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Research Article

Methods of Accelerated Distribution of Winter Wheat Varieties and Lines by their Primary Resistance to Extreme Temperatures

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Abstract

We provided experiments with the seeds of 73 varieties and lines of hexaploid wheat (2016 harvest), reproduced in the Kharkiv region of Ukraine and in the Chisinau area of Moldova, to elucidate the efficiency of the elaborated accelerated method of assessing genotypes' primary resistance (excluding the adaptation processes induced during plant ontogenesis) to high temperatures and frost. We performed tests under laboratory controlled conditions to evaluate the seeds' germination capacity after exposure to specific doses of heat shock (HS) with a high temperature of +50°C time for 30 minutes or frost shock (FS) with a negative temperature of -7°C for 8 hours. Based on the determination of the seed germination percentage of different varieties and lines of common winter wheat exposed to HS or FS, their distribution according to their primary resistance to frost and heat stress was evaluated. The obtained results demonstrated, that by their primary resistance to HS or FS, conditionally wheat varieties and lines can be divided into three groups: with low, medium and high primary resistance to extreme temperatures, including genotypes that after exposure to a chosen dose of temperature stress germinated up to 30%, between 30 and 70%, and more than 70% of seeds, respectively. The data demonstrate that seeds' resistance to HS or FS is specific for different wheat varieties and lines. Among all of them with low, medium and high resistance to FS, respectively 38, 44 and 80% simultaneously showed increased resistance to HS. The summary number of genotypes equivalently distributed in mentioned three groups is equal to 25, which constitutes 50% of the total number of genotypes included in our research. Twelve genotypes were identified (1088, 87, 1101, Kolichuga, Toulouse, 126, 91, Odesskaia 267, 21, 1013, Ovidii and 781) which characterized by a very high plasticity and high summary primary resistance to HS and FS.

Keywords: *Triticum aestivum* L; Germinating Seeds; Accelerated Methods; Primary Heat and Frost Resistance

Introduction

Due to the global warming trend, 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 globally decrease by 6% [2].

Wheat has been and remains to this day the main food of mankind. At present, wheat is grown on more land area than any other commercial crop and continues to be the most important food grain source for humans [3-5]. For this reason, increasing the wheat crop is one of actual task. Identification of genotypes with high resistance to abiotic factors helps to solve this problem.

A clear understanding of the mechanisms of plant resistance and adaptation to stress factors is crucial for agricultural specialists since the rational use of varieties and hybrids, optimization of the methods for breeding new genotypes, as well as testing the effects of biologically active compounds on plant resistance and productivity under stress conditions [6,7]. The mechanisms of resistance to extreme temperatures are complex and depend on plants' morphological, physiological, biochemical, and genetic characteristics. These features' values can vary from genotype, phase of ontogenesis [7,8], and the specificity of environmental conditions [9]. In this regard, it is essential to develop methods for accelerated and separate assessment of the primary (initial) and adaptive plants' resistance to extreme temperatures [7,9]. Also, plant resistance and productivity are determined by various avoidance components, leading to a decrease in the exposure dose of the stress factor [6,7,10].

Various approaches make it possible to assess the resistance of plants to various stress factors. Bringing a single common denominator of the abundant data concerning plant responses to stressors obtained on a different level of organization is not always possible. In addition to the effects caused by stressors of varying nature and intensity, there are also specific features for plant reactions at the subcellular, cellular, tissue, and organism [6-8] and ecological levels [10]. The variety of stress factors and the specificity of plant responses at different levels of organization complicate developing consistent methods for assessing their resistance to stress factors. We believe that the principles of systems theory [7,11] and comprehensively developed mathematical models for the accelerated assessment of the stress stability of technical systems [9] can largely contribute to solving this complex problem.

Precise separation of the primary and adaptive resistance components indicators is necessary to systematize experimental data and methods for assessing plant resistance to extreme temperatures. It is also essential to consider the input of avoidance (reduction of the exposure dose of the stress factors [6,7,10] to the overall plant resistance to the action of one or another stressor. First of all, it is necessary to determine the primary plant resistance and then identify adaptation components and avoidance phenomena to the total plant resistance to stress factors. In practice, this turns out to be not easy since, depending on the stage of ontogenesis, various adaptation phenomena superimposed on the primary resistance of plants, the contribution of which is difficult to assess accurately.

The use of plant seeds simplifies the mentioned problems. The germination parameters of wheat seeds previously exposed to extreme temperatures characterize the initial plant tolerance to extreme temperatures. By exposing the seeds of varieties and lines of wheat to a specific dose of HS or shock with FS, it was possible to distribute them according to their primary resistance to positive or negative temperatures.

