

The Impact of Natrium Humate on Anatomical Organization, Yield and Content of Heavy Metals in Spring Wheat

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Abstract: The impact of natrium humate on peculiarities of spring wheat anatomical organization was studied and the connection between the wheat morpho-anatomical characteristics and productivity was revealed. It was determined the positive impact of natrium humate on reduction of heavy metals (Cd, Pb, Cu, Zn) in wheat grain, the dependence of grain productivity on the methods and doses of natrium humate application.

Key words: Natrium Humate • Spring Wheat • Morpho-anatomical Characteristics • Heavy Metals

INTRODUCTION

At the present time, the application of natrium humate, obtained from brown coal as a plant growth regulator, is one of the methods to increase the yield and quality of the obtained products [1].

Being absorbed by plants at the cell level, natrium humate fulfills the following functions: increases the seed productivity, speeds up the plant general growth and root system development. Physiological activity of humine substances is explained by their impact on bioenergetic system of the organism. The increase of the organism energy resources speeds up protein synthesis, which is the main composite material [2-4].

To study the impact of natrium humate on growth and development of spring wheat, the field investigations were carried out on experimental station of North-Kazakhstani Agricultural research and Scientific Institute. The subject of investigation was the spring wheat of Kazakhstani early-ripe sort.

The purpose of the work was the experimental justification of effectiveness of humine substances application for spring wheat cultivation. It was studied the impact of natrium humine on morpho-anatomical peculiarities, yield volume of spring wheat grain of Kazakhstani early-ripe sort and the role of humine substances in obtaining the ecologically pure products.

MATERIALS AND METHODS

The soil of the test field was typical black soil, carbonate, medium-thick and low-humic. The square of

the test plot was 100,8 sq.km. the record plot was 64 sq.km. fourfold replicate. Agricultural engineering for wheat cultivation of Kazakhstani early-ripe sort corresponded to the recommendations, approved in the zone. The seeds were treated by natrium humate in concentration of 0,005 % in a day of sowing; the seeds were fertilized in tillering stage and soil application in a dose of 60 kg/ha was done before sowing. Humine substances were used without phosphorous background and at ρ_{60} background and were compared to the control variant.

Anatomical investigations were carried out as per standard practice. To study the peculiarities of the anatomical structure the works of Prozin M.L. and Braune W. were used [5,6]. Morphometric indices were statistically analyzed as per Lakin G.F. methodology (1990) [7].

Yield consideration was done by the method of complete gathering of the plot by grain combine. Yield data are modified to basic conditions. Disperse and correlation analyses are fulfilled as per Dospekhov B.A. (1982).

Heavy metal content (Cd, Pb, Cu, Zn) was determined as per GOST D 51301-99 on the device AAA -1-03 on the laboratory of the branch of "Akmolinsk Agricultural Expertise" of Republican Governmental Enterprise "Kazagroex".

RESULTS AND DISCUSSION

The experiments were held to compare the stalk anatomical organization of the spring wheat of Kazakhstani early-ripe sort, selected from the control

variant without humates, with anatomical organization of stalks, selected from the variants with natrium humate.

Internodes cross section of the spring wheat of Kazakhstani early-ripe sort consists of three ordinary systems of tissues: covering, mechanical and conductive [8].

Covering tissue consists of one raw of epiderm cells with thickened walls. In bottom part of epiderm cell layer there is a mechanical tissue, comprising circular sclerenchyma and sclerenchyma sheaths of conducting bundles. Thin-walled chlorenchyma cells are distinctly seen in sclerenchyma fiber.

Conducting bundles are closed, collateral, comprising xylem, phloem and do not have cambium.

The location of the conducting bundles has a particular importance: small bundles are located in the sclerenchyma fiber and are finished by sclerenchyma sheaths of conducting bundles. In the second raw large vascular bundles are located in parenchyma. Cell walls are thin; parenchyma cells create 4-5 rows. In the stalk center the cells of medullar parenchyma are destroyed and the air pocket is formed [9, 10].

In the internal composition of the wheat stalk, selected from the control variant without humates, the mechanical tissue consists of 5-6 cell rows, the quantity of conducting bundles is 26, including 16 large conducting bundles and 10 small conducting bundles; parenchyma cells consist of 5-6 rows (Table 1).

