Fagopyrum esculentum in vitro

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SUMMARY

Fagopyrum esculentum in vitro
Common buckwheat is a valuable source of the flavonoid rutin. Its *in vitro* culture was derived from a young seedling plant and the content of flavonoids in the callus culture was compared with their content in an intact plant. The optimal nutrient medium for the cultivation of the *in vitro* culture and for the production of flavonoids is Murashige and Skoog medium which contains, during the cultivation in the normal light regime, the growth regulators combination of 2,4-D 1 mg/l and kinetin 1 mg/l.
Key words: buckwheat – *Fagopyrum* – rutin – callus culture

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SOUHRN

Fagopyrum esculentum in vitro
Pohanka obecná je cenným zdrojem flavonoidu rutinu. Z klíční rostliny byla odvozena kultura *in vitro* a porovnán obsah flavonoidů v kalusové kultuře a v intaktní rostlině. Pro kultivaci kultury *in vitro* a produkci flavonoidů je optimální živné medium Murashigeho a Skooga s obsahem kombinace růstových regulátorů 2,4-D 1 mg/l a kinetinu 1 mg/l při kultivaci za normálního světelného režimu.
Klíčová slova: *Fagopyrum esculentum* – pohanka – kultura *in vitro* – rutin – flavonoidy

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Introduction

Common buckwheat (*Fagopyrum esculentum* Moench.) is a dicotyledon, a member of the *Polygonaceae* family [1]. A related plant is *Fagopyrum tataricum* Gaertner [2]. Buckwheat comes from Asia, most likely from the region between Lake Baikal and Manchuria. It is one of the youngest cultivated plants and was imported to Europe from the East in the 13th century. In our country, the cultivation of this plant became more widespread in about the 16th century, namely in the poor soils of mountain regions – in the Beskydy Mountains, in the Carpathian region, and in East Slovakia. At present, buckwheat farming in our country goes through a revival [3]. Some authors distinguish between two types of cultivars. One type comes from Japan, Korea, southern China, Nepal, and India and these plants are of tall growth, heavily infoliated, and later-ripening. To develop generative organs, these types of cultivars need days with at least ten-hour daylight. Another type of buckwheat cultivars is grown in Europe and northern China where the nine-hour daylight provides ample daylight for the plant to develop its generative organs. These cultivars are shorter in growth, less infoliated, and earlier-ripening [1]. The blooming herb (*Fagoryri herba*) and/or hull are used for medical purposes. Rutin is extracted form the plant's haulm. Buckwheat achenes (*Fagopyri semen*) provide valuable nourishment and sufficient amount of fibre [2]. Buckwheat contains the flavonoids rutin, isoorientin, orientin, vitelin [4]. Rutin is the most important substance found in the blooming herb and in the seed hull. The seeds also contain the B-group vitamin complex, vitamin E, and a whole line of chemical elements, namely potassium, phosphorus, magnesium, calcium, and traces of iron, copper, manganese, and zinc. From the medical point of view, the content of choline is interesting; so is

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the fact that the buckwheat achenes contain many proteins of full value. Fagopyrin present in buckwheat causes oversensitivity to light [2]. The protein content in the buckwheat seeds amounts to 10–14 %, the starch content is 55–70 %, the seed oiliness is 1.5–3.7 %, and the total fibre content in seeds is 3.4–5.2 %. The linolic acid content [5] is relevant among the content of several olefinic fatty acids. Buckwheat lipids contain 0.2 % of physiologically active plant sterols: sitosterol and compesterol [6]. Rutin, 2-epicatechin, hyperosid, and quercetin are the most important phenolic substances found in the hull and in buckwheat flour [7].

Buckwheat is also a source of selenium. The selenium content in buckwheat seeds was increased by approximately 8.5 % after selenium solution had been applied to leaves during the blooming period. This method could make buckwheat a rich source of selenium and a useful material to enrich food industry products [8].

