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Plant Physiology

Formation of the Phenolic Compounds in the Tissue Culture of *Rhododendron Caucasicum* Pall.

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ABSTRACT. Callus cultures are obtained from the leaf and stem of *Rhododendron Caucasicum* PALL. growing at passages. The primary callus was formed in 10-15 days on Heller's modified nutrient medium. For selection of the best conditions of growth for the callus, different concentrations of some nutrient medium components were tested. It was found that 5 and 10mg/l of 2,4D supported tissue growth, while 0.5mg/l suppressed it. Kinetin suppressed growth in both 1 and 5mg/l concentrations. The yeast extract of 1000–2000mg/l and 20mg/l of mioinositol increased the yield of biomass, while 100 mg/l of yeast extract and 2mg/l of mioinositol decreased it. Three phases of callus growth were identified: monotonous (up to 20 days), linear (20-40 days) and reduced (40-50 days). Besides, the stem callus outstripped the leaf tissue in growth. Preliminary analysis showed that the Rhododendron leaf and stem cultures maintain the ability to synthesize biologically active phenolic compounds typical of an intact plant. However, their amount and composition were considerably less in callus tissues, which is characteristic of most cultures. The content of phenolic compounds is 4-5 % of dry weight. Besides, the main part (85-90%) consists of (+) catechin, (-) epicatechin and proanthocyanidines. © *2014 Bull. Georg. Natl. Acad. Sci.*

Key words: *Callus cultures, Rhododendron leaf and stem, nutrient medium, phenolic compounds.*

The use of tissue and cell cultures of higher plants as the instruments of various investigations in biotechnology is of interest. The main function of a secondary metabolism in intact plants is to solve the ecological problems [1]. This indicates that synthesis of the secondary compounds is strongly controlled by the organism. Consequently, proceeding from their properties the tissue and cell cultures appear to be the alternative model determining the probability of a secondary metabolism decrease [2]. There are a lot of data confirming that [3]. Different growth regulators can increase the maintenance of secondary compounds in the tissue and cell cultures.

Rhododendron Caucasicum Pall. is widely spread in Georgia. It contains a great amount of biologically active phenolic compounds: catechins, proanthocyanidines and flavonols [5].

In literature, there are no data about introduction of rhododendron into tissue culture. The goal of the present work was to obtain rhododendron callus culture growing at passages and select the nutrient medium of optimal composition to provide the best

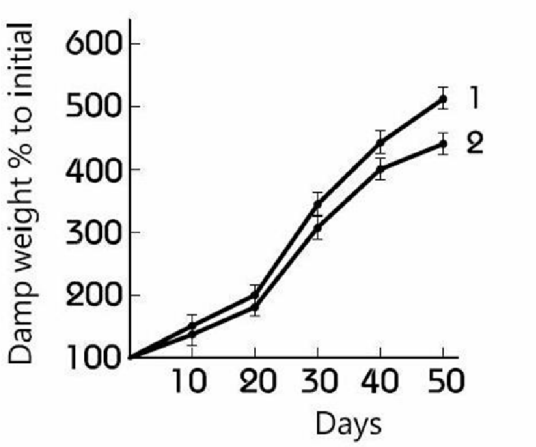

Fig. 1. Curves of the leaf and stem callus of rhododendron growth: 1 - stem, 2 - leaf.

growth of the callus and to synthesize phenolic compounds.

Materials and Methods

Callus tissue was obtained from young leaves and stems of *Rhododendron Caucasicum* growing in the hothouse. Initial material was sterilized by diacid. The primary callus was formed in 10 -15 days on Heller's modified nutrient medium with the following composition (mg/l): mineral salts according to Heller; Fe–citrate 3.5; glucose 25 000; vitamins according to White; 2,4-D 5.0; adenin 5.0; Ca-panthothenat 0.1; mioinositol 20.0; yeast extract 1000; agar 7000; pH 5.2 [6]. The medium for further cultivation of the obtained callus had the same composition. Tissue was grown in test tubes in darkness at 26 ^{0}C with relative humidity of 70 % and was transplanted every 6 weeks.

