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PHENOTYPIC PLASTICITY AND PLANT RESISTANCE TO ABIOTIC STRESS

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Summary. The phenotypic plasticity of cultivated plants exceeds that characteristic of spontaneous plants. Concerning this, the question arises as to why a commensurate level of genome volume did not accompany the high level of crop plant phenotype changes. More and more data are emerging, demonstrating that, due to the rapid activation of phylogenetic adaptation systems stored in the genome, phenotypic plasticity generates non-random adaptation changes. We present the results of assessing the resistance of plants to the action of extreme temperatures and the influence of the biostimulator *Reglalg* on the viability, resistance, plasticity, and productivity of wheat and pedunculated oak plants.

Keywords: wheat, oak, phenotypic plasticity, resistance to extreme temperatures, productivity

Introduction

Charles Darwin [1] stated that in different species, phenotypic variation is generated randomly, and natural selection chooses the most adapted forms. After discovering the molecular mechanisms of heredity, the idea was launched that genetic mutations represent the main source for selection of the variability, which manifests itself over generations [2]. Links between phenotypic variation, genetic variation, and adaptive evolution have been highlighted. Current research demonstrates that phenotypic variation, induced by environmental conditions, through phenotypic plasticity, is subject to selection, thus expanding the ways of adaptation in ontogenesis and evolution [3, 4]. These arguments were important for recognizing that not all variations are randomly generated, a view that covered a gap that hampered the understanding of the unfolding of the adaptation processes of organisms in ontogeny and evolution.

Plasticity manifests as a reaction norm in populations and lines of clones obtained from the population [5]. The plastic response can be appreciated by analyzing the course of development, which is an important source for selection. Thus, random mutations and changes in reaction norms induced by environmental conditions serve as a source for selection and evolution, both sources of variation being involved in natural and artificial selection. This concept has not been extensively analyzed concerning artificial selection, or the influence of biostimulants (BS) on plant viability, adaptive capacity, and productivity. Even in the definition of phenotypic plasticity through the ability of a genotype to respond to environmental conditions due to the formation of variable phenotypes, only the property of genes is emphasized, without mentioning the importance of the specific influence of developmental processes on plasticity. The joint analysis of these characteristics would allow the appreciation of the influence of phenotypic variability on ontogenesis and evolution, a view supported by data that demonstrate that developmental processes in ontogenesis can be influenced not only by genotype but also by transgenerational effects, transduced from predecessors. They can modify the reaction norms, including the plastic response [4, 5]. Moreover, developmental systems are modifying the influences of genetic mutations on the phenotype, as the adaptation mode of biological systems varies depending on the interactions between signaling pathways encoded in different genes. The interactions between different developmental systems, being functionally redundant, channel the phenotypic variations towards a similar result [6]. The random influence of genetic mutations, and the non-random ones of developmental processes, on phenotypic plasticity causes distortions in development, since only some of the non-random variations of developmental systems, generated by mutations, can be adaptive. In this article, we present the results of the influence of BS *Reglalg* [7] on the phenotypic plasticity of wheat and pedunculated oak genotypes that beneficially influence viability, resistance to abiotic stress factors, and plant productivity.

Materials and methods

The experiments were carried out over several years in the laboratories and the experimental field of the Institute of Genetics, Physiology, and Protection of Plants, applying the methods described in [8]. Experimental results were statistically analyzed, determining the mean, standard deviation, and validity of differences between means obtained in at least three experiments [9].

Results and discussions

To evaluate the resistance of different wheat genotypes to the action of extreme temperatures, as well as their level of phenotypic plasticity, we studied different doses of *heat shock* (HS), or *negative temperature shock* (NTS), influence on the germination of seeds of different wheat genotypes in the control variants (seeds moistened in water) and those in the experimental variants (seeds moistened in solution containing BS *Reglalg*) [10, 11]. As a result, the doses of exposure to HS or NTS were determined which gave the possibility to separate the wheat genotypes according to the primary resistance to the action of extreme temperatures. The data obtained are presented in Figure 1. The presented data demonstrate that wheat genotypes differ in their primary resistance to HS, and pre-treatment of seeds with BS *Reglalg* provides increased resistance. Depending on the genotype, the percentage of seed germination in the experimental variants increased by 5.3 – 20 %. The beneficial effect of pre-treatment of the seeds with BS *Reglalg* was all the more significant, the lower the primary resistance of the genotype.

