Modifying the effect of stressed spring wheat seeds on intact ones

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Abstract. Spring wheat seeds react to the effect of stress factors of various natures with a nonspecific adaptive reaction, accompanied by intense emission of stress ethylene in trace amounts during the entire time, while. On the one hand, they remain viable, and on the other hand, have mechanical, radiation-chemical and temperature damage. Damaged air-dry seeds are not able to regenerate damaged organs and tissues and, being in a state of chronic stress, permanently induce ethylene biosynthesis. The effect of stressed seeds on intact ones is achieved remotely when they are stored together in the air environment and are blocked by active aeration, air-insulating materials and a decrease in temperature. The stronger the damaging effect on seeds is caused by the stress factor, the more intense is the accumulation of stress ethylene. An increase in the duration of exposure of intact by stressed seeds is accompanied by a more pronounced physiological modifications in seedlings of intact seeds with a sequential change in time in their stimulation of the initial growth processes and a sharp deterioration in sowing qualities.

1. Introduction

The study of the mechanism of remote interspecific communication of plant organisms has been and continues to be a rather complex and urgent problem of modern agricultural biology and ecology [9, 2, 3]. This is due to the fact that the identification of connections and the mechanism of the impact of some biological objects on others has not only theoretical, but no less important applied value, allowing to predict and manage the development of processes occurring in agroecosystems. The mutual influence of plants as a result of their release into the environment of various physiologically active substances that affect other plants of the same or other species growing nearby is a fairly widespread and well-studied phenomenon [1, 10]. But in recent years, a series of experimental studies have established the phenomenon of remote influence of air-dry stressed plant seeds on intact ones [4-6]. The experiments revealed previously unknown processes for the formation of cascade intraspecific effects in air-dry seeds in the system: from irradiated seeds to unirradiated ones and from them to unirradiated seeds [7], a method for diagnosing the stress level in cereal seeds was developed, which showed a close dependence of the stress level on the concentration of ethylene in the inter-seed air [8]. However, until now there is no information explaining the mechanism of the effect of air-dry stressed seeds on intact ones in scientific publications characterizing physiological processes and sowing qualities in damaged (injured, irradiated, hyperthermic) seeds. In addition, there are still no studies of factors that can modify the degree (strength) of the effect of stressed seeds on intact ones.

In this regard, the purpose of this work was to study the ability of air-dry stressed seeds to modify at a distance the germination and sowing qualities of intact seeds, depending on conditions of their...
post-harvest storage. The task was to prove experimentally the properties of stressed seeds to produce physiologically active volatile compounds that affect the physiological state and sowing qualities of intact cereal seeds.

2. Materials and methods

The studies were carried out with seeds of soft spring wheat (*Triticum aestivum* var. *lutescens*) cultivar Agata.

The state of stress in air-dry seeds of spring wheat used in the experiment was achieved by the damaging effects of γ radiation, through mechanical shocks with violations of the integrity of the caryopses and temperature from +50° C to +70° C. The seeds were exposed to γ radiation in the dose range from 8 to 400 Gy at γ-unit Kolos and GUPOS-8 with a radiation source of 137Cs and an Investigator unit with a radiation source of 60Co at a radiation dose rate of 8.0; 4.8 and 27.0 Gy/min, respectively. Mechanical injury (similar to damage to cereal grains during mechanized harvesting) was carried out by means of shock mechanical impacts, with the formation of 15-20 % of macro- and micro-damages of seed shells, cracks, dents in tissues of intact seeds. Hyperthermia was through exposure to a coolant (air) with a temperature of 50-55° C for 2-4 hours and up to a day, and 70° C for 1-2 hours. As a rule, conditioned seeds were used in all series of experiments. Remote exposure of stressed seeds to intact (undamaged) ones ranged from 1 to 12 months and was carried out through a common air medium with a volume of 1 to 5 dm³ at a distance of 1 to 100 cm from each other. The exposure of stressed seeds to intact ones took place in lab conditions at a temperature from +18° С to 25° C and in the seed storage at a variable temperature from -15° C to +18° С. To store seeds and establish the nature of the agent that determines the effect of stressed seeds on intact ones, fabric, Kraft paper, quartz glass and ordinary, galvanized iron were used as insulating materials. The mass of experimental samples of stressed and intact seeds ranged from 100 to 500 g, with a ratio of 1:1 and 1:2, 1:3, 1:4, 1:5, 1:8, respectively.

