17th INTERNATIONAL MULTIDISCIPLINARY SCIENTIFIC GEOCONFERENCE
S G E M 2 0 1 7

CONFERENCE PROCEEDINGS

VOLUME 17

ECOLOGY, ECONOMICS, EDUCATION AND LEGISLATION

ISSUE 52

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ECOLOGY AND ENVIRONMENTAL PROTECTION

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29 June - 5 July, 2017

Albena, Bulgaria

17th International Scientific Conference on EARTH and GEOSCIENCES, SGEM 2017
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RESISTANCE OF WHEAT INTROGRESSIVE FORMS TO HEAVY METALS

Assoc. Prof. Ph.D. Tamara Tazhibayeva[1]

Prof. Dr. Aigul Abugalieva[2]

Dr. Kenebay Kozhakhmetov[2]

[1] SRI of Ecological Problems, Al-Farabi Kazakh National University, **Kazakhstan**
[2] Kazakh Scientific Research Institute Agriculture and Plant Growing, **Kazakhstan**

ABSTRACT

Wheat resistance to heavy metals (HM) is an urgent ecological problem and it can limit crop production around the many countries in the world. HM is considered as an environmental factor of the anthropogenous nature which influences growth and development of wheat. New approaches are focusing on using of a genetic pool of wild-growing types of crops as an introgressive genetic material for increasing wheat adaptability to HM.

In the model, laboratory tests of HM influence on wheat growth used the grain yield of 2014-2016, which is grown up on the field of Kazakh Scientific Research Institute Agriculture and Plant Growing. Wilkins's coefficient to quantitative comparison of metals resistance was applied. The level of free proline in the wheat seedlings, which are grown up on the nutrient medium containing different salts of HT, are determined. Data was processed by method of variation statistics. The paper presents the results of experimental researches on the effect of HM on the growth rates of seedlings of 10 introgressive forms of wheat. Introgressive forms obtained by interspecific hybridization of varieties of winter wheat *(Triticum aestivum L.)* of Kazakh breeding with wild species of *Triticum timopheevii, Triticum militinae, Aegilops cylindrical, Aegilops triaristata.* It showed that HM cause inhibition of growth processes of wheat introgressive forms. However, there was heterogeneity in the degree of response of roots and seedlings, as well as HM effect on various introgressive forms of wheat. Significant growth inhibition effect caused by Cu^{2+} and Cd^{2+}. Inhibition of root growth occurred on average by 40-60% under the impact of these metals. Zn^{2+} salt even stimulated the germination of some introgressive forms.

The introgressive form *PEG 304 x Komsomol 1*, allocated with higher level of a proline response for action of zinc and copper ions had received. The sample *Zhetysu's x T.timopheevii* differed by 2-3 times in lower indicators of free proline in response to a metal stress. The maximum accumulation of free proline was observed under the influence of Cd^{2+} and Cu^{2+}, smaller – under the influence of zinc salt (Zn^{2+}).

These phenomena were established: wide norm reaction for the introgressive forms of wheat on HM influence (salts Zn^{2+}, Cu^{2+}, Cd^{2+}); genotype specificity in response to the environmental stress caused by ionic toxicity; specificity of influence of various HM on the growth of studied forms; prospects using of free proline to determine of resistance of wheat introgressive forms to HM. The hybrid combinations with *T.militinae* participation showing resistance to HM, for example, wheat introgressive form *Zhetysu x T.militinae,* were revealed. The received results will promote wheat breeding on adaptability to ecological stresses.

Keywords: heavy metals, wheat, introgressive forms, growth, proline.

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INTRODUCTION

Currently, the presence of heavy metals (HM) in the environment is being considered as one of the severe environmental stresses of abiotic nature caused by irrational human economic activity. In connection with the increasing anthropogenic pressure on natural communities, the increase of the resistance of agricultural crops, particularly, wheat, to TM influence has become a very urgent problem. Emissions of mining, metallurgy and most chemical industries, road transport and pesticides may be the sources of HM. The most of metals are biophilic, but in concentrations exceeding the maximum permissible, they become "heavy", hinder growth and development, cause mutagenic and carcinogenic effects. They settle in the soil and actively affect agricultural crops showing ionic toxicity.

