

# The New Effective Biostimulator for Agroecological Engineering

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**Abstract**—New biostimulator from wheat seeds which by its chemical composition relates to fusicoccin is presented in this article. New biostimulator could be used as powerful hormonal substance that has ability to increase productivity and salt tolerance of agricultural plants. Also on the basis of biostimulator we have developed vegetative method for fast reproduction of perennial plants as desert plant - *Tamarix gracilis*.

**Keywords**—Biostimulator, crop productivity, ecology, fusicoccin, salt tolerance.

## I. INTRODUCTION

THE global warming complicates the ecological situation over the world, especially in Kazakhstan. More desertification and salinization occur. There comes strong desertification and salinity of the earth. In this reason the application of new biostimulators allow to soften the ecological situation and to receive the good productivity of agricultural plants in hard environment conditions.

In Laboratory of structure and regulation of enzymes of Aytkhozhin's Institute of Molecular biology and biochemistry was developed the new biostimulator. The new biostimulator was purified from germinating wheat seeds by method of Sultanbaev et al. But the preparation was received in very small quantities because only analytical methods were used for purification. Small quantities of a biostimulator are enough only to conduct physiological and biochemical tests. And the given quantity of a preparation is not enough for realization for ecological and field experiments. That is why the first task of our investigation is the development of new method which allow to receive pure biostimulator in preparative quantity. For this aim we decided to use the method of nanotechnology. In Institute of Combustion problem of Al-Farabi's Kazakh

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National University under the supervision of Prof. Z.A.Mansurov for the first time the new nanostructured carbon sorbent which is convenient for separation of bioorganic substances was developed [1]. Now this sorbent is produced in Scientific-industrial technological center "Zhalyn" (Almaty, Kazakhstan) under the trademark "Nanocarbosorb" ARK type. These carbon sorbents have advantages in comparison to earlier existing sorbents. The sorbent is highly strong and stable for many years. Traditional sorbents which were made from dextran gels are easily attacked by fungies and microorganisms. In this reason time of exploitation of this gels is only several months. Due to the soft properties of traditional gels they don't allow to receive substances in preparative quantity whereas new "Nanocarbosorb" has not this disadvantages. The task of our investigation the development of method of preparative purification, characterization of biostimulator and the development of method of it's application.

## II. RESULTS AND DISCUSSION

### A. Isolation of Biostimulator and Investigation of its Structure

We took B.E.Sultanbaev's method [2] as a basis in purifying biostimulator, but we have made some changes. Experiments were carried out as follows: 3 litres of boiled water was lead up to room temperature and 0,1 mM of 6-benzylamine purine (6-BAP) was dissolved in it. Adding of 6-BAP is necessary for formation of our biostimulator. Then 2kg of wheat grains "Steklovidnaya-24" cultivar was added to the solution and it was left for 2 days. After the expiration of this time under influence of 6-BAP in germs of wheat the biostimulator was formed. Grains of wheat were dried up and homogenized in 4 litres of 80% ethanol. Then homogenate was centrifuged in a centrifuge K-70 (Janetzki, Germany) at 5000 x g during 10 minutes.

The supernatant ethanol extract was used for the further purification. Instead of hydrophobic on octyl-Sepharose (B.E. Sultanbaev et al) we have introduced purification of biostimulator by using nanostructured carbon sorbent "nanocarbosorb" ARK type.

Ethanol extract was introduced on a column with "nanocarbosorb". For elution of column from non-sorbed substances column was eluted by 10% ethanol. Further elution by 50% ethanol was carried out to isolate the fraction

containing a biostimulator. Chromatography of isolation of the biostimulator on a column with "nanocarborb" is presented at Fig. 1. As it shown from Fig. 1, the biostimulator eluates in the second symmetric peak. The purified preparation was used for the further studies.

