Effect of short peptides on seed germination and morphometric parameters of flax seedlings

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Abstract. The use of peptide preparations is one of the current trends in modern agriculture. These preparations can be used to increase the efficiency of sowing treatment of agricultural crops seeds. The article presents the results of a laboratory experiment aimed at studying the effect of a complex of short peptides AC-3 (glutamic acid, aspartic acid, leucine) on seed germination and morphometric parameters of flax seedlings (Linum usitatissimum L.) - Tverskoy variety. The maximum germination energy (68, 68 and 74%, 50% at the control) and seed germination values (95, 95 and 100%, 75% at the control) were obtained in variants with seed treatment with a complex of short peptides at concentrations of 1*10-12, 1*10-13, and 1*10-15 g/l, respectively. According to the totality of the maximum values of seed quality indicators and morphometric characteristics of flax seedlings, the Tverskoy variety (the length of roots and sprouts, the yield of raw and dry biomass of seedlings), the optimal concentration of a complex of short peptides for pre-sowing seed treatment is 1*10-15 g/l.

1 Introduction

Flax has been grown all over the world for centuries and serves as a raw material for the production of food, medicines, textiles and, therefore, is of great importance for the culture and mankind development. Flaxseed oil is well known for its health benefits, mainly due to the high content of fatty acids in the form of alpha-linolenic acid (about 45-55%). The addition of crushed flax seeds to the diet increases the nutritional value, since they are also a rich source of lignans with antitumor properties [1-2]. Also, flax is a source of industrial fibers. During its processing, long and short fibers can be obtained. Long fiber is used in the production of high-quality linen clothing, while short fiber is the basis for the production of cheaper products. Also, due to its performance characteristics, linen is in demand in design and other industries [3-4]. In the last decade, the fiber industry has made some efforts to develop high-quality products from flax stalks for their use in the pulp industry, the production of technical fibers, as well as biofuels [5].

According to the Rosselkhoznadzor, the sowing of flax in the Russian Federation in 2023 was carried out in 15 regions. According to Rosstat, the area under commmon flax in 2023 increased to 37 thousand hectares from 35 thousand hectares in 2022. Due to the

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increased demand for flax products in recent years, the issue of increasing the yield of flax on existing areas, as well as improving the quality of the products obtained, is acute. Currently, the use of innovative methods provides opportunities to achieve these goals, contributing to the development of this branch of agriculture, in particular, the possibility of cultivating flax in the Central region of Russia.

One of such methods is the use of various biologically active substances that promote the growth and development of flax plants, in particular, short peptides, which are a system of signaling molecules that regulate the body functions at the molecular, genetic, subcellular, cellular, and tissue levels [6]. In recent years, numerous biochemical and genetic studies have demonstrated that peptide signaling plays a larger role than expected in various aspects of plant growth and development, and they are currently being considered as a new class of plant hormones. As important physiological regulators, peptides are used in many fields, including medicine, cosmetics, nutrition, and animal health, as well as plant nutrition and their protection. In recent years, peptides have become a popular subject of research in the field of plant protection as inducers of antimicrobial and immune drugs, plant growth regulators, insecticides and herbicides due to their extensive sources of raw materials, excellent activity and perfect compatibility with the environment. They are used as antimicrobials and immune inducers, plant growth regulators, insecticides, and herbicides to protect plants from bacteria, viruses, pests, and weeds [7-8].

Plant growth regulators are an important tool in plant cultivation, thanks to which they play a key role in agriculture and food security, contributing to an increase in crop yields worldwide. At the same time, to meet agriculture needs, there is a growing need to create new growth regulators that meet the requirements of environmental safety and pose minimal risk to the environment [9-10].

Plant growth and development are influenced by plant hormones, including auxin and others, which mediate intercellular communication during development. Nevertheless, recent studies have shown that short peptides also play an important role in various processes of plant development, such as the differentiation of meristematic stem cells, the formation of tissues and individual organs, fruit ripening, falling off, and also contribute to resistance to adverse environmental factors, their adaptation to biotic and abiotic stress. Thus, short peptides represent a new class of plant hormones with high biological activity at very low concentrations (10^{-7}-10^{-9} M). These results indicate the importance of peptides in the plant growth regulation [11].

