# Efficiency of using biostimulants Agrinos 1 and Agrinos 2 in adaptation of microplants of garden strawberry to *ex vitro* conditions

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**Abstract.** This article presents the results of experimental studies of the effect of biostimulants Agrinos 1 and Agrinos 2, containing strains of microorganisms *Azotobacter vinelandii* and *Clostridium pasteurinum*, etc., as well as a complex of nutrients (macro-, microelements, protein, amino acids, chitin, chitosan) on the activation of metabolic processes and reduction of stress effects on strawberry plants obtained by *in vitro* method. The effectiveness of the reaction of biostimulants on the adaptive processes of growth and development of microplants of garden strawberry varieties Alba, Kemiya, Clery has been studied. In the course of the research, a positive effect of biological products on the growth of the vegetative mass of plants was established: an increase in the height of the plants compared to the control by 20-25%, the number of leaves by 25-28% and an increase in the root system of plants by 30-40%. In the Clery variety, the use of the biostimulants promoted the formation of the runners. In addition, the introduction of biostimulants into the soil contributed to the improvement of the quality indicators of the soil. The content of nitrate nitrogen in the soil increased by 18%.

## 1 Introduction

Micropropagation of garden strawberries *in vitro* is becoming one of the most widely used methods in plant growing [1]. However, its obvious advantages can be easily leveled out due to problems and limitations arising in the process of work.

One of the main and problematic stages in the process of obtaining healthy strawberry planting material using micropropagation methods is the transfer of rooted plants obtained *in vitro* to non-sterile *ex vitro* conditions. A significant number of plants cultivated *in vitro* do not tolerate transfer from *in vitro* conditions to a greenhouse or open field. Plants grown *in vitro* are very delicate as the micropropagation process takes place under conditions of high humidity, controlled temperatures and low light intensity. In this regard, such plants lack protective mechanisms: waxy cuticle, stomata regulation, leaf hairs, which makes them very vulnerable to drying out under the influence of environmental conditions after plant transplantation from in vitro to *ex vitro* conditions [2].

Most plants grown under *in vitro* conditions require some acclimation period to ensure survival and vegetation growth when transferred to soil.

In this regard, it is relevant to use natural biostimulants to improve the general health, growth and vitality of plants grown *in vitro*, as well as not causing any risks to the environment. In addition, improving the absorption of nutrients is one of the advantages of using biostimulants associated with their ability to increase the microbiological and enzymatic activity of the soil, due to the effect on the root structure, as well as the solubility and transportability of micronutrients [3-5].

Nitrogen bacteria are one of the components of biostimulants for horticultural crops. Biostimulants contain microorganisms that live freely in the soil, or are symbiotic with plants, and make a direct or indirect contribution to the nitrogen and phosphorus nutrition of plants. Nitrogen bacteria are capable of producing hormones, vitamins, and other substances necessary for the growth and development of plants. Microorganisms that are symbiotic and non-symbiotic in nature fix atmospheric nitrogen. According to a number of literature sources, the usage of *Azotobacter chroococcum* during the transplantation of plants from one condition to another, leads to a significant increase in the growth and productivity of crops. [6-15]

Considering these facts, this study was initiated to study the effect of biostimulants Agrinos 1 and Agrinos 2 on the growth, development, and adaptation of garden strawberries grown by *in vitro* method. as well as to study the effect of biostimulants on agrochemical characteristics of soil used for adaptation of garden strawberry plants to non-sterile *ex vivo* conditions.

## 2 Materials and methods

The studies were carried out in the adaptation phytotron of the Virology laboratory of the FSBSI NCFSIHVW in 2020-2021. Objects of research: microplants of garden strawberry varieties Kemiya, Alba and Clery, grown by *in vitro* method

The main characteristics of the used biostimulants: Agrinos 1 - is a product containing microorganisms, such as *Azotobacter vinelandii* and *Clostridium pasteurinum*, capable of producing and releasing organic acids as waste products, dissolving mineralized salts, and converting K +, Mg +, and Ca ++ from organic forms to mineral forms, more accessible to plants. Agrinos 2 is a microbial fermentation product containing L - amino acids (4%), chitin, chitosan, glucosamine (4%), enzyme complex, lactic acid, polysaccharides, carbon, nitrogen, and other essential trace elements used by plants to improve health and productivity during different stages of growth and development, as well as under different growing conditions. The biostimulants improve the tolerance of plants to various stresses (abiotic, biotic, and physiological) and supports the process of photosynthesis.

The introduction of biostimulants into containers with soil, where strawberry microplants were planted after transplanting them from sterile conditions *in vitro* to non-sterile *ex vitro*, was carried out once, by irrigation with an aqueous solution of the biostimulants Agrinos 1 at a concentration of 5 ml / l, and after 7 days - Agrinos 2 (5 ml / l, also once).

In the course of the experiment (45 days after watering), the indicators of plant growth were recorded. The length of the plants, the number of formed leaves, the length and the intensity of the root system formation were determined. Soil analysis was carried out by methods generally accepted in agrochemistry. The content of organic matter in the soil was determined by ashing in a muffle furnace at a temperature of 525C to constant weight.

## 3 Results and discussion

As a result of the experimental studies, it was found that the length and the number of leaves of the strawberry plants in *ex vitro* 45 days after usage of biostimulants was significantly increased comparing to control plants. The maximum length of the most threated plants for strawberry variety Clery was 10.8 cm, Kemiya - 9.8 cm, Alba - 6.4 cm. In the control variants, this indicator was lower by 25%, 23%, and 20%, respectively (Fig. 1, 2).