The control was intact (whole, unaffected) seeds, separately stored from stressed ones and meeting the requirements of sowing standard GOST R 52325-2005.

The effect of remote exposure was assessed by the intensity of the initial growth processes of 3-, 5- and 7-day old seedlings. When germinating seeds in rolls of filter paper, the following morphometric parameters were determined: the length of primary roots, the mass of primary roots and their number, the length and mass of sprouts; growth correlations; germination energy and laboratory germination in accordance with GOST 12038-84 and growth force in accordance with GOST 10340-84.

The identification and content of released volatile compounds in the inter-grain air space of stressed and intact seeds were investigated using chromatograph analyst “Crystal - 2000 M” manufactured by CJSC SKB "Khromatek". Samples of seeds of the control and experimental (irradiated, mechanically injured, hyperthermic) variants weighing 250-500 g were placed in glass flasks with a volume of 1 l with ground-in lids and stored at positive temperature (laboratory conditions). Air samples from the flasks were taken with all-glass syringes.

The study of the effect of stressed seeds on intact ones, depending on the intensity of gas exchange between them when passive aeration, was carried out by placing these seeds in open boxes made of galvanized iron at a distance of up to 10 cm between seeds, where there was a natural flow of air. When active aeration, a compressor was used to eliminate volatile physiologically active compounds, providing air supply to the bottom of the box.

All experiments were reproduced many times over several years of research. Statistical data processing was performed using Statistica 6.0 software package. The significance of differences between the studied indicators was assessed by the Student’s test. Differences were considered statistically significant at P<0.05. Results were presented as mean and standard error of the mean.

3. Results and discussion

In accordance with the working hypothesis, the phytohormone stress ethylene is the agent providing the effect of stressed seeds on intact ones. It is known that stress in biological objects is generated by
damaging factors of different nature. The study examined typical widespread stress factors: ionizing radiation and mechanical injuries that inevitably occur in caryopses when combine harvesting of grain.

A significant similarity in the response of intact seeds to remote exposure of stressed ones, having the stress induced by mechanical damages and \( \gamma \) irradiation, was experimentally established (Table 1). The result of the experiment indicates that the effect of remote exposure is observed not only in \( \gamma \)-irradiated seeds, but also in injured ones, which do not tend to induce electromagnetic radiation. The effect of stressed seeds on intact ones was not significant at early stages of germination (1st - 3rd days).

When exposed for 1 month at a distance of 1-10 cm, only a tendency to accelerate the formation and size of embryonic roots and sprouts was noted. It has been shown that remote storage of intact wheat seeds of cultivar Agata with stressed seeds is accompanied by significant suppression of the initial growth processes after exposure for 6 months. The inhibitory effect from stressed seeds increased as the storage period increased to 9 months. The most pronounced suppression of seedlings was observed in the variant of storing intact seeds with \( \gamma \)-irradiated ones compared with damaged ones. This is probably due to the stronger damaging effect of radiation on the tissue cells of the seed embryos (damage to all structures of the caryopsis) than with mechanical damage, where there is a damage of only individual tissues and organs. Thus, after 6 months of exposure to \( \gamma \)-irradiated seeds, all the studied parameters: the length and weight of sprouts, as well as the number, weight, length of embryonic roots were less than the control by 24.6 % and 34.1 %, 12.1 %, 20.2 % and 18.1 %, respectively.

With an increase in exposure to 9 months, these indicators continued to fall in relation to the control by 70.5 % and 60.3 %, 20.9 %, 61.1 % and 35.5 %. Whereas, with the same exposure to damaged seeds, reliable inhibition of seedlings was noted only by the criterion of the mass of the sprout and the embryonic root. But after 9 months of storage of seeds in this variant, there was a decrease in all indicators of the sprout and embryonic roots, respectively, by 56.6 % and 53.1 % and by 17.2 %, 44.3 % and 24.2 % in relation to the control.

The more vivid effect of remote suppression of intact seeds in the variant with \( \gamma \)-irradiation, in comparison with damaged ones, is probably explained by the stronger damaging effect of radiation on cells and tissues of the seed embryo (damage to all structures of the caryopsis), inducing a strong development of stress reactions than with mechanical damage, where the integrity of only individual tissues and organs is violated.

