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Biotechnology

Formation of Phenolic Compounds in Callus Culture from *Rhododendron Caucasicum* Pall. and Influence of Hormonal Effectors on The Process

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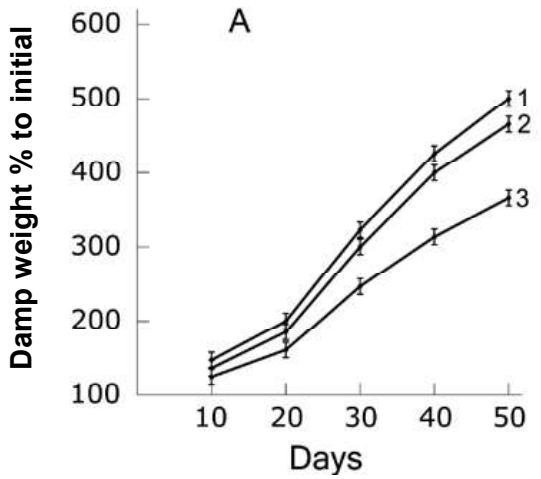
ABSTRACT. Effect of various concentrations of 2,4-D, Kinetin and NAA on the growth and formation of phenolic compounds in stem callus culture from *Rhododendron Caucasicum* Pall. was studied. 5mg/l and 10mg/l 2,4-D favorably effected on the growth of tissue culture and formation of summary phenolic compounds and flavans (catechins + proanthocyanidines). Kinetin 1mg/l and 5mg/l suppressed the growth of *Rhododendron* culture and formation of polyphenols (summary phenolics and flavans). 5mg/l and 10mg/l NAA stimulated the synthesis of summary phenolic compounds and summary flavans. The best result was shown by 10mg/l NAA. At the same time both concentrations of NAA visually did not change the growth of callus culture. Our data confirm that the growth of *Rhododendron* tissue culture and the formation of phenolic compounds in it are under hormonal control. The components of nutrient medium that increase the yield of biomass (2,4-D and NAA) also increase the formation of summary phenolic compounds and flavans, and vice versa. Kinetin, suppressing callus growth, reduces the amount of phenolic compounds. *© 2015 Bull. Georg. Natl. Acad. Sci.*

Key words: *callus cultures, stems of Rhododendron, hormonal effectors: 2,4-D, Kinetin and NAA, phenolic compounds and flavans.*

The phenolic compounds are widespread representatives of secondary metabolism in plants synthesized practically in all cells. They perform different functions in the processes of photosynthesis, respiration, energy transfer, protection of cells from a number of stress influences. Recently, the interest to these compounds significantly increased due to their high biological activity [1].

The characteristic peculiarity of *Rhododendron Caucasicum* Pall. is that it synthesizes a big amount of biologically active phenolic compounds, especially catechins, proanthocyanidines and flavonols [2]. That's why *Rhododendron Caucasicum* is widely used in pharmacology and medicine.

Callus and suspension cultures of majority of plants keep ability to produce phenolic compounds characteristic of intact plants [3, 4]. Besides, the level of their accumulation in isolated cultures, as a rule, is



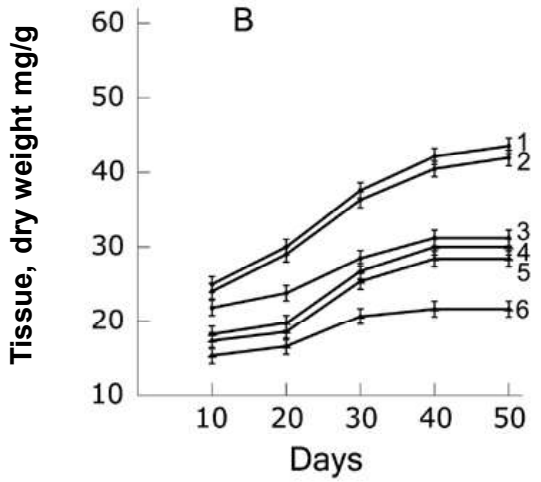
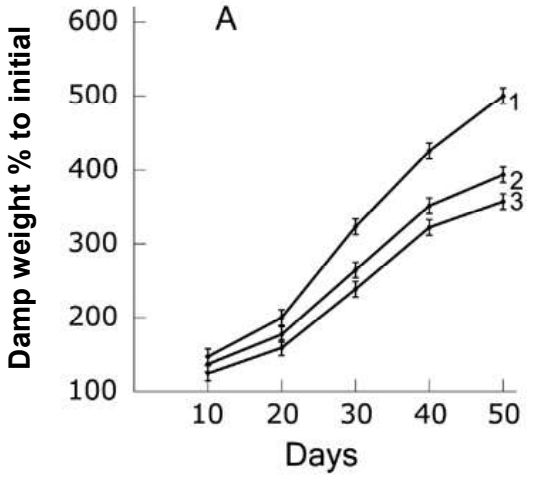


Fig. 1. Influence of different concentrations of 2,4-D on *Rhododendron* callus culture growth (A) (1, 2, 3); phenolic compounds summary content (B) (1, 2, 3) and flavans summary content (B) (4, 5, 6).
1 and 4 - 5mg/l; 2 and 5 - 10mg/l;
3 and 6 - 0,5 mg/l 2,4-D.



