International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 13, Issue 3-2026

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2026.13.03.001

The effect of growth regulators on some physiological parameters of Rhododendron regenerants (Rhododendron L.) *in vitro* conditions

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Abstract

The paper present the results of experimental research concerning the influence of epibrassinolide, emistim C and quartazine on content of photosynthetic pigments *a, b, a+b,* carotinoids in regenerants of four *Rhododendron* species: *Rh. ponticum*, *Rh. fortunei, Rh. japonicum, Rh. Smirnowii* in the aceptic culture. It was found the content of photosynthetic pigments in regenerants of *Rhododendron* L. *in vitro* depends on the selectivity action of the regulate growth, their concentration in nutritive medium and plant species.

Keywords: *Rhododendron* species, photosynthetic pigments, epibrassinolide, quartazine, emistim C

Introduction

It is well known that the growth and development of plants is regulated by endogenous phytohormones synthesized in the plant itself. Biologically active compounds (growth regulators) obtained artificially affect the change in the endogenous level of natural phytohormones. This allows the researcher to regulate the growth and development of plants in the direction he needs.

One of the indicators of the reaction of plants to the action of growth regulators is the content of photosynthetic pigments in them. The content of green and yellow pigments in plant leaves is one of the essential signs of their photosynthetic activity and, to a certain extent, the intensity of the supply of assimilates to plants (Herzog1982, Hoet al., 1987). At the same time, the chlorophyll content in the leaves serves as evidence of the age-related condition of plants, since with their aging, the level of chlorophyll in the leaves decreases significantly (Tamas et al., 1981; Zhdanova and Koryagina,1997).

Currently, a large number of growth regulators are known, tested on many plant species *ex vitro* (Wellburn, 1994; Titova et al., 2016; Cui et al., 2016; Malyarovskaya and Belous, 2017; Anwaret al., 2018; Naziret al., 2019; Belous and Platonova, 2019; Deryabin et al., 2021; Deryabin and Suvorova,2022; Gözel and Gökbayrak, 2022; Verma et al., 2024; Chernobrovkina et al., 2025; Kalashnikova et al., 2026). Unfortunately, there is no information in the literature available to us about the effect of epibrassinolide, emistim C and quartazine on the content of photosynthetic pigments in the regenerants of any plants in sterile culture (*in vitro*) and rhododendrons, including.

The purpose of our research is to study the effect of different concentrations of epibrassinolide, emistim C and quartazine on the content of chlorophyll a, b, their sum (a+b) and carotenoids in regenerants of four species of rhododendrons. Depending on the effect of growth regulators on the content of photosynthetic pigments, they can be used as stimulators for clonal micro-reproduction of rhododendrons or as inhibitors for depositing a collection of sterile cultures.

Materials and Methods

The objects of the study were four species of rhododendrons: Pontic (*Rhododendron ponticum* L.), Fortune (*Rh. fortunei* Lindl.), Japanese (*Rh. japonicum* L.), Smirnova (*Rh. smirnowii* Schneid) from a collection of sterile cultures represented by more than 30 species and varieties belonging to the Ericaceae Juss family. Rhododendrons are beautifully flowering shrubs with high decorative properties, as well as medicinal, essential oil, soil protection, water–regulating and gas-absorbing properties. This allows them to be used in the medical industry, landscaping of large cities and industrial centers.

The same-aged regenerants of four types of rhododendrons (Pontic, Fortune, Japanese, Smirnov), 10 mm high, were planted in flasks of the same volume, 25 pcs. in each, on an agarized medium WPM (Kutas, 1997) containing 0.0001; 0.001; 0.05; 0.2 mg/l of epibrassinolide; 1, 2, 4, 8 mg/l of emistim C; 2, 4, 8, 16 mg/l of quartazine and a control variant that does not contain growth regulators in the nutrient medium. All components of the nutrient medium of domestic production and CIS countries.

The cultivation of regenerants was carried out at a temperature of 25 ° C, relative humidity of 70%, photoperiod of 16 h, illumination of 4000 lux. The content of chlorophyll a, b, their sum (a+b) and carotenoids were determined 6 weeks after planting regenerants on the nutrient medium using the generally accepted method (Shlyk, 1971; Ladygina et al., 1975). By this time, the growth curve of regenerants was in an exponential phase. Three independent experiments with three-fold analytical repetition each were set. The tables show arithmetic averages and their standard errors.

Results and Discussion

The data given in Table 1 indicate the maximum content of green (a, b, a+b) and yellow (carotenoids) pigments in two species of rhododendrons (Pontic and Fortune) at an epibrassinolide concentration equal to 0.0001 mg/l of nutrient medium. This suggests that epibrassinolide in such a concentration contributes to an increase in the synthesis of pigments in these species of rhododendrons. In Japanese rhododendron, regardless of the concentration of epibrassinolide in the nutrient medium, there was a decrease in all the studied pigments: chlorophyll a, b, their sum (a+b), carotenoids in comparison with the control (Table 1). In this case, epibrassinolide contributed to a decrease in the synthesis of photosynthetic pigments. Indirect evidence of a decrease in synthesis, and not destruction under the influence of epibrassinolide in regenerants of Japanese rhododendron, can be a decrease in their growth by 2.2 times and the preservation of the green color of the leaves both in the control variant and in the experiment (Garaninova and Kutas 2001).