It is observed the increase of indices in anatomical organization of plants, selected from the variants with natrium humate. The variant with seeds treatment and sowing fertilization by natrium humate has an increased thickness of mechanical tissue and the mechanical tissue consists of 6-7 cell rows. The quantity of big and small conducting bundles increases up to 36. The area of parenchyma cells is increased and they consist of 6-9 rows.

The increase of the stalk anatomical indices is observed on the plants, selected from the variants with seeds treatment with humate, $\rho_{60}+$ seeds treatment with humate, $\rho_{60}+$ sowing fertilization by humate, $\rho_{60}+$ application of humate to soil, $\rho_{60}+$ seeds treatment and sowing fertilization by humate.

The results of correlation and regression analysis show close conjugation and straight-line dependency between one of the elements of the stalk internal organization, quantity of conducting bundles and yield of spring wheat grain. Correlation index is $r=+0,748\pm 0,541$ and the regression index is $b_{yx}=0,443\pm 0,320$.

Thus, natrium humate changes the indices of the stalk internal organization. The thickness of the mechanical tissue, the quantity and the area of conducting bundles and the quantity of parenchyma cell rows increase. Due to increase of the stalk mechanical tissue, the plants become more resistant to drowning.

Table 1: The impact of natrium humate on the anatomical organization of the spring wheat stalk.

| No | Variants | Quantity of mechanical tissue cell rows | Quantity of conducting bundles | Quantity of big conducting bundles | Quantity of small conducting bundles | Quantity of parenchyma cell rows |
|----|--|---|--------------------------------|------------------------------------|--------------------------------------|----------------------------------|
| 1 | Without humate (control) | 4-5 | 26±0,7 | 16±0,6 | 10±0,5 | 5-6 |
| 2 | Seeds treatment by humate | 5-6 | 31±1,2 | 18±1,0 | 13±0,7 | 5-7 |
| 3 | Sowing fertilization by humate | 5-6 | 28±1,1 | 19±1,1 | 9±0,4 | 6-7 |
| 4 | Application of 60 kg/ha of humate to soil | 5-6 | 27±0,6 | 17±0,5 | 10±0,6 | 6-9 |
| 5 | Seeds treatment and sowing fertilization by humate | 6-7 | 36±0,8 | 24±0,6 | 12±0,4 | 6-7 |
| 6 | P_{60} | 5-6 | 30±1,3 | 19±0,9 | 11±0,7 | 6-7 |
| 7 | $P_{60}+$ seeds treatment with humate | 6-7 | 35±1,0 | 23±0,8 | 12±0,6 | 6-7 |
| 8 | $P_{60}+$ sowing fertilization by humate | 5-6 | 30±0,6 | 19±0,4 | 11±0,3 | 6-7 |
| 9 | $P_{60}+$ application of 60 kg/ha of humate to soil | 5-6 | 30±0,5 | 20±0,3 | 10±0,4 | 5-6 |
| 10 | $P_{60}+$ seeds treatment and sowing fertilization by humate | 6-8 | 32±1,2 | 20±0,9 | 12±0,4 | 6-8 |

Table 2: The impact of natrium humate on the anatomical organization of the spring wheat leaf

| No | Variants | Area of conducting bundles ($\times 10^{-3}$ mm ²) | | |
|----|--|---|----------------|---------------|
| | | Tillering stage | Shooting stage | Heading stage |
| 1 | Without humate (control) | 35,27±2,17 | 44,27±1,15 | 44,06±1,06 |
| 2 | Seeds treatment by humate | 36,10±1,76 | 45,21±1,16 | 44,26±1,13 |
| 3 | Sowing fertilization by humate | 37,05±2,17 | 44,27±1,15 | 43,33±0,94 |
| 4 | Application of 60 kg/ha of humate to soil | 38,83±2,17 | 46,05±1,95 | 45,10±2,05 |
| 5 | Seeds treatment and sowing fertilization by humate | 45,21±1,88 | 52,78±1,64 | 51,92±1,42 |
| 6 | P_{60} | 45,21±1,87 | 50,23±1,27 | 49,29±2,00 |
| 7 | $P_{60}+$ seeds treatment with humate | 46,56±2,26 | 51,7±1,14 | 50,55±1,39 |
| 8 | $P_{60}+$ sowing fertilization by humate | 46,56±2,25 | 50,55±1,40 | 49,29±1,99 |
| 9 | $P_{60}+$ application of 60 kg/ha of humate to soil | 48,14±1,95 | 51,28±1,04 | 51,26±0,94 |
| 10 | $P_{60}+$ seeds treatment and sowing fertilization by humate | 49,19±2,09 | 52,77±1,64 | 51,40±1,14 |