Buckwheat haulm is administered to increase blood vessel walls' strength and elasticity. It is utilized in the treatment of varicose veins, hemorrhoids, venous ulcerations, in cases of vascular deficiencies of the limbs which are characterized by the appearance of red capillary blood vessels, and as prevention against blood vessel rapture (for instance, sudden brain stroke prevention). Medicine utilizes the buckwheat achenes to lower the blood cholesterol level, to treat bowel ailments, and during organism detoxification [2]. Buckwheat is used to curb arthritis [4] and strengthens the immune system [9]. Buckwheat is one of the staples to eat during pregnancy; consuming buckwheat is also recommended to people who suffer from diabetes since it contains no gluten, and is suitable for celiac patients. Buckwheat consumption protects against heart diseases as it decreases LDL cholesterol and increases HDL cholesterol [10].

The study aimed to derive a callus culture *Fagopyrum esculentum*, find ideal conditions for the culture's growth in *in vitro* culture, and compare the content of flavonoids in the buckwheat herb with the content of flavonoids in the callus culture. To carry out the experiment, the authors used the callus culture which was derived from the root parts of young seedlings of the *Fagopyrum esculentum* plants in the 3rd–10th passage.

EXPERIMENTAL PART

Materials and methods

The tissue culture was derived from the root parts of young seedlings of the *Fagopyrum esculentum* plants. Before cultivation, seeds were sterilized on the surface. After sterilization, the seeds were individually transferred into Erlenmayer flasks containing Murashige and Skoog (MS) culture medium [11] into which agar and the growth regulator 2,4-dichloro-phenoxy-acetic acid in a concentration of 1.0 mg/l were added. The cultivation took its course at a temperature of 25 °C, during the normal light regime, which is 16 hours of light and 8 hours of darkness. Callus formed from the young seedlings was transferred to a paper bridge placed inside a sterile Erlenmayer flask with MS medium supplemented with the 2,4 D growth regulator of a 1.0 mg/l concentration. The cultivation of the culture continued under these conditions until the 3rd passage. Starting with the 3rd passage, the MS culture medium was enriched with the following growth regulators: 6-benzyl amino purin (BAP) in concentrations of 0.1, 1, 10 mg/l; α-naphthalene acetic acid (α-NAA), 0.1, 1, 10 mg/l; 2,4 dichloro-phenoxy-acetic acid (2,4 D), 0.1, 1 mg/l; a combination of growth regulators: 2,4 D 0.1 and 6-furfurylaminopurin (K), 0.1 mg/l; 2,4 D 0.1 and 6-furfurylaminopurin 1 mg/l; 2,4 D 1 and 6-furfurylaminopurin 0.1 mg/l; 2,4 D 1 and 6-furfurylaminopurin 1 mg/l.

The culture was allowed to grow for 5 weeks at 25 °C under different light conditions – daylight photoperiod (16 hr daylight and 8 hr darkness), in darkness when the culture was blocked from light during the entire growth period, and in light when the culture was continuously exposed to light coming from a 100 W light fixture suspended 1 m over the culture. After 5 weeks of cultivation, the calluses which had developed were weighed and the growth was evaluated – the growth factor R was utilized [12].

Analyses

A spectrophotometer was used, in accordance with PhB 97, to determine the content of flavonoids [13]. The dried calluses were turned to powder state before the determination process could begin.

RESULTS AND DISCUSSION

When cultivating plant cultures *in vitro*, it is very important to select a suitable culture medium which offers the optimal combination and concentration of growth regulators. It is also necessary to set the growth conditions (light, temperature, pH) and adhere to aseptic principles during work.

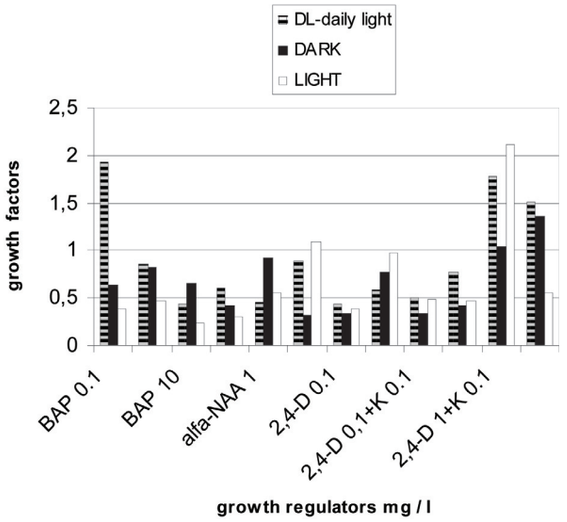

Fig. 1. Growth factors of Fagopyrum esculentum callus culture on MS medium supplemented with various growth regulators and under various light conditions

