The influence of bioregulating adaptogens on the growth processes, development and decorative qualities of an orchid

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Abstract. At present, plant biostimulating adaptogens used in the process of growing a wide range of cultivated plants are artificial analogs of natural stimulants, the chemical composition of which contains phytohormones, amino acids and a vitamin complex, which enhance the growth and development of the root system of plants by several times. The effectiveness of these agents is explained by the creation of a mutually beneficial symbiosis of the mycelium of mycorrhizal fungi and the root system of the plant, thereby providing the roots of the orchid with biologically active elements: mineral salts, enzymes, hormones and vitamins. Mycorrhizal fungi, in turn, receive carbohydrates necessary for their growth and development. Due to the fact that indoor plants contain a small amount of growth regulators in their chemical composition, their synthetic analogs are necessary for the productive growth and development of the orchid, which determines the relevance of the research carried out. The article presents the results of experiments on the effect of biostimulating adaptogens Ribav-extra, Ecopin and Epin-extra on the process of root formation of cuttings and morphological features in the studied species of dendrobium orchid. The studied plants were propagated in three ways: by dividing the bush, pseudobulbs and cuttings. The cuttings were soaked for 18 h before planting in drug solutions at a concentration of 1 ml/10 L of water. Also, vegetative plants were sprayed twice with biostimulants: in the phase of regrowth of the first leaf and two weeks later at a concentration of 0.5 ml/10 L of water. The results obtained showed differences in the experimental variants in terms of the onset of phenological phases: the beginning of the formation and regrowth of roots, leaves and peduncles, as well as the flowering period. The reaction of dendrobium to treatment with Ribav-extra, Ecopin and Epin-extra was revealed. In plants in all variants, the preparations used stimulated the development of the root system, leaf apparatus, and the appearance of peduncles. The phenophase data in the tested plants occurred 7-28 days earlier in comparison with the control variant (without the use of bioregulators). The treatment of the test plants with the growth regulator Ribav-extra promoted earlier regrowth of the first and second roots and leaves (by 20-28 days). Ekopin turned out to be the most passive and did not significantly affect the rate of development of the tested plants. The phenophases of regrowth of the 1st root and 1st leaf began 3-5 days earlier than the plants of the control variant. Ribav-extra and Epin-extra stimulated the appearance of peduncles in plants by the age of 3 years.

1. Introduction

Orhidacece Juss. is the largest family of monocots, numbering about 75 genera and from 20,000 to 25,000 species, and—according to some sources—up to 800 genera and 35,000 species. On the
territory of the Northwestern Caucasus within the Krasnodar Territory, 47 species of families belonging to 20 genera are currently registered [1].

All orchids are herbaceous perennials that form branched shoots, which are valuable medicinal plants with high decorative properties, which makes these species promising for economic use [2, 3].

The use of growth regulators in the cultivation of flower crops is based on improving the growth processes of plants, the germination of seeds and corms, the quality of seedlings, the survival rate of cuttings, and the commercial qualities of plants. Currently, a special role is played by agents of the group: L-alanine+, L-glutamic acid, Ribav-extra R. (produced by ZAO Selkhozekoservis, OOO Biotechnoecological center RIBAV). They are aimed at activating growth processes at different stages of plant growth [4, 5].

The use of growth regulators and physiologically active substances in indoor floriculture corrects the course of ontogenesis, optimizes the intensity of growth and development processes, which ultimately determines an increase in the productivity of ornamental crops. But the use of these drugs requires increased attention, since the substances that various plants contain must receive the appropriate components from growth substances, otherwise the plant will not react to their intake. The activity of growth regulators is largely determined by the influence of external factors (weather, climatic and edaphic conditions, innovations in agricultural technology, etc.) [6, 7, 8].

2. Problem Statement

The use of bioregulators-adaptogens, when growing indoor plants in indoor or greenhouse conditions, gives a positive effect. Anti-stress growth regulators (adaptogens) increase plant resistance to unfavorable environmental conditions (low and high temperatures, moisture deficit), especially at the first stages of the life cycle. The group of such drugs includes Heteroauxin, Kornevin, Ribav-extra, Ukorenit, Epin-extra, Ecopin, under the influence of which the formation of roots is accelerated, and resistance to diseases in plants that are considered very fastidious to the environmental conditions of the environment increases. Spraying of vegetative plants is recommended to be carried out several times, starting from the phase of formation of aerial roots [5]. We used 3 bioregulators-adaptogens: Ribav-extra, Epin-extra, Ecopin, the effect of which we observed during the entire period of research.