Plant resistance to ionic toxicity has been ensured by phenological avoidance (under conditions of seasonal manifestations of the stress factor), prevention of absorption, neutralization (by formation of chelate complexes, localization, dissolution, excretion) and tolerance [1]. On the one hand, the implication of ionic toxicity depends on the concentration of the element at which plant damage occurs, as well as its participation in the processes of growth and development, on the other, is determined by the genetic characteristics of the plant. Therefore, while studying the metal resistance of wheat, the emphasis of ecological and physiological research should be directed to the creation of breeding and genetic resources with increased adaptability to ionic toxicity.

However, the narrow specificity of breeding, the tendency to a partial loss of fitness genes, induces wild species and varieties - donors of resistance to certain unfavorable factors of the environment - to be involved in the breeding of crops. Great perspectives are opening by using a pool of wild species as an introgressive genetic material [2-4]. It is known that introgression (introgression, latin *intro* - in, inside and *gressus* - approaching, crossing, attacking) means the inclusion of individual genes of one kind of organism in the gene pool of another species.

This has been achieved by one of the traditional methods, such as trans-species hybridization, which makes it possible to obtain a highly plastic new genetically original source material for wheat breeding. Wild relatives of wheat are, as a rule, "records" of sustainability, because they were selected by nature. The series of trans-species crossings with wild types of wheat *T.kiharae, T.timophevii, T.militinae* and others, as sources of an immunity, stability and high content of protein in grain, were carried out in the Kazakh Scientific Research Institute Agriculture and Plant Growing within 10 years (1998-2008).

It is established that the type of *T.kiharae* has all genes of immunity, differs in the considerable maintenance of a protein and mineral elements, such as iron and zinc [5]. Increased requirements for the created varieties and significant genetic erosion of existing ones encourage researchers to use wild relatives to improve valuable qualities of wheat more often [6].

The purpose of this work is studying the physiological parameters of the growth of introgressive forms of wheat obtained from trans-species crossing of wheat varieties with wild species, which are regionalized under ionic toxicity conditions in the Republic. Heavy metal salts of copper, cadmium and zinc are the most common pollutants on the territory of Kazakhstan. According to this reason, these metals were investigated.

MATERIALS AND METHODS

The 10 introgressive forms of winter wheat were served as object of research. Introgressive forms, F6-F7, were identified by cytology, breeding methods and selected for homogeneity, evaluated in terms of yield and grain quality, adaptability to local growing conditions [5]. They were formed from introgression of wild wheat germplasm to winter common wheat cultivars (*Triticum aestivum L.*). Wild types of wheat: *Triticum timopheevii, Triticum militinae, Triticum kiharae, Aegilops cylindrical, Aegilops triaristata* and winter wheat varieties of the Kazakhstan breeding - *Karlygash, Eritrospermum 350, Zhetysu, Steklovidnaya 24, Komsomolskaya 1* are involved in the researches. *Bezostaya 1* had been used as the grade (standard) in Kazakhstan for a long period.

Grain was grown up in identical conditions on the field sets of the Kazakh Research Institute of Agriculture and Plant Growing, to carry out laboratory experiments on the HM effect studying of the wheat introgression forms. Researches were conducted on plants of one reproduction, the yield of 2014- 2016. For sprouting of 7-day-old wheat seedlings, experiments have been working with the addition of HM to the nutrient solution: control - 0, 1 mM $CaSO_4$; the experiment -1mM $CaS0_4$ and 20mg / copper (Cu^{2+}), cadmium (Cd^{2+}) and zinc (Zn^{2+}) salts. As the anion of TH salts, $S0_4^{2+}$ was appeared. The same anionic background of the nutrient medium was the best way to identify cationic toxicity. Stressful concentration of the studied HM salts in nutritious solution of 20 mg/l was applied. This one was the most indicative for genotype distinctions and for the effect of plants oppression [7]. The first two days the grain were grown up in a thermostat at a temperature of 26-28 °C (a stage of shoots), and then in laboratory at the room temperature (a stage of the first leaf of a seedlings). Experiments were carried out in triple frequency. In each frequency used 20 plants.