2 Materials and Methods

2.1 Laboratory testing methodology

Studies and evaluation of the effect of the peptide preparation on the early stages of growth and development of flax seedlings were carried out in the laboratory of peptide technologies of the RSAU - MAA named after K.A. Timiryazev.

A medium-ripened common flax variety Tverskoy (Linum usitatissimum L.) was used as an object of research. The seeds were produced at the All-Russian Flax Research Institute, Tver Region, city of Torzhok, Russia.

Ovagen Lingual is a complex of short peptides AC-3 (glutamic acid, aspartic acid, leucine), belongs to the cytogen class (this group of drugs is also called synthetic peptide bioregulators). Cytogens are synthesized from natural amino acids, resulting in a copy of the working part of the most active peptide part from the entire complex contained in the extract, that is, one shortened molecule. This drug was developed by the "Saint-Petersburg Institute of Bioregulation and Gerontology", St. Petersburg, Russia.

The seeds were germinated in Petri dishes with a diameter of 95 mm on filter paper in a climate chamber for permanent conditions with software control (Binder KBW 720) at a temperature of 21°C and a constant humidity of 60%. 20 seeds were placed in each cup, treated with tap water (control variant) and solutions of a complex of short amino acids in concentrations (g/l): $1*10^{-8}$, $1*10^{-9}$, $1*10^{-10}$,$1*10^{-11}$, $1*10^{-12}$, $1*10^{-13}$, and $1*10^{-15}$ (experimental options). The exposure of seed treatment is 1 hour. The repetition of the experiment is 4-fold. The seeds were moistened with tap water every day for 7 days.

The number of germinated seeds was counted on the 3rd and 7th days. The characteristics of seed germination were determined: germination energy on the 3rd day and seed germination on the 7th day (GOST 12038- 84). As germinating seeds, those that had a normally developed root and sprout were considered. In this case, the main root should not be shorter than the seed itself, and the sprout should not be shorter than half of the seed. In addition, the intensity of seed germination along the length of sprouts and roots was measured. The yield of raw biomass and the dry mass of seedlings, respectively, were determined using analytical weights.

2.2 Statistical evaluation

The collected data were subjected to statistical analysis using a statistical package (statistics 8.1). The results represent the average values of measurements on three experimental samples. The Least Significant Difference (LSD) test with a probability level of 5% was used to determine the average comparison.

3 Results and Discussion

Figure 1 demonstrates that the use of the AC-3 short peptide complex had a positive effect on the germination energy and germination of flax seeds at all tested concentrations. When seeds were treated with an amino acid complex in all experiment variants, the germination energy was higher, with the exception of the variants of seed treatment with a concentration of 10^{-8} and 10^{-9}, the values below the control (50%) were 40 and 45%, respectively. It should be noted that the values of the considered indicators varied significantly depending on the drug concentration. The highest germination energy was observed when seeds were treated with a complex of short peptides with concentrations of 10^{-12}, 10^{-13}, and 10^{-15} and amounted to 68, 68, and 74%, respectively, which is 36-48% more relative to the control.

