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DETERMINATION OF *TRITICUM AESTIVUM* L. PRIMARY RESISTANCE TO HIGH TEMPERATURE

A. DASCALIUC, N. ZDIORUC, T. RALEA

Institute of Genetics, Physiology and Plant Protection
20 Padurii St., Chisinau, 2002, Moldova
e-mail: dascaliuca@yahoo.com

The changes in the seeds germination capacity of the hexaploid wheat variety Odesskaya 267 after different periods of immersion in water at a temperature of 4 °C were studied. The seeds immersion in water for up to 104 hours was accompanied by rapid absorption of water in the first 32 hours (phase I), followed by a slow increase in seed moisture over the next 72 hours (phase II). The seeds sensitivity to the application of heat shock (48—52 °C) increased with the immersion time, which, together with the improvement of their germination parameters during phase I and the first part of phase II, suggests the seeds coming out of dormancy and initiating processes of germination. The sensitivity to heat shock of the seminal roots initials, especially of the radicle, gradually increased with the imbibition time expansion. Their meristems initials cells have progressed more in exit from dormancy and germination processes intensification during this period. The elaborated method of assessing the seed resistance to heat shock is prospective to compare the primary resistance (without the involvement of adaptation processes) of different wheat genotypes to high temperatures.

Key words: *Triticum aestivum* L., seeds, germination, high temperature, tolerance.

According to some meteorological models, the global average temperature towards the end of this century will increase by 2.0—4.0 °C [1]. The survival and productivity of plants depend not only on the average temperature but also on the extremes of positive and negative temperatures and their duration. The physiological, genetic, and ecological effects of climate changes on crops, especially wheat, were discussed in the paper [2]. A particular interest represents comparing the primary resistance of different plant genotypes to extreme temperatures. Because this does not include adaptation/acclimation components, it gives the possibility to characterize the input of various features separately into the total resistance [3]. Given this, the need to develop rapid and efficient methods for determining plants' resistance to extreme temperatures and select resistant genotypes suitable for cultivation in specific geographical regions becomes clear.

Several factors influence the correctness of determining the plants resistance to extreme temperatures. Among them of particular importance is the lack of uniformity of the plants physiological state at the time when

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determining their resistance. The physiological state of plants depends not only on the genetic identity, age, and current environmental conditions but also on the equivalence of the history of their adaptation to the environment during ontogenesis. In natural conditions, plants physiological state synchronization occurs during the seeds formation, maturation, and entry at dormancy [4]. In quiescent embryos of wheat seeds, cells are in the G1 phase of the cell cycle [5, 6], the transcription, translation, and mitotic cycle stopped. At the initiation of germination, the physiological states of quiescent embryos are practically identical.

However, several factors can disrupt the synchronization state of embryo cells in dormant seeds. The maturation of grains in the wheat ear is in different time; also, plants physiological state and the external conditions may differ. The plant seeds vary not only in size but also by structural and functional characteristics. The specificity of internal and environmental conditions during maturation and entry at dormancy can perturb cells synchronization due to capturing them into different mitotic stages [4, 6]. Probably, the accumulation of most cells in the G1 phase of the cell cycle is manifested only in optimal for maturation and desiccation of seeds conditions, when embryos humidity is installed gradually at about 10 % by the balance between seeds and air relative humidity.

The heterogeneity of the seeds physiological state can also increase during the creation of favorable germination conditions when gradually restored the metabolic processes and the expression of the genes necessary for germination. Germination of the wheat quiescent embryo is primarily influenced by temperature and seed humidity, which induce the expansion of the embryo axis, finalized with the penetration of seed tegument by the radicle [4]. If exposure to temperature can be uniform and precisely controlled, then the uniform increase of different seeds moisture is much more challenging to ensure. The seeds structural-morphological differences influence the dynamics of water absorption, which determine the heterogeneity of the periods of reaching the moisture level, sufficient for germination initiation.

