Antioxidant action of a nanocomposite biological product Azogran on seeds development of different varieties of barley

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Abstract- *Significant opportunities allow the use of biological products that increase the resistance of plants to adverse stress factors, increase yields and improve product quality. In the present work, the dependence on the suppressing effect of hydrogen on various seeds of different species was established. It was shown that the post-treatment of seed with the nanocomposite bacterial preparation Azogran exerts an antioxidant effect on plant growth at different development phases.*

Index Terms- hydrogen peroxide, oxidative stress, *Bacillus subtilis*, *Azotobacter vinelandii,* Barley

I. INTRODUCTION

Historically, cereals have been one of the main types of plants in agriculture. Barley is one of the most important nutritious crops, 100 kg of seeds is equal to 120 feed units, and has a high starch content (44-51%), which is a valuable raw material for beer and ethanol. Barley is currently the fourth most important cereal crop in the world, growth in more than 100 countries. In the last decade, Europe produced about 60% of the world's barley tonnage, while Asia and America produced 15% and 13% respectively [1, 2]. However, one of the important tasks of modern agricultural science is to increase the resistance of crops to abiotic stresses (soil drought [3], frost [4], and salinization [5], the action of heavy metals (TM), UV radiation, herbicides and flooding). Climatic instability increasing in recent years and an increase in the number of weather anomalies lead to disturbances in plant metabolism and a decrease in their productivity [6]. The plant can give in to stress even at the initial stage of ontogenesis, lose the ability to germinate at the seed stage as a result of the accumulation of degenerative changes. One of the early effects of stress on plants is excessive accumulation of reactive oxygen species (ROS) and the development of oxidative stress [7]. The study of plant reactions to stressful situations periodically occurring during the growing season is necessary to develop methods that reduce their negative impact. The solution to this problem in modern agricultural production is closely related to the use of microbial preparations. According to the literature [8], bacteria, in comparison with plants, are characterized by a wider spectrum of enzymatic and non-enzymatic antioxidant defense systems, which are less sensitive to the influence of stress agents. The use of biological products accompanies the better development of plants and the production of better products. In the department of microbiological processes on solid surfaces of the Institute of Microbiology and Virology. D.K. Zabolotny of the NAS of Ukraine created a highly effective nanocomposite complex bacterial preparation called Azogran. The components of this biological product is the nitrogen-fixing strain *A. vinelandii* IMV B-7076, phosphate-mobilizing bacteria *Bacillus subtilis* IMV B-7023 and the clay mineral bentonite. Earlier, we found that the metabolite complexes of these strains exhibit an antioxidant effect on the germination of wheat and rye seeds [9]. However, the antioxidant effect of Azogran as a whole has not been investigated. In this regard, the aim of this work was to study the effect of barley seed bacterization by the nanocomposite complex bacterial biological preparation Azogran on plant growth under peroxide stress.

II. MATERIALS AND METHODS

In order to verify the effect of the nanocomposite complex bacterial preparation Azogran on the growth of barley plants under conditions of peroxide stress in the IMV named after D.K. Zabolotny of the NAS of Ukraine, his experimental batch was developed. The components of the preparation are strains of *Azotobacter vinelandii* IMV B-7076 [10], *Bacillus subtilis* IMV B-7023 [11] and bentonite nanoparticles. The number of viable cells in the biological product was:

- *A.vinelandii* IMV B-7076 - $(3.1 \pm 0.1) \cdot 10^7$ cells/ml;
- *B. subtilis* IMV B-7023 - $(3.4 \pm 0.1) \cdot 10^7$ cells/ml.

The seeds of spring barley of the varieties Burkhant (Mongolia), Virazh (Ukraine), and Copeland (Canada) were used in the experiment. In the first series of experiments, 50 seeds of each cultivar were selected and treated with hydrogen peroxide (6%, 20%, and 33%) for 30 min. Seeds were washed three times with physiological saline and laid out on filter paper moistened with sterile distilled water for germination at t=20° C. The experiments were carried out in laboratory conditions in triplicate. In the second series of experiments, plants of 3 varieties of barley were grown in a greenhouse. Pre-seed was subjected to processing according to the scheme:

1. Control - seeds treated with sterile distilled water (H_2O);
2. Seeds bacterized by the nanocomposite complex bacterial preparation Azogran (CX);
3. Seeds treated with 33% hydrogen peroxide for 30 minutes. (H_2O_2);
4. Seeds exposed to 33% hydrogen peroxide (30 min.) and bacterialized with 3 ml of the nanocomposite complex bacterial preparation Azogran for 1 hour (H_2O_2 + CX).

