in the amount of 7 mg·dm^{-3} have created the highest growth of callus tissue. According to the research conducted by Kintios and Michaelakis from 1999 using other specimens of cytokines and auxines is also possible. 2,4-D in the concentration of 4,5 mg·dm^{-3} and KIN in the concentration of 0,46 mg·dm^{-3} initiated the most optimal callus growth in all samples. The growth regulators applied in this experiment are a strong subgroup of stimulating compounds, therefore their properties often show unwanted influence on the cells of callus tissues (Kintzios and Michaelakis 1999). A similar group of regulators was used in research on basil (*Ocimum basilicum* L.). Callus obtained by applying 2,4-D, BAP and GA_3 showed a high degree of proliferation and its lifespan was satisfactory. Explants lying onto the nutrition solution enriched in 1 mg·dm^{-3} of 2,4-D and 0.5 mg·dm^{-3} of BAP had the most signifigant influence on the growth of the callus tissue (Krupa-Małkiewicz and Zieliński 2013). Other used growth regulators used to proliferate callus, like chicory, were IAA and 2iP, which concentration 0.1 mg·dm^{-3} IAA and 4 mg·dm^{-3} 2iP has shown the greatest efficiency of the growth of the callus tissue (Doliński and Olek 2013). Applying even IAA auxine itself can lead to callus tissue initiation. Applying only 0.25 mg·dm^{-3} causes the initiation of callus in tobacco, which shows the influence of this regulator on proliferation of the tissue (Hanus-Fajerska and Citkowska 2012). IAA and BAP were also used in proliferation oif the tissues in tomatoes (Rzepka-Plevnes et al. 2006), or the (Gatz and Kowalski 2011). Despite using the same growth regulators by these researchers, their results differed significantly. This variety could be caused by genotype diversity, as well as taxonomic distance between them. An interesting work on proliferation of callus in *in vitro* conditions was conducted by Kumar and Kumar-Kanwar on the specimens of Gerber Jamesonia. Their work covered various influence of IBA, NAA 2,4-D BA and Kinetin on growth and quality of the callus tissue. The preliminary test conducted this way can indicate which of the regulators is the most effective on the explant while maintaining its vitality (Kumar and Kumar-Kanawar 2007). The least used growth regulators is TDZ. The effects of its action indicate strong diminishing of regeneration abilities and vitality of cells. After applying TDZ, crambe tissues significantly did not made division abilities (Furmanek and Banaś 2011). Such important influence of plant regulators, supported by research, depends on the kind of growth regulators, their intensity, state and age of the isolated part.

Despite all the problems researchers encounter, with using appropriate regulators, nutrition solutions and conditions, using callus tissues is possible. Its properties cannot be underestimated in research and industry. The results obtained during research of "Złoty Łan" camonile can lead to obtain new somaclonal varieties and obtaining higher concentrations of secondary metabolites which proves the signifigance of the carried out research.

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