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Research Article

The Influence of the Biostimulator Reglalg on the Primary Resistance of Winter Wheat Genotypes to Extreme Temperatures

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Abstract

In this article, we present the results regarding the influence of the biostimulator (BS) Reglalg on the primary resistance of 3 varieties and 11 lines of winter wheat to heat shock (HS) and frost shock (FS). Exposure to temperatures of +50°C for 30 minutes and -7°C for 8 hours, respectively, was used as HS and FS. These values of HS and FS were obtained experimentally. Their effects made it possible to significantly distinguish the majority of the studied genotypes in terms of primary resistance. The relative level of increasing the primary resistance to HS or FS of the seeds of different wheat genotypes treated with BS Reglalg tends to be inversely related to their primary resistance to extreme temperatures, but the absolut level of influence of BS Reglalg increasing seeds germination after exposure to extreme temperatures on seeds the germination of which after exposure to HS or FS tend to be around 50%.

The proposed accelerated method of determining the wheat genotypes primary resistance to HS or FS, is fast, easy, and low expensive, thus opening new perspectives in optimizing the selection of new varieties and rational distribution zones for the cultivation of existing genotypes according to their genetic resistance to extreme temperatures; and application of biostimulators in agriculture.

Keywords: Wheat; Seed Germination; Genotypes; Bs Reglalg; Extreme Temperatures; Resistance

Introduction

As a result of the influence of global climate warming, significant changes occur in the seasonal calendar of many natural events, including the duration of the ontogenesis period of winter wheat, the final effect being often unfavorable for productivity [1,2]. Periods of extreme temperatures are becoming more frequent, with increasing intensities and durations [3,4]. Phenological observations have shown that due to climate change, the annual rate of plant growth and development changes [5,6]. Considering this, to maintain and increase the reliability of plant cultivation, radical changes in their selection and cultivation technologies are required in the specific conditions for each area [7]. Some of the mentioned problems can be solved with the aid of biostimulators [8].

Apart from the effects of improving soil physical and chemical properties, algae produce a wide range of natural growth regulators and plant protectants [8-10]. Components and biological effects similar to those described for marine algae are highlighted for substances eliminated in lake habitats and in algal extracts [10,11]. Treating seeds before sowing and plants during the vegetation period [11,12] with preparations obtained from them demonstrates beneficial effects on resistance to thermal stress and plant productivity [12]. Unlike synthetic plant protection substances, preparations obtained from algae, such as BS Reglalg, do not negatively affect the environment; therefore, they are compatible with the requirements of organic agriculture [12]. It has been shown that using algae extracts in agriculture can generate several benefits, including stimulation of root system growth, enhancement of photosynthesis, and resistance to abiotic stress factors [12]. These extracts can be applied separately, together with regulators, or with synthetic plant protection substances, reducing the effective dose of HS when used per plant, or introduced into the rhizosphere, independently of the nutrient content of the product, stimulating the natural processes of increasing plant vigor and resistance to thermal stress factors, contributing to increasing the quantity and quality of the crop [12,13].

The resistance of wheat genotypes to the action of extreme temperatures is a genetically determined characteristic. Its manifestation occurs through processes coordinated over time and adapted to specific environmental conditions [13-15]. Although the resistance of plants to the action of stress factors is genetically determined, the adaptation processes can be optimized by treating plants with biostimulators [12,16]. The BS Reglalg is certified for agriculture in the Republic of Moldova [17]. We studied the specificity of the influence of seed treatment of different wheat genotypes before sowing with the BS Reglalg solution on their germination percentage in the norm, as well as after exposure of the seeds to specific doses of HS or FS.

In this article, we present the results of the study of the influence of the pre-sowing treatment of the seeds of different winter wheat genotypes with a solution of BS Reglalg on seed germination, growth, and the primary resistance of the plants to the action of extreme, positive and negative temperatures. The objectives of our research with different genotypes of winter wheat were the following

- Assessing the efficiency of accelerated methods of distributing wheat genotypes according to their primary resistance to the action of HS or FS.
- Elucidation of the possibility of modifying the primary resistance to high temperatures and frost of wheat genotypes due to treatment of seeds with BS Reglalg.

