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Development of the method of obtaining the endogenic biostimulator from wheat green spike glumes

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ABSTRACT

In this paper, a method of obtaining a highly active endogenous biostimulator from green spike glumes of wheat, which play an important role in regulation of donor-acceptor interactions, was considered. The obtained plant biostimulator performed protective functions in mitigating the cold effect as an unfavourable environmental factor. It was observed that the biostimulator obtained from green spike glumes of wheat could be used for vegetative propagation without separation of the plants from different ecological zones, agriculture and forestry.

Key words : Biostimulator, cultivated plants, limiting factor, seeds, wheat

INTRODUCTION

For Kazakhstan with its harsh environmental and climatic conditions, the most characteristic feature is the frost effect a temperature stress factor (Biological and Landscape Diversity of the Republic of Kazakhstan, 1997). The problem is similar to conserving biodiversity is crucial in the world (?khmetova *et al.,* 2018).

Currently, the issue of the impact of cold as a limiting unfavourable environmental factor is one of the important and urgent environmental problems in Kazakhstan, and there are practically no works in this direction which relate to the effect of positive low temperatures on plant growth processes.

The most promising method to combat the unfavourable environmental factor is the use of biostimulators that act in small concentrations (Kakymova *et al.,* 2017), but result in the large changes in such parameters as plant growth and resistance to the environmental stress factors (Ibragimova *et al.,* 2004; Sreevidya *et al.,* 2010).

To date, only chemically synthesized stimulators have been used in practical ecology, which are characterized by high toxicity and carcinogenicity, as well as biostimulators obtained on the basis of an expensive imported reagent, which respectively increased the cost of the obtained biostimulator by dozens of times (Gilmanov *et al.,* 2006; Basygaraev, 2007).

This study is devoted to the development of a method of obtaining a biostimulator from winter wheat green spike glumes of the variety “Vitreous - 24” to improve the resistance of seeds of cultivated plants to cold stress factors.

MATERIALS AND METHODS

The research subject included green spike glumes of wheat (*Triticum aestivum*), “Vitreous-24” variety, which contains a highly active stimulant.

To solve the above problems, adsorption chromatography on a nanostructured carbon sorbent of the “Nanocarbosorb” type was used, which was obtained by the method of carbonization of apricot kernels in the “Zhalyn” STPC, Institute of Combustion Issues (Almaty, Kazakhstan). To control the chromatographic separation, a Uvicord S II type UV monitor manufactured by LKB (Sweden) was used. For comparative analysis of the obtained

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biostimulator with commercial fusicoccin, high-performance high pressure chromatography was performed by using HPLC chromatograph 616 (USA) type produced by Waters Company. Fluorimetric determination of the conjugation of the fluorochrome-marked specific peptide from proton ATPhase with 14-3-3 proteins and fusicoccin was carried out in the Professor Albert de Boer's laboratory at the Free University of Amsterdam (the Netherlands).

The obtained quantitative results were processed by a standard computer statistical method using the Origin program. The devices used in the work meet all the requirements of modern metrology.

RESULTS AND DISCUSSION

During the seeds maturation, very intensive physiological and biochemical reactions occur, among which it is necessary to point out the processes of mobilization of plastic substances from dying organs - leaves and stems and their transportation into maturing seeds. It is natural that these donor-acceptor interactions must be controlled by very effective bioregulators. Probably, the seed fruit covering should be the center of their synthesis. Assuming that the center of the bioregulators synthesis can be green spike glumes, which enormous role in the formation of wheat productivity was proved in the course of the experiment of Professor A. N. Pavlov (Pavlov and Kolesnik, 1974), the task was to extract the regulator from green glumes taken from wheat ears in the phase of milky wax ripeness.

Ten g of green glumes from winter wheat spikes of "Vitreous-24" variety, collected in the phase of milky wax ripeness, were taken to obtain a biostimulator. Glumes were ground in a porcelain mortar in the cold, in a pre-cooled 70% ethanol. The homogenate was filtered through a red paper filter and the filtrate was used for further purification. The obtained alcohol filtrate was subjected to purification on a column with "Nanocarbosorb" of a size 3 cm - 20 cm, which was previously equilibrated with distilled water. Seventy ml of alcohol extract was applied to the column. After that, to completely remove the unbound substances, the column was washed with 200 ml of 10% ethanol. Desorption of biostimulator was done by using 50% ethanol, and then, to completely remove all substances, the column was finally washed with 96% ethanol.

Fig. 1 shows the results of chromatography of the alcohol extract obtained from the green spike glumes of wheat on a column with "Nanocarbosorb". As can be seen from the figure, the biostimulator emerged with a clear symmetrical peak, which was eluted with 50% ethanol.