It is logical to assume that the decrease in growth is associated not only with a decrease in the synthesis of pigments, but also with a decrease in the photosynthetic activity of regenerants and the intensity of their assimilate supply, because these physiological processes can be interconnected. In comparison with the control, Smirnov's rhododendron had the maximum pigment content at an epibrassinolide concentration of 0.2 mg/l (Table 1).

Therefore, epibrassinolide can be recommended to reduce the growth rate of Japanese rhododendron, which is important to keep in mind when depositing regenerants of this species.For the rhododendrons of Pontic, Fortune and Smirnov, this growth regulator, depending on the concentration, should be used both for clonal micro-reproduction and deposition.

From the Table 2 it can be seen that emistim C had the greatest effect on reducing the content of photosynthetic pigments in Japanese rhododendron regenerants, regardless of its concentration in the nutrient medium. There was a decrease in chlorophyll a, b, their sum (a+b) and carotenoids in regenerants of this species at all tested concentrations of emistime C (1, 2, 4, 8 mg/l, Table 2). The opposite pattern was observed in Smirnov's rhododendron. In this case, emistim C contributed to an increase in the content of chlorophyll a, b, their sum (a+b) and carotenoids. In rhododendron Fortune, the content of green and yellow pigments fluctuated in comparison with the control and depended on the concentration of emistim C present in the nutrient medium (Table 2).

Consequently, emistim C had an ambiguous effect on the synthesis of green and yellow pigments in the studied rhododendron species, which resulted in both an increase and a decrease in their content depending on the type of rhododendron and the concentration of emistim C in the nutrient medium.

The figures presented in Table 3 indicate that quartazine also influenced the content of photosynthetic pigments in the regenerants of the studied rhododendron species. Thus, the content of chlorophyll a, b, their sum (a+b), as well as carotenoids decreased in Fortune and Pontic rhododendrons in comparison with the control (Table 3). The opposite effect was exerted by quartazine on the content of photosynthetic pigments in Japanese and Smirnov rhododendrons. They increased the content of chlorophyll a, b, their sum (a+b) and carotenoids in comparison with the control (Table 3). This is proof of the species specificity of the reaction of rhododendrons to the presence of quartazine in the nutrient medium.

Table 1 − The content of photosynthetic pigments in rhododendron regenerants at different concentrations of epibrassinolide in the nutrient medium

Species	Indicator, mg/g of raw mass	Control	Epibrassinolide, mg/l			
			0,0001	0,001	0,05	0,2
Rhododendron ponticum	Chl*a*	0,656±0,014	0,800±0,021	0,487±0,011	0,454±0,023	0,514±0,027
	Chl*b*	0,259±0,009	0,333±0,017	0,303±0,012	0,348±0,021	0,354±0,024
	Chl*a+b*	0,915±0,023	1,133±0,038	0,790±0,022	0,802±0,044	0,868±0,051
	Carotenoids	0,196±0,008	0,233±0,003	0,208±0,010	0,098±0,013	0,030±0,002
Rh. japonicum	Chl*a*	0,731±0,027	0,585±0,019	0,472±0,013	0,425±0,023	0,431±0,019
	Chl *b*	0,596±0,018	0,317±0,014	0,287±0,017	0,163±0,008	0,187±0,016
	Chl *a+b*	1,327±0,045	0,902±0,033	0,759±0,030	0,588±0,031	0,618±0,035
	Carotenoids	0,149±0,014	0,103±0,009	0,121±0,005	0,139±0,009	0,131±0,010
Rh. fortunei	Chl*a*	0,724±0,030	1,222±0,041	0,668±0,028	0,969±0,037	0,607±0,029
	Chl *b*	0,316±0,025	0,587±0,032	0,217±0,019	0,370±0,022	0,227±0,020
	Chl*a+b*	1,040±0,055	1,809±0,073	0,885±0,047	1,339±0,059	0,834±0,049
	Carotenoids	0,131±0,007	0,363±0,025	0,094±0,014	0,283±0,025	0,091±0,010
Rh. smirnowii	Chl*a*	0,096±0,004	0,073±0,001	0,114±0,010	0,670±0,031	0,848±0,043
	Chl *b*	0,060±0,002	0,055±0,004	0,059±0,005	0,277±0,012	0,372±0,016
	Chl *a+b*	0,156±0,006	0,128±0,005	0,173±0,015	0,947±0,043	1,220±0,059
	Carotenoids	0,010±0,001	0,014±0,002	0,022±0,004	0,153±0,009	0,191±0,013

Table 2 − The content of photosynthetic pigments in rhododendron regenerants at different concentrations of emistim C in the nutrient medium

