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THE EFFECT OF ENVIRONMENTAL FACTORS ON SEED DORMANCY AND PREHARVEST SPROUTING OF WINTER TRITICALE

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Abstract: the effect of temperature and soil moisture deficiency on seed dormancy is studied in a greenhouse experiment with two triticale lines having various level of prehan'est sprouting resistance. The influence of treatment with both chlormequat chloride (chlorocholine chloride) and urea on seed dormancy after plant anthesis, has been investigated besides. It has been discovered that grain ripened at 15°Cpossesses more prolonged dormancy period than grain ripened at 25°C. Treatment with both chlormequat chloride and urea induces deeper dormancy only in triticale line having frank dormancy period in a nontreated variant (without any treatment).

Key words: triticale, seed dormancy sprouting in spike, temperature, soil moisture, chlormequat chloride, urea.

Preharvest sprouting is a serious problem of triticale in most regions of cultivation. In the process of germination hydrolytic enzymes degrade starch and storage proteins of the grain, which determine the quality of farinaceous products. Respiration during germination results in dry weight loss. The sowing value of the grain germinated in spike rapidly decreases during storage [17]. The vitality of the sprouted grain lasts about a month if it does not perish from drying at once and does not damage during harvesting, cleaning and storage [2]. The feeding value of the sprouted grain is not reduced significantly, but feeding on it is dangerous due to accompanying microflora development, which produces toxins [13].

Precocious grain sprouting is restrained mainly by seed dormancy [24, 7]. Seed dormancy of wheat and triticale is classified as non-deep physiological dormancy. Biochemical processes in seed with non-deep physiological dormancy are under the control of the balance of phytohormones - gibberellins and abscisic acid [10]. Abscisic acid (ABA) restrains germination and promotes the production of substances that let the embryo cells to survive in desiccated condition. Gibberellins, on the contrary, stimulate the processes of germination: cell growth, hydrolytic enzymes synthesis and etc. When seeds are in dormant state, abscisic acid synthesis and degradation of active forms of gibberellins is prevailing. When the shift to germination happens the opposite situation can be observed - the synthesis of gibberellins and catabolism of ABA. Environmental factors influence seed dormancy mainly through alteration of phytohormone balance or changing the sensitivity of target tissues to them [11].

The plant breeding is considered to be the main method of resolving the problem of preharvest sprouting [2]. Cultural practices that can prevent this unfavorable phenomenon are being searched at the same time. The experiments with sorghum *(Sorghum bicolor)* [21] and maize *(Zea mays)* [25, 26] have revealed that suppression of gibberellin synthesis at a period of seed development prevents their precocious germination in mutant lines with low ABA production or sensitivity.

The effect of mineral nutrient supply on seed dormancy is nearly unstudied to date. It was shown that the lack of molybdenum weakens the preharvest sprouting resistance in wheat. Foliar application of this microelement at the stage of six leaves and at flag leaf emergence makes the seed dormancy deeper, protein and nitrate content in the grain being increased [9]. Nevertheless, it is known that nitrates can release the seed dormancy of mature wheat seeds and seeds of other plants [5, 8].

It is common knowledge that foliar application of urea favors wheat grain quality increasing the gluten content [27]. But the effect of such an additional fertilizing on seed dormancy of grain crops has not been studied.

It is known about the effect of temperature during seed ripening on seed dormancy of wheat. Seeds formed and ripened at low temperature possess more prolonged period of afterripening and corresponding deeper dormancy at harvest time [18]. The effect of soil water deficiency together with the effect of temperature on seed dormancy was studied in common *(Triticum aestivum* L.) wheat [6]. The plants grown under condition of drought in combination with high air temperature as well as under condition of sufficient water supply in combination with low temperature produced the grain with well-expressed seed dormancy and sensitivity to ABA. However in a variety susceptible to preharvest sprouting low temperature during a period of ripening, on the contrary, weakened seed dormancy.

There is some information concerning the connection between the quantity of atmospheric precipitations and preharvest sprouting in the field. Larger amounts of precipitations under condition of relatively low temperature during grain formation and filling, as a rule, result in deeper seed dormancy at harvest [3, 19,22]. At the same time, there are exclusions from a rule caused by the genotypic peculiarities of separate lines and varieties.

The specific investigations concerning the effect of environment during vegetation period on seed dormancy of triticale have not been conducted yet. The information on the environmental control of seed dormancy of triticale is important for breeding for preharvest sprouting resistance, in particular - for adequate interpretation of the variety trial data.