For definition of influence of HM, salts on intensity of wheat samples seedlings measured the total length of roots and shoots, comparing them to similar indicators of control plants. Growth indicators of control plants had been taken for 100%. Applied Wilkins's coefficient calculated by a formula K = by Lme/Lc where L – the total length of roots to quantitative comparison of metals tolerance.

A quantitative definition of proline content was provided by the method of L.S. Bates et all [8].Conclusion of HM resistance seedlings could be fined by the coefficient of proline accumulation in correlation experience/control. Data processed by method of variation statistics.

RESULTS AND DISCUSSION

Ionic toxicity of metals has already shown on initial stage of ontogenesis of examinees wheat samples - a stage of shoots of grain. Two-days introgressive forms slightly differed on viability in the nutrient solution contained salts of various HM (table 1).

The obtained data testify in favor of turning on of the mechanism of plants avoiding by of the specified TH dose in various degree at the compared wheat forms. High level of seeds's growing-up indicates the start of metabolic adaptation processes, which have to provide the stability of wheat under conditions of more prolonged metal stress, that is confirmed in the paper [9].

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Table 1 – Influence of TM on wheat viability, % of number of the seeded grain

Wheat introgressive forms	Control	Cu^{2+}	Cd^{2+}	Zn^{2+}
PEG231 x Karlygash	100±0,02	92±0,03	88±0,02	96±0,02
Eritrospermum 350 xT.militinae	98±0,03	96±0,04	86±0,05	100±0,03
PEG 1718	100±0,01	82±0,03	79±0,03	100±0,03
PEG 304 x Komsomolskaya 1	95±0,02	89±0,02	82±0,04	95±0,03
Zhetysu x T.timopheevii	97±0,04	90±0,02	81±0,03	97±0,04
Steklovidnaya 24 x T.militinae	99±0,01	99±0,05	90±0,03	100±0,03
Zhetysu x T.militinae	98±0,03	97±0,04	91±0,01	98±0,02

Seven - day seedlings of wheat were characterized by wide variability in response to ionic toxicity. Scope of variability of growth indicators of the studied wheat forms on each of metals is presented in the Figure 1.

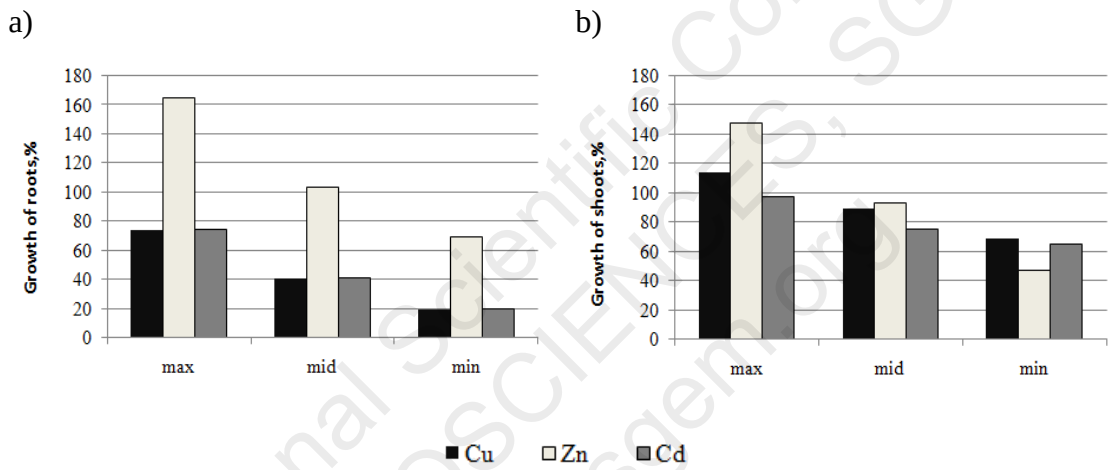

Figure 1 – Scope variability of growth indicators for wheat introgressive forms under the HM action: a) roots, b) shoots

The seedlings root system of introgressive forms was presented by 3-5 roots, and their length significantly decreased under influence of Cu^{2+} salts + and Cd^{2+} that would be coordinated with researches [9, 10]. Variability in reaction of root systems of wheat introgressive forms on action of HM fluctuated over a wide range from 54 to 96% in relation to control and was built in the sequence: $Cu^{2+} > Cd^{2+} > Zn^{2+}$.