Species	Indicator, mg/g of raw mass	Control	Emistim C, mg/l			
			1	2	4	8
Rhododendron ponticum	Chl*a*	0,656±0,014	0,377±0,010	0,686±0,010	0,534±0,026	0,481±0,019
	Chl*b*	0,259±0,009	0,226±0,006	0,354±0,010	0,266±0,030	0,430±0,021
	Chl*a+b*	0,915±0,023	0,603±0,016	1,040±0,020	0,800±0,056	0,921±0,040
	Carotenoids	0,190±0,008	0,072±0,004	0,190±0,003	0,161±0,002	0,069±0,003
Rh. japonicum	Chl*a*	0,731±0,027	0,496±0,016	0,370±0,008	0,505±0,019	0,544±0,022
	Chl *b*	0,596±0,018	0,182±0,012	0,209±0,007	0,246±0,009	0,345±0,019
	Chl *a+b*	1,327±0,045	0,678±0,028	0,579±0,015	0,751±0,028	0,889±0,041
	Carotenoids	0,175±0,017	0,149±0,014	0,101±0,006	0,151±0,011	0,162±0,013
Rh. fortunei	Chl*a*	0,724±0,030	0,596±0,024	0,419±0,040	0,576±0,021	0,456±0,018
	Chl *b*	0,316±0,025	0,425±0,031	0,133±0,005	0,268±0,004	0,258±0,010
	Chl *a+b*	1,040±0,055	1,021±0,055	0,552±0,045	0,844±0,025	0,714±0,028
	Carotenoids	0,131±0,007	0,094±0,003	0,177±0,004	0,161±0,001	0,091±0,004
Rh. smirnowii	Chl*a*	0,376±0,015	0,765±0,027	0,820±0,010	0,930±0,030	0,621±0,029
	Chl *b*	0,201±0,009	0,383±0,011	0,510±0,020	0,580±0,010	0,283±0,007
	Chl *a+b*	0,577±0,024	1,148±0,038	1,330±0,030	1,510±0,040	0,904±0,036
	Carotenoids	0,070±0,024	0,109±0,003	0,206±0,010	0,160±0,007	0,139±0,014

Table 3– The content of photosynthetic pigments in rhododendron regenerants at different concentrations of quartazine in the nutrient medium

Species	Indicator, mg/g of raw mass	Control	Quartazine, мг/л			
			2	4	8	16
Rhododendron ponticum	Chl*a*	0,679±0,019	0,602±0,013	0,656±0,014	0,497±0,021	0,391±0,012
	Chl*b*	0,477±0,017	0,337±0,011	0,371±0,011	0,337±0,011	0,259±0,009
	Chl*a+b*	1,156±0,036	0,939±0,024	1,027±0,024	0,834±0,032	0,650±0,021
	Carotenoids	0,190±0,008	0,171±0,007	0,156±0,010	0,083±0,001	0,069±0,003
Rh. japonicum	Chl*a*	0,596±0,018	0,705±0,032	0,724±0,017	0,893±0,021	1,049±0,036
	Chl *b*	0,371±0,027	0,445±0,021	0,462±0,019	0,566±0,018	0,611±0,013
	Chl *a+b*	0,967±0,045	1,150±0,053	1,186±0,036	1,459±0,039	1,660±0,049
	Carotenoids	0,093±0,007	0,110±0,001	0,145±0,011	0,191±0,009	0,224±0,014
Rh. fortunei	Chl*a*	0,724±0,030	0,670±0,028	0,394±0,014	0,657±0,010	0,538±0,031
	Chl *b*	0,345±0,023	0,316±0,025	0,119±0,009	0,121±0,010	0,210±0,006
	Chl *a+b*	1,069±0,053	0,986±0,053	0,513±0,023	0,778±0,020	0,748±0,037
	Carotenoids	0,134±0,003	0,103±0,005	0,082±0,001	0,094±0,007	0,072+0,006
Rh. smirnowii	Chl*a*	0,760±0,014	1,039±0,056	1,121±0,022	1,175±0,014	1,198±0,012
	Chl *b*	0,401±0,020	0,523±0,021	0,660±0,023	0,682±0,012	0,692±0,019
	Chl *a+b*	1,161±0,034	1,562±0,077	1,781±0,045	1,857±0,026	1,890±0,031
	Carotenoids	0,100±0,001	0,129±0,011	0,145±0,009	0,175±0,007	0,214±0,009

Conclusion

Thus, the analysis of the results of experimental studies showed that the tested growth regulators (epibrassinolide, emistim C, quartazine) influenced the content of photosynthetic pigments in the regenerants of the studied rhododendron species (Pontic, Japanese, Fortune, Smirnov). The manifestation of the stimulating or inhibitory effect of growth regulators on the content of photosynthetic pigments in rhododendron regenerants *in vitro* depends on their selective action, concentration in the nutrient medium and the species of the plant.

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Quick Response Code	Subject: Plant Physiology
DOI:10.22192/ijarbs.2026.13.03.001	

How to cite this article:
Elena Kutas. (2026). The effect of growth regulators on some physiological parameters of Rhododendron regenerants (Rhododendron L.) *in vitro* conditions. Int. J. Adv. Res. Biol. Sci. 13(3): 1-8.
DOI: http://dx.doi.org/10.22192/ijarbs.2026.13.03.001