The seed germination began with the activation of metabolic processes. In wheat embryos, protein biosynthesis occurs in 30 minutes after immersion in water [7]. The proteins synthesized in the first 9 hours after embryo germination initiation are necessary for DNA replication. Still, the final activation of DNA biosynthesis performed 6—8 hours later, accompanied by the transition of cells from the G1 phase to phase S (phase of DNA synthesis) of the mitotic cycle [8]. During the germination of *Arabidopsis thaliana* seeds, the initiation of DNA replication occurs only after the roots appearance, simultaneously with the transcription of most genes involved in the cell cycle, i.e., after germination [5]. 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 prepare the initiation of cell divisions that occur after the radicle has protruded tegument. The activation of the cell cycle in wheat embryos begins with the induction of mitosis at the initials of the radicle, and then it gradually spreads to all meristematic cells [5]. The start of phase S and G2 (post-synthesis phase) in wheat radicle meristematic cells occure in

12—14 hours after germination [9]. Thus, under favorable conditions, the germination of wheat embryos will occur with 24 hours lag period. Typically, the uniformity of seeds imbibition dynamics can substantially influence the heterogeneity of seed germination. At the initiation of wheat seed germination, mentioned triggering of DNA replication by proteins encoded by mRNA, stored in mature seeds, was not sufficient for the cells to commence mitosis [10].

A rational way to reduce germination heterogeneity due to the different seeds imbibition speed is to immerse them for a long time in the water at low temperature. In these conditions, the physiological and biochemical processes slowed down.

So, the aim of our work was to study the imbibition dynamics of wheat seeds immersed in distilled water at 4 °C, the parameters that characterized the germination capacity, and resistance to heat shock of wheat seeds with different humidity levels.

Materials and Methods

In the studies, we used seeds of the wheat variety Odesskaya 267 multiplied in 2016—2017 on the experimental field of the Institute of Genetics, Physiology and Plant Protection, Chisinau, Moldova. We calibrated the wheat seeds by passing through a sieve with a diameter of 2.4—2.6 mm, than incubated them for 10 minutes in 0.1 % potassium permanganate solution. Finally, we washed the seeds thoroughly with a tap water, then with distilled water.

During immersion in water at temperature +4 °C, the dynamics of water accumulation by wheat seeds determined by weighing at different periods of imbibition up to 130 hours. In the experimental variants, the seeds were subjected to heat shock (HS) by immersion during different periods in the water at different temperatures, maintained with an accuracy of ±0.05 °C, using the ultratermostat U10 (Germany). Before germination, the seeds of control variants were immersed in water with a temperature of +24 °C during periods equal to applying HS in experimental variants.

Subsequently, the seeds from the experimental and control variants were placed for germination in Petri dishes, 25 seeds each, in three repetitions, in the dark, at 24 °C and relative air humidity 75—85 %. The reaction of wheat seeds to HS was evaluated based on the percentage of seeds germinated in 5 days, the average number of seminal roots per seedlings, and the portion of seedlings with inhibited growth of the radicle. We elucidated the influence of immersion time and that of HS application on different wheat seeds germination parameters. The experimental variants relative values were determined compared to the respective values in the control variants. The data were statistically processed, determining the mean value, standard deviation, and credibility of the mean values differences [11].

Results and Discussion

The data presented in Fig. 1 shows the kinetics of water accumulation by seeds of wheat variety Odesskaya 267. The dependence of dynamics of

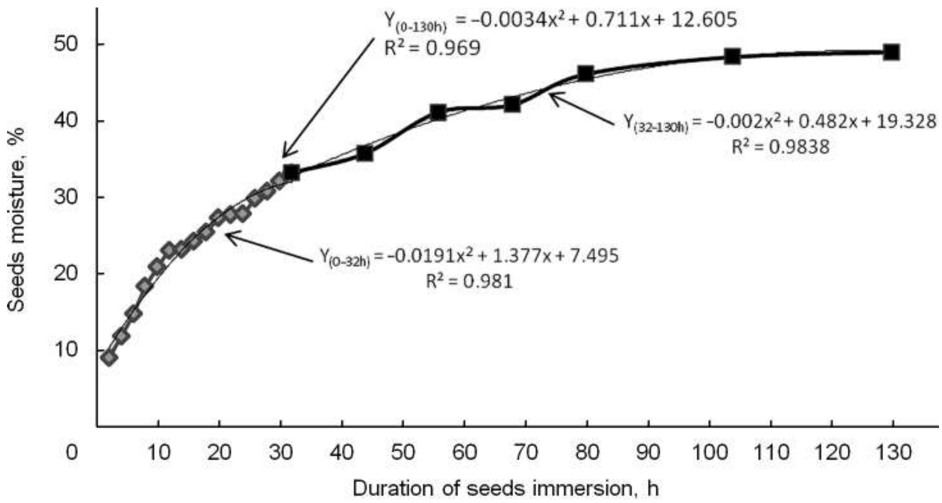

Fig. 1. Dynamics of increasing the humidity (I) in the wheat seeds, variety Odesskaya 286, depending on the duration of immersion in water at a temperature 4 °C. $Y_{(0-32h)}$, $Y_{(32-130h)}$, and $Y_{(0-130h)}$ — the polynomial approximation of increasing seeds humidity on intervals of 0—32, 32—130,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