Variability in reaction of root systems the introgressive forms of wheat on action of HM fluctuated over a wide range from 54 to 96% in relation to control and was built in the sequence: $Cu^{2+} > Cd^{2+} > Zn^{2+}$. On average values of growth indicators of roots the inhibition of ions Cu^{2+} occurred more than twice, ions Cd^{2+} - by 1,4 times for all studied wheat forms . For all forms of the growth up, inhibition was being observed under the influence of Cd^{2+} - at 1,35 times. However, ions Cu^{2+} and Zn^{2+} on average values did not cause essential decrease in growth parameters of a vegetative biomass. The greatest scope of variability, which characterized by the maximum deviation from average (σ), was traced for Zn^{2+} both in experiences with roots and shoots. Forms, which adaptation to Zn^{2+} ions stimulated growth up were allocated: *Eritrospermum 350 x T.militinae* (roots) for 15%; *Zhetysu x T.militinae* (roots and shoots) for 65% and 48% respectively; *Steklovidnaya 24 x T.militinae* (shoots) for 13%.

Variability tendency to response extents comparing wheat forms to effect of various metals is presented in the figure 2.

Cu2+ and Cd2+ salts caused the considerable effect of oppression of growth. The inhibition of roots's growing-up occurred on average for 40-60% at effect of these metals. Sometimes ions Zn^{2+} even stimulated growth up of some introgressive forms. In general, growth reactions of wheat introgressive forms demonstrate broad norm of reaction of genotypes to action of a metal stress. In general, growth reactions of wheat introgressive forms demonstrate broad norm of genotypes reaction to influence of a metal stress.

Introgressive forms are subject to the greatest inhibition of growth processes under the influence of HM: *PEG 231 x Karlygash; PEG 304 x Komsomolskaya1; Zhetysu x T.timopheevii.* Forms with growth reactions to the metal stress are opposite to the general tendency of inhibition. They are noticed: *Zhetysu x T.militinae* (shoots) against the background of Cu^{2+}; *Eritrospermum of 350 x T.militinae* (roots) against the background of Cd^{2+} and Zn^{2+}, *Steklovidnaya 24 x T.militinae* (shoots) against the background of Zn^{2+}; *Zhetysu x T.militinae* (roots and shoots) against the background of Zn^{2+}. There is a competition for use of nutrients, and influence of ionic toxicity differently affects growth of root and vegetative biomass systems despite high coherence in functioning of roots and vegetative biomass of plants. Moreover, manifestation of the "barrier", the "accumulative" and "filtering" functions of roots is characteristic for plants with function of "an exception", in particular wheat and barley. At the same time vegetative part "suffers" much less that is caused by "effect of detention" of metals ions in roots and is coordinated with works [7, 10]. In this series of experiences this effect is shown most