Taking into account the fact that, after exposure to HS, in the control version the percentage of seed germination of the most sensitive genotype (genotype 372) was quite high (32%), in the research on the influence of NTS, for exposure we chose the dose that inhibited seed germination of the most sensitive genotypes (genotypes 160 and 466, figure 1 B). We observe that, quantitatively, the treatment with BS *Reglalg* showed the most pronounced beneficial effect on the seeds of the genotypes, whose germination inhibition after exposure to NTS, in the control variant, was moderate (between 40 and 60%). From this, it follows that the maximum beneficial effect of treating seeds with the BS *Reglalg* solution on the resistance to the HS or NTS dose can be achieved in all genotypes, but only after their exposure to doses that are specific for each genotype; which in the control variant inhibits seed germination up to 40 - 60 %.

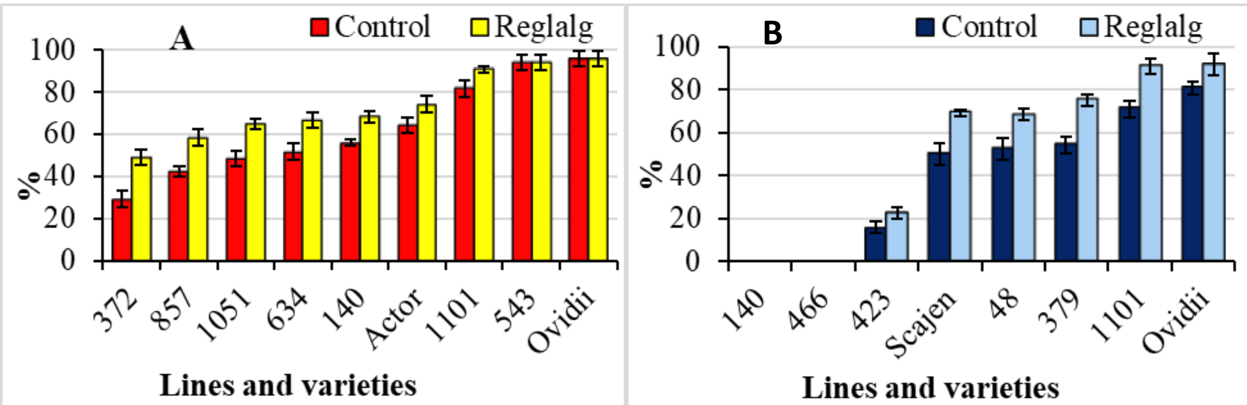

Figure 1. Change in the germination capacity of the different wheat genotypes seeds treated with water, or solution of BS *Reglalg* diluted with water in ratio (1/200), then exposed to HS (+50ºC for 30 minutes - A) or NTS (-7ºC for 8 hours - B)

To analyze the later effects of seed treatment with BS *Reglalg* on the physiological state of the obtained plants, samples of plants that passed the second stage of frost hardening during the winter were taken from the experimental field. Plants were exposed at -12ºC, for 8 hours, to NTS. Later, after cutting them at the first internode level, the plants were incubated in favorable conditions to initiate adventitious roots. The assessment of plant resistance was carried out based on the determination of the percentage of plants in which, during 7 days after exposure to NTS, adventitious roots were formed from the tillering node (Figure 2). The obtained data demonstrate that after the second hardening phase, in the variants in which the seeds were not exposed to NTS, the percentage of plants that formed adventitious (coronals) roots reached the maximum possible value, 100%. At the same time, regardless of the variety, after exposure to NTS, the ability of plants obtained from seeds treated with BS *Reglalg* to form adventitious roots tended to be higher compared to that characteristic for plants obtained from seeds moistened in water. Kuialnik cultivar plants showed a higher resistance to NTS than the Moldova 5 and Missia cultivars. Together, the previously presented data suggest that plants of the Kuialnik variety are more resistant to NTS than those of the other two varieties, and treating the seeds before sowing with BS *Reglalg* ensures the increase of the adaptive capacity of the obtained plants to the action of negative temperatures.