Ribav-extra is a biostimulating adaptogen, characterized by a wide spectrum of action, useful for vegetables and berry bushes, fruit, ornamental and coniferous plants: it improves seed germination, increases root growth, has a healing effect when part of the root system dies off, stimulates flowering of plants. The effectiveness of this drug has been proven for transplanting indoor plants, planting trees and shrubs. Epin-extra is a unique broad-spectrum agent that increases plant stress resistance and the ability to quickly adapt to changes in environmental factors, repairs damage, stimulates growth, improves yield and flowering, and is recommended for use in different phases of plant growth. Ecopin is used to stimulate the growth and flowering of all types of plants. This preparation contains beneficial bacteria and contains essential nutrients. It is recommended to use it throughout the entire life of the plant. This bioregulator has a beneficial stimulating effect on the sprouts helping them to develop better. Natural compounds that make up Ribav-extra, Epin-extra and Ecopin act on plant cells, activate stress resistance genes [9, 10].

According to the results of numerous studies, when indoor plants were treated with these bioregulating adaptogens, an acceleration of the intake of water and nutrients into the plants was observed, the activity of the photosynthesis process increased. The mechanism of action of these complex preparations is to regulate the synthesis of other phytohormones by the plant itself: auxins, gibberellins, cytokinins, abscisic acid and ethylene. Moreover, this regulation depends on the phase of plant development and the conditions of its cultivation. Thus, these bioregulating adaptogens stimulated the production by the plant itself of those hormones that it needs at every stage of development: they increased the content of antioxidant enzymes in plants, increasing their resistance to unfavorable environmental factors (drought, frost) and diseases, exhibiting the properties of nonspecific immunomodulators; prevented the accumulation of heavy metals and radionuclides in plants [8, 11, 12].
Our research included the use of the safest hormonal stimulants based on epibrassinolide, a phytohormone from the brassinosteroid group, to treat daughter plants, pseudobulbs and orchid cuttings by soaking them before planting and further foliar feeding with solutions of these drugs. The relevance of the research is given by its theoretical and practical focus on solving the issue of agrobiological substantiation of the expediency of using orchid species for reproduction using solutions of these agents.

3. Research Questions

3.1. Research objects
The objects of research were plants of the dendrobium orchid species.

Dendrobium Orchid (Dendrobium Nobile) is a thermophilic tall hybrid, up to 75 cm high, with a thick stem, consisting of segments (pseudobulbs), and two-row leaves. Flowers are bicolor with ovoid petals that form on leafless last-year shoots. The advantages of this type of orchid include: the duration of flowering (from November) of one inflorescence is up to 30 days; in general, that of one plant is up to 12 weeks; the flowers have a delicate aroma, are 4.0-5.0 cm in diameter (depending on the care of the plant) and have not falling foliage.

Plants of the studied orchid species were propagated in three ways: by dividing the bush, pseudobulbs, and cuttings. The planting of daughter plants, pseudobulbs and orchid cuttings was carried out in the first decade of February 2018 in transparent containers (0.7 liters) with a substrate similar to the habitat of their external environment, and consisting in equal parts of the following components: pine bark, sphagnum moss, charcoal, pine cones, fern bark.

3.2. Research subject
The subject of our research was the use of biostimulating adaptogens Ribav-extra, Ecopin and Epin-extra, which contribute to the rooting of the objects of our research, adaptation, the development of the aerial part, the manifestation of peduncles and their further flowering.

The scheme of our experiment included 4 options: 1) control (without the use of biostimulating adaptogens); 2) soaking cuttings, pseudobulbs and daughter plants for 18 hours before planting in a solution of the Ribav-extra preparation (1 ml/10 l of water), then twice spraying vegetative plants in the phase of the appearance of the first leaf and in the phase of the appearance of the first peduncle with the preparation at a concentration of 0.5 ml/10 l of water; 3) soaking cuttings, pseudobulbs and daughter plants for 18 hours before planting in a solution of the Epin-extra preparation (1 ml/10 l of water), then twice spraying vegetative plants in the phase of the appearance of the first leaf and in the phase of the appearance of the first peduncle with the preparation at a concentration of 0.5 ml/10 l of water; 4) soaking cuttings, pseudobulbs and daughter plants for 18 hours before planting in a solution of the Ecopin preparation (1 ml/10 l of water), then twice spraying vegetative plants in the phase of the appearance of the first leaf and in the phase of the appearance of the first peduncle with the preparation at a concentration of 0.5 ml/10 l of water [13, 14].

