Differences in the spring wheat varieties response to herbicide as a stressor

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Abstract. When assessing the sensitivity level, some difference was found, probably genetically determined, in the response of plants of different varieties to the herbicide as a stress factor. One of the analyzed varieties shows a decrease in growth rate of axial organs against the background of lower mitotic activity of apical meristem and increased frequency of cell division abnormalities associated with increased herbicide dosage. In another variety, there were no significant dependencies in growth and development with this factor. The most sensitive was the average total length of the seedling roots. In a more sensitive variety, in the absence of a stressor, its level of variability was initially lower and increased at a double dose of herbicide. In the same variety, there was less connectivity of the parameters studied, which, however, increased with the action of the stress factor.

In modern agriculture, the use of pesticides, including herbicides, on crops remains a popular and quite effective method of preserving the crop of cultivated plants [1]. According to the mechanisms of their effects on harmful organisms, they can be unsafe for the cultures themselves [2].

Modern preparations have a certain selectivity level, but the negative effects on crops cannot be completely excluded. Besides the obvious crop losses, there may also be hidden ones when the crop increase from weed elimination is lower than possible.

When assessing the pesticides effectiveness and safety, in particular herbicides, it is mandatory to determine whether this preparation will be toxic to the protected crop.

Plant resistance to herbicide is ensured by various mechanisms: barrier properties of the covers, the cytoplasm ability to detoxify, biochemical or physiological immunity to the active substance, which is primarily due to the genotype, including varietal properties. There are a number of papers showing different degrees of resistance of wheat, barley, maize and some other crops to negative effects of herbicides [3-6].

Therefore, it is necessary to take into account the possibility of varietal differences in the response to a particular preparation. Since up to a dozen or more varieties of each crop are zoned and used in each region, it becomes technically and economically difficult to identify these varietal features in relation to each chemical defense preparation.

Thus, it seems appropriate to develop rapid methods for assessing the crops and their varieties sensitivity using a complex of fairly easily identifiable features. Based on these methods, potentially sensitive varieties can initially be selected in vitro for subsequent field testing.
The aim of the study was to determine the level of sensitivity (resistance) of spring wheat varieties to herbicide in vitro.

To do this, the following tasks were set: to study the individual varietal reactions of seeds of the studied varieties to various concentrations of the preparation in aqueous solution by germination energy and germination; assess the effect of Banvel on plant growth and development at the earliest stages of ontogenesis; to identify cytological features of the sprout rootlets meristem manifested by the herbicide action.

In this research, an attempt was made to identify a complex of sensitivity signs to the herbicide of two varieties of spring wheat of different types using the example of the Banvel herbicide (Syngenta, Switzerland), created on the basis of dicamba, which is a synthetic auxin. Banvel is a selective systemic herbicide for use against annual and some perennial broad-leaved weeds on cereals and corn. There are known cases where this herbicide, when used in the recommended doses on cereal crops, showed phytotoxic action, which resulted in a decrease in plant height and yield [6].

The research used seeds of two varieties of spring medium-ripe wheat Minusa (a variety of erythrosernum) and Zemlyachka of Siberia (a variety of lutescens), samples of which were provided by Krasnoyarsk Research Institute of Agriculture.

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For the test, one sample of each wheat variety of 100 seeds was taken, three repetitions in each test variant. At assessment of direct influence of herbicide on test seeds of varieties in vitro we used its water solutions in the concentration corresponding to a half working dose (0.5WD), a single working dose (1WD) and a double working dose (2WD). Seed germination was carried out in a rolled crop under normal conditions. Control seeds were germinated in tap water.

In accordance with State Standard (StSt) 12038-84 "Seeds of crops. Germination methods" [7] we assessed germination energy and germination. Besides, in 30 seedlings in each repetition, the number of roots was counted to account for plant growth and development, the sprout length was measured, and the total root length was assessed. For cytological evaluation, 50 seeds of each variety were used separately, solutions according to the above concentrations were added to Petri dishes. Settled tap water was also used as a control. Seed germination was carried out in a thermostat at a temperature of 22\(^{0}\) C, the exposure time was 72 hours. At the end of this time, the tips of the rootlets were cut off in 30 germinated seeds and fixed in acetic alcohol (3:1). Additionally, 5 germinated seeds were treated with colchicine 0.01% solution for two hours in each test variant. The tips of the rootlets were fixed similarly. Prior to analysis, the fixed material was stored in a refrigerator.

Semi-permanent pressurized preparations stained with 2% acetoorsein and encased in glycerol were prepared to analyze the cytological features of the sprout rootlets meristem. The analysis took into account the total number of cells in the division zone, the number of cells at different stages of division; in the biomaterial after exposure to colchicine - the number of cells with abnormalities of the interphase nucleus and plasmalemma, with division disorders (bi-nuclearity, chromosome lag, etc.).

The obtained data were subjected to standard statistical processing procedures; the validity of the differences was evaluated by single-factor variance analysis of the MS Excel analysis package.

When analyzing the effect of the herbicide on the ability to germinate seeds, no statistically significant effect was found with respect to the germination energy and seeds germination of both varieties, which seems quite logical in view of the preparation characteristics.

Prolonged exposure of experimental solutions to seedlings of tested varieties revealed the following patterns of their reaction to herbicide. The results are shown in table 1.

The average roots number in Minusa wheat seedlings compared to the control decreased slightly with an increase in the herbicide concentration, and in the 2WD version they become 8% truly less. In seedlings of Zemlyachka of Siberia variety, a true decrease in this indicator occurred only in experiment with a middle concentration.
of the varieties Minusa and Zemlyachka of Siberia, however, the organism was a decrease in the sprout length compared to the control. The double dose contributed to a 40% - 11.9%, with a double dose - already 22%. However, the effect of this factor on the sprout length was several times lower than on the total root length.