increasing seeds humidity on the duration of immersion in water from 0 to 130 hours we described by the polynomial equation: $Y_{(0-130h)} = -0.0034x^2 + 0.7114x + 12.605$. 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 hours (initial active water adsorption), and phase II — from 30 to 130 hours (plateau phase). The increasing of the seeds humidity in interior of mentioned phases is described by equations $Y_{(0-32h)} = -0.0191x^2 + 1.3766x + 7.4948$, and $Y_{(32-130h)} = -0.002x^2 + 0.4822x + 19.328$. Comparing the three equations, we can observe that in all the increase of the humidity percentage with the increase of the imbibition time, the linear components 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 (1,377 and 0.482; ratio equal to 2.886). During immersing in the water a period between 0 and 130 hours, as expected, this coefficient of linear proportionality was lower compared to that characteristic for the interval I and higher than in the interval II (in interval III equal to 0.711). 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 time of immersion in water was equal to 0.29 % per hour when in phase I and II were respectively equal to 0.77 and 0.16 % per hour.

During 130 hours of imbibition at 4 °C, the germination of the seeds did not occur. Therefore, there was no transition to a new phase III, characterized by a further increase in the rapidity of water imbibition and radicle emergence [4, 12]. The seeds imbibition in water during 130 hours 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

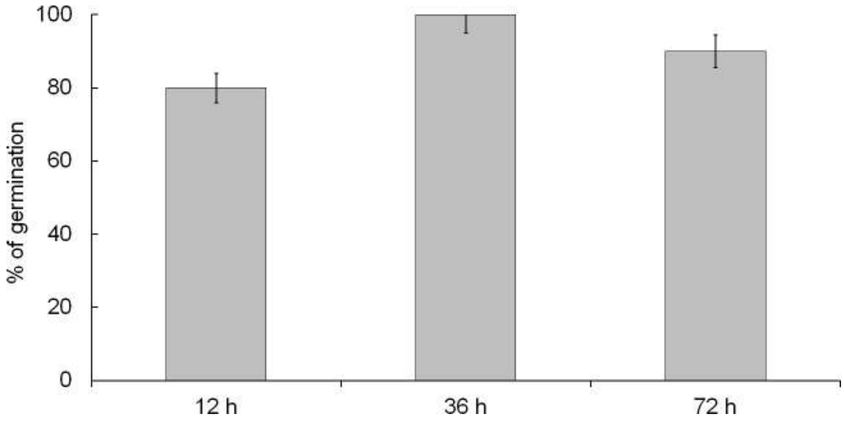

Fig. 2. Percentage of germination of the wheat seeds, variety Odesskaya 286, previously immersed in distilled water at 4 °C for 12, 36, and 72 hours

dormancy, described in the specialized literature, following which the germination and the appearance of the radicle take place only in phase III of germination [4, 8]. In our experiments, the radicle formation was not detected even after 130 hours of seeds immersion in water.

Considering the dynamics of water accumulation in seeds, we determined the wheat seeds germination parameters previously immersed in water during 12, 36, and 72 hours. The seeds were placed for germination in a thermostat at a temperature of 24 °C, considered germinated if, on the fifth day of incubation for germination, the radicle was prominent, reaching a length of 2—3 mm. The obtained data demonstrate that germination of the seeds previously immersed in water for 36 hours was practically equal to 100 % when the germination of those imbibed for 12 and 72 hours peaked 90 and 80 % (Fig. 2). Mass germination (more than 50 % of seeds) of preliminary water-immersed wheat seeds during 12, 36, and 72 hours occurred 24, 12, and 32 hours after incubation at 24 °C. The data confirm the information that at a temperature of 4 °C, in wheat embryos, start the germination and growth processes slowly [13].

Although seeds germination did not occur during 130 hours of immersion in water, the percent of germination in favorable conditions of seeds previously immersed 12 hours in water at 4 °C was lower than those imbibed 36 hours (Fig. 2). These results suggest the activation during immersion in water of metabolic processes necessary for the initiation of germination. Thus, the wheat seeds imbibition at 4 °C improved the synchronization of seeds physiological state by accumulating them for germination in identical starting positions. The imbibition of seeds during 12 hours was for a too-short time to reach equal humidity of all wheat seeds; during 72 hours, it was too long (probably due to oxygen consumption processes during long time immersion) and inhibits germination [5]. Considering the kinetics and percentage of germinated seeds, the immersion of wheat seeds for 72 hours negatively influenced their germination. Therefore later, we provided researches only with the seeds immersed in water for 12 and 36 hours.