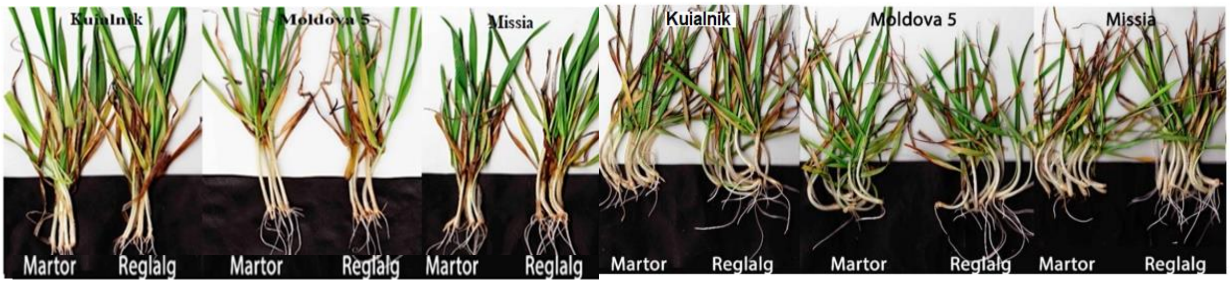

Figure 2. The influence of NTS, applied by exposure to -12ºC for 8 hours, on the plants after the second phase of hardening of the wheat varieties Kuialnik, Moldova 5, and Missia, obtained from the seeds treated with water before sowing, or with a solution of BS *Reglalg* diluted with water in a ratio of 1/200 about the percentage of formation of adventitious roots in plants cut at the level of the first internode

Another important parameter that determines the influence of extreme temperatures on wheat plants is the level of location of the tillering node in the soil. It is well known that in the upper layers of the soil, during the winter the temperature increases proportionally with the depth of the soil [12].

Table 1

Epicotyl length of wheat cultivars plants obtained from seeds treated before sowing with water or BS Reglalg solution

Variety	Variant	Epicotyl length, $\bar{x}$ *(cm)± DS*	Δ	CV	P	δ
Moldova 5	Control	3.12 ± 0.28	0.08	9.03	-	-
	Reglalg 1/200	1.35 ± 0.27	0.08	20.34	0.00E-01	56.7
Kuialnik	Control	4.25 ± 0.16	0.05	3.86	-	-
	Reglalg 1/200	2.58 ± 0.21	0.06	7.96	0.00E-01	39.3
Missia	Control	2.89 ± 0.41	0.11	14.13	-	-
	Reglalg 1/200	1.74 ± 0.44	0.12	25.05	1.39E-24	39.8

Analyzing the data presented in Table 1, we can mention that in the plants obtained from the seeds treated with BS *Reglalg*, regardless of the variety, the epicotyl (first internode) length is 1.15 – 1.67 cm lower than that of the control plants. Due to this, in the experimental plants, the tillering node is located deeper in the soil, which during the snowless winters causes the temperature increase in these soil layers up to 5.2 – 7.5°C. As a result, the plants in the experimental variants are better protected from the action of extreme temperatures during the winter.

The previously presented data suggest that wheat plants obtained from seeds treated with BS *Reglalg* show increased vigor and the ability to adapt to stress factors throughout the growing season due to the induction of phenotypic plasticity. Considering this, we studied the influence of seed treatment with BS *Reglalg* on the productivity of different wheat cultivars. In Table 2 we present the results obtained on the experimental field of the IGFPP in 2016, for the wheat varieties Moldova 5, Kuialnik, and Missia. They demonstrate that from plants grown from seeds treated with BS *Reglalg* solution, potential productivity, recalculated for one hectare equal to 48, 58, and 56 q/ha was obtained, respectively. On the control plot, the yield was equal to 41, 50, and 51 q/ha, respectively. Therefore, the recalculated yield was 7, 8, and 5 q/ha in favor of the plants obtained from the seeds treated with BS *Reglalg*.