The seeds of each grade of barley were sown in 4 replicates of 50 pcs. in a row. During the growth of barley, the following phases were noted: GS-24 tillering, GS-45 booting, GS-61 flowering, GS-87 dough development, GS-91 ripening. The onset of the developmental stages of barley plants was noted according to the method described in [12]. In each phase, the height of the plants was investigated. Harvesting and recording of crops was carried out in the phase of full ripeness in the plots in accordance with traditional methods manually [13]. The data obtained were processed using standard statistical methods [14] and using standard computer programs Microsoft Office Excel. The figures and tables show the arithmetic mean values and standard deviations. The significance of differences between the mean values was evaluated by student t-test.

III. RESULTS AND DISCUSSION

At the first stage of experiment, the parameters of peroxide stress were experimentally selected. Hydrogen peroxide is a natural metabolite of cells and is formed during the disputation of the superoxide anion radical and during the oxidation of various reduced cellular components (iron–sulfur-containing proteins, flavoproteins).

However, H_2O_2 can accumulate in excess in the cells of prokaryotes, eukaryotes and act as one of the most aggressive promoters of lipid peroxidation (LPO), as well as contribute to the development of oxidative stress. This stress agent activates the lysis of cell membranes, which in turn is accompanied by a decrease in cell viability and causes necrosis in general, and also acts as a factor in abnormal ontogenesis [15-17].

It was shown that 6% hydrogen peroxide was not an aggressive oxidant and accordingly, did not inhibit the similarity of seeds, but rather increased it (Table 1). From literary sources it is known [18] that dilute aqueous solutions of H_2O_2 are used as an environmentally friendly growth stimulant and disinfectant for vegetables and grain crops. However, under the action at 20% and 33% H_2O_2, the values of this indicator decreased significantly. The development of oxidative stress was especially pronounced under the influence at 33% of hydrogen peroxide. In particular, when incubating barley seeds of Virazh variety in 33% H_2O_2, their germination rate decreased by 31.7%, Burkhant variety - by 66.9% and Copeland variety - by 41.2%, relative to the control.

During ontogenesis, plants are exposed to changing environmental conditions, to which they are forced to adapt, developing protective mechanisms to reduce their negative impact and preserve life potential [19].

However, the implementation of such mechanisms is accompanied by significant energy costs, and this in return leads to a decrease in productivity [20].

TABLE 1. THE EFFECT OF DIFFERENT CONCENTRATIONS OF HYDROGEN PEROXIDE ON THE GERMINATION OF SEEDS OF DIFFERENT VARIETIES OF BARLEY

Barley variety	Seed germination (%) under the action of different concentrations of H_2O_2			
	Control	6%	20%	33%
Virazh	50,4 ± 1,1	186,5 ± 0,9	36,1 ± 1,8	18,7 ± 0,2
Burkhant	75,6±0,6	98,1±0,5	56,1±2,0	8,7±0,5
Copeland	68,0±1,4	99,4±1,1	65,6±1,6	26,8±1,2

Notes: 1. - control - seeds treated with sterile distilled water; 2. - the treatment time with hydrogen peroxide was 25 minutes

Among the responses of plants to the influence of various stress factors are mutually beneficial bonds of plants with rhizospheric microorganisms that are able to synthesize a wide range of antioxidant metabolites [21].

Such soil microbes are of interest in creating biological plant protection products. Since they are characterized by the formation of long-term protection of the macroorganism from stressful environmental factors [22].

According to the results of experiments in the greenhouse, it was found that the post-treatment with a nanocomposite complex bacterial preparation of seeds of rape barley, strains Virazh, Burkhant and Copeland, stressed by hydrogen peroxide, significantly stimulated plant growth at different stages of development (Fig. 1, 2, 3). So, at the tillering stage, the height of the Virazh barley plants in the pre-sowing seed treatment variant 33% H_2O_2 + Azogran increased by 20.5%, the Copeland cultivar - by 9.6%, compared with the variant where the stress agent acted on the seeds (Fig.3). However, stimulation of the studied parameter was not observed for the Burkhant variety (Fig.2).