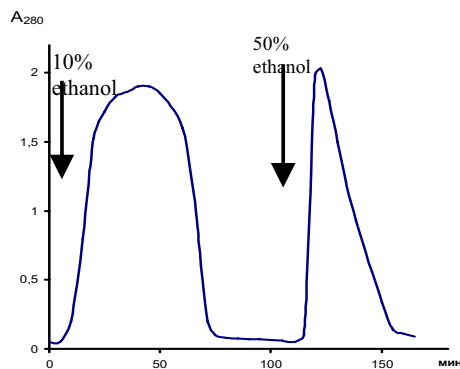


Fig. 1 Chromatography of a cell-free extract isolated from wheat on a column with "nanocarborb" ARK type

Nowadays mass spectrometry is the most suitable method for research of the chemical structure of substances. The structure of the biostimulator isolated by us was investigated in V.N.Orehovich Institute of biomedical chemistry of Academy of medical sciences of Russia on device Agilent 1100 Esquire 3000 plus. The device was equipped with an ionic trap. The received results are shown in Fig. 2.

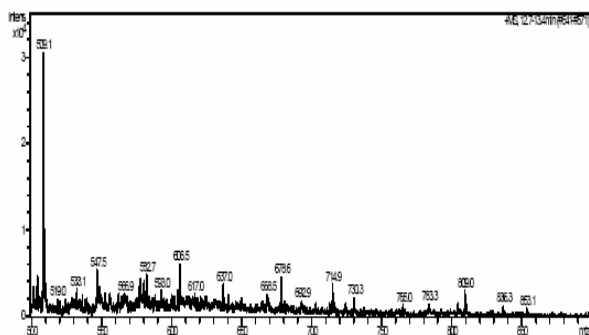


Fig. 2 Mass spectrum of a preparation of a biostimulator

Comparison of the received data with mass-spectra database has shown, that the received spectrum, namely peak at 509,1 corresponds to the basic ring of fusicoccin [3]. Thus obtained mass-spectra speak about purity of preparation. On the basis of this data it is possible to make a conclusion that the biostimulator has fusicoccin nature and a used method of purification of a biostimulator on a column with nanostructured sorbent - "nanocarborb" type ARK allows isolating the pure preparation which does not contain other substances.

### B. Using Biostimulator for Reproduction of Perennial Plants Shanks

For softening of an ecological condition of Kazakhstan one of the major questions requires the decision is the question on gardening and forestry of territories of the republic. For planting shanks in the big quantities introduction of new effective technologies is necessary. For reproduction of perennial plants by vegetative reproduction is more preferable in comparison with generative reproduction. The plants which have been brought up from seeds because of weakness have the big degree of loss whereas the plants which have been brought up by a vegetative method are stable against environmental influences. It is necessary to note one more important advantage of a vegetative way of reproduction. The sprouts received by a vegetative method are a copy of an adult plant which, in turn, is adapted to the given climatic and ecologic conditions. Whereas the plants received by the generative way have not this property. For this reason introduction of new technologies of vegetative reproduction is undoubtedly actual.

We investigated applicability of our biostimulator as a hormone for acceleration of vegetative reproduction of perennial plants. For this purpose we have taken shanks of desert plant *Tamarix gracilis* have left on 12h soaked in a solution of a biostimulator of concentration 50 ng/ml. After that shanks have taken out and have lowered them in drink water. Water was changed each 4-5 days and experiment was carried out within 1 month and observed formation of roots and leaves at shanks. Results of experiments are given in Fig. 3.



Fig. 3 Effect of a biostimulator on rooting of shanks *Tamarix gracilis*. 1 - an experimental plant processed by a solution of a biostimulator (50 ng/ml). 2 - the control plants. Time of an exposition – 1 month

For research of activity of a biostimulator, also shanks of *Eleaegnus argentea* and a yellow acacia were investigated. *Eleaegnus argentea* is a valuable plant, which acquire atmospheric nitrogen through roots microorganisms and increases nitrogen substances in soil [4]. Reproduction of such plants will allow to improve ecology of Kazakhstan.

Experiments on rooting were carried out with *Tamarix gracilis* too. Results are presented in Figs. 4 and 5. It is necessary to note that the biostimulator causes formation of roots and also the formation of leaves. The given fact is distinctive feature of our new biostimulator. All other phytohormones have polarity of action. For example, auxine stimulates formation of only lateral roots and stops growth of leaves. Cytokinin stimulates growth of leaves, and growth of roots is not observed.