In the control variant, the plants were watered at the onset of the same growth and development phases.

4. Purpose of the Study
The purpose of our work is to establish the feasibility of using biostimulating adaptogens that affect the development and decorative qualities of orchid species.

This goal was achieved by solving the following tasks:
– to identify the features of the growth processes of the studied orchid species in dynamics;
– to determine which of the used biostimulating adaptogens contributes to an increase in the cold resistance of the studied orchid species in different variants of the experiment in the autumn-winter and winter-spring periods.
5. Research Methods
We used the following research methods: vegetation, laboratory and statistical. The studies were carried out according to the methods generally accepted in plant growing. The experiment was laid in a tropical greenhouse of the botanical garden of the Kuban State Agrarian University under conditions of constant humidity in the greenhouse in 4-fold repetition. Each of the replicates consisted of 30 plants (10 cuttings, 10 pseudobulbs, and 10 daughter plants).

In the research work, the following counts and observations were carried out:
1. Biometric observations. A month after planting, in dynamics, the height of the experimental plants was determined, the number of roots and leaves was counted and the size of the leaf blade was measured, the number of peduncles, flowers, and the intensity of flowering were measured. These observations were carried out on all plants of each replication of the experiment.
2. Phenological observations. We marked the dates of planting, mass (75%) regrowth of leaves, the formation of peduncles of the studied species of orchids in all variants of the experiment.
3. The area of the assimilation surface of cabbage plants was calculated using the formula of N.F. Konyaev.
4. The reliability of differences in the results of biometric abductions was assessed using the analysis of variance according to B.A. Dospekhov.

6. Findings
Ontogenesis of any plant consists of successively passing phenological phases, during which changes are observed in the processes of its growth and development. The phenological observations in the experiment made it possible to determine the timing of the appearance of external changes occurring with the studied plants. Knowledge of the phenological phases of orchids and the timing of their onset makes it possible to choose agrotechnical methods of cultivation that contribute to obtaining high quality plants [5, 7, 13, 14].

The dynamics of observation of the growth of the studied species of orchids revealed that until mid-March, plants kept in a solution of the biostimulator Ribav-extra had some advance in the beginning of the formation of leaves and roots (Table 1).

Table 1. Influence of biostimulating adaptogens on the timing of the onset of phenological phases in the dendrobium after propagation by dividing the bush, 2018 (planting on 09.02.18).

| Variant           | beginning of formation of leaves and roots | growth of the 1st root | growth of the 2nd root | growth of the 1st leaf | growth of the 2nd leaf |
|-------------------|-------------------------------------------|------------------------|------------------------|------------------------|------------------------|
| Control (water)   | 06.04.                                   | 18.04.                 | 03.05.                 | 27.05.                 | 04.06.                 |
| Ribav extra       | 10.03.                                   | 26.03.                 | 11.04.                 | 22.04.                 | 08.05.                 |
| Epin-extra        | 19.03.                                   | 02.04.                 | 26.04.                 | 05.05.                 | 17.05.                 |
| Ecopin            | 02.04.                                   | 15.04.                 | 30.04.                 | 20.05.                 | 28.05.                 |

In the context of the variants of the experiment, we see that the timing of the onset of phenophases in the studied species of dendrobium, propagated by dividing the bush, depending on the drug used on the plants, has different terms. For instance, in the control variant, the phase of the beginning of the formation of leaves and roots began 2 months after planting the studied plants, while Ribav-extra showed the best results (a month after planting, the first rudiments of the root system and leaves appeared). Ekopin showed results close to the control variant.

The biostimulants Ribav-extra and Epin-extra had a positive effect on the growth processes of pseudobulbs and cuttings of dendrobium; however, in these experiments, there was some advantage of the second option (Ribav-extra) over others (Tables 2 and 3).
Table 2. Influence of biostimulating adaptogens on the timing of the onset of phenological phases in dendrobium pseudobulbs, 2018 (planting on 09.02.18)

| Variant      | Phenological phase                      |
|--------------|----------------------------------------|
|              | beginning of formation of leaves and roots | growth of the 1st root | growth of the 2nd root | growth of the 1st leaf | growth of the 2nd leaf |
| Control (water) | 11.04.                                | 13.05.                | 20.05.                | 25.05.                | 02.06.                |
| Ribav extra   | 16.03.                                | 31.03.                | 09.04.                | 21.04.                | 03.05.                |
| Epin-extra    | 23.03.                                | 11.04.                | 21.04.                | 07.05.                | 13.05.                |
| Ecopin        | 03.04.                                | 09.05.                | 15.05.                | 15.05.                | 26.05.                |