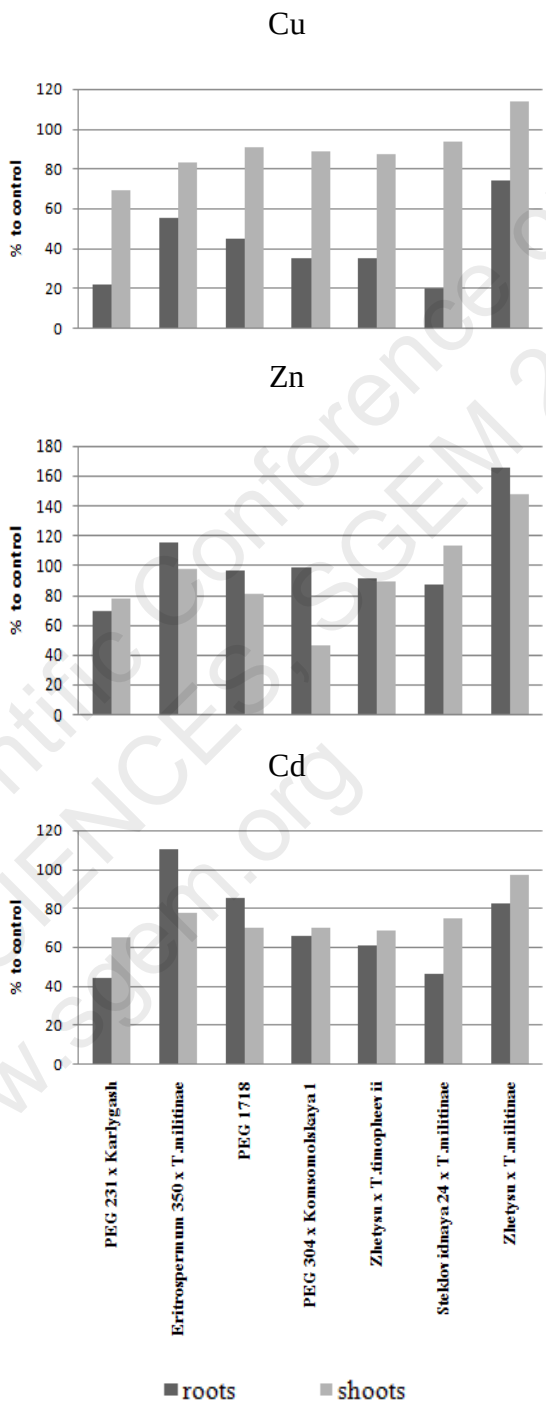

Figure 2 – Influence of salts of various HM to the growing up of wheat introgressive forms

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accurately at adaptation of wheat introgressive forms to action of ions Cu^{2+}, and the ratio of growth reactions an escape/root is the highest (1,5-2 σ).

An accordance of Wilkins coefficient the wheat forms are formed the following ranks:

Cu^{2+}: *Steklovidnaya 24 x T.militinae < PEG 231 x Karlygash < Zhetysu x T.timopheevii < PEG 304 x Komsomolskaya1 < PEG 1718 < Eritrospermum 350 x T.militinae < Zhetysu x T.militinae*.

Cd^{2+}: *PEG 231 x Karlygash < Steklovidnaya 24 x T.militinae < Zhetysu x T.timopheevii < PEG 304 x Zhetysu < Komsomolskaya 1 x T.militinae < PEG 1718 < Eritrospermum 350 x T.militinae.*

Zn^{2+}*: PEG 231 x Karlygash < Steklovidnaya 24 x T.militinae < Zhetysu x T.timopheevii < PEG 1718 < PEG 304 x Komsomolskaya1 < Eritrospermum 350 x T.militinae < Zhetysu x T.militinae.*

The comparison of Wilkins coefficients for wheat genotypes has confirmed a different quality of introgressive forms in response of root system to the metal stress created by various salts. Forms, which roots more "suffer" under the influence of ions of metals, for example *Steklovidnaya 24 x T.militinae, PEG 231 x Karlygash* are allocated. Along with this, introgressive forms, which root system actively developed in the conditions of a metal stress, are found: *Zhetysu x T.militinae, Eritrospermum 350 x T.militinae*.

Against the background of cultivation of seedlings on solutions of salts of the heavy metals (HM), the increasing of free proline level for wheat introgressive forms in different degree is established. Data on the most characteristic genotypes, the reflecting changes of free proline of wheat introgressive forms under the influence of a metal stress are submitted in the figure 3.

According to data of 2015 the introgressive form *PEG 304 x Komsomolskaya 1*, was allocated with higher level of free proline in response to HM action, especially ions of zinc and copper has been established. The sample *Zhetysu's x T.timopheevii* differed by 2-3 times in lower indicators of free proline content in response to metal stress. In experiments of 2016 the maximum accumulation of free proline was observed under the influence of cadmium (Cd^{2+}) and copper (Cu^{2+}), smaller – under the influence of zinc (Zn^{2+}). For example, the introgressive form *(Bezostaya 1 x T.militinae) x T. militinae-9* observed increasing of proline concentration against the background of cadmium (Cd^{2+}) in more than 4 times, copper (Cu^{2+}) – by 3,5 times. The sample *(Bezostaya11 x Ae.triaristata) x Karlygash* is marked out the fact of proline accumulation at effect of cadmium salt (Cd^{2+}) by 7 times, and copper (Cu^{2+}) – by 5 times.