For testing the physiological state of seeds, they were initially immersed in water for 12 and 32 hours, subjected to HS by incubation for

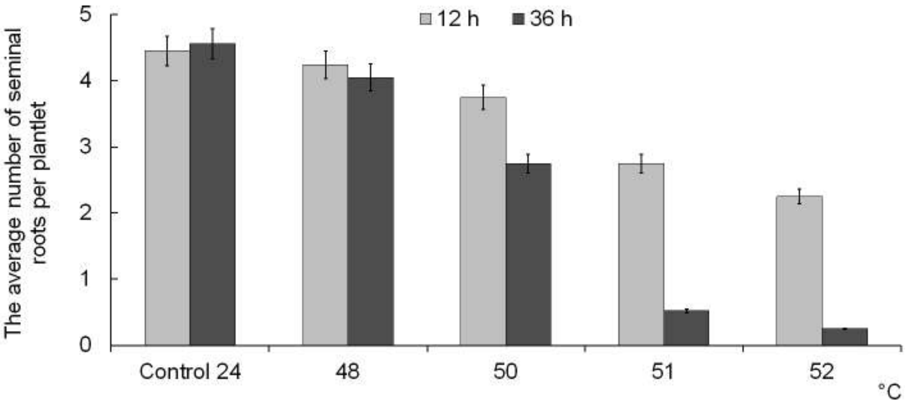

Fig. 3. The number of seminal roots on wheat seedlings, variety Odesskaya 286, germinated after immersion in distilled water at a temperature of 4 °C during 12 and 36 hours (control 24 °C) and subjected to heat shock of 48, 50, 51, and 52 °C during 30 minutes (experiment)

30 minutes at the temperature of 48, 50, 51, 52 °C, and placed at germination. Following the dynamics of seminal roots appearance immediately after germination, we can mention that first emerged the radicle (the wheat central seminal root), then practically simultaneously the first pair and then the next couple of seminal roots, located symmetrically to the radicle. In Fig. 3 presented the data about the influence of HS with different temperatures on the number of seedling roots formed in five days after placement at germination. After five days of germination, five roots appearance was expected (1 + 2 + 2). In two control samples, the mean number of roots per wheat plantlet reached 4.5. Although in two control variants, the average number of roots per plantlets tended to be equal, HS influence on the number of seedling roots was more pronounced on seedlings obtained from the seeds immersed in water for a longer period (36 hours). Application of HS during 30 minutes at temperature 48 °C caused the retention of the formation of the 3rd order roots more pronounced in the sample of seedlings obtained after 36 hours of immersion.

Decreasing the number of roots at the seedlings obtained from the seeds immersed in water during 36 hours and then exposed to HS with temperatures of 48, 50, 51, and 52 °C, was more pronounced than those imbibed only during 12 hours. Increasing the HS temperature applied in the range from 48 °C to 52 °C in phases I and II caused the average number of roots to decrease from 4.2 to 2.3 (1.83 times) and from 4.0 to 0.2 (20 times), respectively. The HS temperature had a more pronounced influence on the number of roots in seedlings obtained after HS application to seeds immersed in water 36 hours, compared to those imbibed only 12 hours. Their higher resistance to HS after 12 hours of imbibition suggests that their seminal roots initials were at a deeper dormancy state. The quiescence levels may influence the HS sensitivity of seeds in phases I and II of imbibition supplementary enhanced by the protective effects of HS proteins accumulated during maturation and persisted during seeds germination. These proteins can lead to increased resistance to HS of the wheat seeds imbibed in water less than 12 hours [15]. In the first 12 hours of immersion in water, exposing wheat seeds to HS could