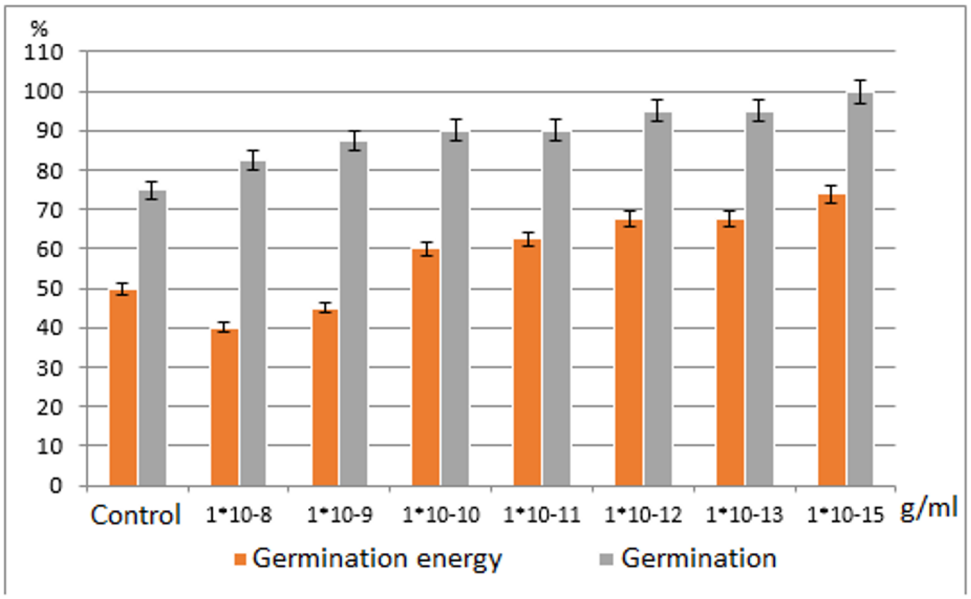

Fig. 1. The effect of different concentrations of a complex of short peptides on the germination of common flax seeds of Tverskoy variety

Laboratory germination is one of the main indicators by which the quality of seed material can be assessed. The smaller the difference between germination energy and germination, the higher the seed quality. If seed germination is low, it means that there will be a significant discrepancy between the results of laboratory and field tests, which in turn can lead to a decrease in yield.

The results of our studies showed that the use of the AC-3 short peptide complex had a positive effect at all tested concentrations. The highest percentage of germination of flax seeds was also observed in variants with seed treatments with a concentration of 10^{-15} and amounted to 100%, which is 33.3% more relative to the control (75%).

Amino acids have an important property to stimulate cell division and growth, which contributes to an increase in the size and volume of plant organs. In addition, short peptides also contribute to accelerating root growth and increasing their surface area, which leads to increased absorption of water and nutrients. As a result, the plant develops a longer and branched root system, which promotes the absorption of nutrients from the soil. This effect was especially evident when studying the length of the common flax sprouts and roots.

Thus, the treatment of seeds with a complex of short peptides increased the intensity of seed germination, having a positive effect on the morphometric parameters of common flax seedlings of the Tverskoy variety according to all variants of the experiment (Figure 2).

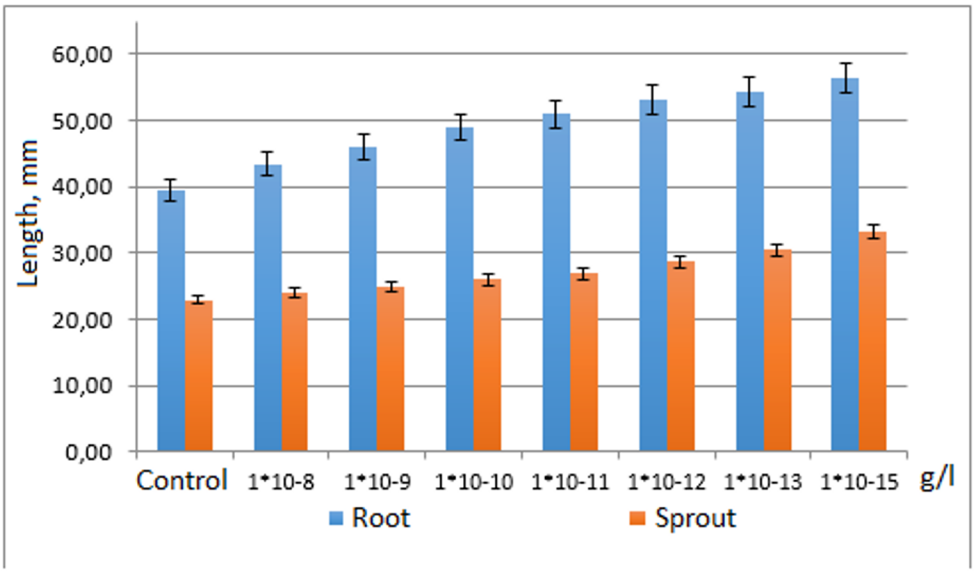

Fig. 2. The effect of different concentrations of the short peptide complex on the morphometric parameters of seedlings.

