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Chapter

Accelerated Methods of Determining Wheat Genotypes Primary Resistance to Extreme Temperatures

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Abstract

Several morphological and functional mechanisms determine the resistance of plants to extreme temperatures. Depending on the specificity of mechanisms of action, we divided them into two groups: (1) the mechanisms that ensure the avoidance/reduction of the exposure dose; (2) functional mechanisms which increase plant resistance and ability to recover damages caused by stress through regulation metabolic and genes expression activity. We developed theoretical and practical methods to appreciate the contribution of parameters from both groups on the primary and adaptive resistance of different wheat genotypes. This problem became more complicated because some properties are epigenetically inherited and can influence genotypes' primary (initial) resistance to stressors. The article describes results obtained by the accelerated determination of the initial resistance of wheat (*Triticum aestivum* L.) genotypes to temperature stress and the prospects for their implementation in the selection and development of methods for rational choosing wheat varieties for cultivation under specific environmental conditions.

Keywords: wheat genotypes, temperature stress, primary resistance, adaptive resistance, accelerated stress resistance tests

1. Introduction

As a result of the global warming trend, environmental conditions are changing; the frequency of periods with extreme temperature and their intensity is increasing. Between 1990 and 2015 the earth's global average surface temperature has linearly risen relative to the average surface temperature of 1961–1990 by 0.9°C [1]. At the same time, according to expectations, if the average temperature rises by one degree, the expected wheat production will decrease by 6% [2]. Environmental changes influence the rate of growth, development, survival, and productivity of plants. That determines the necessity to develop effective breeding methods and technologies for growing hybrids and genotypes following the specific conditions of each plant cultivation region. The success of solving these problems depends on elucidating the mechanisms

that influence the resistance of plants to the action of extreme temperatures. Their knowledge allows specialists to select plant varieties well adapted to the new conditions and rationally distribute the existing genotypes for cultivation in the appropriate areas, thus achieving their biological potential. However, depending on the duration of exposition, the temperatures outside the optimal range, specific to each genotype, can have serious consequences, causing a decrease or complete loss of the harvest. The resistance of plants to the action of temperature stress factors depends on integrating processes at the molecular, subcellular, cellular, tissular, and whole plant levels [3]. The dynamics and integration of these processes determine the primary resistance and the capacity of plants to adapt to changes in environmental conditions [3–5]. The primary (initial) resistance and adaptive capacity of plants to stressors vary [6, 7]. The morphology of plant organs [4], seed vigor, and viability [8] change under environmental conditions. They influence both the mechanisms of avoiding/reducing the dose of the stress factor, determined by multiplying the temperature value (the intensive factor) to the duration of exposure (the extensive factor), and the efficiency of the genetic and epigenetic mechanisms of inheriting the primary and adaptive plants resistance to the action of the stress factor [3, 9]. We should consider that some epigenetically inherited characteristics can be gradually eliminated [9, 10]; therefore, they do not necessarily remain inherited in the next generation. These mechanisms may cause the changes in primary and adaptive resistance of genotypes to stressors.

Given the complexity of the mechanisms that determine the resistance of plants to temperature stress and the specific manifestation in biological systems with different levels of organization and stage of ontogenesis [3, 4], it becomes clear the need to elucidate their interactions. To clarify the specificity of the response of autumn wheat plants to extreme positive and negative temperatures, we introduced in research the seeds of different wheat genotypes reproduced in Moldova and Ukraine. The survival and productivity of winter wheat plants depend on the complex processes of their adaptations to winter frosts, summer heat, and drought. Therefore, the specificity of the resistance inheritance and developing the adaptation processes to environmental conditions of wheat during winter and summer is of theoretical and practical interest. Furthermore, the reaction of biosystems to thermal stress complexly depends on the exposure dose. With increasing the value of stress-inducing temperature, the biological effect of the particular dose also increases [3, 4]. Therefore, to compare the resistance of different wheat varieties to extreme temperatures, we chose the temperature level and duration of exposure, which applied to varieties with medium resistance will cause a 50% decrease in the value of the studied parameter. The parameters can be different: percentage of seed germination, accumulated plant biomass, crop yield, etc. By applying this procedure, we were able to arrange wheat genotypes according to their resistance to stress. The objectives of our researches with different winter wheat genotypes were the following:

1. To develop the accelerated methods of determining the wheat genotypes' primary resistance to the action of high temperatures and frost.
2. To use the elaborated methods for evaluation of the wheat genotypes' primary resistance to the action of high temperatures and frost.
3. To determine the influence of the environmental conditions of seeds reproduction and their storage during 1 year on wheat genotypes' primary resistance to heat or frost.

2. Materials and methods

Our studies used the seeds of different varieties and lines of hexaploid wheat reproduced in Ukraine and Moldova. In the beginning, we calibrated the wheat seeds by volume, passing them through sieves with 2.4–2.6 mm diameter. Next, they were immersed in a 0.1% potassium permanganate solution for 20 min, thoroughly washed with a tap, then with distilled water. After soaking in water at +4°C, we exposed the seeds to heat shock (HS) by immersing them in the water at a specified temperature for a particular time, maintained with an accuracy of $\pm 0.05^\circ\text{C}$ using an ultrathermostat U10 (Germany). The shock with negative temperatures (SNT) was provided by seeds incubation in an air thermostat Rumed 3401 (Germany) for 8 h, maintaining the air temperature with an accuracy of $\pm 0.5^\circ\text{C}$. In the studies, we installed two control variants: first for experiments with determining the wheat genotypes' resistance to frost, and second, for determining their resistance to high temperatures. Before applying for germination, we incubated the seeds of control variants at a temperature of +24°C for an additional 8 h or 30 min, equal to the duration of exposure to SNT or HS in experimental variants. Seeds of experimental and controls variants were then germinated in Petri dishes, 25 wheat seeds each, in triplicate, in the dark, at 25°C, and 75–85% relative humidity. The reaction of seeds of individual wheat genotypes to SNT, or HS, was judged by the percentage of seeds germinating within 5 days. After 5 days of incubation at +25°C, we mentioned that germinated at least 95% of wheat seeds from the control variants. Therefore, the genotype's response to SNT, or HS, was judged directly based on the percentage of seeds germinated in the experimental variants. To determine the statistical validity of the obtained data, we calculated the average value from at least three experiments repetitions, the standard deviations of average, and the credibility of the differences between the average values [11].