The objectives of our study were: 1) to develop the accelerated methods of determining the wheat varieties and lines' primary resistance to the action of high temperatures and frost, 2) to evaluate the discriminating ability of the methods based on seeds exposure to a specific dose of HS or FS as a rapid, simple, repeatable, and inexpensive method of screening in laboratory conditions of the resistance to extreme temperatures of winter wheat.

Materials and Methods

We used the research of 23 varieties of winter wheat seeds multiplied in 2015-2016 in the Chisinau area (Moldova) on the experimental field of the Institute of Genetics, Physiology & Plant Protection (IGFPP) and the seeds of 10 varieties and 40 lines bred in the Kharkiv region (Ukraine). We sowed, cultivated and harvested wheat in accordance with generally accepted technologies [15].

In the preliminary research, we used wheat seeds (*Triticum aestivum* L.), variety Odesskaia 267, with a relative humidity of 14%, uniform in color, shape, and size. Before being investigated, we immersed the seeds in distilled water at a temperature of +4°C. Specifying the kinetics of water imbibition by wetting the seeds, the period of immersion sufficient to ensure the degree of wetting ensuring their uniform and rapid germination was estimated. Before placing them for germination, we disinfected the seeds by immersion in a solution with 0,01% potassium permanganate for 10 min, then washed them with distilled water. After this, we placed the seeds from the control variant for germination and previously subjected those from the experimental variants to HS and FS.

Initially, we elaborated on the optimal procedure of determining the genotype's primary resistance to negative temperatures by exposing the seeds from the experimental variants to FS with temperature of -7°C time for 8 hours. To accomplish this task, we exposed the wheat seeds of experimental variants to FS by incubating them at different negative temperatures (intensive factor) and exposure periods (extensive factor). To choose the optimal exposure dose to FS, we incubated the seeds of the Moldova 5 variety at -5, -6, -7, -8, or -9°C, for 8 or 16 hours. As a result, we applied the dose obtained by incubating the seeds at -7°C for 8 hours for the distribution of genotypes according to their response to FS. Incubation was carried out in the climatic chamber Rumed type 3401 (Germany), maintaining the temperature at ±0.1°C accuracy.

In addition, an optimal procedure was developed for determining the primary resistance of genotypes to high temperatures by exposing the seeds to HS with temperature of +50°C for 30 minutes. To determine the genotype's primary resistance to high temperatures, we exposed the wheat seeds to HS by immersion in water at temperature +50°C (maintenance accuracy ±0.05°C) and incubation time for 30 minutes in the U-10 ultra-thermostat (Germany). We chose the mentioned dose of HS based on testing the effect of HS applied to the seeds of the Odesskaia 267 variety with positive temperatures of +48, +49, +50, +51, +52 or +53°C applied for 30 minutes.

The germination capacity of the seeds and the subsequent growth of the seedlings we determined by the placement of wheat seeds from the control and experimental variants in Petri dishes on wet filter paper and incubating in a thermostat in the dark with a temperature of +25°C and air relative humidity of 75 - 85% during five days. We considered the seed germinated when the radicle reached a length of 2-3 mm.

In each of our studies, both the control and experimental variant were presented in triplicate with 40 seeds each. In other words, 240 units were used from each variety or line. Thus, 4320, 12000, 2160 and 12000 seeds were involved in four experiments.

We determined the germinative capacity of wheat seeds at FS and HS on seeds germination on the fifth day from the moment of placement in the thermostat. We calculated the germination percentage (G) of the seeds from the control and experimental variants according to the formula

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

where Ng and N are the number of germinated and total number of seeds, respectively.

Based on mentioned parameters, determined for the seeds of the Odesskaia 267 variety, the durations, and temperatures of exposure to the HS and FS were selected, which in the experimental variant caused the seed germination percentage to decrease by 50% compared to that in the control variety. These positive and negative temperature shock doses were considered optimal to be subsequently applied to assess the primary resistance to heat or frost of wheat. Based on these data, to determine the primary resistance of wheat varieties and lines to negative temperatures, we applied the temperature shock of -7°C for 8 hours and the resistance to high temperatures - by seeds exposure to +50°C for 30 min. After exposure to these doses of shock with minus or plus temperatures, results were obtained, which allowed arranging the wheat varieties and lines included in the research according to their primary resistance to the action of high or low negative temperatures. We emphasize that the different values of the seed germination percentage of wheat genotypes after applying a certain dose of HS or FS were the basis of the method of accelerated distribution of genotypes according to their initial resistance to high temperatures or frost.