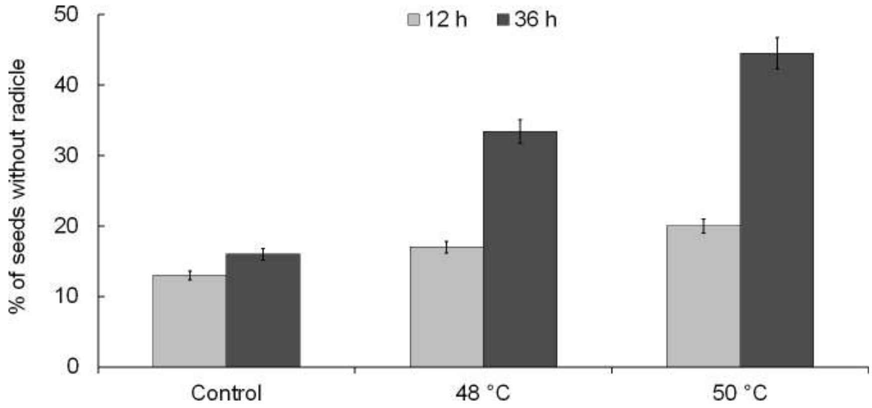

Fig. 4. Percentage of wheat seedlings without radicle, variety Odesskaya 286, obtained from the seeds preliminary immersed in distilled water at a temperature of 4 °C during 12 and 36 hours (control) and subjected to heat shock of 48 and 50 °C during 30 minutes (experiment)

induce new HS protein synthesis, leading to increased thermotolerance in further germination [16].

The data presented in Fig. 4 shows the percentage of seedlings that lacked the radicle in different experimental variants of HS application. The number of plantlets appeared without a radicle increased with rising the HS temperature. Radicle was missed even in 13—15 % of the control samples plantlets, the percentage of which tends to be significantly higher in the seedlings obtained from the seeds previously immersed in water for 36 hours.

Data from the literature suggest that high temperatures and drought, applied apart and combined, on the wheat genotype with low thermotolerance at the period after pollination, causes the formation of seeds that at germination deed not formed radicle. However, the initials of all seminal roots exist in both heat-resistant and tolerant variety. This effect did not occur when the exposure of embryos to these factors provided during meiosis [17].

Exposure of seeds in phase I (immersion duration 12 hours) and phase II (immersion duration 36 hours) to HS caused by the temperature of 50 °C increased the number of seedlings that lacked the central seminal root from 13 to 20 % and from 15 to 44 %, respectively. If compare the data in Figs. 3 and 4, we reveale that exposure to HS at 50 °C of seeds in phase I and phase II caused the decrease in the average number of roots from 4.49 to 3.84 (1.17 times) and from 4.55 to 2.75 (1.65 times), respectively. In the mentioned phases, the percentage of plants without radicle increased from 13 % to 20 % (1.54 times) and from 15 % to 44 % (2.93 times). It follows that exposition of seeds to HS after 36 hours of immersion compared to that after only 12 hours had more pronounced effects on the total number of seminal roots and the number of seedlings that lacked the radicle. Simultaneously, the increase of the water immersion duration of the seeds in phase II influenced more significantly the number of seedlings that lacked the root compared to the influence on the total number of seminal wheat roots. The change of initials thermotolerance demonstrates that their exit from dormancy is gradual, previously of radicle, and later of other

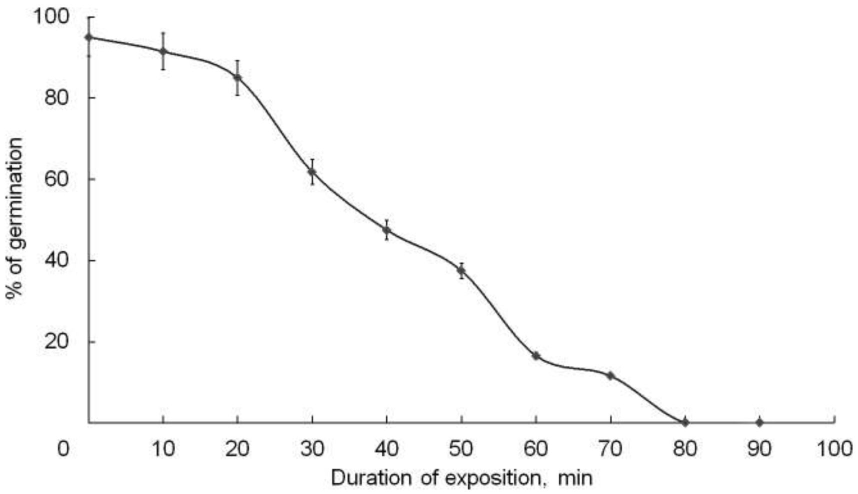

Fig. 5. Percentage of wheat seeds, variety Odesskaya 286, germinated after immersion in distilled water at a temperature of 4 °C for 36 hours and subsequently exposed to heat shock of 50 °C, depending on the exposure duration

seminal wheat roots. The above data show that seeds immersed in water for 36 hours germinate more evenly and quickly than those imbibed only during 12 hours. Therefore, both the radicle initials and other seminal roots that were at an early stage of emergence, being more sensitive to HS exposition. Considering the above, we conclude that immersing the seeds in water at 4 °C for 36 hours ensures the seeds physiological state uniformity and a good position for initiating the germination of wheat seeds in optimal conditions.