Table 3: The impact of natrium humate on spring wheat grain yield, c/ha

| No | Variants | Years | | Average index | | |
|---------|--|-------|------|---------------|----------------|------|
| | | 2010 | 2011 | Yield | Yield increase | |
| | | | | | c/ha | % |
| 1 | Without humate (control) | 17,3 | 16,5 | 16,9 | - | - |
| 2 | Seeds treatment by humate | 20,8 | 19,5 | 20,1 | 3,2 | 18,9 |
| 3 | Sowing fertilization by humate | 19,7 | 19,3 | 19,5 | 2,6 | 15,4 |
| 4 | Application of 60 kg/ha of humate to soil | 20,2 | 19,8 | 20,0 | 3,1 | 18,3 |
| 5 | Seeds treatment and sowing fertilization by humate | 21,6 | 23,1 | 22,3 | 5,4 | 31,9 |
| 6 | P ₆₀ | 21,2 | 22,0 | 21,6 | 4,7 | 27,6 |
| 7 | P ₆₀ + seeds treatment by humate | 23,6 | 23,4 | 23,5 | 6,6 | 39,0 |
| 8 | P ₆₀ + sowing fertilization by humate | 22,0 | 21,0 | 21,5 | 4,6 | 27,2 |
| 9 | P ₆₀ + application of 60 kg/ha of humate to soil | 21,5 | 23,9 | 22,7 | 5,8 | 34,3 |
| 10 | P ₆₀ + seeds treatment and sowing fertilization by humate | 24,1 | 25,5 | 24,8 | 7,9 | 46,7 |
| HCP 0,5 | c | 2,47 | 2,18 | | | |

It was determined the positive correlation and straight-line dependency between the index of the stalk anatomical organization, quantity of conducting bundles and grain yield.

Cross section of the leaf anatomical organization of the spring wheat of Kazakhstani early-ripe sort consists of covering, main and conductive tissue systems. Covering tissue is an epidermis, made of two types of cell forms. Long and comparatively flat cells are located in parallel to the long leaf axis. Epidermis comprises a lot of one-celled trichomes and they are located closely to each other [11].

Stomatoes are located closely on the adaxial leave side as compared to the abaxial one.

Parenchyma cells in the central part have the loose connection. All cells of the leaf blade have chloroplast.

Conducting bundles are collateral, xylem is located in the top part of the leaf blade, phloem is located in the bottom. The conducting bundles are surrounded by two cell rows. Sclerenchyma mechanical tissue is located in the bottom and top part of the conducting bundles.

The results of the impact of natrium humate on the area of the central conducting bundle of the leaf blade are given in Table 2.

The area of the central conducting bundle of the leaf blade, selected from the control variant in the tillering stage, was $35,27 \pm 2,17 \times 10^{-3} \text{ mm}^2$, in the shoot stage it was $44,27 \pm 1,15 \times 10^{-3} \text{ mm}^2$, in the heading stage it was $44,06 \pm 1,06 \times 10^{-3} \text{ mm}^2$. In the variant with seeds treatment and sowing fertilization by natrium humate in the tillering stage the area was $45,21 \pm 1,88 \times 10^{-3} \text{ mm}^2$, in shooting stage it was $52,78 \pm 1,64 \times 10^{-3} \text{ mm}^2$, in heading stage it was $51,92 \pm 1,42 \times 10^{-3} \text{ mm}^2$. The improvement of the anatomical indices is observed on the variant ρ_{60+} seeds treatments and sowing fertilization by humate (tillering- $49,19 \pm 2,09 \times$

10^{-3} mm^2 , shooting - $52,77 \pm 1,64 \times 10^{-3} \text{ mm}^2$, heading - $51,40 \pm 1,14 \times 10^{-3} \text{ mm}^2$).

As per the obtained results, it can be concluded that the area of the central conducting bundle increases under the impact of natrium humate. Our vegetation investigations show positive changes of spring wheat growth and development processes under the impact of natrium humate. The combination of these changes forms the spring wheat yield.

The results of the investigations determine the dependency of the grain yield on the methods and doses of natrium humate application.