For selection of the best conditions for callus growth different concentrations of some of the components of nutrient medium were tested. Experiments were carried out for 42 days. Damp and dry weights of culture were determined (mass grown during the passage in test-tube). Experiments were repeated 5 times.

Phenolic compounds from lyophilized material were extracted by hot methanol up to color disappearance with 1%-vanillin solution in concentrated HCl. The content of phenolic compounds in the obtained extracts was determined by using Follin-Dennis reagent [7] followed by the measurement on the spectrophotometer at 725nm. Calibration curves were constructed using recrystallized (-)-epicatechin as a standard.

Chromatographic analysis of polyphenols was conducted in fractions obtained after separation of the polymeric phenolic compounds on the polyamide column. The methanol extract from lyophilized material was evaporated in vacuum; the residue was dissolved in water and passed through the polyamide column (2.5g; 2.5x3cm). The column was washed by water and then phenolic compounds were eluted by 96% ethanol. The process of elution was controlled according to the color with 1%-vanillin solution in concentrated HCl. Ethanol elution was evaporated in vacuum and the residue was dissolved in a small volume of 96%-ethanol. The latter was used for plotting on the paper.

For separation of phenolic compounds we used the paper Filtrak 3 and the dissolvent system *n*-buthanol–vinegar acid–water (40:12:28 according to the volume). Substances were identified by virtue of their fluorescence in UV-light (before and after treatment by NH_3 steam), comparing their Rf with markers [7].

Results and Discussion

As a result of our investigation we introduced a culture and obtained the callus culture from the leaf and stem of *Rhododendron Caucasicum* growing at passages. Tissues were compact and light becoming considerably darker at aging. The difference between the leaf and stem calluses was not observed. Tissue growth was rather slow, though in the following passages it accelerated slightly. Fig.1 shows the growth curves of the rhododendron leaf and stem callus. One can distinguish three phases of growth: monotonous (up to 20 days), linear (20-40 days) and reduced (40-50 days). Besides, the stem callus growth outstrips the growth of the leaf tissue.

For selection of the best conditions for callus

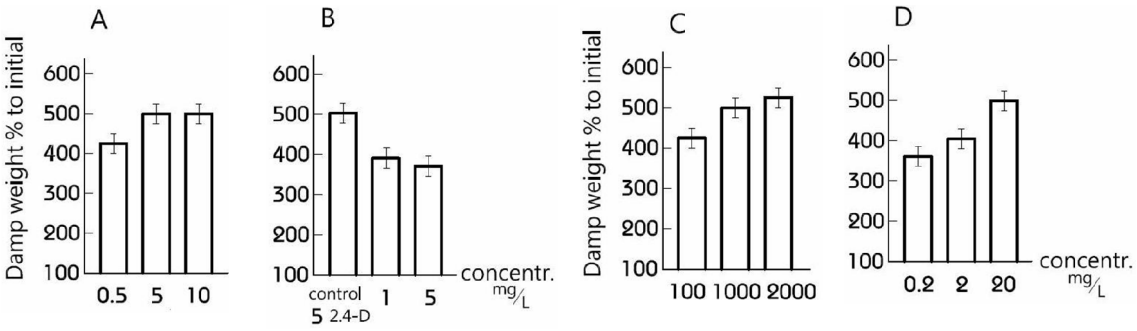

Fig. 2. Dependence of the Rhododendron callus culture growth on different concentrations. A - 2.4-D; B - kinetin; C - mioinositol; D - yeast extract.

growth, the experiments for optimization of the nutrient medium composition were conducted. Different concentrations of 2,4-D, kinetin, mioinositol and yeast extract were tested (Fig. 2). Data obtained allowed choosing the most favorable composition of nutrient medium for growing the rhododendron callus cultures. It was found that 5 mg/l and 10 mg/l of 2,4-D support tissue growth, while 0.5 mg/l suppress it (Fig. 2A). Kinetin suppresses growth in 1mg/l and 5mg/l concentrations (Fig. 2B). Concentrations of 1000 mg/l and 2000 mg/l for yeast extract and 20 mg/l for mioinositol proved to be the most suitable for callus growth (Fig. 2C, D). Obtained results almost coincide with the data of the tea callus culture [6].

