Dependence of germination of wheat grains after the treatment by impulse pressure and long-term storage on the vitreousness of endosperm

E E Nefedieva¹, Ya I Khramova¹, V N Khramova¹,², I F Gorlov¹,², V I Lysak¹ and M I Slozhenkina¹,²
¹ Volgograd State Technical University, 28, V.I. Lenin Av., Volgograd, Russia
² Volga Region Research Institute of Manufacture and Processing of Meat-And-Milk Production, 6, Rokosskogo Str., Volgograd, 400131, Russia

E-mail: nefedieva@rambler.ru

Abstract. The aim was identification of patterns of changes in the physical and chemical state of biopolymers in durum and soft wheat seeds after the treatment of impulse pressure (IP) and during the storage of seeds. It was found that durum wheat seeds accumulated microcracks over the structure of the glassy endosperm after the IP treatment, while soft wheat seeds reacted to IP treatment by "collapsing" air gaps in the loose endosperm, as well as by vitrification of amorphous areas that were in a highly elastic state and the transition of crystalline areas to vitreous ones. The vitreous content of durum seeds decreased, while that of soft seeds increased linearly after the treatment by IP and storage. Treatment of soft wheat seeds with IP and storage increased their germination, growth and quality of seedlings due to vitrification of the seed endosperm. The decrease in the vitreousness of durum wheat grain after the treatment with IP and storage led to deterioration in the quality of grain and sprouts during storage. So the glassy state in seeds is suggested to serve as a stabilizer. The rapid progress of deterioration may represent a weakening of the glassy state.

1. Introduction
Seeds are an important life cycle stage because they guarantee plant survival in unfavourable environmental conditions and the transfer of genetic information from parents to offspring. However, similar to every organ, seeds undergo aging processes that limit their viability and ultimately cause the loss of their basic property, i.e., the ability to germinate. Seed aging is a vital economic and scientific issue that is related to seed resistance to an array of factors, both internal (genetic, structural, and physiological) and external (mainly storage conditions: temperature and humidity). Researchers investigating seed aging claim that the effective protection of genetic resources requires an understanding of the reasons for senescence of seeds to long-term storage [1].

Seed aging tolerance and rapid seedling growth are important agronomic traits for crop production; however, how these traits are controlled at the molecular level remains largely unknown. There are provide evidence that some genes regulate the longevity of seeds by stimulating the production of raffinose while simultaneously acting to limit auxin-mediated cell expansion [2]. The aging effects may be alleviated by seed priming with certain chemicals, such as salicylic acid (SA) [3]. The effects of
gibberellic acid (GA(3)) and stratification on germination characteristics of seeds were demonstrated [4].

The vitreous quality of wheat seeds is one of the indicators of their quality [5]. In the process of industrial crushing of seeds, there is an increase in the hardness of the seeds, but then plastic deformations and accumulation of micro-destructions until the actual destruction of the seeds occurs [6].

Powdery seeds have a loose endosperm structure with minute voids. It consists of tightly closed starch grains with faces and attached protein, and the intermediate protein fills the narrow space between them. In vitreous seeds, starch and protein are solidly bonded; the endosperm of those seeds is represented by rounded starch grains with large gaps filled with smaller starch grains and free protein. Mechanical action on the powdery grain leads to the formation of cracks. Cracks will run along the intermediate protein between the starch grains, without affecting them, while the glassy grain will be destroyed throughout the volume [6].

We proposed the method of presowing treatment of seeds by impulse pressure (IP) generated by a shock wave. It is characterized by high intensity and accuracy of dosage. IP has unique physical properties due to the excellent parameter control that determine the intensity of the influence [7].

Seeds are not crushed by ID treatment, but the endosperm structure changes depending on their vitreous state. Vitreous seeds can be susceptible to micro-cracking of the endosperm with a violation of the bonds in the starch grains, as well as the bonds between starch and protein. Powdery seeds due to the loose structure of the endosperm can be compacted, acquiring the properties of glassy polymers and resistance to aging [5]. In this case, the crystal bonds in starch molecules can break down, and the starch molecules pass into a glassy state due to spatial restrictions for the restoration of the crystal lattice [8].

The aim was identification of patterns of changes in the physical and chemical state of biopolymers in durum and soft wheat seeds after the treatment of impulse pressure (IP) during the storage of seeds.

2. Materials and methods
The first object of research was wheat grains of the durum cultivar, Saratovskaya zolotistaya. That cultivar of spring soft wheat was created at the research Institute of Agriculture of the South-East. Genealogy of the cultivar: Leucurum 1838/ Leucurum 1830. Variety is Leucurum. The cultivar is medium.

The second object of research was wheat grains of the soft cultivar, Saratovskaya 73. That cultivar of spring soft wheat was created at the research Institute of Agriculture of the South-East. Genealogy of the cultivar: Lutescens 2014/ Tr. timopheevii. Variety is Graecum. The cultivar is medium-late.

Seeds were treated by impulse pressure (IP) 11 and 29 MPa. IP was generated by an impact wave created by the detonation of a water resistant explosive and propagated in an aqueous medium, resulting in a volume compression of the seeds for 15–25 μs.

The pressure in the wave front was calculated by formulae for spot charge

\[ P = 53.3 \cdot \left( \frac{Q^{\frac{1}{3}}}{R} \right)^{1.33} \]

where P is the pressure, MPa; Q is the weight of the explosive, kg; R is the distance between the center of the explosion and the seed surface, m [9].

The seeds were treated as follows (figure 1).