3. Results and discussions

3.1 Preparation of seeds for the appreciation of hexaploid wheat genotypes' primary resistance to high temperatures and frost

The data presented in **Figure 1** shows the kinetics of water accumulation by the seeds of wheat variety Odesskaya 267. We described the dynamics of increasing seeds humidity with the duration of immersion in water from 0 to 130 h by the polynomial equation of dependence $Y_x = -0.0034x^2 + 0.7114x + 12.605$, where Y_x is the moisture content of seeds depending on the duration of incubation- x . In general, there was a tendency to decrease water accumulation speed with increasing duration of immersing the seeds in water. By the rate of water accumulation, we separated the imbibition into two phases: phase I—from 0 to 30 h (initial active water adsorption), and phase II—from 30 to 130 h (plateau phase). **Figure 1** shows the data corresponding to these phases marked in blue and dark blue, respectively. The increasing of the seed's humidity in interior of phase I is described by equation $Y_x = -0.0191x^2 + 1.3766x + 7.4948$, and phase II—by equation $Y_x = -0.002x^2 + 0.4822x + 19.328$. Comparing the three equations, we can observe that in all the increases of the humidity percentage with the increase of the imbibition time, the linear component influence dominates due to the high value of the coefficient. As expected, the value of the linear coefficient in phase I of imbibition is much higher than in phase II (1.3766 and 0.4822; the ratio is equal to 2.886). For the total period of immersing in the water, in a period between 0

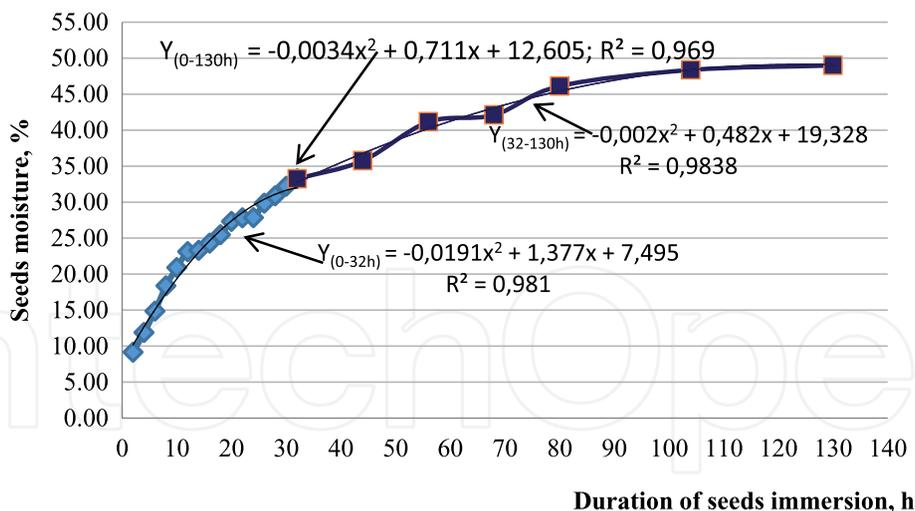


Figure 1. Dynamics of increasing the humidity (I) in the wheat seeds, variety Odesskaya 286, depending on the duration of immersion in water at a temperature of 4°C. $Y_{(0-32h)}$, $Y_{(32-130h)}$, and $Y_{(0-130h)}$ —The polynomial approximation of increasing seeds humidity approximation on intervals of 0–32, 32–130, and 0–130 h of imbibition, respectively.

and 130 h, as expected, this coefficient of linear proportionality was lower than that characteristic for interval I and higher than in interval II, being equal to 0.7114. The average values of the seed moisture growth rate in each phase confirm these data. The mean growth rate of seed humidity during the total immersion time in the water was equal to 0.29% per hour when phases I and II were equal to 0.77 and 0.16% per hour.

During 130 h of imbibition at 4°C, the germination of the seeds did not occur. Therefore, there was no transition to phase III, characterized by a further increase in the rapidity of water imbibition and prominence of the radicle [8, 12]. The seeds' imbibition in water during 130 h at 4°C was not associated with finalizing the embryonic axis extension up to a level that ensures germination expression. The mentioned legitimacies correspond to the stages of water accumulation in the seeds in a state of dormancy described in the specialized literature. The germination and the appearance of the radicle take place only in phase III of germination [4, 9]. The radicle's formation by seeds was not detected even after 130 h of immersion in water in our experiments.

The conditions developed for preparing seeds for germination and determination of the integral kinetics of wheat seeds imbibition with water, shown in **Figure 1**, were the necessary steps for appreciating the wheat genotypes' primary resistance to high temperatures and frosts. The resistance of biological systems to biotic and abiotic stressors depends on the processes at different levels of their organization. In general, external factors can induce stress, specifically influenced by the nature, dose of the stressor, and specificity of the mechanisms determining the plant's resistance. The higher the exposition dose that initiates the stress state, the higher the biological system's resistance to a specific stressor. Stress tolerance can also be induced by external factors and allow organisms to survive the action of lethal doses when previously exposed to moderate levels of the stressor [3, 4]. This type of induced high level of extreme temperatures tolerance is maintained only for a limited period. It corresponds to the processes defined as acclimation [4]. From the above, it results that at any stage of ontogenesis, the total resistance of the plant depends on two components: (1) the initial (primary) resistance, and (2) the secondary resistance [13], induced by environmental factors, superimposed on the plant's primary resistance.