This work was conducted on purpose of investigation the effect of environmental factors on seed dormancy of triticale and determination the opportunity of regulation of seed dormancy for increasing their sprouting tolerance.

The tasks were as follows: 1) to determine the pattern of the effect of temperature and drought on seed dormancy of triticale during grain formation and filling; 2) to check the opportunity of regulation the seed dormancy using the gibberellic acid synthesis inhibitor - chlormequat chloride; 3) to estimate the effect of urea foliar application on seed dormancy of triticale.

Materials and methods

Abbreviations: TC - total moisture capacity, ABA - abscisic acid, GI - germination index, CCC - chlormequat chloride (chlorocholine chloride), ant. - anthesis, m.r. - milky ripeness.

Two winter triticale *(Triticosecale* Wittm.) lines were used. The line 313 was created by self-fertilization of individual spikes of triticale sample 21759/97, which possesses 2D(2R) chromosome substitution [1]. The line 435 was produced in the same way from semi-dwarf Polish cultivar Fidelio. Following testing the material at Plant-breeding station named after PI. Lisitsin in 2009-2010 the line 313 revealed it as resistant to preharvest sprouting (0% germinated grains in spike on the 8-th day of moist chamber test), while the line 435 was the most susceptible (14% of germinated grains in spike).

The research work was performed by means of pot experiments in greenhouses and climatic chambers of Plant physiology laboratory in Russian State Agrarian University named after Timiryazev. The mixture of upper layer of sod-podzol cultivated loam soil with neutralized high-moor peat in equal proportion by volume was used as a substrate. The seeds were sown in 2,5-liter plastic pots. After seedling emergence 4 plants were left in each pot. The vernalization was conducted at tillering stage in a refrigerated room at 4°C under constant lighting during 48 days. From seedling emergence up to the tillering and after vernalization before flowering all the plants were grown in a greenhouse under constant lighting. After flowering the pots were separated for use in two experiments.

Three-factor designed experiment for estimation of temperature and drought effect on seed dormancy was done using climatic chambers. The first factor was temperature regime (variants: 25°C day, 15°C night; 15°C day, 13°C night), the second factor was watering rate (up to 70% TC 3-4 times a week; up to 40% TC 2-3 times a week). The third factor was the genotype of triticale line. Lighting was given 18 hours a day.

Two-factorial experiment for estimation the effect of CCC and foliar urea application was performed in a greenhouse. The first factor was the treatment, the second - the genotype of the line. In one of the variants spraying the plants with CCC solution was done immediately after anthesis, in another variant - at milky ripeness of the grain. CCC was applied at a concentration of 1 g/1 as water solution. In the third variant spraying the plans with 10% (w/w) solution of urea was performed immediately after anthesis. Control plants were sprayed with pure water.

All the experiments were done in four replicates (4 pots per variant).

After the spike yellowing (for each pot the time of color change was determined separately) the plants left in their pots without watering were dried in a room of the laboratory for two weeks. The grain was hand-threshed and put into the freezer at -20°C to stop the afterripening [4], where it was stored until the end of harvesting.

The germination index (GI) based on the dynamics of the grain germination in Petri dishes during 14 days was used as a measurement of dormancy level [23]. Index is calculated according to the formula: $GI = \frac{14 \times n_1 + 13 \times n_2 + \ldots + 1 \times n_{14}}{D \times N}$, where n1, n2, … nl4 - numbers on newly germinated grains on the first, the second and subsequent days; N - the total number of viable grains; D - the total number of days; 14, 13, ..., 1 - the weights given for sprouted grains on the first, the second and subsequent days respectively. The GI varies between 0 and 1, the deeper the dormancy the lower the index.

The grain collected from main spikes of plants in a separate pot was germinated in a separate Petri dish. Sixty grains were laid crease down on double layer of filter paper wetted with distilled water in each dish. The Petri dishes were placed in a thermostat without lighting at 20°C. The germinated grains were removed from after counting each day. The filter paper was moisturized additionally as required. On the 15th day the Petri dishes with non-geminated grains were placed into a refrigerator at 4°C to break the dormancy. Three days later the residual viable grains were counted, which did not germinate during two previous weeks of testing.

The resulting data were processed using the analysis of variance analysis of covariance.