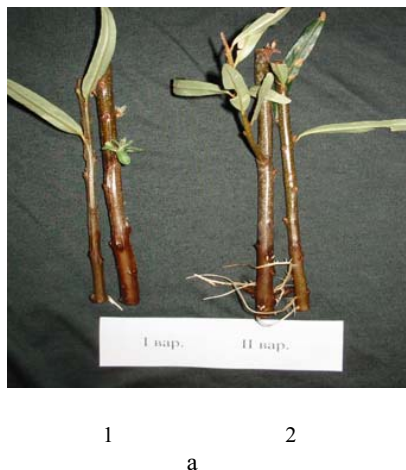


Fig. 4 Effect of a new biostimulator on root and leaves formation of shanks of plants *Eleaegnus argentea*. 1 - the control; 2 - plant treated by a solution of a biostimulator (50 ng/ml). Time of an exposition 1 month



Fig. 5 Effect of a new biostimulator on formation of root and leaves of shanks of yellow acacia (*Acacia fornesiana*).; 1 - plant treated by a solution of a biostimulator (50 ng/ml). Time of an exposition 1 month. 2 - the control

Thus, the new biostimulator activates growth roots and leaves. The given property allows using new biostimulator as a preparation for obtaining new shanks. As a result of the done work we have established, that only the biostimulator isolated by us answers the purpose for vegetative reproduction of deserte plants.

*C. Using Biostimulator to Increase Salt Tolerance of Wheat and to Increase Crop Productivity*

The majority of territory of Kazakhstan is desert and also is salted. Because of anthropogenic factors the secondary salting caused by irrigation [5]. Deep earth salts go up and result the second salinity. In this reason the desert territory undergoing to salinity. This resulted the essential decreasing of productivity of agricultural plants. To solve this problem it is necessary to involving innovational technologies with application of new biostimulators capable to increase salt tolerance. We tested our biostimulator for increasing of tolerance of wheat.

The wheat seeds of "Steklovidnaya-24" (*Aestivum Triticum*) cultivar were taken for the experiments. They were soaked during 12 h in a solution of our biostimulator in concentration of 10 nanogramm per 1 ml. After soaking grains were dried up and germinated in Petry dishes with 2 % NaCl solution. The control untreated by biostimulator wheat seeds also were grown in 2% NaCl. The results of experiment are illustrated in Fig. 5.



(a)



(b)

Fig. 6 Effect of a biostimulator on wheat seeds germination ("Steklovidnaya-24" cultivar) in a solution of 2 % NaCl. a) 2 % NaCl; b) 2 % NaCl pretreated by a solution of a biostimulator (50 ng/ml)

As it is shown from Fig. 6 untreated seeds did not germinate whereas the grains pretreated by a biostimulator have sprouted very well. The results of our experiment also are represented at Table I.

TABLE I  
EFFECT OF A BIOSTIMULATOR ON INCREASING OF SALT TOLERANCE OF  
WHEAT SEEDS "STEKLOVIDNAYA-24" CULTIVAR

	Control, water		2 % NaCl, seeds untreated by biostimulator		2 % NaCl + 10 ng/ml of biostimulator	
	steam, mm	root, mm	steam, mm	root, mm	steam, mm	root, mm
Quantity of grains	50		50		50	
Average value	3,06	3,54	0	0	1,42	1,75
Standard deviation	0,15	0,56	0	0	0,28	0,21

As you can see from Table I, despite of presence of salt wheat grains germinated under action of a biostimulator. Thus, new biostimulator essential increases salt tolerance of germinated wheat seeds.

As our experiments have shown a new biostimulator operates nonpolar and promotes development of both ground parts and underground parts of plants. On the basis of these data we have made the assumption that action of a biostimulator should increase biological productivity of plants. For this purpose we carried out large-scale field experiments from 2005 to 2007. Dry winter wheat seeds of "Steklovidnaya-24" cultivar were treated for 12 h in solution of biostimulator (concentration 10 ng/ml). Further fields were sowed with pretreated and untreated wheat grains. Action of a biostimulator on productivity of a winter wheat was studied in the following year. Results of field experiments are presented in Table II.