Materials and Methods

We used in the research 3 varieties (Actor, Ovidii, Scajen) and 11 lines (372, 857, 1051, 634, 140, 1101, 543, 466, 423, 48, 379) of winter wheat seeds multiplied in 2017–2018 of the Experimental Field of the Institute of Genetic, Physiology and Protection of Plants in the Chisinau area (Moldova). We carried out wheat sowing, cultivation, and harvesting per generally accepted technologies [18].

To study the effect of BS Reglalg on wheat, we selected genotypes that showed low, medium and high primary resistance to HS and FS based on the results of evaluating 10 varieties and 40 lines of winter wheat in our experiments. For this reason, different lines and varieties were represented in both experiments. Although variety Ovidii and line 1101 showed high plasticity and for this reason were involved in both experiments.

For the analysis in the laboratory conditions, seeds were calibrated by volume, passing through sieves with a hole diameter equal to 2,4-2,6 mm. Then we incubated them in 0,1% potassium permanganate solution for 20 minutes and thoroughly consecutively washed seeds with distilled water. After soaking in water at +4°C for 36 hours [23], we exposed wheat seeds to HS by immersing them for a particular time in the water for 30 minutes at temperatures +50°C, maintained with an accuracy of ±0,05°C using an ultra-thermostat U10 (Germany). Also, after soaking, the seeds were exposed to FS by incubation in an air thermostat, Rumed 3401 (Germany), at a temperature of -7°C for 8 hours, maintaining the air temperature with an accuracy of ±0,5°C. All of the above values of exposure and seed soaking temperatures, as well as time durations, were selected from many values obtained after a series of experiments, as the most optimal for assessing the primary resistance of wheat genotypes.

Our studies installed two control variants without exposure to thermal stress, including determining the percentage of seeds germinating after water immersion (1) and in the BS Reglalg solution (2) for 5 minutes. The number of experimental variants with thermal shock was equal to four: the first and second - seeds previously immersed in the water were treated with HS or FS, and the third and fourth - wheat seeds were initially submerged in the solution of the BS Reglalg and then treated with HS or FS. The wheat seeds of experimental and control variants were then germinated in Petri dishes, 40 seeds each, in three repetitions, in the dark, at a temperature of +25°C and air relative humidity of 75 - 85%. We considered the seeds germinated when the radicle reached a length of 2 - 3 mm [19].

To determine the influence of FS and HS on the germination of seeds, on the fifth day from the moment of placement in the thermostat for germination, the germination percentage (G) of the seeds from the control and experimental variants we calculated according to the formula

$G = (N_g/N) * 100\%$,

where N_g and N- are the number of germinated seeds and the total number of seeds sown, respectively.

Statistical data analysis, graphical, tabular, and textual representation performed using Microsoft Word and Microsoft Excel programs. The values obtained for all the parameters for the seedlings of the control group were considered reference values, which reported the results of the determinations carried out in the experimental groups, and each experiment we repeated at least three times. In the figures, the date presented as Mean ± SD [20].

Results

We included the results regarding the influence of BS Reglalg on the resistance of the seeds of different wheat genotypes to the action of shock with a temperature of +50°C for 30 minutes in figure 1. We mention that in the control variants without exposure to thermal shock, the seeds of all wheat genotypes, previously immersed in water or a solution of BS Reglalg, germinated at a level higher than 98%. At the same time, the germination percentage of the genotypes treated with BS Reglalg before being exposed to HS was 9 - 24% higher than those previously immersed in water. It is important to note that in the genotypes of the low resistance group, the treatment of seeds with BS Reglalg before the application of HS ensured the increase of the seed germination percentage by approximately 24% and in those of the medium resistance group by 9 - 17%. In one of the three genotypes with high primary resistance to HS (line 1101), treatment with BS Reglalg stimulated the germination percentage by 9%, and in the other two genotypes (line 543 and variety Ovidii), after prior exposure to HS of the seeds soaked in water, or in a solution of BS Reglalg, the germination percentage was practically equal, reaching respectively 93 and 96%. The absence of a statistically significant difference in the values of the percentage of germination between the control and experimental samples of line 543 and variety Ovidii under HS is explained by the weak influence of BS Reglalg on highly resistant genotypes. This phenomenon was observed in our other experiments using BS Reglalg [25].