During ontogenesis, due to adaptation processes, the resistance of genotypes to stress factors varies considerably. The induced level of adaptation depends on both the genotype and the dose of stress factor [12–14]. Therefore, the correct distribution of genotypes by resistance requires their synchronization in an identical physiological state. In each moment, the entire plant's stress resistance depends on the primary stress resistance, superimposed by the induced adaptations, named the secondary resistance, and the mechanisms that assure stress avoidance [4, 12, 14]. Thus, the plants' total resistance to abiotic stress depends on three groups of mechanisms: primary resistance, resistance by avoidance, and resistance due to adaptations performed in ontogenesis. Considering this, when preparing wheat seeds for germination, the results of which are presented in **Figure 1**, the seeds imbibition with water were provided under conditions excluding the induction of adaptations. The use of seeds prepared for germination in this way opens up the possibility of directly assessing the primary resistance of the wheat genotype to extreme temperatures, excluding the influence of adaptation and avoidance mechanisms. Because of the variability of adaptive resistance, determined functionally and by avoidance, the correct determination of the genotype primary resistance is an important and challenging problem. The physiological state of plants depends not only on the genetic identity, age, and current environmental conditions but also on their previous adaptations to the environment during ontogenesis. The specially designated experiments showed that the hexaploid wheat genotypes' primary frost resistance constitutes only 14–33% of the value of total frost resistance achieved after inducing the maximum level of hardening [15, 16].

In natural conditions, the synchronization of seeds' physiological state occurs during their formation, maturation, and entry into the dormancy [8]. In the quiescent embryos of wheat seeds, cells are in the G_1 phase of the cell cycle, the transcription and the mitotic cycle are stopped [8, 13, 16, 17]. At the initiation of germination, in all seeds, the physiological states of quiescent embryos are practically identical. However, several factors can disrupt the synchronization state of embryo cells in the transition of wheat seeds from a quiescent state to the germination stage. The period of different grains maturation in the wheat plant's ear is non-uniform. In addition, the environmental conditions which can be different during the plants ripening influencing the size, structural and functional characteristics of the grains. The specificity of internal and environmental conditions during seed maturation and emergence from dormancy can disrupt the synchronization of embryonic cells due to their capture at different stages of mitosis [8, 13, 17]. Probably, the accumulation of most cells in the G_1 phase of the cell cycle manifests itself only under conditions optimal for their maturation and desiccation of the grain, when the moisture content of the embryos gradually decreases to about 10%. These factors can generally disrupt the uniformity of the release time of wheat seeds from dormancy during the germination period. Germination starts with moistening and is completed when the extending radicle penetrates the structures that surround the seed [12].

The heterogeneity of the seeds' physiological state can also increase when creating favorable conditions for germination. In this period, the metabolic processes are restored; the expression of the genes necessary for germination is gradually activated. Germination of the wheat quiescent embryo is primarily influenced by temperature and seed moisture. If exposure to temperature can be uniform and precisely controlled, then the uniform increase of different seeds moisture and readiness for germination is much more challenging to ensure. The seeds' structural-morphological differences influence the dynamics of water absorption, which determine the

heterogeneity of the time of reaching the moisture level, sufficient for germination initiation. The seed germination begins with the activation of metabolic processes. In wheat embryos, protein biosynthesis initiates 30 min after immersion in water [16]. The proteins synthesized in the first 9 h after imbibition are involved in the activation of the DNA replication. Still, the final activation of DNA biosynthesis is performed only 6–8 h later, accompanied by the transition of cells from the G₁ phase to phase S (phase of DNA synthesis) of the mitotic cycle [17]. The G₁ phase of the mitotic cycle is like the gateway through which cells transit from the non-proliferative (quiescent) to the proliferative period, accompanied by the synthesis of a limited number of proteins functionally involved in the cell cycle. The biosynthesis of DNA is induced or activated. These processes precede the initiation of cell divisions that occur after the radicle protrude tegument. Activation of the cell cycle in wheat embryos begins with the induction of mitosis in the cells of root apex cells then gradually spreads to all meristematic cells [17]. The start of phase S and G₂ (post-synthesis phase) of the cycle in wheat radicle meristematic cells is observed 12–14 h after germination initiation [16]. Thus, under favorable conditions, the germination of wheat embryos will occur with 24 h *lag* period. Typically, the uniformity of the dynamics of seeds imbibition with water can substantially influence the heterogeneity of their germination. As demonstrated experimentally, at the initiation of wheat seed germination, replication of DNA triggered by proteins translated from mRNA stored in mature seeds are not sufficient for the cells to commence all stages of mitosis [16, 17].

The need to synchronize the physiological state of seeds in the pre-germination stage is due to many reasons. First, the penetration at this stage of water into the seeds causes temporary structural perturbations of membranes, which lead to a leakage of solutes and low molecular weight metabolites into the surrounding water for imbibition. Second, the diminution of the processes of metabolites leakage occurs after a transition of the membrane phospholipid components from the gel phase achieved during maturation drying to the hydrated liquid-crystalline state, after which the low molecular weight metabolites leakage is reduced [18]. A rational way to reduce the heterogeneity of seed germination time, influenced by different water absorption rates in seeds, was achieved by extending the duration of seed saturation with water at low temperatures, **Figure 1**. Under these conditions, the rate of wheat seeds saturation with water became high, but germination processes slowed down substantially. As a result of the long duration of the wheat seeds immersion in water, the reparation of structures damaged during their maturation and rehydration were completed, and the uniformity of seeds' germination increased. Data presented in **Figure 1** shows that during 130 h of water imbibition in wheat seeds at +4°C, their transition to faze III of germination deed did not occur. We noticed that after the pre-imbibition under the mentioned above conditions and the subsequent exposure of the seeds in optimal germination conditions, the seeds were characterized with the highest speed and the highest percentage of germination when immersed in water with temperature + 4°C, during 36 h. At optimal temperature, 90, 100, and 80% of seeds were germinated, previously submerged in water with +4°C, for 12, 36, and 72 h. Mass germination of seeds (more than 50% of the wheat seeds) occurred 24, 12, and 36 h after the transition to optimal conditions for germination. Thus, the immersion of the seeds in water for 12 h was too short of reaching the level of moisture and physiological transformations necessary for the rapid germination of the wheat seeds. On the contrary, more than 72 h of immersion in water was too long-lasting and inhibited seed germination (probably due to anoxia caused by excessive oxygen consumption during prolonged immersion). So, the wheat seeds previously incubated in water at a temperature of

+4°C for 36 h were the best prepared for germination. Therefore, for further research on the accelerated assessment of the primary resistance of wheat genotypes to high temperatures and frost, we used seeds prepared for synchronized germination by their immersion in water for 36 h, at temperature + 4°C.