The tillering phase is a very important stage of organogenesis, at which regenerative organs are laid and formed. The future harvest depends on their normal development. In addition, in this phase of development, young plants are in great need of nitrogen and phosphorus nutrition [23].

Highly effective nitrogen-fixing and phosphate-mobilizing microorganisms, in particular, bacteria-components of the biological product Azogran, can take part in eliminating this problem [24, 25].

Analysis of data on the height of barley plants in the phase of exit to the tube (stem) for the variant using hydrogen peroxide and the preparation Azogran also showed an increase in this indicator. Accordingly, for the Virazh variety - by 16.7%, for the Burkhant variety - by 10.4% and for the Copeland variety - by 7.7%, in comparison with the option where the plants developed from seeds treated with a 33% H_2O_2 solution (Fig. 1, 2, 3). It should be noted that the barley plants of the Copeland variety did not develop in subsequent stages. Presumably this could be due to greenhouse conditions, which may not have been optimal for growing this variety. Accordingly, further studies were carried out on 2 varieties of barley: Virazh and Burkhant.

In the flowering phases - wax ripeness, differences in the height of barley plants between the treatment options for seed 33% H_2O_2 and at 33% H_2O_2 + Azogran were preserved. Correspondingly, the studied indicator for barley of the Virazh variety during these development phases increased in the variant with post-treatment of seeds with a nanocomposite complex bacterial preparation by 14.7 - 18.6%, and for the Burkhant variety-by 7.6 - 14.7%, respectively, to the variant where the seeds were exposed to hydrogen peroxide (Fig.1, 2).

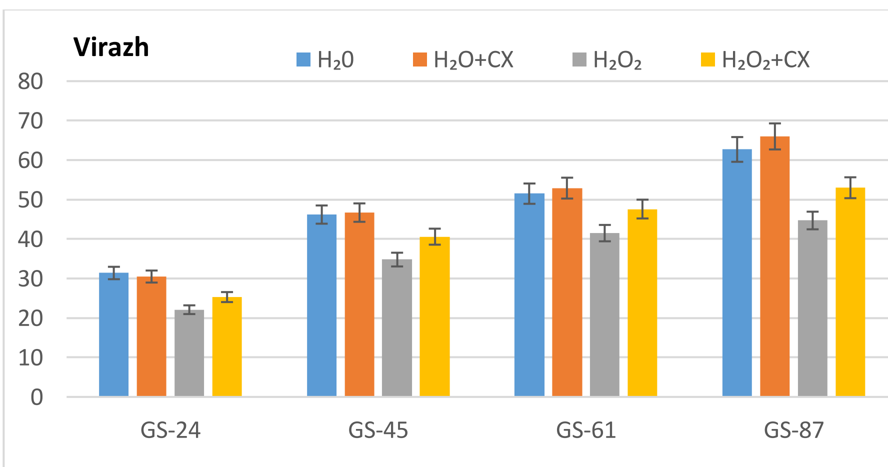

Fig. 1. The effect of a nanocomposite complex bacterial preparation on the height of Virazh barley plants in different phases of development, after seed treatment with hydrogen peroxide

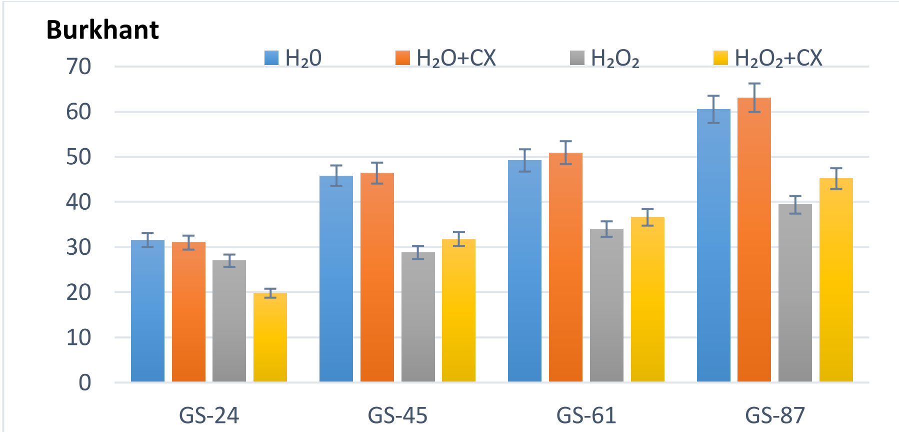

Fig. 2. The effect of a nanocomposite complex bacterial preparation on the height of Burkhant barley plants in different phases of development, after seed treatment with hydrogen peroxide