Statistical data analysis, graphical, and textual representation performed using software package Statistica, 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. We provided each experiment at least three times. Mean values, standard deviation, and reliability of differences between variants were determined [13]. In the figures, the date presented as Mean ± SD. In each of our experiments, there is a statistically significant difference between the three groups means with a probability of 95%.

Results

In previous research [14], we have demonstrated that wheat seeds germinate quickly and synchronously if, before incubation in optimal conditions for germination, they have been soaked in water at a temperature of +4°C for 36 hours. After washing, the humidity of the seeds reaches 35%. Precisely for this reason, before assessing their response to FS or HS action and subsequent germination incubation, we soaked the wheat seeds of all varieties and lines in the mentioned conditions.

In supplementary, we determined the optimal parameters of FS or HS doses, making it possible to separate wheat genotypes according to their primary resistance to extreme temperatures. In multiple experiments, we determined that the exposition of the moderately resistant to high temperatures or frost seeds of wheat variety Odesskaia 267 to HS at a temperature of +50°C for 30 minutes or FS at a temperature of -7°C for 8 hours, causing a diminution of their germination by 50%. We applied the mentioned doses of extreme temperatures to seeds of the different wheat varieties and lines to assess their primary resistance to frost or heat. We emphasize that the total resistance of the wheat seeds prepared for germination in the mentioned way depends only on the initial (primary) resistance of the genotype, to which in ontogenesis, due to adaptation processes, the secondary resistance is added [6,7], induced under the influence of environmental factors. The physiological state of plants depends not only on their genetic identity, age, and current environmental conditions but also on their previous adaptations to the environment during ontogenesis [6,7,9]. Because of the variability of adaptive resistance, determined functionally and by avoidance, correctly choosing the genotype primary resistance is an important and challenging problem.

Differentiation of wheat varieties and lines according to their primary resistance to the action of heat shock

After applying temperature shock +50°C for 30 minutes on the wheat seeds reproduced in Moldova, Figure 1, in the group of genotypes with high thermotolerance, those after exposure to the shock with high temperatures germinated more than 70% of seeds. We found the varieties Ephoros, Skagen, Moldova 11, Moldova 5, and Chevalier among them. Average thermotolerance was shown by the varieties Auriu, Odesskaia 51, Avantaj, Kuialnik, Căpriana, Antonovka, Matrix, Plai, Odesskaia 267, and the line H333, whose seed germination, after exposure to the shock with high temperatures, was distributed between 30 and 70%. The seeds of the Vdala, Missia, and Epoha varieties showed low tolerance, which after exposure to the shock with high temperatures, germinated below 30% of the wheat seeds.

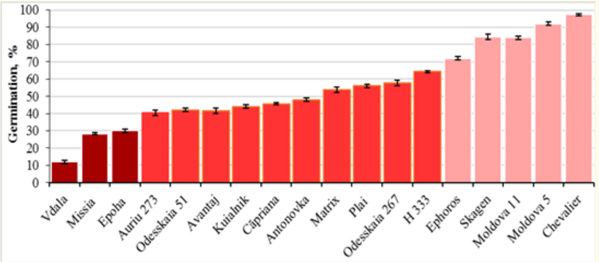

Figure 1: The percentage of seed germination of wheat varieties propagated in the experimental field of the IGFPP, exposed before incubation for germination to the HS of +50°C for 30 minutes.

The data on the influence of HS on the germination of seeds of 50 wheat varieties and lines reproduced in Ukraine are presented in figure 2. It is necessary to emphasize that during five days in each control variant (without the application of HS), no less than 95% of seeds germinated. According to the response of the wheat seeds to HS, wheat genotypes with high, medium, and low resistance, respectively, 25, 22, and 3 genotypes, were identified, constituting 50, 44, and 6% of the total number of tested genotypes.

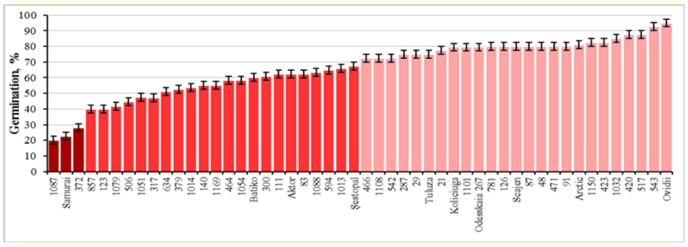

Figure 2: The percentage of seed germination of wheat varieties propagated in the Kharkiv region of Ukraine, exposed before incubation for germination to the HS of +50°C for 30 minutes.