### Table 1. The remote effect of stressed seeds of spring wheat cultivar Agata on the initial growth processes of intact seeds (3-day old seedlings).

| Variant                  | Months of exposure | Sprout         | Embryonic root |
|--------------------------|--------------------|----------------|----------------|
| K – separate storage of  | 1                  | 16.6±0.55      | 3.82±0.08      | 3.73±0.05 | 3.24±0.07 | 46.7±1.7 |
| seeds                    |                    |                |                |          |          |          |
| Intact with \( \gamma \)- | 18.0±0.46          | 3.94±0.12      | 3.81±0.08      | 3.16±0.08 | 49.4±1.1 |
| irradiated               |                    |                |                |          |          |          |
| Intact with damaged      | 17.2±0.44          | 3.91±0.09      | 3.75±0.07      | 2.93±0.03 | 47.9±1.4 |
| K – separate storage of  | 17.1±0.81          | 4.69±0.11      | 3.76±0.06      | 3.32±0.06 | 50.5±2.0 |
| seeds                    |                    |                |                |          |          |          |
| Intact with \( \gamma \)- | 12.9±0.39*         | 3.09±0.17*     | 3.32±0.09*     | 2.65±0.05* | 41.4±1.7* |
| irradiated               |                    |                |                |          |          |          |
| Intact with damaged      | 14.2±0.54*         | 3.39±0.13*     | 3.55±0.05*     | 2.83±0.07* | 43.5±1.9* |
| K – separate storage of  | 16.0±0.92          | 4.71±0.13      | 3.79±0.09      | 3.07±0.04 | 48.2±1.5 |
| seeds                    |                    |                |                |          |          |          |
| Intact with \( \gamma \)- | 4.72±0.16          | 1.87±0.08*     | 3.00±0.04*     | 0.92±0.09* | 31.1±0.7* |
| irradiated               |                    |                |                |          |          |          |
| Intact with damaged      | 6.95±0.57          | 2.21±0.22*     | 3.14±0.05*     | 1.71±0.06* | 36.5±1.1* |

* differences with the control are statistically significant when \( P<0.05 \)
Remote storage of intact seeds with $\gamma$-irradiated ones for a month in cloth bags at positive temperature was accompanied by an increase in the length of the sprout and embryonic root relative to the control by 33.6 % and 21.7 %, respectively (Table 2). An increase in exposure to 9 months caused a sharp inhibition of the initial growth processes in intact seeds, so these indicators were 38.3 % and 70.1 %, respectively, compared to the control. Weakly expressed stimulation of growth processes in intact seeds during storage for a month was noted in the variant with damaged ones. After 9 months of exposure this variant of the experiment had pronounced inhibition instead of weak stimulation, while the length of the sprout and embryonic root was 55.0 % and 79.9 %, respectively, compared to the control. Whereas storage of seeds in metal containers with stressed ones caused an increasing stimulating effect in intact seeds. So, the length of the sprout and the embryonic root after 9 months of exposure to $\gamma$-irradiated and damaged ones exceeded the control by 20.1 % and 34.9 %, 15.0 % and 27.7 %, respectively. That is, storage of intact stressed seeds in metal containers for 9 months with limited contact with external influences (air exchange, light, atmospheric moisture) was accompanied by an increase in the effect of growth stimulation in intact seeds, which was most vivid in the variant with $\gamma$-irradiated ones. The mass of sprouts and embryonic roots in intact seeds did not change significantly.

**Table 2.** The intraspecific effect of stressed seeds of spring wheat on the initial growth processes of intact seeds depending on storage conditions.