TABLE II  
EFFECT OF PRESEEDING PRETREATMENT BY A BIOSTIMULATOR OF SEEDS OF A  
WINTER WHEAT «STEKLOVIDNAYA - 24»

Allotment №	Variants	Productivity, centner/hectare	Growth in productivity, % from control
1	Control	11,40	38,60
	Experiment	15,80	
2	Control	12,60	28,60
	Experiment	16,20	
3	Control	12,00	25,00
	Experiment	15,00	
4	Control	12,00	33,30
	Experiment	16,00	
Average	Control	12,00	33,30
	Experiment	16,00	

As it shown from this table the pretreatment of seeds by solution of biostimulator 10 ng/ml gives the increasing of productivity on 33,3 %.

Also we tested the effect of a new biostimulator on productivity of winter rye cultivar "Sholpan". The experiment was carried out on fields of the individual agricultural

enterprise «Integrated poultry farm Bishkol». Results of experiments are showed in Table III.

TABLE III  
EFFECT OF A NEW BIOSTIMULATOR ON PRODUCTIVITY OF RYE "SHOLPAN"  
CULTIVAR

Variants	Productivity, centner/hectare			
	From allotments			Average
	1	2	3	
Control	19,9	20,0	20,1	20,0
Experiment	27,9	28,0	28,1	28,0

As it shown from this table the pretreatment of the seeds of winter rye gives the increasing of productivity on forty percent.

Also we tested the effect of the biostimulator on increasing of productivity of the important agricultural plant - sugar beet. Experiments were carried out in territory of agricultural industry "Zher-Ana" nearby Taldykurgan city on the area in 2 hectares. Results of experiments are showed in Table IV.

TABLE IV  
EFFECT OF A BIOSTIMULATOR ON PRODUCTIVITY OF SHUGAR BEET

Productivity, centner/hectare		Growth in productivity, %
Control	Experiment	
250	300	20

As it shown from this table pretreatment of seeds of the shugar beet by a solution of a biostimulator (concentration of 10 ng/ml) raises productivity of beet on the average by 20 %.

### III. CONCLUSION

It was established that our new biostimulator by its chemical composition relates to fusicoccin. It was shown that new biostimulator causes fast formation of main and collateral roots and it's also causes fast growth of leaves and stems of plants shanks. Biostimulator is very perspective for vegetative reproduction of trees plants at various ecological regions. Experiments show that biostimulator increases resistance of agricultural cultures to salt stress. Field experiments on sowing of pretreated by biostimulator winter wheat, beet and rye show that this pretreatment led to increasing of cultures productivity.

### REFERENCES

- [1] Z.A. Mansurov, "Some Applications of nanocarbon materials for novel devices", *Nonoscale-Devices - Fundamentals*, Springer, vol. 233, pp. 355-368, 2006.
- [2] U.Takahama, "Effects of fusicoccin and indole acetic acid on the levels of ascorbic acid and dehydroascorbic acid in the apoplast during elongation of epicotyl segments of *Vigna angularis*," *Physiologia Plantarum*, vol.98, issue 4, pp. 731-736, 1996.
- [3] B.E. Sultanbaev, M.K. Gilmanov, N.Yu. Abramycheva, V.V. Stolpakova, L.M. Ginodman, G.S. Muromtsev, "An embryonal factor

- and fusicoccin induce NADP-specific glutamate dehydrogenase in germinating wheat seed”, *Plant Science*, vol.88, pp.19-24, 1993.
- [4] A. Nelson, “Rocky Mountain Herbarium Studies. III. The Elaeagnaceae-A Mono-Generice Family”, *American Journal of Botany*, vol. 22, No. 7, pp. 681-683, 1935.
- [5] A. Maggio, S. De Pascale, G. Angelino, C. Ruggiero, G. Barbieri, “Physiological response of tomato to saline irrigation in long-term salinized soils”, *European Journal of Agronomy*, vol. 21, Issue 2, pp. 149-159, Aug. 2004.