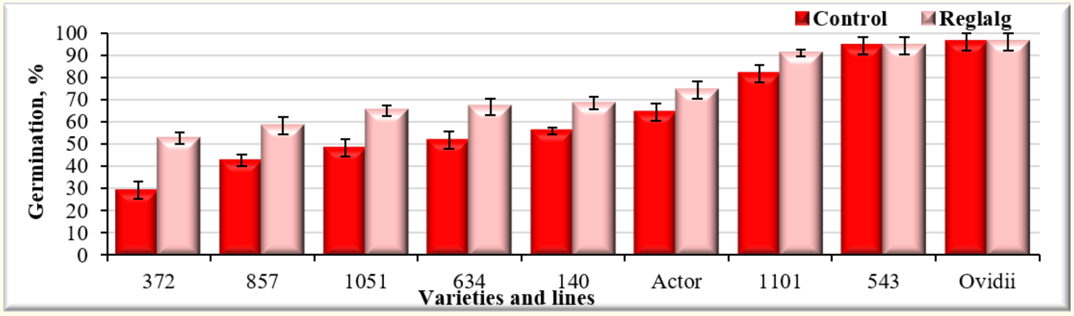

Figure 1: Seed germination percentage of winter wheat lines and varieties after five days of germination at +25°C, which were pre-treated with water (Control) or BS Reglalg solution (1/200), and then subjected to HS of +50°C for 30 minutes.

The beneficial effect of treating the seeds of line 372 with BS Reglalg before exposure to HS was manifested by increasing the germination percentage from 29% of the wheat seeds previously moistened in water to 53% - of those moistened in the solution of BS Reglalg. In general, the obtained data demonstrated that following the treatment of seeds with BS Reglalg, the tendency to increase the primary resistance of the wheat seeds to HS was more pronounced, and the more sensitive the wheat seeds of the given genotype were to the action of HS. For example, under the influence of treating the seeds of wheat lines 372, 1051, and 543, respectively, with low, medium, and high resistance to HS, due to the treatment with BS Reglalg, the germination percentage increased respectively by 1.828 (53/29), 1.354 (65/ 48), and 1.003 (94.0/93.7) times.

In conditions of exposure to the temperature shock of -7°C for 8 hours (Figure 2), the results of the investigations demonstrate that the seeds of the control samples, before the exposure to the shock with negative temperatures, were not treated with BS Reglalg, the percentage of seed germination of different genotypes was inhibited differently. In lines 140 and 466, seed germination was completely stopped, while in the variety Ovidii, 80% of the seeds germinated. Pretreatment of lines 140 and 466 seeds with BS Reglalg, lines 140 and 466 highly sensitive to temperature shock of -7°C, did not contribute to their survival. The influence of BS Reglalg on seed resistance of other genotypes was specific. The absolute value of the increase in the percentage of seed germination was the highest in the seeds of line 48 (68,3% - 52,7% = 15,6%), the line being characterized by medium resistance to FS, and the lowest in wheat seeds of the variety Ovidii (91% - 81% = 10%). The seeds of line 423, the genotype with low resistance, the seeds of which, after soaking in water, still survived exposure to FS, under the influence of pre-soaking in BS Reglalg solution, the percentage of seeds that germinated increased by 15% (23% - 8%). In this way, under the influence of BS Reglalg, the relative value of the germination percentage of the seeds of lines 423 and 48, and the variety Ovidii increased respectively by 1,88 (15/8), 0,30 (15,6/52,7) and 0,12 (10/81) times. In this way, as in the case of the reaction to HS, the relative beneficial effect of BS Reglalg tended to be the higher, the lower the resistance of the genotype to the action of FS (excluding the genotypes that did not survive exposure to the shock dose given). It is clear that for lines 140 and 466, the dose of shock obtained by exposure to -7°C for 30 minutes was too high.