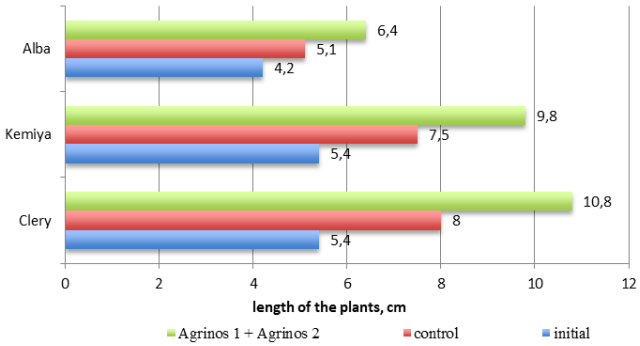


**Fig.1**. Influence of biostimulants Agrinos 1 and Agrinos 2 on the length of garden strawberry in the process of adaptation to non-sterile conditions

**Control** **Agrinos 1 + Agrinos 2**

**Fig. 2.** Appearance of strawberry plants: control variant and variant with Agrinos 1 + Agrinos 2, variety Kemiya

The increase in the number of leaves in the variants with the use of biostimulants was observed in all varieties 45 days after the application of the biostimulants. The average number of leaves in varieties Clery and Kemiya ranged from 6.6 to 7.3 leaves per bush, Alba - 7.6. In the control variants, this indicator was lower by 25-28% (Fig. 3, 4).

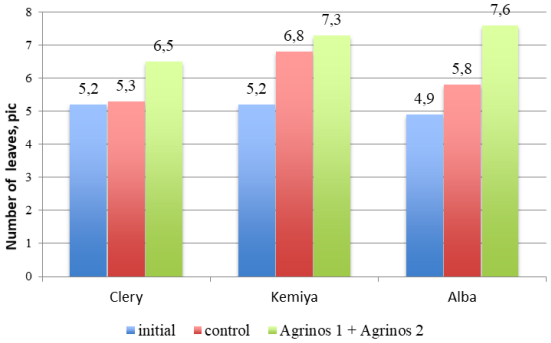


**Fig. 3.** Influence of biostimulants on the formation of vegetative mass of garden strawberry plants after 45 days

It should be noted that all control variants of strawberry microplants had tendency of the leaf apparatus to dry out and die during the growth under *ex vitro* conditions, due to increased sensitivity to new environmental conditions, while this was not observed in the treated plants.

**Control**

**Agrinos 1 + Agrinos 2**

**Fig. 4.** Appearance of garden strawberry plants: control variant and variant with processing Agrinos 1 + Agrinos 2, variety Alba

The increase in green mass in treated plants can be associated with the formation of a large amount of chlorophyll upon the introduction of nitrogen fixers and with the production of plant growth regulators in the rhizosphere by bacteria, which are absorbed by the roots. Consequently, the enhancement of vegetative growth can be caused by increased biological nitrogen fixation. In the literature resources, there is information about an increase in the height of strawberry plants and the number of leaves when using *Azotobacter* [11].

In our studies, it was noted that the use of a complex of biostimulants Agrinos 1, containing active microorganisms and Agrinos 2, containing amino acids, a complex of enzymes and basic microelements, led to a significant improvement in root formation. The length of the root system in the variant with the use of biostimulants averaged 15-20 cm, in the control variant the length of the roots varied within 4-10 cm, depending on the variety (Fig. 5).

**Control**

**Agrinos 1 + Agrinos 2**

**Fig. 5**. Root system of garden strawberry plants: control variant and variant with treatment Agrinos 1 + Agrinos 2, variety Clery

In addition, in strawberries of the Clery variety, in the variant with biostimulants, an early onset of runners formation was noted. As early as 3 months after transplanting plants from *in vitro* conditions to *ex vitro* conditions, each plant of the Clery variety in the experimental variant had 2-3 runners, while in the control variant they were completely absent (Fig. 6).

**Control** **Agrinos 1 + Agrinos 2**

**Fig. 6**. Runner formation in garden strawberry, depending on the use of biostimulants, variety Clery

According to the literature, nitrogen fixation is one of the most important biological processes and is considered an interesting microbial activity on the soil surface, since it provides nitrogen recirculation and plays an important role in nitrogen homeostasis in the biosphere [14]. What's more, biological nitrogen fixation also helps maintain soil fertility and increase crop yields. It has been found that azotobacteria are useful organisms for use as bioinoculants and for studying the process of nitrogen fixation due to their ability to grow rapidly and quickly fix large amounts of nitrogen. Azotobacter is capable of converting atmospheric nitrogen into ammonia, which, in turn, is absorbed and utilized by plants [13].

In our experiment, the analysis of the control soil and the soil with the sequential introduction of biostimulants Agrinos 1 and Agrinos 2 was carried out. (Table).

**Table 1.** Soil analysis depending on the use of the biostimulants

| Varuant | pH | Organic matter, % | Nitrate nitrogen, mg/kg | $P_2O_5$mg/kg | $K_2O$mg/kg |
|---|---|---|---|---|---|
| Agrinos 1 and Agrinos 2 | 7,18 | 32,34 | 300,8 | 118,50 | 772,19 |
| Control | 7,12 | 29,21 | 253,5 | 110,06 | 899,24 |

The chemical analysis showed that introduction of biostimulants into the soil increased the content of nitrate nitrogen by 18%.

## 4 Conclusion

Based on the obtained data, a significant effect of the biostimulants Agrinos 1 and Agrinos on the adaptive ability of microplants of garden strawberries grown *in vitro* conditions. The studies showed that the intensity of vegetative growth in the treated variants was 20-25% higher than in the control. The plants had a stronger root system (5-10 cm longer than in the control), and, consequently, a higher adaptive ability to *ex vitro* conditions. The usage of these biostimulants influenced the content of mineral nitrogen available to plants in the soil. The amount of nitrate nitrogen increased by 18%

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