The presented ones suggest that the accelerated distribution of wheat genotypes according to their primary resistance to high temperatures was applied a single dose of the HS on the wheat seeds well prepared for germination. However, this method we considered a preliminary one. To generally assign a wide range of genotypes according to thermotolerance, applying relatively moderate temperature shock (+50°C, 30 min) is promising. If there are only genotypes with relatively high or low resistance in the group under analysis, then we recommend exposure to HS of +51°C or +49°C, for 30 minutes, respectively.

Differentiation of wheat varieties and lines according to their primary resistance to the action of shock with negative temperatures

In research on the resistance to negative temperatures, we tested the effectiveness of the shock dose achieved by exposure for 8 hours to the temperature of -7°C. In new research, we have included nine varieties reproduced on the experimental field of the IGFPP. The data presented in Figure 3 suggest that after applying the temperature shock of -7°C for 8 hours, only the wheat variety Moldova 66 was distributed in the group of genotypes with high tolerance, and with average-Blagodarca, Pisanka, Nufic, Moldova 5, Kuialnik, Moldova 79, and Moldova 11, the germination of seeds of which after exposure to this dose of FS was between 38 and 55%. The wheat seeds of the variety Missia were characterized by low thermotolerance. After exposure to FS, seed germination of this variety reached only 26%.

In the additional research, we demonstrated that the distribution of the mentioned genotypes after exposure to -6°C temperature shock during 16 hours at FS remains analogous to that shown in figure 3. Still, the resolution capacity for the distribution of genotypes with a low resistance to FS increases substantially and decreases for those with high resistance to FS. From this, it follows that for the distribution of wheat genotypes according to their primary resistance to negative temperatures, the FS dose can be optimized by varying within reasonable limits the temperature and the duration of exposure of the seeds to the shock.

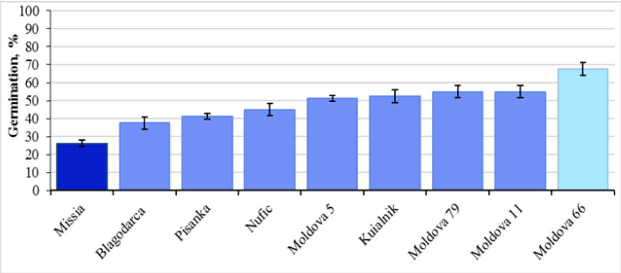

Figure 3: The seed germination percentage of wheat varieties propagated on the Experimental Field of the IGFPP, exposed before incubation for germination to the FS with -7°C for 8 hours.

To distribute according to the resistance to the action of negative temperatures of wheat genotypes reproduced in the Kharkiv region (Ukraine), the same conditions of exposure to FS as those bred in Moldova were used. The obtained data is presented in Figure 4. The classification of wheat varieties and lines according to the increasing value of the percentage of germinated seeds in the experimental variant provides the opportunity to arrange them according to their primary resistance to the action of FS. According to the response of wheat seeds to shock with negative temperatures, as in the case of the reaction of wheat seeds to shock with high temperatures, wheat varieties and lines were distributed conditionally into three groups: with high resistance (I), more than 70% of wheat seeds germinated; medium resistance (II), 30-70% of wheat seeds sprouted; and low resistance (III), only up to 30% of seeds germinated. In mentioned groups were distributed 10, 27, and 13 genotypes, respectively.

We mention two important particularities of the proposed methods

- Although after different exposure doses to HS or FS, the ranking of genotypes according to resistance would practically not change, their distribution in groups with specific resistance would vary. For example, when decreasing the exposure temperature to HS by 1°C, Figure 2, some genotypes from among those with medium or high resistance to HS would move into the group of those with low or medium resistance. At the same time with the action of FS, Figure 4, the change of the exposure temperature from -7°C to that -6°C would cause the transition of some genotypes from the group of those with low or medium resistance to the group of those with medium or high resistance, respectively.
- Since the proposed methods of distributing genotypes according to their response to HS or FS are fast and relatively easy to perform, depending on the purpose of the research, with a high level of reliability, genotypes with low or increased resistance to HS or FS can be highlighted, as well as increasing the effectiveness of the selection of genotypes by analyzing the deviation of their distribution from the normal one.