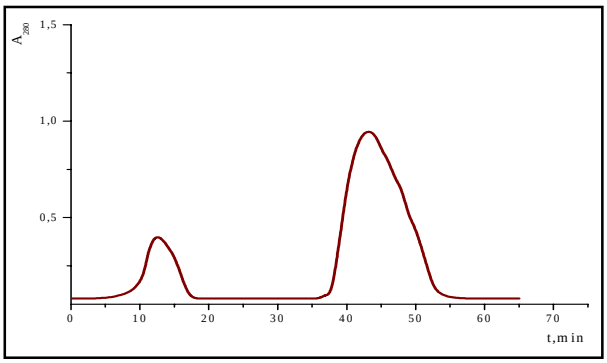

Fig. 1. Chromatography of an alcohol extract of green spike glumes of wheat on a column with "Nanocarbosorb" from apricot kernels.

Thus, we developed a simple, inexpensive and effective method of obtaining a regulator from green spike glumes, which is different from the method of Basygarayev *et al.* (2004) by the source of regulator isolation and another type of sorbent.

Having a highly purified biostimulator preparation, it was necessary to determine whether it was similar to the fusicoccin stimulator that was extracted by Basygarayev *et al.* (2007). With this purpose, we carried out high-performance chromatography using a high-pressure HPLC chromatograph, type 616 produced by Waters Company (USA). The HPLC chromatography data are shown in Fig. 2.

As can be seen from Fig. 2, the biostimulator is shown in an absolutely different peak (2A) on the chromatogram than the standard fusicocin (2B). These data indicated significant differences in chromotographic behaviour of the biostimulator and fusicoccin. Important evidences, that our biostimulator is not fusicoccin, have been obtained at the laboratory of the Free University of Amsterdam (Netherlands) under the guidance of Professor Albert de Boer, who showed interest in our biostimulant.

According to the technique of Professor Albert de Boer, the level of fluorescence of the triple complex formed by binding of a proton