Considering that the difference of frost resistance between wheat varieties increases with the adaptation processes' accomplishments, the traditional methods of determining frost resistance ordinarily are performed after induction of adaptive resistance at the maximal level [15, 16, 19]. Although the ability to differentiate genotypes in this state by frost resistance appears to be the greatest, the widespread use of the method is difficult. The long duration of adaptation processes fulfillment and the dependence of the kinetics of adaptation from the level of genotype resistance to frost [15, 19], are among the main arising problems. Furthermore, the complexity of mentioned processes determines the low productivity of the noted method, high costs of testing, and the uncertainty of the results that depend on the influence of a large complex of factors. In particular, studies have shown that the maximum difference between the primary frost resistance of wheat varieties does not exceed 3°C, and after their complete hardening reaches 12°C [15, 16]. Despite this, the relative simplicity of the procedures providing uniform preparation for germination of seeds of different varieties before determining the primary resistance of wheat varieties to frost inspired us to assess the possibility of separating wheat genotypes by their resistance to extreme temperatures (positive and negative) in this identical state. In our experiments, in the control variant, a similar physiological state was confirmed by the practically 100% seeds germinating during the first 24 h of incubation in favorable conditions. Therefore, after reaching this equivalent physiological state, differences in the response of seeds to HS or SNT will mainly depend on genetic differences between genotypes.

3.2 Determining the wheat genotypes primary resistance to high temperatures and frost

In multiple experiments, we determined that the exposition of the moderately resistant to high temperatures or frost seeds of wheat variety Odesskayia 267 to HS at temperature 50°C during 30 min, or SNT at a temperature -7°C during 16 h, causes diminution of their germination by 50%. Based on these results, we proposed to arrange the wheat genotypes by their primary resistance to extreme temperatures by their germination reaction after seeds exposure to the mentioned dose of HS or SNT. In **Figure 2**, as an example, we provide the results that support the correct choice of HS dose caused by exposing wheat seeds to a temperature of +50°C over 30 min as one effective to separate genotypes by their primary resistance to high temperature rapidly. Analyzing the data presented in **Figure 2**, we notice that after exposure to the mentioned dose of HS, the distribution according to the percentage of germinated seeds, genotypes were uniformly occupying the area between 11.5% (variety Vdala) and 98.5% (variety Chevalier). Of the 20 wheat varieties included in the analysis, 9—showed low, 5—medium, and 6—high thermotolerance. After HS exposure, the seeds of genotypes constituting groups with low, medium, and high thermotolerance, respectively germinated up to 50%, between 50 and 70%, and more than 70%.

The data on the primary resistance to SNT or HS of the seeds of 10 wheat genotypes multiplied in Ukraine or Moldova are presented in **Figure 3**. We considered that the genotypes, whose seeds after exposure to SNT or HS germinated up to 50%, between 50 and 70%, and more than 70%, can be divided into groups with low,

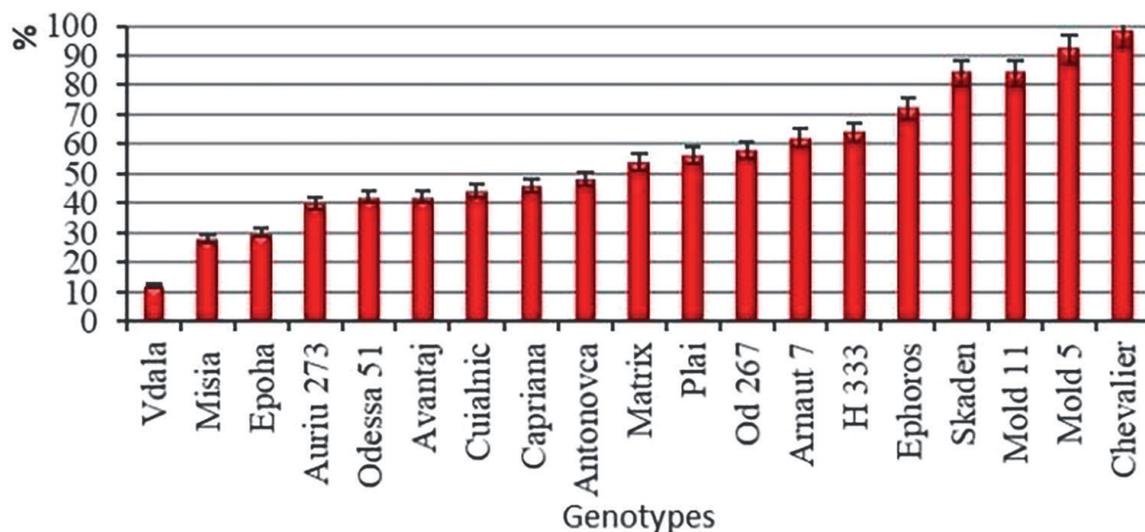


Figure 2. Influence of seed exposure of different wheat varieties to HS at 50°C for 30 min on germination percentage over 5 days.