The herbicide tested did not have a notable effect on this indicator of wheat seedlings of the Zemlyachka of Siberia variety, only in the concentration of 1WD there was a decrease in the sprout length compared to the control, but this difference was statistically inaccurate.

Correlation analysis of the tested marks showed that at the dosage corresponding to half of the herbicide working dose, in both varieties the link tightness between the parameters increases, and most significantly in plants of the Minusa variety due to the initially low level of their correlation. An increase in dose led to a change in the balance between the marks in both varieties, however, the organism integrity in the Zemlyachka of Siberia variety was less disturbed. This may be due to the genetic determinacy of plant resistance to herbicide [8].

It is known that auxin at high concentrations sharply inhibits root elongation. This is mainly due to inhibition of cell division, apparently caused by a violation of the relationship between total auxin and endogenous cytokinin.

The main root growth inhibition in Minusa variety seedlings was quite naturally accompanied by a decrease in the mitotic activity of the apical meristem of the rootlets. Already at minimum and average concentrations, the mitotic index decreased by almost 50% and with a further increase in Banvel concentration, the situation worsened (figure 1).

In Zemlyachka of Siberia variety, a lower concentration of herbicide caused the activation of apex cell division by 1.5 times. At an average herbicide concentration corresponding to the working dose, the mitotic index was approximately equal to that one in the control. The double dose contributed to a 40% reduction in this parameter compared to the control.

### Table 1. Wheat seedlings development indicators of the varieties Minusa and Zemlyachka of Siberia, depending on the Banvel herbicide concentration.

| Experiment variant | Minusa | Zemlyachka of Siberia |
|--------------------|--------|-----------------------|
|                    | X ± Mx | σ        | Cv, % | X ± Mx | σ        | Cv, % |
| 0                  | 4.9±0.05 | 0.51   | 10.42 | 4.5±0.09 | 0.86   | 19.3  |
| 0.5WD              | 4.8±0.06 | 0.67   | 12.73 | 4.4±0.08 | 0.74   | 16.97 |
| 1WD                | 4.7±0.06 | 0.60   | 12.71 | 4.0±0.09 | 0.82   | 20.63 |
| 2WD                | 4.5±0.07 | 0.66   | 14.54 | 4.5±0.08 | 0.75   | 16.6  |
|                    | Average total roots length (cm) |         |        |         |        |       |
| 0                  | 43.4±1.09 | 10.41 | 23.98 | 25.6±1.44 | 13.69 | 53.6  |
| 0.5WD              | 41.1±1.01 | 9.59  | 23.35 | 30.0±1.38 | 13.05 | 43.5  |
| 1WD                | 39.5±0.45 | 9.01  | 22.79 | 22.0±1.27 | 12.01 | 54.6  |
| 2WD                | 24.5±1.09 | 10.33 | 42.14 | 30.1±1.04 | 9.84  | 32.7  |
|                    | Average sprout length (cm) |         |        |         |        |       |
| 0                  | 11.8±0.36 | 3.38  | 28.78 | 8.0±0.19 | 1.81  | 22.76 |
| 0.5WD              | 10.8±0.29 | 2.83  | 26.14 | 8.5±0.18 | 1.67  | 19.56 |
| 1WD                | 10.4±0.23 | 2.19  | 21.08 | 7.4±0.21 | 1.98  | 26.87 |
| 2WD                | 9.2±0.37  | 3.49  | 37.72 | 8.0±0.23 | 2.22  | 27.86 |
Figure 1. Mitotic activity of the wheat seedlings rootlets meristem of the varieties Minusa and Zemlyachka of Siberia depending on the concentration of Banvel herbicide.

Cell abnormalities frequency analysis of the Minusa seedling rootlets meristem showed a linear direct dependence of this indicator on Banvel concentration. Thus, no cell abnormalities were detected in the control samples. In preparations prepared from herbicide-treated rootlets at maximum concentration, the abnormalities frequency increased to 6.8 ‰ (figure 2).

Figure 2. Abnormalities frequency dependence among cells of the wheat seedlings rootlets meristem of the varieties Minusa and Zemlyachka of Siberia on the Banvel herbicide concentration.

In Zemlyachka of Siberia variety, the cell abnormalities proportion was relatively low and practically independent on the herbicide concentration.

Characteristically, most abnormalities are associated with the membrane apparatus disorders of cells. This is the increased number of cells destroyed during making of the preparation and shape distortion of the nucleus, apparently associated with disorders in the nuclear envelope formation, its ruptures and the bi-nuclearity of the cells arising from incomplete cytokinesis, that is, the lack of restoration of the plasma membrane. Mitosis pathologies were extremely rare.

Some difference was found, probably genetically determined, in the reaction of plants of different varieties to the herbicide as a stress factor. One of the analyzed varieties shows a decrease in growth rate of axial organs against the background of lower mitotic activity of apical meristem and increased cell division abnormalities frequency associated with increased herbicide dosage. In another variety, there were no significant dependencies in growth and development with this factor.

The most sensitive was the average total length of the seedling roots. In the experimental versions, this figure was almost halved at maximum concentration. Interestingly, in a more sensitive variety in
the absence of a stressor, its level of variability was initially lower and increased at a double herbicide
dose.

In the same variety, there was less connectivity of the parameters studied, which, however, increased
with the action of the stress factor.

The found regularities require further research, which seems to us very promising for to create a
system of indicators for diagnosing the resistivity of cultivated plant varieties to used herbicides.

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