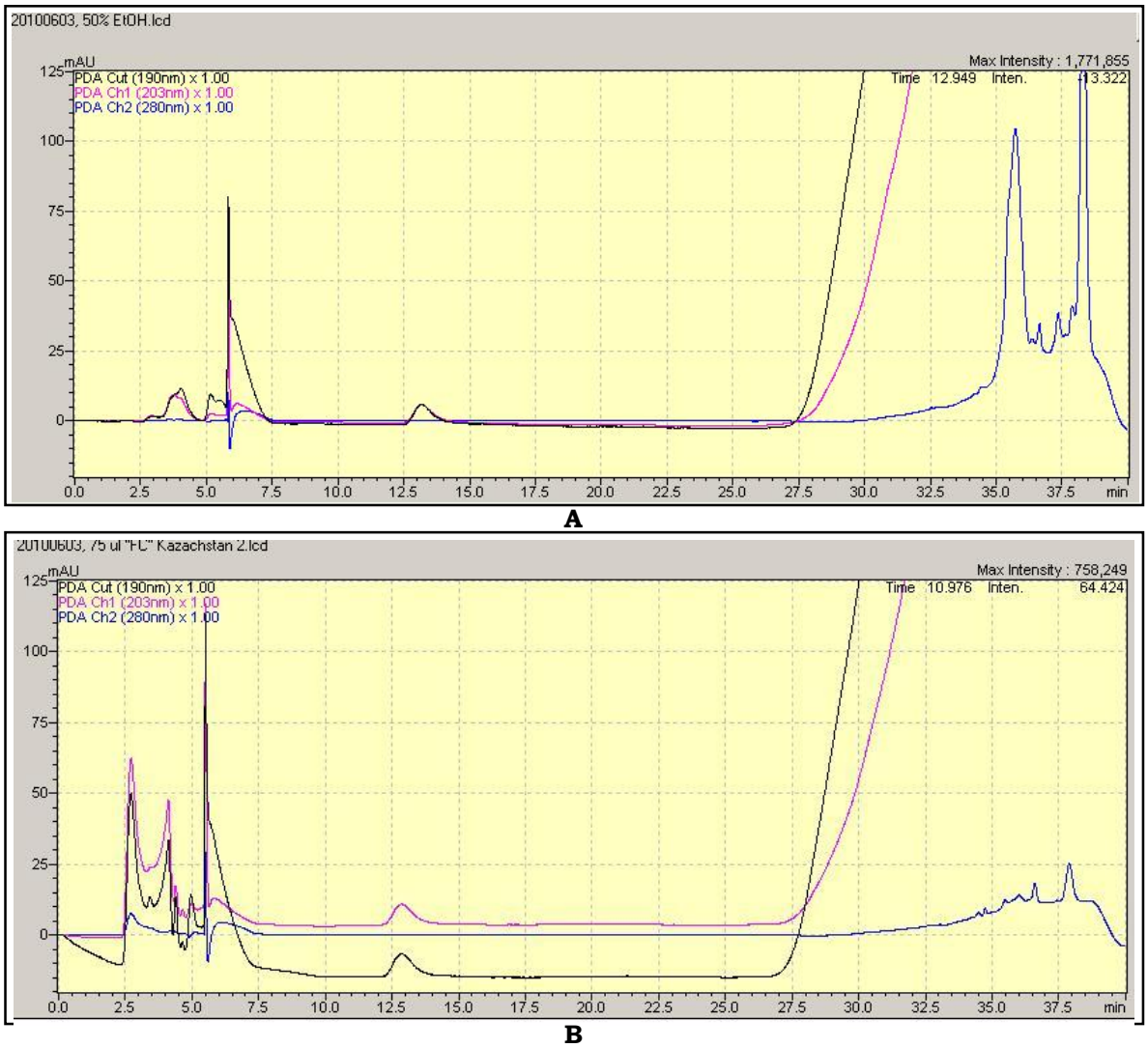

Fig. 2. HPLC chromatography of purified biostimulator in ethanol and standard fusicoccin : A : HPLC chromatography of purified biostimulator; B : HPLC chromatography of standard fusicoccin.

ATPhase specifically marked with fluorochrome peptide with 14-3-3 protein and fusicoccin was studied, whereas the biostimulator we obtained was not capable of participating in the formation of this complex, as seen in Fig. 3.

As can be seen from Fig. 3, the formation of a triple complex - a peptide with 14-3-3 protein and with fusicoccin results in fluorescence quenching. Whereas the peptide with 14-3-3 protein could not combine with the biostimulator purified by us, and there was no quenching of fluorescence in this experiment.

Thus, important results were obtained that indicated that the explored biostimulator was not a substance of fusicoccinic nature and it fundamentally differed in its properties from the stimulator studied by Basygarayev Zh. M. and staff members (Basygarayev *et al.,* 2004).

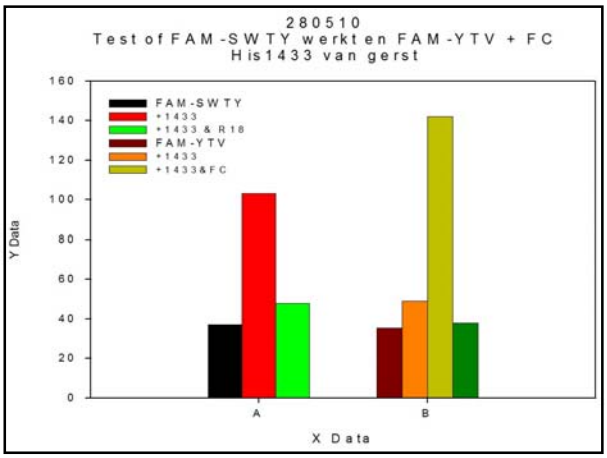

Fig. 3. Fluorescence test for the formation of a triple complex of fluorochrome-marked peptide of proton ATPhase with 14-3-3 protein and with fusicoccin or purified biostimulator: A : Experiment with fusicoccin; B : E xperiment with biostimulator.

Determination of the structure of biostimulator is a future task for specialists in the field of studying the chemical structure.

As a result of the conducted work, a method of obtaining a biostimulator from green spike glumes of wheat was developed which aimed at increasing the plants' resistance to unfavourable environmental factors.

The biostimulator was extracted from green glumes taken from wheat spikes of the Vitreous-24 variety, collected in the phase of milky wax ripeness, which is a cheaper source for obtaining a biostimulator that does not require the use of expensive imported reagents, in contrast to the biostimulator obtained by induction method with the use of the reagent 6-benzylaminopurine, which increases the cost of the biostimulator dozen times.

The biostimulator was purified by chromatography method using a nanostructured carbon sorbent of the "Nanocarbosorb" type, obtained by carbonization of apricot kernels.

It was found that the regulator purified by us was not able to participate in the formation of a triple complex, similar to how a specific proton ATPhase peptide forms a strong complex with 14-3-3 protein and fusicoccin (Basygaraev *et al.,* 2007). Based on which, it was found that the obtained biostimulator was not a substance of fusicoccinic nature.

In conclusion, it is proved that our biostimulator belongs to fusicoccin with determining of structure by mass spectrometry. At present, fusicoccin is separating by a very complex method to fungus, its price is about $ 200- $ 500 per milligram. The price of fusicoccin can be cheaper 10 times by using our separation method. The price of fusicoccin is at least $ 200 or 70,000 tenge in Kazakhstani condition. The price of our biostimulator is 70 times less than the world price.

Recommendations for the Use of Findings in Specific Locations

This research shows that nanogram of biostimulator is highly active in concentrations, which is two to three times smaller using than other phytogormones. It's safe to say that the biostimulator is widely used in agriculture, forestry and ecology. In fact, our experiments have shown that this biostimulator is an effective preparation for the rootstock of various plant roots, and the auxins, which are the principal regulator of the root, are suitable only for the rootstock of the plants with indirect root system. The exact details of the biostimulator can also be used in the following fileds :

- To teach theoretical and practical lectures on biochemistry, biotechnology and plant physiology or using as educational material.
- As using a biostimulator for vegetative reproduction of perennial plants adapted to ecology, agriculture, forestry, fruiting and ecology.
- To recommend biostimulator to increase productivity and stress resistance of agricultural crops adapted to different environmental zones.

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