By the end of the experiment, the best effect was observed in seedlings whose seeds were treated with the drug in concentrations of 10^{-12}, 10^{-13}, and 10^{-15}. Nevertheless, there was no significant difference when using these concentrations. Thus, the average length of sprouts and roots of flax in the variant with a concentration of 10^{-15} was 33.2 mm and 56.5 mm, respectively. In the variant without treatment – 23.1 mm and 39.5 mm, respectively. The increase was 30.4 and 30%, respectively.

The data in Figure 3 show that the use of an amino acid complex significantly affects not only morphometric parameters, but also the biomass of flax seedlings. The results of studies showed that the use of the AC-3 short peptide complex had a positive effect at all tested concentrations. The raw mass of common flax seeds in the variant of seed treatment with a concentration of 10^{-15} was the highest - 1.61 g, whereas in the control variant it does not exceed 1.1 g, the increase was 46.3%. In other variants, this indicator varied from 1.15 g to 1.15 g.

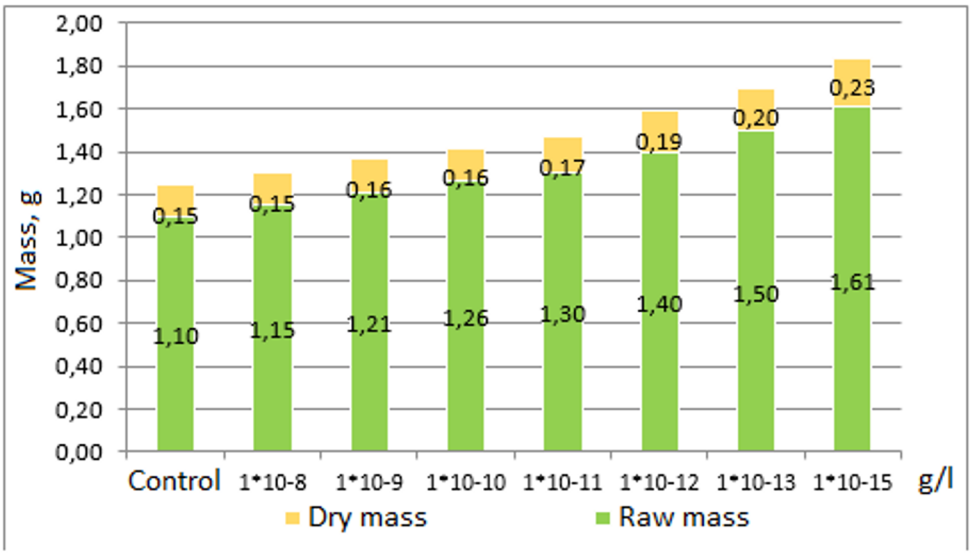

Fig. 3. The effect of different concentrations of the short peptide complex on the weight of seedlings.

The dry mass of seedlings after a certain time interval is an indicator of the efficiency of using endosperm reserves for the formation of primary morphostructures. A batch of seeds with a higher germination percentage and higher dry weight values is considered as a batch of seeds with greater strength. When seeds were treated with a complex of short peptides AC-3 with a concentration of 10^{-15}, the maximum values of the dry weight of seedlings were also observed – 0.23 g, compared with the control variant – 0.15 g. Seed treatment in the highest concentration (from 10^{-8} to 10^{-11}) had no significant differences regarding control.

4 Conclusions

The results of laboratory studies show that different concentrations of Ovagen Lingual drug, which is a complex of short peptides AC-3, has a different effect on the germination of common flax seeds, the Tverskoy variety. It was found that the highest concentrations of the short peptide complex did not show significant differences compared to the control variant, while lower and average concentrations contributed to an increase in germination energy, laboratory germination of seeds and morphometric parameters of seedlings. The highest results were observed when using Ovagen Lingual at a concentration of $1*10^{-15}$ g/l. These data will be used in the future for conducting field and production experiments.

This study confirms that short peptides act as the most important plant growth stimulators. It is necessary to expand the list of plant species and varieties of agricultural crops so that conclusions can be drawn about the exact application of the complex of short peptides in agriculture.

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