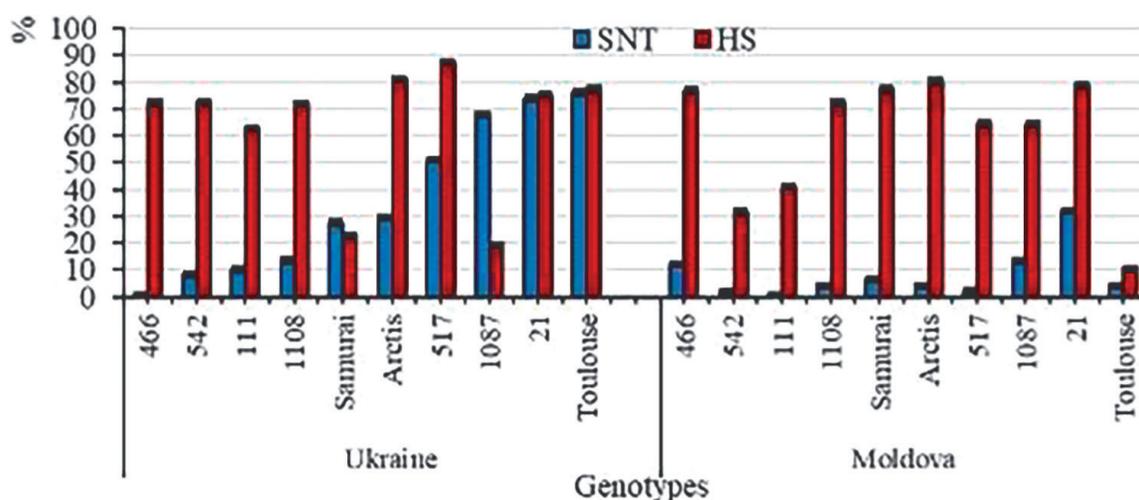


Figure 3. The percentage of seeds of the 10 winter wheat genotypes germinated after their exposure for 16 h SNT at -7°C (blue), and that of the percentage of germinated after exposure for 30 min to HS with 50°C (red). On the left are presented the data obtained with seeds multiplied in 2015–2016 in the Kharkiv region (Ukraine), and on the right—Those obtained with the seeds multiplied in 2016–2017 in the Chisinau area (Moldova).

medium, or high resistance SNT or HS. The data presented in **Figure 3** shows that the seeds of genotypes 466, 542, 111, 1108, Samurai, and Arctis, after cultivation in Ukraine, were characterized with low resistance; those of the genotypes 517 and 1087—with medium resistance, and seeds of genotypes 21 and Toulouse—showed high resistance to SNT action. After multiplication in Moldova, the seeds of all genotypes, except those of the variety Toulouse, with medium resistance to SNT, have shown low resistance to SNT. In contrast, except for seeds of lines 21, 542, and 111 with a low resistance to HS, the multiplied in Moldova seeds of the other seven genotypes showed medium or high resistance to HS. Among them, the seeds of lines 466 and 1087, varieties Samurai and Toulouse, obtained from the plants grown in Moldova, showed relatively high resistance to HS. After cultivation in Moldova, the correlation coefficient between the resistance of the genotypes seeds to HS and SNT reached a value of 0.434. We mention that the correlation coefficient of the resistance

to SNT of the seeds obtained from the plants cultivated in Moldova and Ukraine was equal to 0.481 when the respective correlation of the seed's resistance to HS was negative to -0.314 . Thus, the resistance to SNT of seeds obtained from plants grown in Moldova was lower than those multiplied in Ukraine. Nevertheless, the positive values of the correlation coefficients of resistance to SNT of seeds grown in Ukraine and Moldova suggest that a tendency towards a decrease in resistance to SNT of propagated in Moldova seeds were manifested concomitantly in most genotypes. The negative correlation coefficient between the HS resistance of the seeds multiplied in Ukraine and Moldova suggests the opposite direction of changing the HS resistance of seeds obtained from plants grown in Moldova compared to those of the seeds multiplied in Ukraine. For example, the seeds HS resistance of the lines 542, 111, and the variety Toulouse was higher for propagated in Ukraine seeds, when the resistance of the seed of the genotypes Samurai, Arctis, and 1087, on the contrary, was higher in the case of their multiplication in Moldova.

3.3 Influence of the conditions of reproduction on the plasticity of wheat genotypes resistance to extreme temperatures

The plasticity of genotype response to extreme temperatures can be characterized by their primary resistance to high temperatures and negative ones. Theoretically, all possible values of the sum obtained for seeds of the different genotypes exposed to SNT and HS occupy the range between 0 and 200%. Therefore, we considered that this sum is the limit of the maximum value of the sum of seeds percentage that will germinate after exposure to HS or SNT. By comparing the values characterizing the wheat genotypes in the experimental variants, we can conclude about the relative plasticity of genotypes resistance to extreme temperatures: the lower the value of this sum, the lower is genotype plasticity in response to extreme temperatures.

The results of the experimental determination of 10 winter wheat lines and varieties' plasticity are included in **Figure 4**. Thus, in specially designed experiments, based on the normalized response of wheat seeds to SNT or HS, we estimated each genotype's plasticity in response to the action of extreme temperatures. Based on the percentage of seed germinated after SNT or HS, we divided the wheat genotypes into three groups: genotypes with low, medium, and high plasticity. They included genotypes characterized by the value of sum less than 100%, between 100% and 140%, and higher than 140%, **Figure 4**.

Let us analyze the level of plasticity of the response to SNT and HS of the wheat genotypes seeds reproduced in Ukraine. We can mention that high plasticity was specific to seeds of the variety Toulouse, medium—those of lines 21 and 517; the seeds of other genotypes have low plasticity, **Figure 4**. The seeds of the Toulouse variety obtained from plants cultivated in Moldova have medium plasticity. In contrast, the plasticity of reaction to excessive temperatures of the seeds of nine other wheat genotypes was low. It is interesting to note that, as a rule, the higher plasticity level of multiplied in Ukraine seeds was due to their higher resistance to SNT compared to the seeds obtained from the plants cultivated in Moldova. At the same time, in general, the HS resistance of seeds reproduced in Moldova tended to be higher. After reproduction in Ukraine, only the seeds of line 21 showed high plasticity and high resistance to SNT and HS. After reproduction in Moldova, they were characterized with a low resistance to SNT, HS, and plasticity.

The original goal of our researches was to elaborate one the accelerated method for determining the primary resistance of wheat genotypes to extreme positive and