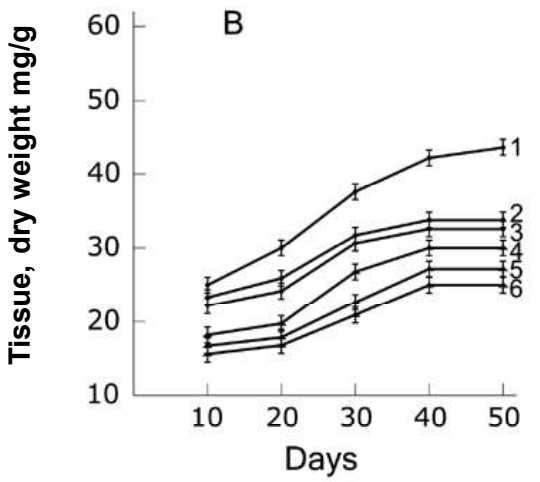


Fig. 2. Influence of different concentrations of Kinetin on *Rhododendron* callus culture growth (A) (1, 2, 3); phenolic compounds summary content (B) (1, 2, 3) and flavans summary content (B) (4, 5, 6).
1 and 4 - 5 mg/l 2,4-D (control); 2 and 5 - 1mg/l;
3 and 6 - 5 mg/l Kinetin.

lower than in intact tissues and their composition changes that can be the result of genetic and biochemical changes of cells *in vitro* [5].

Previously we obtained callus tissue from leaves and stems of *Rhododendron Caucasicum*, which kept ability to synthesize phenolic compounds, and selected optimal nutrient medium for callus culture growth [6].

Hormonal effectors occupy significant place among the factors effecting the formation of so-called secondary compounds in plant sell and tissue cultures. However, the effect of such compounds is difficult to prognose. For example, auxin and citokinin analogues 2,4-D, 1-naphtylacetic acid (NAA) , Kinetin et al. formations of phenolic compounds in some cultures stimulate, but in others suppress the synthesis of these compounds [7, 8].

The goal of the present work was to study the influence of different concentrations of 2,4-D, NAA and Kinetin on the tissue growth and formation of phenolic compounds in stem callus culture of *Rhododendron Caucasicum*.

Materials and Methods

In our experiments callus cultures from leaves of *Rhododendron Caucasicum* cultivated during one year

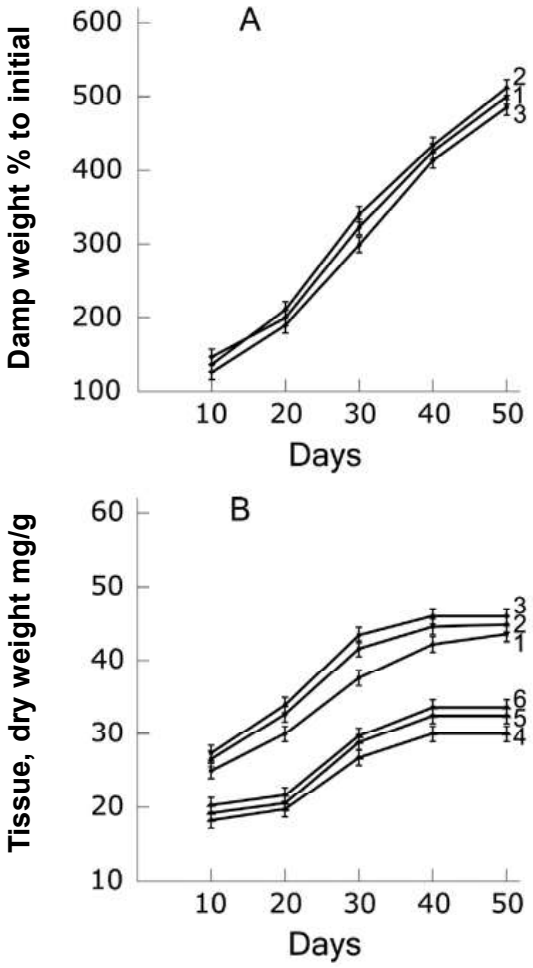


Fig. 3. Influence of different concentrations of NAA on *Rhododendron* callus culture growth (A) (1, 2, 3); phenolic compounds summary content (B) (1, 2, 3) and flavans summary content (B) (4, 5, 6).
1 and 4 - 5 mg/l 2,4-D (control); 2 and 5 - 5mg/l; 3 and 6 - 10 mg/l NAA.

according to [6] were used. Nutrient medium had the following composition (mg/l) : mineral salts according to Heller; Fe–citrate – 3.5; glucose – 25000; vitamins according to White; 2,4-D – 5.0; Adenin – 5.0; Ca-panthothenat – 0.1; Mionositol -20.0; yeast extract - 1000; agar – 7000; pH – 5.2 [9]. Tissue was grown in test-tubs at 26^0 C and relative humidity 70% in darkness and was transplanted in every 7 weeks. After the ending of exposition the tissue was lyophilized.