Depending on natrium humate application methods, yield increase of spring wheat grain, as compared to control at the average for the years of investigation, was equal to 2,6-5,4 c or 15,4-31,9 %, calculated per one hectare.

When natrium humate was applied with phosphorous background, this index rose, grain yield increase was equal to 4,6-7,9 c or 27,2-46,7 %, as compared to control.

At the average for the years of investigation the maximum grain yield increase was obtained on the variant with seeds treatment and sowing fertilization by natrium humate, as compared to control variant, the yield increase was equal to 5,4 c or 31,9 %. With this method of natrium humate application with phosphorous background the increase was equal to 7,9 c or 46,7 %.

Thus, the results of the field studies show the increase of the spring wheat grain yield under the impact of natrium humate. On the ordinary heavy-loamy black soil the maximum grain yield increase was obtained on the variant with seeds treatment and sowing fertilization by natrium humate. When natrium humate was used with phosphorous background, the yield increased.

At the present time, mineral fertilizers have a positive impact on yield, however, it was determined that they decrease the biological value. The investigation results show the content of heavy metals in all mineral fertilizers. Every year 10...50 hazardous substances penetrate to the soil with each kilo of mineral fertilizers [12].

The results of experiments show that when the soil saturates with poisonous elements, the function of selective absorption decreases in the plants [12]. There is an increase of danger of heavy metals penetration to the human body through plants and animals.

That is why the application of substances, reducing the content of heavy metals in plant bodies, becomes very urgent. Among such substances are the humine growth regulators. A property of natrium humate to form in soil the slightly soluble complexes with ions of other metals enables to make the forms, inaccessible for plant bodies, from the water-soluble salts of heavy metals.

Our investigations show the positive impact of natrium humate on reduction of heavy metals (Cd, Pb, Cu, Zn) content in spring wheat grain.

Grain samples, selected from the variants with high indices of biological, economical and bioenergetic effectiveness, were compared to the grain samples of the control variant. The grain samples, selected from the control variant, did not contain Cd element; the indices of other elements content were at the following level: Pb - 0,520 mg/kg, Cu - 4,964 mg/kg, Zn - 15,561 mg/kg.

As per MPC requirements for the ecological assessment of the grain quality, heavy metals content shall not increase the following: Cd - 0,1 mg/kg, Pb - 0,5 mg/kg, Cu - 10 mg/kg, Zn - 50 mg/kg. The content of Pb on the control variant equals to 0,520 mg/kg and exceeds MPC at 0,02 mg/kg. The grain samples, selected from the variant with seeds treatment and sowing fertilization by natrium humate, did not contain Cd element; the indices of other elements content were at the following level: Pb - 0,455 mg/kg, Cu - 4,519 mg/kg, Zn - 16,174 mg/kg. There was no departure from MPC. The grain samples, selected from the variant ρ_{60} + seeds treatment and sowing fertilization by natrium humate, did not contain Cd element; the indices of other elements content were at the following level: Pb - 0,307 mg/kg, Cu - 4,901 mg/kg, Zn - 12,919 mg/kg. There was no departure from MPC.

Thus, the grain seeds, selected from the variants with seed treatment and sowing fertilization by natrium humate, ρ_{60} + seed treatment and sowing fertilization by natrium humate, the reduction of heavy metals (Cd, Pb, Cu, Zn) content is observed, as compared to the control variant, proving the ecological safety of natrium humate application for wheat spring.

CONCLUSIONS

Positive impact of humine substances on the anatomical organization of the spring wheat stalk was determined. Under the impact of natrium humate the anatomical organization of stalk and leaf has the increased quantity and size of the conducting bundles, thickness of mechanical tissue, sizes of parenchyma cells and quantity of their layers. The plant resistance to drowning grows with the increase of the mechanical tissue.

The correlation between the morpho-anatomical characteristics of the wheat and productivity was determined. The highest correlation connection was determined between the grain yield and quantity of conductive bundles in the anatomical organization of the stalk ($r=0,748$).

The significant impact of humine substances on the grain yield was determined. The maximum increase of spring wheat grain yield was provided by seeds treatment before sowing and during sowing fertilization in tillering stage by natrium humate; the yield increase for two years was equal to 7,9 c/ha, when the yield on the control was 16,9 c/ha.

It was determined the positive impact of natrium humate on reduction of heavy metals (Cd, Pb, Cu, Zn) content in spring wheat grain and its role in obtaining the ecologically pure products.

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