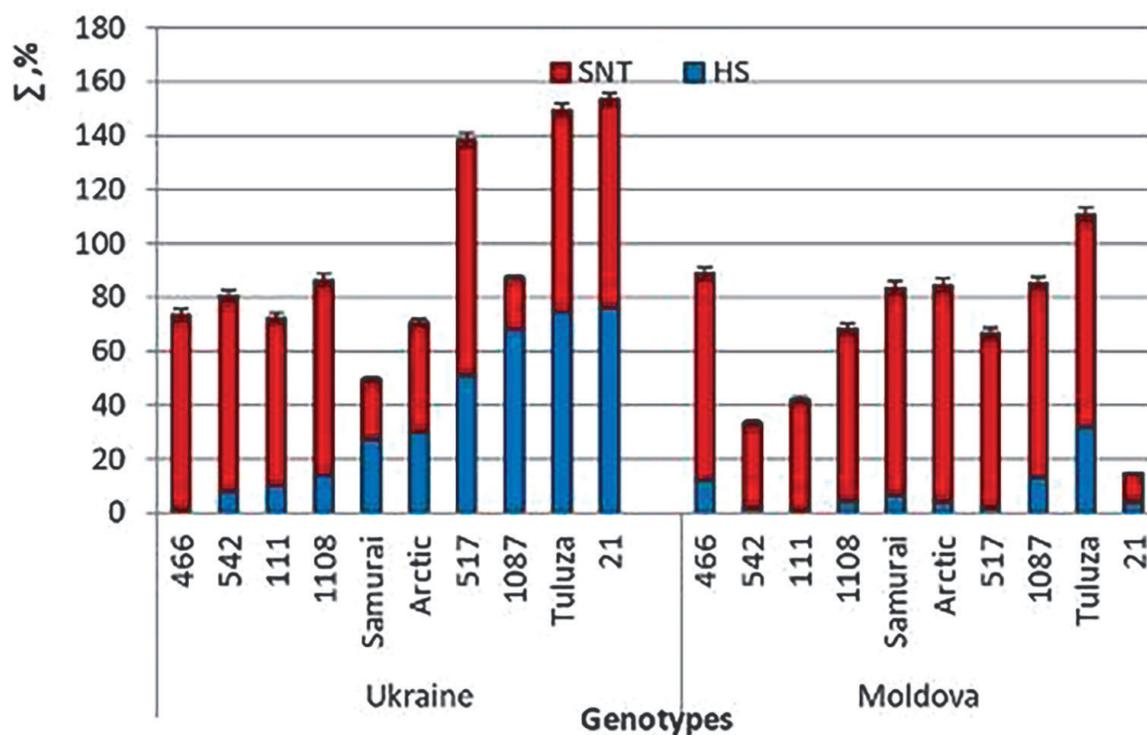


Figure 4. The sum of the percentage of the 10 winter wheat genotypes seeds germinated after exposure to SNT for 16 h SNT at -7°C plus that of germination percentage after seeds exposure for 30 min to HS at 50°C . On the left are presented the data obtained with seeds multiplied in 2015–2016 in the Kharkiv region (Ukraine), and on the right are those obtained with seeds multiplied in 2016–2017 in the Chisinau area (Moldova).

negative temperatures. Hexaploid wheat cultivars are characterized by adaptation to a broad range of environmental conditions. The trials in artificial conditions with well-organized systems and testing by cultivation in different regions were accomplished [20, 21]. Hexaploid wheat cultivars respond to environmental stressors by adaptations to a large temporal seasonal variability across regions and years. The wheat cultivation in different ecological conditions indicates genetic systems that allow genotypes to cope with rapid shifts of the temperature values. The contemporary breeding programs are mainly based on the extensive and expansive testing of cultivars in different environments or climatic chambers with well-controlled temperature, illumination, and nutrition conditions. These researches demonstrated that the combined effects of multiples genes that influence morphology, growth, and development of plants, in combination, can assure the survival of wheat cultivars in conditions with extreme temperatures. Under the exposition to extreme temperatures, the adaptive changes in general plant traits: organ dimensions, shape, anatomical structure, photoperiod, vernalization, etc., are observed. It is known that enhancing wheat productivity in the Green Revolution was mainly based on the selection of semi-dwarf, insensitive to photoperiod, and disease-resistant genotypes [22]. In fluctuating environmental conditions, we must implement agrotechnical procedures that can ensure the harvest's economically convenient quantity and quality. Mechanisms of stress avoidance formed the basis of the principles that influenced the improvement of productivity of wheat plants genotypes that actually are cultivated in stressful conditions. They have influenced plants' resistance to temperature stress by changing the morphological and anatomical structure of plants. Among these changes, we note the development of the root system, penetrating more profound and more moist soil layers [20, 21], the coating of leaves with a layer of wax, the composition of pigments,

the angle and shape of the leaves, which increase the refraction of light and the efficiency of transpiration [21]. The complexity of the influence of the mechanisms mentioned above of avoiding stressors caused a diversion of the attention from the genotypically determining physiological and biochemical processes, influencing the response of plants to stressors. We have made efforts to appreciate the involvement of these mechanisms in achieving by different wheat genotypes the primary resistance to extreme temperatures by exposing them to shock with positive or negative temperatures. We determined the primary resistance of wheat genotypes to the action of high temperatures using seeds well prepared for germination but which have not yet completed it. By excluding the influence of avoidance mechanisms and ontogenetic adaptations to extreme temperatures stress, we assured the possibility of comparing the primary resistance of different wheat genotypes. However, the data presented in **Figures 2–4** shows that, for the well-prepared for germination seeds, the value of the differences between the resistance of genotype to the action of extreme temperatures is relatively low compared to those obtained after induction of plant adaptation under long-time exposition to optimal for adaptation temperatures [15, 16]. Nevertheless, our experiments can differentiate the 10 wheat genotypes by their primary resistance to extreme positive or negative temperatures.

The ratio between the percentage of seeds germinated after HS and SNT in genotypes with high summary resistance to both types of shock showed a general tendency of approaching the value equal to one. Let us compare the trend of changing the values of this balance for the 10 genotypes reproduced in Ukraine and Moldova, **Figures 3 and 4**. We notice that for seeds multiplied in Moldova, the value of the primary resistance to HS reported to that of resistance to SNT, in general, is higher than this ratio evaluated for seeds reproduced in Ukraine. The shift in this ratio was primarily caused by declining the resistance to SNT of the seeds multiplied in Moldova. After reproduction in Moldova, only the variety Toulouse fell into the category of genotypes with medium summary resistance to extreme temperatures, and the others demonstrated low resistance. When reproduced in Ukraine, seeds of line 517 showed medium summary resistance, those of the Toulouse variety and the line 21—high resistance. Unexpected were the results obtained with seeds of line 21 reproduced in Moldova. If, after breeding in Ukraine, they concomitantly showed the increased resistance to HS and SNT, then after producing in Moldova, on the contrary, they were susceptible to both HS and SNT.