Kalini et al. pointed out the interrelations between the callus growth intensity and the size of the explant. The authors discovered that the larger the explant in size, the more varied composition of cells. The growth intensity

of the same type of callus may differ. This variability is caused by the fact that the inoculum is collected not only from different parts of callus cells growing uncontrolled, but also from different calluses [14].

During cytodifferentiation, as well as cell aggregation, and morphologic organization stages, the growth slows down, which directly correlates with the synthesis of secondary metabolites in the tissue culture. It has been demonstrated that the factors which are known to slow down the tissue cultures' growth also stimulate the production of secondary metabolites, namely during the secondary metabolism phase. This fact suggests certain antagonism of the primary and secondary metabolism, which is represented at the molecular level by different utilization of common precursors. If the conditions are set to promote fast growth and division, these substances are utilized, for instance, for the synthesis of proteins; but if the conditions are set to inhibit the growth, the substances are utilized for the synthesis of secondary metabolites [12].

The results of the experimental work indicate that the maximum growth of culture during the cultivation occurred when the light and the regulator 2,4-D 1 mg/l+K 0.1 mg/l (RF 2.109) were used (Fig. 1). The maximum production of flavonoids in the culture was achieved during the normal daylight regime cultivation with s 2,4-D 1 mg/l+K 1 mg/l (0.069 %) (Fig. 2).

When the BAP growth regulator was used, the highest growth was recorded when the BAP in a concentration of 0.1 mg/l was used under normal light conditions. The growth factor increased in this case approximately 3.5 times. The growth was also significant with BAP in a concentration of 1 mg/l under normal light regime and in darkness (Fig. 1). When α-NAA was used the highest growth was recorded with the use of α-NAA 10 mg/l concentration under normal light regime and also under light, and with the use of α-NAA 1 mg/l in darkness. The growth factors under these conditions were approximately twice as high as with α-NAA 0.1 mg/l (Fig. 1). When the 2,4-D growth regulator was used, higher growth was achieved with 2,4-D 1mg/l compared to 2,4-D 0.1 mg/l. When the combinations of the growth regulators were used, the highest growth of culture was observed when 2,4-D 1+K 0.1 mg/l and 2,4-D 1+K 1 mg/l combination was used where the growth factor roughly tripled compared to the use of the 2,4-D 0.1+K 0.1 mg/l and 2,4-D 0.1+K 1 mg/l combinations (Fig. 1). The test results show that the growth regulators selected had a significant impact on the culture's growth.

The results in the summary of growth factors suggest that the *Fagopyrum esculentum* grew best under normal light regime. The highest growth of the culture under normal light regime was achieved during the cultivation in which with the BAP 0.1 mg/l; 2,4-D 1+K 0.1 mg/l and 2,4-D 1+K 1mg/l regulators were used. Under these conditions, the growth factor roughly tripled. In light, the highest growth was achieved with the 2,4-D 1+0,1K 1mg/l regulators; and in darkness with 2,4-D 1+0.1 K 1 mg/l (Fig. 1).

The results obtained indicate that statistically important increase in the flavonoid content under normal light condition was achieved when cultivating with the growth regulators BAP 0.1, 1, 10 mg/l; α-NAA 0.1, 10 mg/l; 2,4-D 0.1, 1 mg/l; 2,4-D 0.1+K 1; 2,4-D 1+K 0.1; 2,4-D 1+K 1 mg/l (Fig. 2). When cultivating in darkness, a statistically important increase was recorded in the flavonoid content when BAP 0.1 mg/l; α-NAA 1 mg/l; 2,4-D 0.1, 1 mg/l; 2,4-D 0.1+K 1; 2,4-D 1+K 1 mg/l regulators were used. Statistically important increase in the flavonoid content was observed when the following growth regulators were used: BAP 1, 10 mg/l; α-NAA 0.1, 1, 10 mg/l; 2,4-D 0.1 mg/l; 2,4-D 0.1+K 0,1; 2,4-D 0.1+K 1; 2,4-D 1+K 0.1; 2,4-D 1+K 1 mg/l (Fig. 2). The results obtained from the test allow the authors to state that the growth regulators which had been selected had a significant impact on the production of flavonoids in the callus culture.