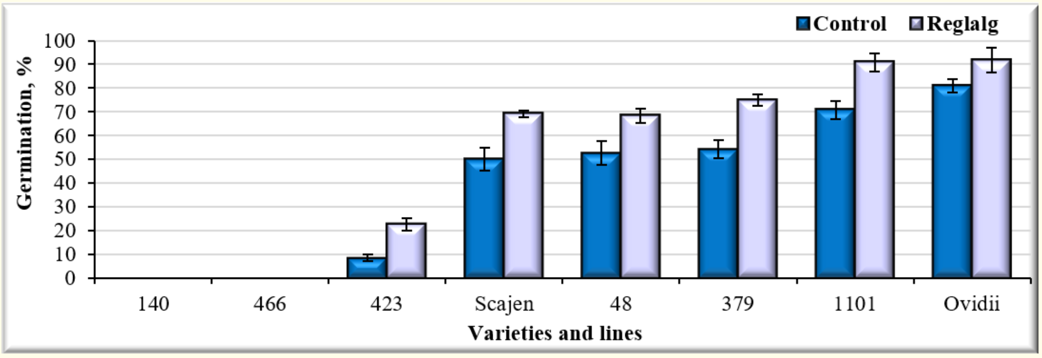

Figure 2: Seed germination percentage of winter wheat lines and varieties after five days of germination at +25°C, which were pre-treated with water (Control) or BS Reglalg solution (1/200), and then subjected to FS of -7°C for 8 hours.

Discussion

The data presented in figures 1 and 2 suggest that the wheat genotypes included in the research differ significantly in their primary resistance to both HS and FS action. This result confirms the effectiveness of the accelerated method of distributing wheat genotypes according to their resistance to temperature stress factors. The result is significant because it is known that the range of differences between the initial frost resistance of different wheat genotypes does not exceed +4°C when after completed adaptations to frost, the range of differences between genotypes extends to 13°C [21], Taking into account the high productivity of the accelerated method of distribution genotypes both according to their resistance to HS and FS, we can foresee the broad prospects of using this method in practice,

Interestingly, treatment seeds of different wheat genotypes with the solution of the BS Reglalg before germination and then exposing them to HS or FS provide increased resistance to HS or FS. These results suggest that BS Reglalg components have action characteristics of protectors, multilaterally studied in case of exposure of plants to the action of ionizing radiation [8]. It has been shown that plants' frost hardening rate tends to be faster in genotypes with higher adaptability. It is known that at the initial stages of adaptation to frost, the hardening rate is taller, the higher the final resistance of the genotype [21]. The data in Figures 1 and 2 suggest that the relative protective effect of the substances in the BS Reglalg composition against HS or FS is higher the lower the primary resistance of the genotype. From this, it follows that the BS Reglalg contains components that stimulate the allocation of reserves from the embryo and endosperm of the seeds, increasing the flexibility of plants to acclimate morphologically and physiologically to changing environmental conditions, optimizing the marginal yields for germination and resistance [12,13,22]. The fact that the marginal gain for resources is already optimized at the start of seed germination positively influences the changes that occur during plant development and their adaptation to environmental conditions. Probably, precisely these adjustments are manifested during the entire period of ontogenesis due to their impact on plants' vigor and resistance to the action of extreme temperatures, continually adjusting through plant ontogenesis, that in standard, leads to increasing crop growth.

The variation in the composition of components with characteristic effects for protectors, concomitantly with the genetic specificity of the genotypes, plays an essential role in determining the variability of the primary resistance of the seeds of different wheat genotypes, as well as of the same genotype reproduced in different climatic zones [23]. It is crucial to elucidate the role of protective substances characteristic of seeds in determining their vigor during long-term storage under various conditions and their revitalization by seeds treatment with the BS Reglalg.