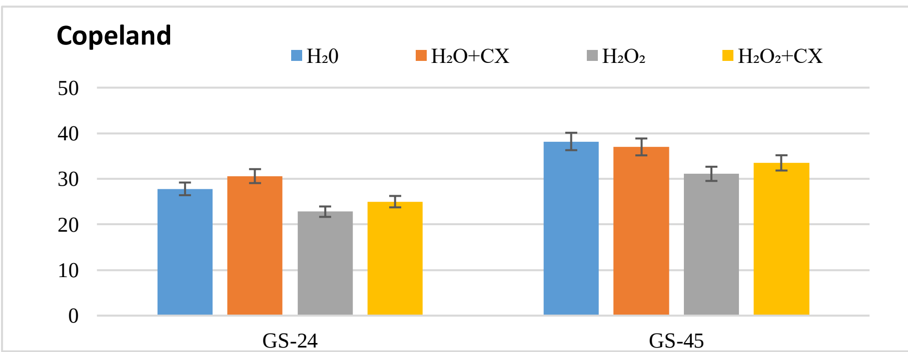

Fig.3 The effect of a nanocomposite complex bacterial preparation on the height of Copeland barley plants in different phases of development, after seed treatment with hydrogen peroxide

The number of grains in an ear is important when selection plant varieties for their productivity and is a prerequisite for a high yield. This indicator is determined by both the genetic characteristics of the variety and the environmental conditions in which it is cultivated [2].

Also, the number of grains in an ear is the main factor of its attracting ability - activation of transport of nutrients to the organ with the highest concentration of phytohormone stimulants [26].

It was shown that post-stress treatment of barley seed with a nanocomposite complex bacterial biological preparation Azogran accompanied an increase in the number of grains per ear for the Virazh variety by 22.8%, and for the Burkhant variety by 29.9%, compared with this indicator for plants grown from stressed seeds (Fig. 4).

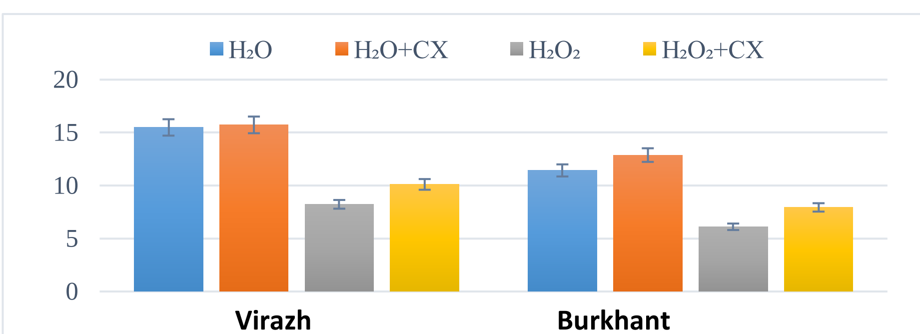

Fig. 4. The effect of hydrogen peroxide and nanocomposite complex bacterial preparation on the number of grains in the ear of plants of varieties between Virazh and Burkhant

IV. CONCLUSION

The obtained results allow us to conclude that the inhibitory effect of hydrogen peroxide on the germination of seeds of different varieties of barley increases with increasing the concentration of this stress agent. Post-treatment with a nanocomposite complex bacterial preparation of stressed barley seed stimulated the growth of plants at different phases of their development, as well as the formation of a larger number of grains in the ear. This indicates the ability of the bacteria-components of Azogran to synthesize antioxidant compounds. The studies allowed us to recommend a nanocomposite complex bacterial preparation, created on the basis of nitrogen-fixing and phosphate-mobilizing strains, to increase the resistance of barley plants to an increase in the content of hydrogen peroxide in cells as a stress agent.

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