Results and discussion

In an experiment in which the effect of climatic factors on seed dormancy of triticale was studied, at a day temperature of 25°C and optimal (70% TC) soil moisture the line 313 produced grain with more prolonged period of dormancy, then the line 435 (fig. 1). However due to considerable variation of GI from pot to pot the differences between lines in this experiment were not statistically significant. At the same time the difference of seed dormancy level between these lines grown under natural weather conditions may be taken proved by aggregate results of number of previous trials.

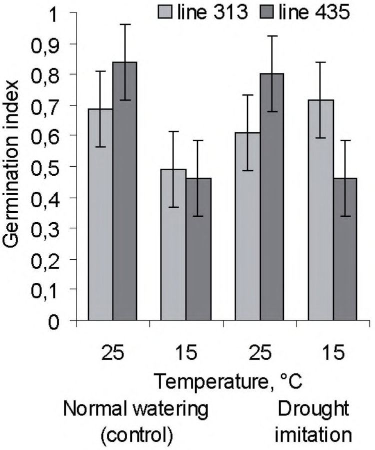

Fig. 1. The effect of temperature and watering rate on seed dormancy of triticale. Vertical bars denote 0,95 confidence intervals

Multifactor analysis of variance has shown the significance of the temperature effect on seed dormancy and the significance of the genotype X temperature interaction. The soil moisture regime on average of two temperature variants did not substantially influence the seed dormancy. However, judging by graphics of the germination dynamics, the imitation of soil drought under high day temperature tended to enhance seed dormancy, while under low temperature - to weaken it (fig 2). This observation is in an agreement with the results of research conducted with common wheat by Biddulph et al. [6]. Other possible interactions of factors in our experiment happened to be statistically insignificant.

By viewing the partial differences it was revealed that the triticale line 435 independently on soil watering regime responded the exposure to low day temperature

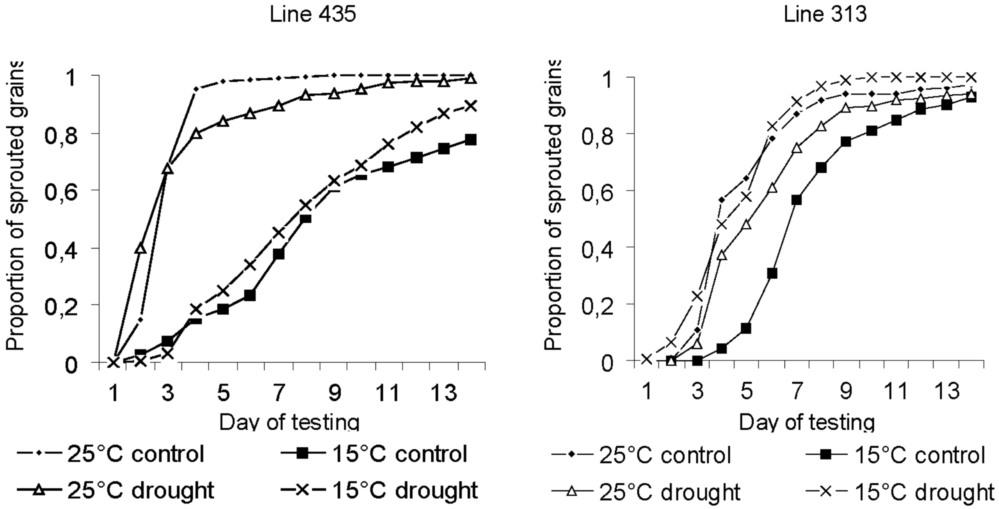

Fig. 2. The dynamics of grain germination of triticale lines 313 and 435 grown under different temperature conditions (day temperature of 15 and 25°C) and under different watering rate (control - up to 70% TC, drought - up to 40% TC) during the period after anthesis until ripening

(15°C) at a period of grain ripening by appearing of the we 11-expressed period of dormancy after harvest, which was nearly absent under natural weather conditions and under condition of 25°C in the experiment. Similar results were obtained by Reddy et al. [18] in an experiment with soft wheat and also in a row of related experiments. The molecular mechanism of this phenomenon is unknown. However the data obtained by Biddulph et al. suggest that deeper seed dormancy provoked by low temperature at ripening is partially due to increased embryo sensitivity to abscisic acid.