Dry seeds (3) were placed in porolone cells (2), which were situated at the bottom of a stainlesssteel container (1) filled with water (4). Beneath the water surface, we clamped a water-resistant explosive (5) with the weight of Q and at the distance from the seed surface equal to R using plank (6), and then the explosive was detonated by electric detonator (7). This technique of seed treatment is covered by a patent [7]. An individual cell contained up to 500 g of seeds, that is, more than 15000 individual seeds, and this amount represented one replication.
Figure 1. Device for seed treatment.

After such treatment, the seeds were kept for 24 h at room temperature, bringing them to an air-dry state. Control seeds were soaked in water for the same time used for soaking seeds during the IP treatment, and then air dried [10].

Grains treated with IP, as well as control ones, were stored in a paper package in a dry, dark place at room temperature and relative humidity of 30-50%. After 4 years, the grains were sorted with the DSZ-2M diaphanoscope into 2 following batches, batch V (vitreous) and batch P (powdery).

The vitreousness of endosperm of seeds was determined by near-IR spectroscopy (NIRS).

Treated seeds were germinated on filter paper at the temperature of 20°C in a humid atmosphere. The numbers of germinated seeds at the age of 4 days and 8 days were determined, and the results obtained were expressed as a percent of total number of seeds from four groups of 100 seeds.

When we calculated the germination power and germination, normally germinated seeds, abnormally germinated seeds and non-germinated (dead) seeds were counted separately. The result of the analysis was taken as the arithmetic mean of the results of all analysed samples.

The length of shoots and roots was determined with an accuracy of 0.5 mm.

3. Results and discussion

The measurement of vitreousness was performed immediately after the treatment by IP, as well as after 4 years of storage. The results are shown in figure 2 and figure 3.

Figure 2 shows that the treatment of durum wheat seed by IP resulted in a significant decrease in vitreousness after the exposure. This change is due to the initial high density of the endosperm and the absence of air gaps in it that can be reduced. This effect led to the emergence of cracks in the endosperm that was caused by plastic deformation upon impact [8].

Treatment of soft wheat seed by IP led to an increase in their vitreousness after the exposure (figure 3). This phenomenon may be associated with compaction of the loose endosperm and reduction of air gaps in its structure. Seeds of batches Soft V and Soft P had a loose endosperm, so the treatment led to a proportional increase in vitreousness with an increase in IP. Apparently, even vitreous seeds of batch Soft V have a different, looser endosperm structure than seeds of durum wheat, which allows them to avoid plastic deformations.
However, the IP 29 MPa was excessive for the seeds of batch Soft V (figure 3), and the increase in vitreousness after the treatment was less than it was after IP 11 MPa. This fact indicates a non-linear effect of IP treatment on the vitreousness of seeds; durum wheat seeds are destroyed, while for soft wheat seeds the optimal IP allowed to get the maximum possible vitreousness.

Figure 4 demonstrates changes in germination of seeds of the soft spring wheat cultivar Saratovskaya 73 after the treatment of IP and after 4 years of storage. Seeds of control part as well as were sorted with a diaphanoscope into 2 batches, batch V (vitreous grains) and batch P (powdery grains). The germination (figure 4) of the control seeds of the powdery batch was lower than that of the control of the vitreous batch, and the amount of dead seeds was 80% and 65%, respectively. Also, seeds with higher vitreous content had a higher percentage of normal seedlings. So, the glassy state in seeds is suggested to serve as a stabilizer. The rapid progress of deterioration may represent a weakening of the glassy state, combined with a facile hydrolysis of sugar components and the unleashing of an array of oxidative processes [5].
Figure 4. Germination of seeds of the soft spring wheat cultivar Saratovskaya 73 after the treatment of IP and after 4 years of storage. $V$ is vitreous grains, $P$ is powdery grains.

Figure 5. Length of shoots and roots of seedlings (8 days) of the soft spring wheat cultivar Saratovskaya 73 after the treatment of IP and after 4 years of storage. $V$ is vitreous grains, $P$ is powdery grains.

After the treatment with IP, the seed germination of both batches increased. This increase in germination may be due to the impact on biopolymers and their vitrification as a result of compression by IP (figure 3).

Treatment of IP 11 MPa and 29 MPa had a stimulating effect [11] on the germination of seeds of the soft spring wheat cultivar in both batches. The length of seedlings and their parts such as roots and leaves significantly exceeded the control parameters (figure 5) in powdery grains treated with IP. Seeds with higher vitreous content in control formed seedlings of greater length than seeds with powdery grains. The length of seedlings and their parts after the treatment of IP was comparable to the control one. So, IP influenced significantly on powdery grains of seeds of the soft spring wheat cultivar Saratovskaya 73.
4. Conclusion

Non-lethal doses of IP had different effects on the vitreous properties of seeds of the durum spring wheat cultivar and soft wheat cultivar. The vitreous content of durum seeds decreases, while that of soft seeds increases linearly.

Treatment of soft wheat seeds with IP and storage increased their germination, growth and quality of seedlings. It increased the vitreousness of the seed endosperm. The improvement was due to the transition of seeds from "abnormal" to "normal", which was more significant after the treatment with IP 11 MPa.

Figure 6 demonstrates changes in germination of seeds of the durum spring wheat cultivar *Saratovskaya zolotistaya* after the treatment of IP and after 4 years of storage. V is vitreous grains, P is powdery grains.

Figure 7. Length of shoots and roots of seedlings (8 days) of the durum spring wheat cultivar *Saratovskaya zolotistaya* after the treatment of IP and after 4 years of storage. V is vitreous grains, P is powdery grains.
The decrease in the vitreousness of durum wheat grain after the treatment with IP and storage led to deterioration in the quality of grain and sprouts during storage.

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