Table 3. Influence of biostimulating adaptogens on the timing of the onset of phenological phases in dendrobium cuttings, 2018 (planting on 09.02.18)

| Variant      | Phenological phase                      |
|--------------|----------------------------------------|
|              | beginning of formation of leaves and roots | growth of the 1st root | growth of the 2nd root | growth of the 1st leaf | growth of the 2nd leaf |
| Control (water) | 19.04.                                | 21.05.                | 30.05.                | 29.05.                | 08.06.                |
| Ribav extra   | 25.03.                                | 07.04.                | 18.04.                | 27.04.                | 07.05.                |
| Epin-extra    | 30.03.                                | 16.04.                | 30.04.                | 10.05.                | 18.05.                |
| Ecopin        | 13.04.                                | 06.05.                | 14.05.                | 19.05.                | 29.05.                |

Plant cuttings do not have a root system and leaf apparatus, therefore, for the plant not to lose its vitality, it is very important to achieve precisely these growth processes. The phase of the beginning of the formation of leaves and roots in pseudobulbs and dendrobium cuttings was the earliest in variants with the biostimulator Ribav-extra. Further development of the root system and leaf apparatus in the studied plants under the influence of biostimulating adaptogens proceeded in the same sequence. Epin-extra preparation also showed good results. In the control variants, the phases of development of the root system and leaf apparatus occurred 5-30 days later than in other variants.

In order to increase the decorativeness of the orchid, in all variants of the experiment, a study was carried out on the effect of biostimulating adaptogens on the development of plants by spraying them with solutions of these drugs in the phases of the appearance of the first leaf and the first peduncle (Figures 1A and 1B).

The results of the experiment carried out during 2018-2019 showed that the preparations Ribav-extra and Epin-extra had the greatest effect on the indicators “plant leafiness”, “plant height”, “leaf length” and “number of stems per plant”. Orchids, propagated by dividing the bush, stood out according to the above indicators. So, when using the Ribav-extra preparation, the average leaf length of these plants as of 01.02.2020 was 9.4-11.8 cm, and the number of leaves varied within 3.5-4.8 pieces, the plant height averaged 8.2 cm and the number of stems was 3.3-4.9 pcs. (Table 4).

The results of further observations of the growth and development of the orchid, when propagated by daughter plants, pseudobulbs and cuttings, carried out for two years, are shown in Table 4. Evidently from data shown in Fig. 1 and Table 4, dendrobiums grow rather slowly and the height of specimens does not exceed 8.2 cm. The average growth rate of leaves in orchid species of the genus Dendrobium averages 2.34 cm per year, but they have not yet reached their maximum length, and on 01.02.2020, their length, on average, was 10.5 cm. Of all the methods of accelerated reproduction of orchids, the best results were shown by reproduction by daughter plants and pseudobulbs. In these variants, the indicators determining the decorativeness of plants are much higher (by 45-111%), in comparison with the variant where orchids were propagated by cuttings. Also, the survival rate of
daughter plants and pseudobulbs in the experimental variants was from 77.2 to 85.4%. Biostimulating adaptogens facilitated the test plants at the beginning of the third year of life to move to the generative period of development.

![Figure 1](image1.png)

**Figure 1.** Influence of biostimulating adaptogens on the dynamics of plant growth parameters of Dendrobium Nobile grown in the tropical greenhouse of the botanical garden of the Kuban State Agrarian University: A) dynamics of the length of the sheet, cm; B) dynamics of plant height, cm.

**Table 4.** Biometric characteristics of Dendrobium Nobile orchid plants, average as of 01.02.2020 (planting on 09.02.18)

| Variant         | Propagation type | daughter plants | pseudobulbs | cuttings |
|-----------------|------------------|-----------------|-------------|----------|
|                 | Number of stems, pcs. | Number of leaves, pcs. | Number of flowers per plant, pcs. | Number of stems, pcs. | Number of leaves, pcs. | Number of flowers per plant, pcs. | Number of stems, pcs. | Number of leaves, pcs. | Number of flowers per plant, pcs. |
| Control (water) | 1.8              | 7.0             | 4.1         | 1.1      | 6.7      | 3.7                      | 0.2                     | 4.3                  | 0.5                      |
| Ribav extra     | 2.5              | 8.6             | 9.3         | 2.0      | 8.1      | 8.6                      | 1.5                     | 5.4                  | 3.3                      |
| Epin-extra      | 2.2              | 8.4             | 8.1         | 1.6      | 7.8      | 7.3                      | 1.0                     | 5.1                  | 2.6                      |
| Ecopin          | 2.0              | 7.3             | 6.9         | 1.4      | 7.1      | 5.2                      | 0.8                     | 4.7                  | 1.9                      |
7. Conclusion
The formation of decorative products in flower crops is the result of photosynthesis, during which simple substances form energy-rich, complex and chemically diverse organic compounds, the accumulation rate of which depends on the size of the leaf surface, determined by the biometric parameters of plants, as well as on the diet and duration of active activities of the leaves.