| Variant | Storage conditions | Months of exposure |
|---------|--------------------|--------------------|
|         |                    | **Sprout** | **Embryonic root** |
|         |                    | Length, mm | Weight, g | Length, mm | Weight, g | Number, pcs |
| K – separate storage of seeds | Cloth bags | 22.0±1.5 | 2.70±0.04 | 35.8±2.2 | 3.38±0.09 | 3.5±0.03 |
| Intact with $\gamma$-irradiated | | 25.6±1.4 | 2.91±0.05 | 39.4±2.4 | 3.14±0.09 | 3.5±0.02 |
| Intact with damaged | | 29.4±0.9* | 3.05±0.05 | 43.6±1.4* | 3.70±0.05 | 3.7±0.02 |
| Intact with damaged | | 9.8 ±1.3* | 0.92±0.06* | 27.6±1.9* | 1.15±0.06* | 3.3±0.01 |
| K – separate storage of seeds | Metal containers | 25.6±0.8 | 2.86±0.09 | 36.6±1.8 | 3.41±0.05 | 3.6±0.02 |
| Intact with $\gamma$-irradiated | | 14.1±1.5* | 1.05±0.07* | 31.5±1.8* | 1.60±0.07* | 3.4±0.02 |
| Intact with damaged | | 23.8±1.1 | 2.68±0.07 | 36.1±1.9 | 3.40±0.07 | 3.7±0.02 |
| Intact with damaged | | 22.9±1.1 | 2.72±0.04 | 35.8±2.5 | 3.23±0.05 | 3.6±0.03 |
| Intact with $\gamma$-irradiated | | 27.5±1.2* | 2.74±0.06 | 40.2±2.0* | 3.67±0.06 | 3.8±0.03 |
| Intact with damaged | | 28.7±1.2* | 2.95±0.09 | 48.3±2.1* | 3.61±0.05 | 3.8±0.02 |
| Intact with damaged | | 25.3±1.3 | 2.69±0.07 | 38.4±2.1 | 3.49±0.06 | 3.8±0.01 |
| Intact with damaged | | 27.5±0.9* | 2.81±0.08 | 45.7±2.0* | 3.46±0.09 | 3.8±0.01 |

* Differences with the control are statistically significant when $P<0.05$

The laboratory germination of intact seeds of the experimental variants did not differ significantly from the control (Table 3). Whereas the storage of stressful seeds in cloth bags for up to 9 months was accompanied by deterioration in the sowing quality of the seeds, as a result of which they became unsuitable for sowing. Considering that the laboratory germination of intact seeds in the variant with damaged ones decreased to 80 % and with $\gamma$-irradiated ones it fell to 65.2 %, such seeds do not meet the requirements of the sowing standard.

**Table 3.** The effect of stressed spring wheat seeds on sowing qualities of intact ones depending on storage conditions.

| Variant | Storage conditions | Months of exposure |
|---------|--------------------|--------------------|
|         |                    | **Germinating energy, %** | **Laboratory germination, %** | **Germinating energy, %** | **Laboratory germination, %** |
| K – separate storage of seeds | Cloth bags | 63.7±2.7 | 90.5±3.0 | 68.4±3.0 | 91.4±3.2 |
| Intact with | | 75.4±2.0* | 92.1±3.1 | 41.6±2.4* | 65.2±1.9* |
Based on the assumption that the remote effect on intact seeds is caused by gaseous physiologically active compounds released by stressed seeds, an experiment was carried out to eliminate (wash out) these substances by changing air exchange rate (Table 4). As a result, the germinating energy and laboratory germination of intact seeds with passive aeration after 6 months were significantly lower than the control. With an increase in the duration of exposure to 9 months, the deterioration of the sowing qualities of seeds continued. The germinating energy and laboratory germination in variants with γ-irradiated and damaged seeds became lower than the control by 29.6 % and 23.9 %, 17.8 % and 13.15 %, respectively. The active aeration during the entire storage period excluded the inhibitory effect of γ-irradiated and damaged seeds on intact ones, providing germinating energy and laboratory germination at the control level.

No less convincing evidence of the volatile nature of physiologically active substances released by stressed seeds is the use in the process of their exposure for isolation from intact substances with different properties. The experiments did not establish the remote effect of stressed seeds in glass flasks and metal containers on intact ones. The parameters of germinating energy and laboratory germination in the experimental variants did not significantly differ from the control during 9 months of storage. Whereas after 9 months of storage of intact and stressed seeds in cloth bags, the germinating energy and laboratory germination of the first ones were lower than the control by 42.7 % and 26.6 %, respectively.