Previously presented data were obtained by studying the influence of HS with different temperatures (intensive factor, variable), at a single exposure duration (extensive factor, constant). We aimed to study the influence of the period of seeds exposure to HS on their germination. If we consider the data shown in Fig. 4, the temperature 50 °C is most suitable for HS exposure with different durations. The HS with exposure during 30 minutes at 50 °C demonstrated moderate effects on the wheat seeds germination. The data obtained after exposure to HS with different duration, at 50 °C, are shown in Fig. 5. Increasing the period of seeds exposure to HS at 50 °C in the interval from 20 to 60 minutes leads to linear decreasing germination by 1.67 % to each minute. Considering that the wheat variety Odesskayia 267 is moderately resistant to high temperatures, we propose to arrange the wheat genotypes by their primary resistance to high temperatures, comparing the germination rate of seeds exposed to HS at 50 °C over 30 minutes. In these conditions are avoided the influence of acclimatization phenomena.

It can be concluded that seed dormancy is a complex phenomenon that influences the resistance of plants to stressors. Their germination depends on the specifics of the physiological state installed during maturation under environmental factors. Because germination depends on seeds humidity, we elucidated the differences in dormancy level at different periods of their imbibitions. The complexity of the interactions between the environment conditions during seed maturation and moistening complicates dormancy levels elucidation during germination. To simplify and unify the method for determining the primary genotypes resistance to high temperatures, we first optimized the immersing time in water of wheat seeds before placing them at germination or exposure to HS. After immersion for 36 hours, at a temperature of 4 °C, wheat seeds pass into phase II of water imbibition, germinate uniformly and quickly, which suggests their physiological state synchronization. This procedure ensures uniform moistening and reduces the seeds physiological heterogeneity, established during their maturation. Due to the determination of the optimal conditions the parameters that characterize the germination of wheat seeds improved, and allow quickly appreciate the seeds primary resistance to high temperatures by their exposure in a uniformly moist state to specific doses of HS.

Thus immersion of wheat seeds in water at a temperature of 4 °C for up to 130 hours is accompanied by quick absorption of water during the first 32 hours (phase I), followed by a slow increase in seed moisture the next 72 hours of immersing (phase II). The improvement of the seeds germination parameters realized by extending the imbibition period in cold water up to 36 hours leads to increased sensitivity to HS, suggesting their exit from dormancy and the initiation of germination processes. The increase of seminal roots sensitivity to HS, especially of the radicle, with extending the duration of immersion suggests that the initial cells of their meristems continually progressed in exit from dormancy concomitantly with the development of germination processes.

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ВИЗНАЧЕННЯ ПЕРВИННОЇ СТІЙКОСТІ *TRITICUM AESTIVUM* L. ДО ВИСОКИХ ТЕМПЕРАТУР

А. Даскалюк, Н. Здіорук, Т. Ралеа

Інститут генетики, фізіології та захисту рослин
2002 Кишинів, вул. Падурія, 20, Молдова
e-mail: dascaliuca@yahoo.com

Вивчали зміну схожості насіння гексаплоїдний пшениці сорту Одеська 267 після різних періодів занурення у воду за температури 4 °С. Занурення насіння у воду на 104 години супроводжувалося швидким вбиранням води протягом перших 32 годин (фаза I), а потім повільним збільшенням вологості насіння протягом наступних 72 годин (фаза II). Чутливість насіння до дії теплового шоку (48—52 °С) підвищується із часом занурення, що разом із поліпшенням параметрів схожості протягом фази I та першої частини фази II передбачає виведення насіння з періоду спокою та ініціювання процесів проростання. Зі збільшенням тривалості всмоктування води чутливість до теплового шоку насіннєвих коренів, особливо корінця, стає більш вираженою. Клітини ініціалів їх меристеми значно прогресували у виході з покою та активізації процесів проростання. Розроблений метод оцінки стійкості насіння до теплового шоку є перспективним для порівняння первинної стійкості (без участі процесів адаптації) різних генотипів пшениці до високих температур.

Ключові слова: *Triticum aestivum* L., насіння, схожість, високі температури, толерантність.