Phenolic compounds were extracted from lyophilized material by hot methanol up to disappearance of color with 1% vanillin solution in concentrated HCl. The content of total phenolic compounds in obtained extracts was determined by using Follin-Dennis reagent [10], followed by measuring on spectrophotometer at 725 nm. The content of total flavans (catechins+ proanthocyanidines) was determined by 1% vanillin solution in 70% H_2SO_4, followed by measuring on spectrophotometer at 500 nm. Calibration curves were constructed using recrystallized (-) – epicatechin as standard.

Results and Discussion

2,4-D is the auxsin type effector most commonly used in plant cell and tissue cultures. It induces callus formation, supports cell division and active growth. 2,4-D is one of the components of nutrient medium for obtaining *Rhododendron Caucasicum* callus culture. Fig. 1 shows the influence of different concentrations of 2,4-D on *Rhododendron* callus culture growth, summary phenolic compounds and summary flavans content. 5mg/l and 10mg/l 2,4-D favorably effect on the growth of tissue culture and formation of summary phenolic compounds and flavans, while 0.5 mg/ml suppresses callus growth and the synthesis of total phenolic compounds and flavans.

Fig. 2 shows the effect of two Kinetin concentrations on the growth of callus culture from *Rhododendron Caucasicum* and the formation of phenolic compounds in it. 5mg/l 2,4-D serves as control. The experiments showed that both concentrations of Kinetin (1mg/l and 5mg/l) suppressed the growth of rhododendron tissue culture as well as the formation of summary phenolic compounds and flavans. These results coincide with the data obtained for tea callus culture where Kinetin also suppressed tissue culture growth and the formation of summary phenolic compounds [11].

Fig. 3 shows the influence of NAA concentration on the culture growth and the formation of phenolic compounds in *Rhododendron Caucasicum* callus culture. 5mg/l 2,4-D serves as control. It turned out that both of the tested NAA concentrations (5mg/l and 10mg/l) stimulated the synthesis of sum-

mary phenolic compounds as well as summary flavans. The best result was shown by 10mg/l NAA. Moreover, both concentrations of NAA visually did not change the growth of callus culture.

The data obtained in this study testify that the growth of *Rhododendron* tissue culture and the formation of phenolic compounds in it are under hormonal control. Components of nutrient medium increasing the yield of biomass (2,4-D and NAA) increase the formation of summary phenolic compounds and flavans as well, and vice versa, Kinetin, suppressing callus growth, reduces the amount of phenolic compounds. Thus, it can be concluded that the callus growth is accompanied by the increase of polyphenolic content. Besides, their highest amount is formed during the phase of intensive growth.

ბიოტექნოლოგია

ფენოლურ ნაერთთა წარმოქმნა დეკას (*Rhododendron Caucasicum* Pall.) კალუსის კულტურაში და ჰორმონალური ეფექტორების გავლენა ამ პროცესზე

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

(წარმოდგენილია აკადემიის წევრის მ. გორდეზიანის მიერ)

შესწავლილ იქნა 2,4-D-ს, კინეტინისა და ნაფთილძმარმჟავას (ნძმ) სხვადასხვა კონცენტრაციების გავლენა დეკას (*Rhododendron Caucasicum* Pall.) ღეროს კალუსის კულტურის ზრდასა და მასში ფენოლურ ნაერთთა წარმოქმნაზე. 5 მგ/ლ და 10 მგ/ლ 2,4-D კეთილმყოფელ გავლენას ახდენს ქსოვილის კულტურის ზრდასა და მასში ფენოლურ ნაერთთა და ფლავანების (კატექინები+პროანტოციანიდინები) წარმოქმნაზე. 1მგ/ლ და 5 მგ/ლ კინეტინი თრგუნავს დეკას კულტურის ზრდას და პოლიფენოლების სინთეზს. 5 მგ/ლ და 10 მგ/ლ ნძმ ასტიმულირებს როგორც ფენოლურ ნაერთთა, ისე ფლავანების ჯამის წარმოქმნას. საუკეთესო ეფექტი ჰქონდა 10 მგ/ლ ნძმ-ს. ამასთან, ნძმ არ ახდენდა მნიშვნელოვან გავლენას ქსოვილის კულტურის ზრდაზე. ცდებმა აჩვენა, რომ დეკას კალუსის კულტურის ზრდა და მასში ფენოლურ ნაერთთა სინთეზი განიცდიან ჰორმონალური კონტროლის გავლენას. საკვები არის კომპონენტები, რომლებიც ზრდიან ქსოვილის ბიომასას (2,4-D, ნძმ), ასევე ასტიმულირებენ ფენოლურ ნაერთთა სინთეზს, და პირიქით, კინეტინი თრგუნავს როგორც კალუსის ზრდას, ისე ფენოლურ ნაერთთა წარმოქმნას.

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