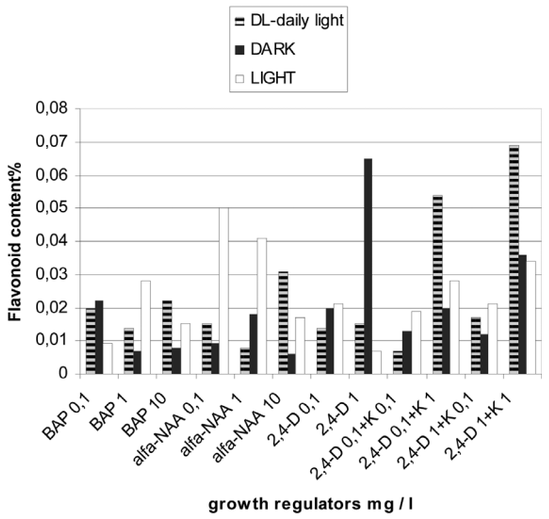

Fig. 2. Flavonoid content in Fagopyrum esculentum callus culture on MS medium supplemented with various growth regulators and under various light conditions

The summary of the flavonoid contents shows that the *Fagopyrum esculentum* culture produced flavonoids best in light. The production increased 3.5 times under the normal light regime with 2,4-D 1+K 1 mg/l and in darkness with 2,4-D 1 mg/l. The production of flavonoids increased roughly 2.5 times during the normal light regime with 2,4-D 0.1+K 1 mg/l and in light with α-NAA 0,1 mg/l; and approximately doubled with the use of α-NAA 1 mg/l (Fig. 2).

The study has revealed that the average content of flavonoids in the *Fagopyrum esculentum* callus culture is 0.023 %. In comparison with intact plants, the plant callus cultures generally produce a smaller number of secondary metabolites. The contents of flavonoids in buckwheat listed below were quoted by different authors: Oshawa and Tsutsumi grew different strains of buckwheat. Employing HPLC to determine the content of rutin in ground seeds, the average rutin content in plants grown under long-day conditions was established at 0.147 mg/g. The plants grown under short-day conditions contained 0.064 mg/g of rutin [15]. Wanatabe stated that the total concentration of flavonoids in buckwheat seeds and hull was 18.8 mg/100 g and 74 mg/100 g [16]. When determining the rutin content in ground buckwhe-

at seeds using the capillary electrophoresis technique, the present authors arrived at the following concentration values of rutin: 131–476 ppm in buckwheat bran, 19–168 ppm in flour, and 29 ppm in hull. The buckwheat leaves, stems, and blossoms contain, on the average, approximately 300, 1000, and 46000 ppm of rutin [13].

The evaluation of the results on the culture's growth and production has shown that the best combination of the growth regulators tested is the 2,4-D 1+K 1 mg/l combination. It is the ideal combination for the culture's growth and the production of flavonoids under normal light regime.

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KNIHY

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Z jiných jsou to nová protiinfektiva: polypeptidové antibiotikum daptomycin (CUBICIN) a tetracyklinový tigecyklin (TYGACIL), antivirotikum u hepatitidy B entekavir (BARUCLADE) nebo antimykotikum posakonazol (NOXAFIL), z enzymů jsou to aglukosidasa alfa (MYOZYME) pro Morbus Pompe a galsufasa (NAGLAZYME) pro mukopolysacharidosu nebo z hormonální produkce uterotonikum karbetocin (PABAL) jako syntetické analogon oxytocinu a z gonadotropinů urofollitropin (BRAVELLE).

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