Table 2

The productivity indices of different wheat varieties plants, cultivated from the seeds treated with water, or with a solution of BS *Reglalg* diluted with water in a ratio of 1/200, and grown on the experimental field of the IGFPP in 2015 – 2016

Variety	Variant	The number of ears per m² $\bar{x}$±*DS*	No. of caryopsis in the ear $\bar{x}$±*DS*	The mass of caryopses per ear, g $\bar{x}$(g)± *DS*	The mass of 1000 caryopses, g $\bar{x}$(g)± *DS*	Calculated harvest $\bar{x}$(q/ha) ± *DS*	CV
Moldova 5	Control	353±52	32.3±1.7	1.17±0.09	36.3±1.0	41±0.49	13.1
	Reglalg	387±38	33.7±1.1	1.24±0.03	36.7±0.5	48±0.31	9.3
	p	0,179	0.088	0.089	0.289	0.025	
	δ	9,6	4.3	6	1.1	17.1	
Kuialnik	Control	366±39	37.1±1.0	1.38±0.05	37.2±0.8	50 ±0.46	9.1
	Reglalg	403±45	38.0±1.4	1.44±0.09	37.8±0.7	58±0.60	10.4
	p	0.087	0.143	0.108	0.164	0.011	
	δ	10,1	2.4	4.4	1.6	16	
Missia	Control	407±21	34.0±1.4	1.28±0.08	36.7±0.5	51±0.19	4.1
	Reglalg	425±25	35.2±2.2	1.32±0.09	37.3±0.8	56±0.42	6.0
	p	1.00	0.65	0.06	0.65	0.41	
	δ	4.4	3.5	3.1	1.6	9.8	

The results of the experiments presented above suggest that the extension of phenotypic plasticity by BS *Reglalg* is preserved throughout the ontogeny of hexaploid wheat plants, which are a monocotyledonous field plant species. Concerning these results, it was important to determine whether similar effects would be obtained in perennial, dicotyledonous plants. We provided research with the pedunculated oak (*Quercus robur* L.). The plants were obtained from acorns treated with water, before placement for germination, or with a solution of BS *Reglalg*.

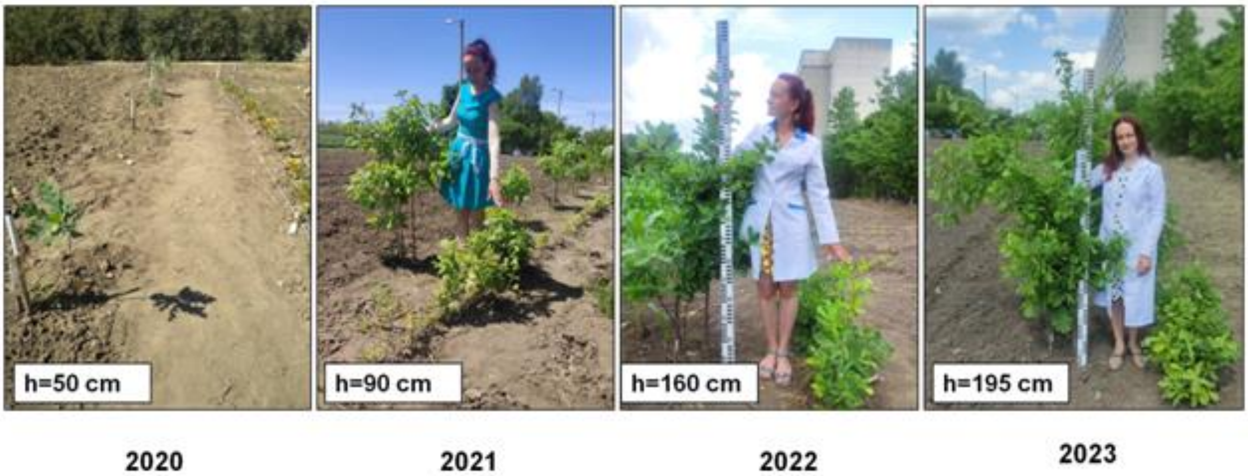

Figure 3. Images of oak seedlings in the first, second, third, and fourth years of growth (2020, 2021, 2022, and 2023) obtained from acorns sown in the field in autumn 2019 (left-hand on each year photos) and from the seedlings obtained and cultivated from November 2019 until May 2020 in artificially created conditions (right-hand on each year photos), then in May 2020 transplanted in the field

The photographs presented in Figure 3, as well as the data in Table 3, demonstrate that in the first four years of life, oak plants obtained from acorns soaked in BS *Reglalg* solution, and subsequently plants treated with BS *Reglalg* solution twice during the active annual vegetation, grew faster compared to the growth detected at plants from the control variant (acorn and plants were treated with water).