Observations of the growth and development of the studied plants growing in the tropical greenhouse of the botanical garden of the Kuban State Agrarian University showed that soaking the orchid planting material in solutions of biostimulating adaptogens promoted good plant survival, especially in variants 2 and 3 (up to 85.4%), as well as an increase in stem by 8-20% in the first month after application.

In the same studies, the effectiveness of spraying plants in all variants with solutions of the Ribav-extra and Epin-extra preparations was established, which positively influenced the dynamics of the growth of the green mass of plants and contributed to the appearance of the first peduncles and the blooming of the first flowers in the third year of life.

References
[1] Shibao Zhang, Yingjie Yang, Hong Hu 2018 Physiological diversity of orchids Plant Diversity 40(4) 196-208
[2] Chengru Li, Na Dong, Junwen Zhai 2021 A Review for the Breeding of Orchids: Current Achievements and Prospects Horticultural Plant Journal (In press, journal pre-proof)
[3] Zuzana Štípková, Spyros Tsiftsis, Pavel Kindlmann 2021 How did the agricultural policy during the communist period affect the decline in orchid biodiversity in central and eastern Europe? Global Ecology and Conservation 26 01498
[4] Hsiangchia Lu, Zhongjian Liu, Siren Lan 2019 Genome Sequencing Reveals the Role of MADS-box Gene Families in the Floral Morphology Evolution of Orchids Horticultural Plant Journal 5 247-254
[5] Chee Keong Chin, Ze Hong Lee, Sreeramanan Subramaniam 2019 Effects of plant growth regulators and activated charcoal on somaclonal variations of protocorm-like bodies (PLBs) of Dendrobium Sabin Blue orchid Biocatalysis and Agricultural Biotechnology 22 101426
[6] Jaime A Teixeira da Silva, Mohammad Mushar of Hossain, Songjun Zeng 2017 Acclimatization of in Vitro-derived Dendrobium Horticultural Plant Journal 3 110-124
[7] Paromik Bhattacharyya, Johannes Van Staden 2016 Ansellia africana (Leopard orchid): A medicinal orchid species with untapped reserves of important biomolecules – A mini review South African Journal of Botany 106 181-185
[8] Warinthon Poonsri 2021 Effects of high CO2 and low O2 on biochemical changes in cut Dendrobium orchids Heliyon 7(2) 06126
[9] Lit Chow Yeow, Bee Lynn Chew, Subramaniam Sreeramanan 2020 Elevation of secondary metabolites production through light-emitting diodes (LEDs) illumination in protocorm-like bodies (PLBs) of Dendrobium hybrid orchid rich in phytochemicals with therapeutic effects Biotechnology 27 00497
[10] Mala B, Kuekgong K, Nontachaiyapoom S 2017 Effect of germination media on in vitro symbiotic seed germination of three Dendrobium orchids South African Journal of Botany 112 521-526
[11] Paromik Bhattacharyya, Suman Kumaria, Pramod Tandon 2016 High frequency regeneration protocol for Dendrobium nobile: A model tissue culture approach for propagation of medicinally important orchid species South African Journal of Botany 104 232-243
[12] Didik Pudji Restanto, Boedi Santoso, Sigit Supardjono 2016 The Application of Chitosan for Protocorm Like Bodies (PLB) Induction of Orchid (Dendrobium sp) In Vitro Agriculture and Agricultural Science Procedia 9 462-468
[13] Reema Vareen Diengdoh, Suman Kumaria, Meera Chettri Das 2017 Asymbiotic germination and seed storage of Paphiopedilum insigne, an endangered lady's slipper orchid *South African Journal of Botany* **112** 215-224

[14] Halyena Indan, Devina David, Jualang Azlan Gansau 2021 Development and characterization of flower and capsule in Borneo jewel orchid Macodes limii J.J Wood and A.L Lamb (Orchidaceae: Asparagales) *Journal of Asia-Pacific Biodiversity* *(In press, journal pre-proof)*