Table 4. The effect of stressed spring wheat seeds on sowing qualities of intact ones depending on the air exchange conditions.

| Variant                  | Aeration mode when storage | Months of exposure 1/6/9 | Germinating energy, % | Laboratory germination, % |
|--------------------------|-----------------------------|---------------------------|------------------------|---------------------------|
| K – separate storage of seeds | Passive                     | 68.5±2.1                 | 92.0±2.4               |
|                          |                             | 71.4±2.7                 | 93.6±1.9               |
|                          |                             | 70.8±2.6                 | 92.5±1.8               |
| Intact with γ-irradiated |                             | 76.7±2.3*                | 93.5±1.9               |
|                          |                             | 52.6±2.0*                | 78.2±2.1*              |
|                          |                             | 41.2±1.7                 | 68.6±2.5*              |
| Intact with damaged      |                             | 73.1±1.8                 | 93.2±2.2               |
|                          |                             | 60.3±1.9                 | 81.0±3.1*              |
|                          |                             | 53.0±2.0*                | 79.4±3.1*              |
| K – separate storage of seeds | Active                     | 71.3±2.5                 | 91.1±1.7               |
|                          |                             | 73.2±2.0                 | 91.5±1.3               |
|                          |                             | 72.9±2.7                 | 92.3±2.6               |
| Intact with γ-irradiated |                             | 70.6±1.7                 | 92.7±2.6               |
|                          |                             | 69.4±2.8                 | 86.3±2.6               |
|                          |                             | 67.8±2.4                 | 90.5±1.9               |
| Intact with damaged      |                             | 72.4±2.0                 | 92.3±1.4               |
|                          |                             | 70.3±2.2                 | 88.7±2.8               |
|                          |                             | 70.6±1.9                 | 91.7±2.3               |

* differences with the control are statistically significant when P<0.05
Studies showed the leading modifying role of the distance between hyperthermic and intact seeds on the effect of the first ones on the latter ones. In addition, the results of the experiments complemented the hypothesis about the volatile properties of the agent, which determines the remote effect of damaged seeds on intact ones. Only the variant with an exposure of 1 month at a distance of 1 cm had a weakly expressed tendency of the stimulating effect of hyperthermic seeds on the sowing qualities of intact seeds. There was an increase in germinating energy by 7 %. A pronounced inhibition in intact seeds was observed at a distance of 1-10 cm from hyperthermic seeds after 6 months of storage and continued with an increase in inhibition during the entire subsequent exposure time.

When increasing the distance between hyperthermic and intact seeds up to 50 cm, there was a consistent weakening of the oppressive effect of the first on the latter ones. In this variant, a significant deterioration in sowing qualities occurred after 12 months, when the germinating energy and the laboratory germination of the intact seeds were 67 % and 88 %, respectively, while in the control, these indicators were 77 % and 95 %.

When increasing the distance between hyperthermic and intact seeds to 1 m or more, the effect of the first on the latter ones was not established. The germinating energy and the laboratory germination of intact seeds were at the control level and were equal to 75 % and 94 %, respectively.

The identification of physiologically active volatile compounds in the inter-grain air environment of seeds showed that irradiated and damaged seeds were characterized by an increased content of ethylene, the concentration of which increased with an increase in the storage period from 1 to 9 months. At the same time, the highest concentration of ethylene was after 9 months of storage in the inter-grain air environment in irradiated seeds and ranged from 0.083 to 0.197 mg/m³. It was from 0.035 to 0.126 mg/m³ in damaged ones, respectively. In the control, during the entire storage period, the ethylene concentration was at the level of 0.016-0.019 mg/m³. Consequently, during the storage of stressed seeds, the concentration of ethylene phytohormone the inter-grain air environment dynamically increased due to metabolic processes. It exceeded the control in the irradiated seeds by more than 10 times and in the damaged ones by 6.5 times, which caused a change in the intensity and direction of metabolic processes in air-dry intact seeds. Weakly expressed stimulation of the initial growth processes in intact seeds was observed after storage for 1 month. A sharp suppression of the initial growth processes, a decrease in the germinating energy and laboratory germination of spring wheat seeds occurred with an increase in the storage time to 9 months with a simultaneous increase in the concentration of stress ethylene.

4. Conclusion
A previously unknown property of air-dry stressed spring wheat seeds has been experimentally established. It induces physiological modifications in intact seeds, depending on the conditions and duration of their exposure to stress. Experiments confirm that stress ethylene is one of the agents providing communication between stressed and intact seeds. With an increase in the duration of exposure from 1 to 9-12 months in intact seeds, there is an increase in the inhibition of the initial growth processes and a sharp deterioration in the sowing qualities of spring wheat seeds. The effectiveness of the influence of stressed seeds on intact seeds is modified by the duration of exposure, aeration or the degree of elimination of the inter-grain air environment, the properties of insulating materials and the distance between seeds.

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