Given results, testify in favor of use of free proline for an assessment of resistance degree of the studied forms of winter wheat to the stress caused by HM. It confirms the universality of this indicator for studying of plants activity enduring influences of extreme factors of the environment, as for example, HM stress. The proline reaction as well as decreasing growth processes of wheat shows that salt of cadmium causes the strongest stress for introgressive forms that correlates with the results of papers [8, 11 and 12].

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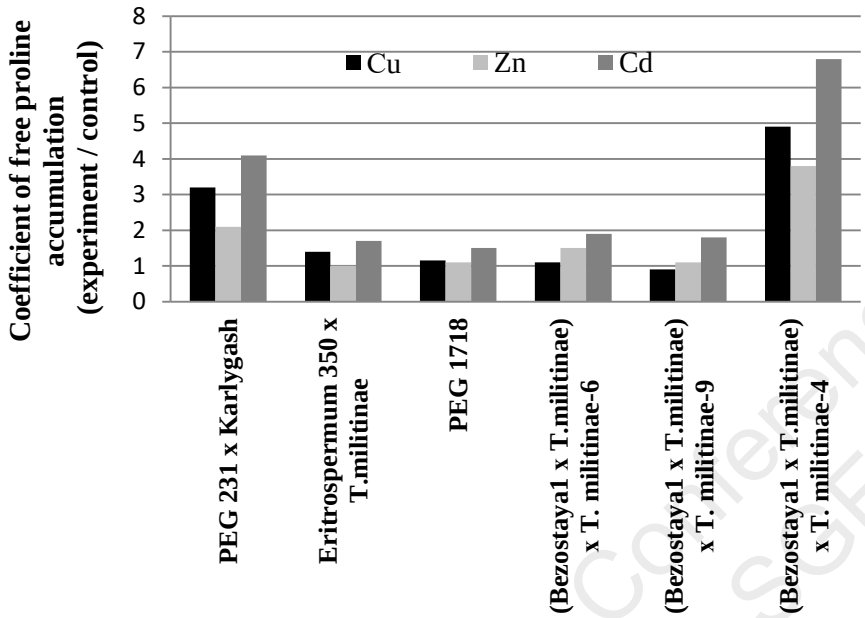

Figure 3 – Coefficient of free proline accumulation in seedlings of wheat unprogressive forms against the background of HM effect

CONCLUSION

The conducted researches have allowed establishing the broad norm of wheat introgressive forms reaction to the action of HM; their genotype specificity in response to the ecological stress caused by ionic toxicity; specificity of influence of various HM on growing up of the studied wheat forms.

An absence of considerable effect of inhibition of viability of grain in the environment containing HM shows the potential of wheat introgressive forms to adaptation for metal stress and the prospects of using the trans-species hybridization with wild types. Wilkins coefficient allows dividing more accurately wheat genotypes on intensity of roots's development in the conditions of metal stress.

The following results have been received:

- Wide norm reaction for introgressive forms of wheat on HM influence (salts Zn^{2+}, Cu^{2+}, Cd^{2+});
- Genotype specificity in response to the environmental stress caused by ionic toxicity;
- Specificity of various HM influence to the growth of studied forms;
- Prospects of using free proline for determination the HM resistance to introgressive forms.

The hybrid combinations with *T.militinae* participation, showing resistance of growth processes to action of HM, for example, wheat introgressive form *Zhetysu x T.militinae*, were revealed.

The received results will promote interpretation of physiological mechanisms of crops's growth and resistance, an intensification of wheat breeding on adaptability to ecological stresses, enrichment of a gene pool of Kazakhstan wheat, a variety of the perspective forms received from crossing with wild relatives.

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ACKNOWLEDGEMENTS

This investigations were carried out by a grant of Kazakhstan Ministry of Education and Science in 2015-2017 " Physiological and biochemical criteria for resistance to abiotic environmental factors of winter wheat genotypes of wild, cultural and introgression forms ", on a task 2011/GF 4.

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