The combined research of the effects of ST or FS on the seeds of different genotypes of control wheat, or pre-treated with a solution of BS Reglalg, demonstrated that different wheat genotypes differ according to their initial (primary) resistance to extreme temperatures. As the germination seeds are in the initial phase of ontogenesis, the expression of genes they have not yet affected the adaptation processes, and due to this, they have not affected the primary resistance of seeds to extreme temperatures. At the same time, the results included in figures 1 and 2 indicate that the active components of BS Reglalg influenced the primary resistance of the wheat genotypes to the action of HS or FS more significantly, the lower their primary resistance was. In previous research, we demonstrated that the primary resistance of the seeds of wheat genotypes varies depending on their breeding conditions [23]. The problem becomes more complicated because the efficiency of the epigenetically inherited primary and adaptive plants' resistance to the action of the stress factor is variable do not necessarily remain inherited in the next generations [10]. Together, these data support the view that genetic and epigenetic factors combine to influence plant resistance to extreme temperatures. Because environmental factors fluctuate widely from year to year, determining the relative role of genetic and epigenetic factors in plant adaptation to extreme temperatures is still an unsolved problem. The elucidation of these relationships depends on optimizing genotype selection methods, their distribution in different areas, and evaluating the cost allocated to plants for genetic and epigenetic adaptations to determine their influence on the quality and quantity of the harvest.

The mentioned effects of BS Reglalg require special attention. Due to the influence of protective substances in the composition of the biostimulator, already during seed germination, plants acquire the ability to adapt more quickly and effectively to the action of excessive temperatures, as well as other stress factors. The presence of mentioned substances in endosperm ensures the increase of the primary resistance and the ability of the plants to recover more quickly from the damage caused by frost and heat. Being applied in optimal doses, BS Reglalg probably complexly amplifies the composition of molecules signaling the action of stress factors. At the right time, they ensure the acceleration of adaptation processes. As a result, the level of damage decreases simultaneously with the acceleration of their recovery processes, which urgently restores the state of homeostasis [12,13]. As a result, achieved rapid germination and uniform growth of healthy plants, organogenesis is modified, and the stages of ontogenesis are optimized, which together ensures an increase in plant productivity. The components of the BS contribute to the harmonization of the passage of heterogeneous processes. Optimizing the interaction between the plant and changing environmental conditions [24]. As a result of treating the seeds with BS Reglalg, the damage caused by stress decreases, accelerating their recovery. Together, the presented data confirm that the fate of biological systems under temperature stress largely depends on the effectiveness of the ontogenetic cycle initiation processes.

Conclusions

The parameters that characterize the influence of specific doses of shock with extreme temperatures on seed germination can be the basis of methods for quickly determining the resistance of wheat genotypes to the action of high or negative temperatures and as well as the influence of biostimulator on these parameters, the methods being simple, sensitive, and reproducible. To determine the relative role of genetic and epigenetic factors in the total primary resistance to extreme temperatures, the study of the resistance of the given genotype seeds obtained in different climatic zones, in combination with the appreciation of the influence of biostimulators on this parameter, is prospective. The relative increase in shock resistance of wheat seeds treated with BS Reglalg tends to be inversely related to the primary resistance of the genotype to extreme temperatures. Still, in absolute terms, after treatment with BS Reglalg and subsequent shock exposure, the germination percentage reached a maximum level in genotypes under the influence of this shock dose surprised in control variants seed germination by 50% and minimum effects in control variant relaces 0 or 100 percents. The accelerated method of distributing wheat genotypes by their primary resistance to extreme temperatures is fast, easy, and much less expensive in comparison with classical ones, thus opening new perspectives in it applying for optimization of the selection, application of biostimulators, and rational distribution zones for the cultivation of existing genotypes according to their genetic resistance to extreme temperatures.

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