The data presented in this article results from efforts to assess the differences between wheat genotypes' primary resistance to extremes temperatures in the accelerated mode. Because the total resistance is the sum of the primary and adaptive resistance at any stage of ontogenesis, their discrimination is an important task. Therefore, we assumed that the summary seeds' resistance to stressors before germination is practically equivalent to the primary one. With this in mind, we have developed a method for synchronizing the physiological state of wheat seeds before the start of germination. Subsequently, using as a model the seeds of the variety Odesskaya 267, characterized by medium resistance to high temperatures and frost, we evaluated the optimal doses of HS or SNT exposure that caused the decreasing seeds germination up to 50%. The mentioned results allowed us to consider that these doses are optimal for the experimental distribution of wheat genotypes according to their primary resistance to high temperatures or frost. Furthermore, the obtained results demonstrated that seed resistance to both types of shock is specific for different genotypes but variable for the seeds of the same genotype but reproduced in other geographical conditions.

We must emphasize that the distribution of genotypes according to their primary resistance to extreme temperatures based on seeds' exposure to a single dose is simplifying. The resistance of biological systems to stressors can be multilaterally characterized by determining the doses of the stressor that causes decreasing the values of the investigated parameter by 16, 50, and 84% compared to that characteristic value for the control variant. After applying different doses of stress factors, some differences can be observed in the distribution of genotypes according to their resistance to extreme temperatures. Among the factors influencing these changes, we note the influence of seeds priming with inorganic salts, plant water extracts, organic molecules [23], and biostimulators [24, 25] on wheat stress resistance and productivity and as well the next generation inherited epigenetically acquired characters [26]. Sometimes these traits are inherited from one generation to the next. Therefore, we cannot exclude that the level of primary resistance, determined by the proposed method, is not influenced by epigenetic inheritance. The latter can be variable in the level of expression and duration of heritage. The practical techniques of elucidating epigenetic processes' integral role in installing plant adaptations to environmental conditions are under development. The preliminary data obtained show that the level of shock dose with extreme temperatures influences the distribution of wheat genotypes according to their primary resistance, which may be a consequence of the specific development of stress-initiated processes of genetic adaptations and simultaneously be influenced by the epigenetic inheritance. We assume that due to the overlapping influence of genetic and epigenetic factors on the wheat varieties' stress resistance, their descendants are characterized by variable primary resistance to HS and SNT and high productivity in very different environmental conditions. Compared to those reproduced in Ukraine, the data obtained with the seeds multiplied in Moldova confirm this suggestion. Genotypes, the seeds of which in our experiments showed essential differences in primary resistance to high or negative temperatures in the case of reproduction in Ukraine or Moldova, are of particular interest to assess the possible influence of the epigenetic inheritance on their primary and adaptive resistance to extreme temperatures depending on the conditions of reproduction.

3.4 Modification of the plasticity of wheat genotypes primary resistance to extreme temperatures during seeds storage

Phenotypic and genotypic analysis has shown that the longevity of wheat seeds is variable, involves different regions of the genome, and is not associated with the genes that determine the state of the seeds' dormancy [27]. Among the genes that influence longevity seeds are those that control the morphological structure of the spike or the response to abiotic and biotic stressors [28]. As the duration of seeds storage increases, their viability [29] and resistance to stress factors decrease [30]. Related to this appears the question of whether the mentioned changes affect the primary resistance of different wheat genotypes seeds. To elucidate this question, we included in the research the seeds of different varieties of hexaploid wheat collected in 2019 in the Chisinau area and kept in the dark for 1 year in a thermostat, at a temperature of 20°C and relative air humidity 67–80%. Throughout the storage period, the seed moisture deed does not exceed 10%. **Figure 5** includes data on the results of determining the germination percentage of seeds of different wheat genotypes after exposure to SNT. The data were obtained for two variants of seeds: the seeds on which the exposure to SNT was carried out 2 months after harvesting and seeds stored for 1 year

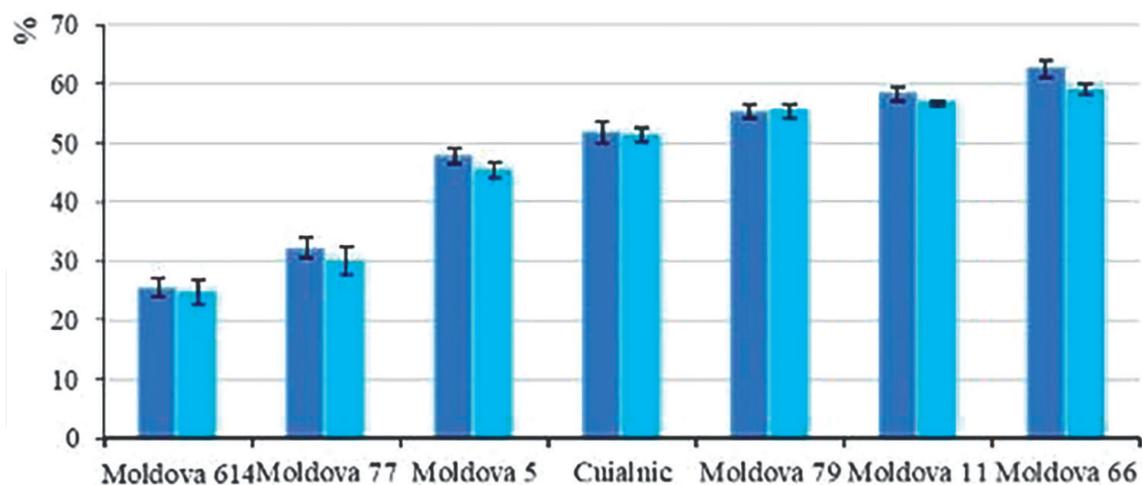


Figure 5. The influence of the shock with temperature -7°C during 16 h on the germination percentage of the seed of different wheat varieties reproduced in 2019 (diagrams marked in dark blue—Left) and those that after collection, the seeds were stored for 1 year (diagrams colored in light blue—Right). After exposure to SNT, the seeds were incubated in the dark, temperature 24°C , for 5 days for germination. In the control variants, the germination percentage of the seeds of all studied varieties varied between 98 and 100%.

before testing. They show that after 1 year of storage, the primary resistance of seeds showed the tendency to decrease for all varieties. The decreasing level varied between 0%, for seeds of the variety Moldova 79 and 3.5%, for the seeds of variety Moldova 66. Although the correlation coefficient between the level of primary frost resistance after 1 year of seeds storage and the resistance before that period was very high, being equal to 0.996, this does not mean that the changes in primary resistance were equivalent at all varieties. This high value of the correlation coefficient is since the storage of seeds of all varieties during 1 year did not significantly influence the absolute value of the percentage of seeds that germinated after exposure to SNT. The correctness of this hypothesis can be confirmed by the fact that the correlation coefficient between the percentage of seeds germinated after exposure to SNT immediately after harvest and the values of the differences between the rate of seeds that grow directly after harvest and a year later was much lower, being equal only 0.190.