The difference of our experiment from previous analogous works was the use of alternating temperature during 24 hours, the plants being exposed to temperature of 15°C at both temperature regimes of experiment. In the first variant the plants were exposed to this temperature during 6 hours in darkness while in the second variant - during 18 hours under lighting. Comparison of our data with data of experiments conducted under controlled conditions with constant temperature and under natural conditions with alternating temperature suggests that seed dormancy depends mainly on maximum day temperature. That is why an action of high temperature during the day, that weakens the dormancy of ripening seeds, is not compensated by exposure to cool temperature at night.

The GI of seeds of the line 313 grown against the background of normal watering rate was slightly decreased, while against the background of imitated drought was insignificantly increased by the action of low temperature. Thus, judging by the results of our and similar works [6, 18, 19], the seed dormancy of resistant to preharvest sprouting varieties is more homeostatic and less sensitive to the environmental changes.

The deepest dormancy of each triticale line was observed in a variant with day temperature of 15°C and normal watering rate, while the shallowest dormancy was observed in seeds grown at 25° C and under normal watering similarly.

There are some contradictions between the investigators regarding the temperature effect on seed dormancy of wheat and triticale. To resolve this problem let us review the publications in order to make a concept of seed response changes to this factor in the process of ripening. It is likely that the ability of seeds to fall into deeper dormancy under influence of low temperature and high humidity keeps almost until full ripeness. Skinnes and Sorrells [20] determined, that the exposure to low temperature (4°C) during 16 days of freshly harvested grain of red-grained wheat provided the moisture content of 25% strengthens its dormancy. However the grain of white-grained wheat under the same conditions, on the contrary, loses the dormancy.

At moisture content higher than 25% the response of the grain to temperature changes [15]. It is known that freshly harvested imbibed in water wheat and triticale seeds under condition of low positive temperatures (5-15°C) rapidly release from a state of dormancy, while under high temperature (25°C and more) their germination is delayed [18, 19, 22,23]. In a course of afterripening the sensitivity of seeds changes once again. Afterripened seeds were reported to have higher GI under higher temperature [12].

As a result of another experiment in which the effect of CCC and urea foliar application on seed dormancy was studied, two-factorial analysis of variance revealed the reliable difference of GI between the lines tested. As was expected, the line 313 possessed deeper seed dormancy than the line 435. Genotype X treatment interaction proved to be significant, which indicate the dissimilar response of different triticale genotypes to CCC and urea. However as a result of high data variability the analysis of variance didn't show significant effect of treatments by themselves. In this connection analysis of covariance was conducted for GI using spikelet fertility as a covariant for each line separately (fig. 3).

As shown in the picture 3, urea foliar fertilization affected the line 313 beneficially, the seed dormancy being prolonged. That can be explained by protein accumulation in seeds that was induced by more nitrogen availability during grain filling [14, 27]. While,

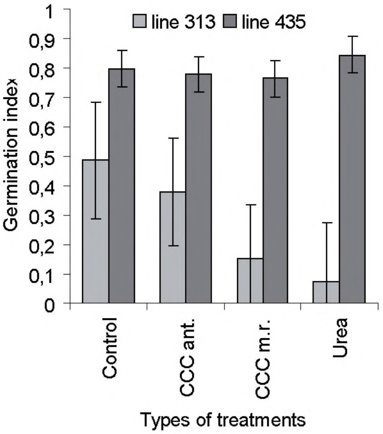

Fig. 3. The effect of treatments of triticale plants with CCC and urea at anthesis and CCC at milky ripeness of the grain on seed dormancy

it is known that proteins can play a role of free radical scavengers, the latter are known to delay the seed dormancy release [11]. Some other explanations can be proposed also.

Spraying the plants of the line 313 with CCC solution enhanced the seed dormancy to a lesser degree than urea. The later CCC was applied the deeper dormancy was induced. More substantial effect of CCC when applied later was caused to all appearance by rapid enough degradation of this substance in a plant, so being applied early it didn't remain in enough for effect quantity in ripened grain.

In an instance with the line 435 treatments with CCC and urea had nearly no effect on seed dormancy (fig. 4). From the latter one may conclude that in a balance of gibberellins and ABA controlling the seed dormancy, the latter substance plays a key role. Nonetheless the sensitivity of embryo tissues to both phytohormones is important.

As a result of our observations there was also noticed that foliar fertilization with urea delayed grain ripening of both lines for two days. The period of time from the beginning of anthesis to wax ripeness of the grain for the line 313 lasted 44 days while for the line 435 - 46 days in control variants. The treatment with CCC didn't influence the period of vegetation significantly. The effect of treatments on the yield structure elements was insignificant.