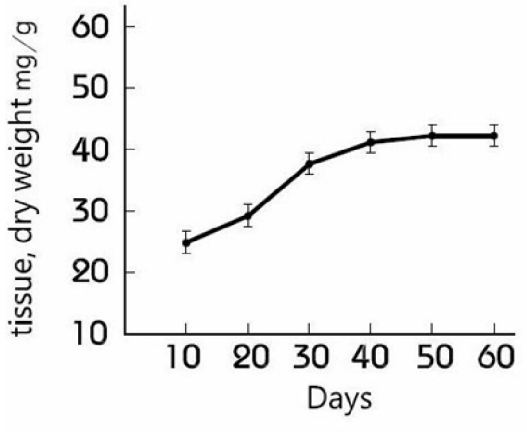

Fig. 3. Change of total phenolic compounds during stem callus culture growth.

Against the background of changes in callus growth, we studied the kinetics of the phenolic compounds content. Fig.3 shows the change of phenolic compounds total content in stem callus culture on its growth. The content of phenolic compounds is 4-5% of dry weight that is considerably less than their content in the intact plant tissue [5]. By preliminary tests (+) – catechin, (-) – epicatechin and proanthocyanidines have been identified in the callus obtained from the stem and leaf of rhododendron, which make 85-90% of phenolic compounds as in the case of the intact plant tissue. Comparison of the phenolic compounds content kinetics (Fig. 3) with the callus growth curve (Fig. 1) shows that the content of phenolic compounds significantly changes according to the tissue growth [8,9]. Moreover, their highest content is formed during the phase of intensive growth.

Conclusion

As it has been shown, we were able to get the tissue culture from the stem and leaf of Rhododendron, which maintained the ability to synthesize biologically active phenolic compounds (+) catechin, (-) epicatechin and proanthocyanidins. However, the amount and composition of those substances in callus culture were significantly less in comparison with the intact plant.

მცენარეთა ფიზიოლოგია

ქსოვილის კულტურის მიღება დეკადან (*Rhododendron Caucasicum* Pall.) ფენოლურ ნაერთთა წარმოქმნის შესწავლის მიზნით

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** სოფლის მეურნეობის სამინისტროს სასოფლო-სამეურნეო ბიოტექნოლოგიის ცენტრი, თბილისი*

(წარმოდგენილია აკადემიის წევრის გ. ნახუცრიშვილის მიერ)

პირველად იქნა მიღებული კალუსის კულტურები დეკას ფოთლიდან და ღეროდან, რომლებიც იზრდებოდნენ ახალ საკვებ არეზე გადატანისას. კალუსის საუკეთესო ზრდისთვის საკვები არის ოპტიმალური შემადგენლობის შესარჩევად გამოცდილ იქნა ზოგიერთი კომპონენტის – 2,4-D-ს, კინეტინის,საფუარის ექსტრაქტის და მიოინოზიტის სხვადასხვა კონცენტრაციები. აღმოჩნდა,რომ 5 და 10 მგ/ლ 2,4 D, 1000 და 2000 მგ/ლ საფუარის ექსტრაქტი და 20 მგ/ლ მიოინოზიტი უზრუნველყოფდნენ კალუსის ქსოვილის საუკეთესო ზრდას. წინასწარმა ანალიზმა აჩვენა, რომ დეკას ფოთლიდან და ღეროდან მიღებული ქსოვილის კულტურები ინარჩუნებდნენ ინტაქტური მცენარისთვის დამახასიათებელი ბიოლოგიურად აქტიური ფენოლური ნაერთების სინთეზის უნარს, თუმცა მათი რაოდენობა და შემადგენლობა კალუსში მნიშვნელოვნად მცირდებოდა, რაც მთლიანად ემთხვევა მონაცემებს სხვა მცენარეთა კულტურებისათვის. ფენოლურ ნაერთთა რაოდენობა მშრალ წონაზე გადაანგარიშებით შეადგენდა 4-5 %. მიღებულ ქსოვილთა კულტურებში იდენტიფიცირებულია (-)-ეპიკატექინი, (+)-კატექინი და პროანტოციანიდინები.

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