Figure 6 includes data on the results of determining the germination percentage of seeds of different wheat genotypes after exposure to HS. From a qualitative point of view, the data on the influence of the different wheat genotypes seeds storage during 1 year on the resistance to HS, shown in **Figure 6**, are similar to those that characterize the impact of seed storage on their resistance SNT, **Figure 5**. After exposure to HS, seed storage during 1 year caused a relatively moderate decrease in germination percentage (minimum value was 3.8% for seeds of the variety Pisanca, and the maximum value was 6.3% for the seeds of variety Moldova 66). The correlation coefficient between the germination percentage of seeds exposed to HS immediately after harvesting (in 2019) and those after 1 year of seeds storage (in 2020) was equal to 0.9980. At the same time, the correlation coefficient between the germination percentage of HS exposed seeds immediately after harvesting, and the value of the difference between the germination percentage of HS exposed seeds directly after harvesting and 1 year later was equal 0.2994.

In general, the above-exposed data give the possibility to suggest that the value of decreasing the percentage of seeds germination after 1 year of storage tends to be practically independent of the level of genotype primary resistance to SNT or HS at the initiation of storage. However, the significance of the small positive correlations

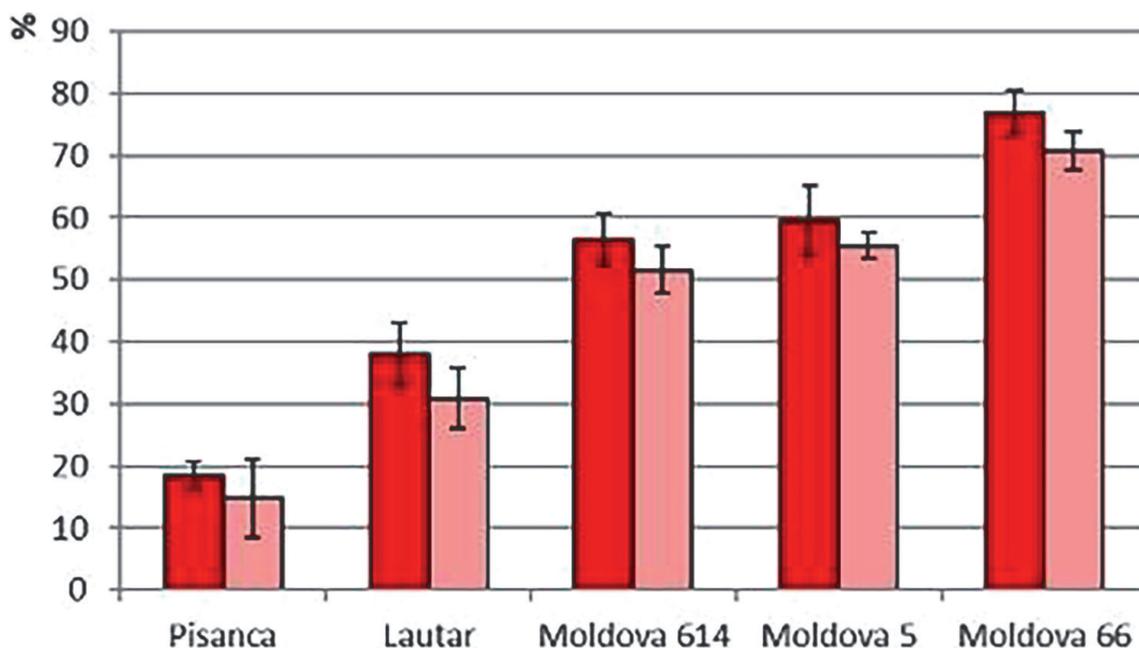


Figure 6. The influence of the shock with temperature 50°C during 30 min on the germination percentage of the seed of different wheat varieties reproduced in 2019 (diagrams are marked in dark red—Left) and those that after collection the seeds were stored for 1 year (diagrams are marked in light red—Right). After exposure to HS, the seeds were incubated in the dark, temperature 24°C, for 5 days for germination.

between SNT or HS resistance, determined at the beginning of storage and the value of its decrease 1 year later, requires further research. In our view, the special attention requires additional experimental analysis of the seeds of wheat varieties with the maximum or minimum values of decreasing the primary resistance to SNT or HS after 1 year of storage. We believe that the key to understanding the significant difference between the rates of decline in these genotypes of seed resistance to HS or SNT after storage for 1 year may be associated with differences in the inheritance of acquired traits.

4. Conclusions

Exposure to HS or SNT of different wheat varieties seeds, prepared for germination in particular conditions, due to the elimination of the influence of avoidance mechanisms and adaptation processes carried out during ontogenesis, give the possibility to distribute genotypes according to their primary resistance to high temperatures frost.

Seeds of different wheat genotypes differ by their primary resistance to HS and SNT. After exposure to HS or SNT, the reduction of wheat seed germination was all the more significant, the lower the primary resistance of the genotype to the action of extreme temperatures. Accelerated HS and SNT resistance determination are practical approaches for managing existing wheat genotypes and optimizing the available germplasm screening methods by laboratory and field testing. Using the proposed guidelines in breeding and agronomy will depend on their application with other physiological and molecular methods. Thus, it is possible to determine the influence of genetic and epigenetic heredity on wheat genotypes' primary and adaptive resistance to high temperatures and frosts.

The environmental conditions of winter wheat reproduction influenced the seeds' primary resistance to HS or SNT. Seeds storage during a year, their resistance to extreme temperatures tended to decrease inversely proportional rate to that of the values of their primary resistance.

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