The data, included in Table 3, indicate that the experimental plants' annual growth in height, and their trunk growth in diameter, on average, were respectively 5.7 and 5.2 times higher than those of control plants. It follows that the beneficial influence of BS *Reglalg* on the phenotypic plasticity and vigor of pedunculated oak plants has manifested over four years.

Table 3

Dynamics of pedunculate oak (*Quercus robur* L.) seedlings growth in the first 4 years of the growing season on the experimental field of the Institute of Genetics, Physiology and Plant Protection, MSU

Year	Trunk height, cm		Trunk diameter, mm	
	Classic method	Laboratory method	Classic method	Laboratory method
2020	7 ± 1.32	52 ± 3.23	1 ± 0.01	7 ± 1.32
2021	19 ± 1.05	95 ± 2.92	4 ± 0.03	19 ± 1.05
2022	24 ± 2.12	120 ± 4.12	5 ± 0.03	24 ± 2.12
2023	31 ± 1.98	176 ± 3.98	6 ± 0.02	31 ± 1.98

The optimization of plant selection and cultivation methods largely depends on the knowledge of ways to increase the performance of organisms under heterogeneous environmental conditions. Because variation in the functional characteristics of organisms is influenced by mutation (largely random) and phenotypic plasticity (largely non-random), knowledge of the relative role of these groups of characteristics as providers of variation that determine genotype specificity is essential for the successful use of the genotype in agriculture. An important aspect consists of exploring the perspectives of applying biostimulators to treat plants to manifest their adaptive capacity due to the expression of the genotype predominantly non-random adaptive plasticity potential. The development systems, dependent on the manifestation of phenotypic plasticity, reflect the selection paths and the evolutionary history of the genotype, therefore, they specifically channel the responses available to environmental factors, and biostimulators accelerate and reduce the energy and material costs allocated to plants for these adaptations. Finally, the mentioned processes ensure the increase of vigor and productive capacity of the corresponding plants to the adaptive potential of the genotype and the specific cultivation conditions.

Conclusions

For the seeds of different wheat genotypes, well prepared for germination, a specific dose of HS, or NTS, can be established, the application of which makes it possible to separate the genotypes according to their primary resistance to the action of high or negative temperatures, determining the percentage of seed germination.

Treatment of the seeds before sowing with the solution of BS *Reglalg*, diluted with water in the ratio of 1/200 ensures the increase of their resistance, the maximum protective effect being highlighted for the wheat genotypes, in which, following exposure to the specified dose of HS or NTS, the germination of the seeds from the control variant reached 40 - 60%.

The maximum protective effect of seed treatment with BS *Reglalg* can be achieved for each wheat genotype provided that the dose of HS, or NTS, applied to the seeds of the control variant, will ensure the germination of only 40-60% of the seeds.

Treating the seeds of different varieties of wheat with a solution of BS *Reglalg* ensures the increase of the viability and resistance of the plants to extreme temperatures during the entire period of ontogenesis, which ultimately leads to increasing of the productivity of the wheat plants, depending on the variety, by 10 - 17%.

BS *Reglalg* showed beneficial effects following the treatment of pedunculated oak acorns before sowing, confirmed by the fact that in the first four years of cultivation the average annual growth rate in height and diameter of the experimental plants, compared to those in the control variant, increased by 5.7 and 5.2 times, respectively.

In common, the data obtained demonstrate that the treatment of wheat seeds, or oak acorns, with BS *Reglalg* before germination ensures the induction of adaptation processes starting from the germination phase, which assures the rapid development of phenotypic plasticity and plant adaptation to the factors of heat stress.

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