The imitation of drought shortened the period of vegetation for 6 days on average, decreased 1000-kernel weight and grain plumpness. The temperature regime had no significant effect on the time period from anthesis to ripening.

In a conclusion, one could say that seed dormancy enhancement using plant growth regulators can be put into practice not for all triticale varieties. The principal way of

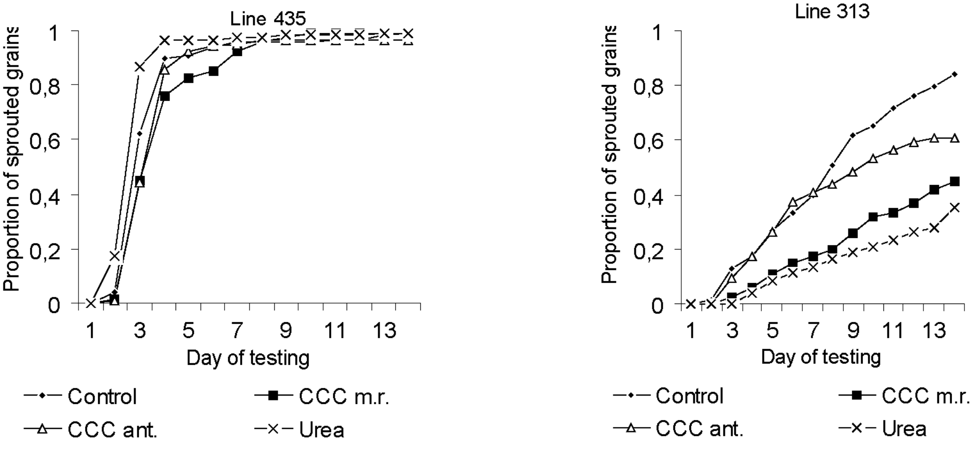

Fig. 4. The dynamics of seed germination of triticale lines 313 and 435, plants treated with CCC and urea immediately after anthesis and with CCC at milky ripeness of the grain in comparison with control (without treatment)

preharvest sprouting prevention should be the plant breeding - synthesis and selection of genotypes with proper seed dormancy level steadily expressed under various environmental conditions.

Resistance to preharvest sprouting based on seed dormancy is an important but not the sole condition of obtaining high quality of the grain. The grain quality depends mainly on initial condition of starch and storage proteins, which forms under the absence of any damaging factors. The quality of the grain may be also reduced as a result of so called "latent sprouting” which is an embryo-independent activation of aleuron layer cells in endosperm accompanied by hydrolytic enzymes release [16].

Conclusions

1. The genotype of the variety is decisive in preharvest sprouting resistance of triticale.
2. Improved nitrogen nutrition and treatment of plants with chlormequat chloride after anthesis can enhance the seed dormancy of triticale.
3. The seed dormancy of triticale depends in grater extent on maximum than on minimum day temperatures in a period from anthesis to harvest. High temperatures more frequently result in weakening of triticale seed dormancy.
4. The effect of soil moisture level on seed dormancy varies with the temperature. At a temperature of 25°C the drought favors high resistance to preharvest sprouting, while at 15°C the same does higher watering rate or atmospheric precipitations.

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ВЛИЯНИЕ ФАКТОРОВ ОКРУЖАЮЩЕЙ СРЕДЫ НА ПОКОЙ СЕМЯН И ПРОРАСТАНИЕ ЗЕРНА В КОЛОСЕ ОЗИМОЙ ТРИТИКАЛЕ

Аннотация: влияние температуры и дефицита влаги в почве на покой семян изучено в вегетационном опыте с использованием двух линий тритикале, имеющих различный уровень устойчивости к предуборочному прорастанию зерна в колосе. Также изучено влияние на покой семян обработок растений хлормекватхлоридом (хлорхолинхлоридом) и мочевиной после цветения. Обнаружено, что зерно, созревшее при +15°С, обладает более продолжительным периодом покоя, чем зерно, созревшее при +25°С. Обработки растений как хлормекватхлоридом, так и мочевиной способствовали появлению более глубокого покоя семян только у линии тритикале, обладавшей заметным покоем в контрольном варианте (без обработки).

Ключевые слова: тритикале, покой семян, прорастание в колосе, температура, почвенная влага, хлормекватхлорид, мочевина.

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