Discussion

The data obtained give the possibility to appreciate whether the tested genotypes were chosen randomly in terms of their primary resistance to extreme temperatures. If the distribution of genotypes according to their resistance to negative or high temperatures had been random, then, taking into account the width of the characteristic percentage interval for each zone (zone I - 70-100%; zone II - 30-70%; zone III - 0-30%), the expected distribution of

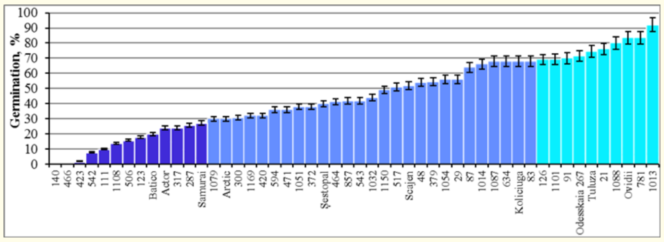

Figure 4: The seeds germination percentage of wheat varieties propagated in the Kharkiv region of Ukraine that were previously exposed before incubation for germination to the FS of -7°C for 8 hours.

the number of genotypes in zones I, II and III would have been the following: 30, 40, 30% of the total number of genotypes included in the experiment, i.e., 15, 20 and 15 genotypes respectively. The analysis of the obtained results (Figures 2 and 4) demonstrates that in reality, in zones I, II, and III, by resistance to high temperatures, 25, 22, and 3 genotypes were highlighted, and by resistance to negative temperatures, respectively 10, 27 and 13 genotypes. It should be noted that the division of genotypes into 3 zones is conditional and is associated with the possibility of the developed method.

Interestingly, out of the 25 wheat varieties and lines with high resistance to HS, only eight simultaneously showed increased resistance to FS, constituting 32% of those highly resistant to HS and 80% resistant to FS. We emphasize that the rest of the genotypes with high resistance to FS (lines 1088 and 1013) simultaneously showed medium resistance to HS. At the same time, of the 13 wheat varieties and lines with a low resistance to FS, Figure 4, only five lines (287, 466, 423, 542, and 1108), Figure 2, showed high resistance to HS, which represents 38% of the genotypes of this group. From this, it follows those 12 genotypes out of the 27 with medium resistance to FS demonstrated high resistance to HS, constituting 44%. Thus 38, 44, and 80% of genotypes with low, medium, and high resistance to FS simultaneously showed increased resistance to HS, the total number of which is 25. These data demonstrate that the selection of genotypes in the Kharkiv region predominantly vectored towards obtaining forms of wheat with high primary resistance to high temperatures.

We note that the sum of the percentage of seeds germinated after exposure to HS plus that of wheat seeds exposed to FS exceeded 140% for varieties and lines 1088, 87, 1101, Kolichuga, Toulouse, 126, 91, Odesskaia 267, 21, 1013, Ovidii, and 781. We emphasize that the consecutiveness of mentioning the genotypes corresponds to increasing the said amount. It gradually increased from 140%, for the seeds of line 1088, to 167%, for the wheat seeds of line 781. These 13 genotypes are characterized by a very high plasticity, showing high summary primary resistance to HS and FS.

The data presented in Figures 1 and 3 indicate that wheat varieties grown in Moldova tend to be more resistant to HS than to FS. After exposure to HS, five genotypes out of 18 analyzed showed high resistance, while after exposure to FS, none of the nine tested varieties and lines showed increased resistance to negative temperatures.

The presented data demonstrate the validity of the methods of rapid distribution of wheat varieties and lines according to their primary resistance (in the absence of ontogenetic adaptations) to extreme positive or negative temperatures. These methods are efficient and rapid, allowing the determination of the primary heat and frost resistance of many genotypes in a reasonable time. However, the data presented in scientific publications suggest a positive correlation between primary and adaptive resistance [6,7,9]. The methods to obtain data in this field are expensive, long-lasting, and of low productivity [9]. With the application of traditional methods, these researches can be contradictory and with much higher expenses. Also, from a quantitative and qualitative point of view, the methods proposed by us represent a step forward, although traditional methods do not offer the possibility to determine in accelerated mode the primary resistance of wheat varieties to heat and frost [7,9,10]. Knowing the level of the genotype's primary resistance to extreme temperature stress, we can, in every moment of plant ontogenesis, correctly and rapidly appreciate the contribution of adaptations depending on the genotype, the stage of ontogenesis, environmental conditions, and the influence of biologically active substances.

Conclusions

In the research regarding comparing the physiological and genetic peculiarities of the seeds of wheat varieties and lines, it is necessary to bring seeds to the same " preparation " phase for germination, avoiding the occurrence of adaptation initiation phenomena. 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 primary resistance of wheat varieties and lines to the action of high or minus temperatures, the techniques being simple, sensitive, and reproducible. To select wheat varieties and lines with a high degree of thermotolerance, it is recommended to test them by applying doses of heat shock, carried out with relatively high temperatures (+50°C or +51°C) during 30 minutes or shock with negative temperatures (- 6°C or -7°C) during 16 or 8 hours. The selection of genotypes in the Kharkiv region, included in our research, was predominantly vectored on obtaining the wheat varieties and lines with increased primary resistance to high temperatures.

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