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ECOLOGY AND ENVIRONMENTAL PROTECTION

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INTRODUCTION

An indicator of the national security of any country is the satisfaction of dietary needs of its population. Under current conditions of growing shortage of wheat, humanity might once again face an acute problem of the food crisis. Annual production of wheat on average is about 600 million tons. It is expected that by 2020 the demand for it may reach more than 840 million tons. Satisfying this need is a rather difficult task, taking into account the fact that the number of cultivating areas decreases, and wheat yields in most developed countries have already reached the maximum level, for example, in Europe, this number reaches more than 8 tons per hectare [1]. Production of high-quality grain in Kazakhstan is an important strategic direction, contributing to stabilization of agriculture, food security of the country and a decent position in the club of grain exporters in the world market [2].

One of the major factors causing significant damage to grain production in Kazakhstan is a leaf (brown) rust of wheat. Epiphytotic diseases occur with a frequency of 2 – 3 times in 10 years with the loss of yield up to 30-50%. The causative agent of brown rust is *Puccinia recondita Rob. Ex Desm f. sp. tritici.* This obligate parasite of wheat and a number of wild cereals, common throughout the world, in places of cultivation of crops and in all areas of cultivation of winter and spring wheat, is a parasite with two host full lifecycle with five types of sporulation [3]. In the vegetative phase of the life cycle there are dikaryotic mycelium aeciospores, teliospores and urediniospores. Number of alternate generations depends on the climatic conditions of the year and the duration of vegetation period in urediostage of wheat and some wild cereal grains. Unicellular uredinia have two haploid nuclei constituting synkaryon. By the end of vegetation plants form telia with teliospores covered by black colored epidermis. Size of urediniospores complies 18 – 26 x 17 – 22 microns, teliospores 30 – 41 x 14 – 17 microns. Latter are unicellular with each cell containing two haploid nuclei. Urediniospores and teliospores are adapted to overwintering. Pathogen winters, mainly in the form of mycelium in leaves of winter wheat and wild cereal grains. In spring teliospores germinate; fusion of haploid nuclei into diploid, meiosis and formation of germ tubes – basidia with four haploid differing in the type of mating basidiospores observed. Condensed moisture is required for spore germination; therefore abundant dew promotes the development of infection. Under favorable temperature conditions (15 – 25°C) and presence of condensed moisture infection is conducted for 6 – 8 hours the next generation urediniospores are formed after 7 – 10 days; during the day up to 3.2 x 10^{13} spores can be produced per 1 hectare. Basidiospores infect intermediate host rue (*Thalictrum minus*, *Thalictrum speciosissium*, *Thalictrum flavum*), resulting on appearance of yellowish-orange spermagony with spermatia (piknospores) of the two mating types on the upper side of the leaf. When transferring spermatia from one spermagony to another mixed mycelium is formed, and as a result of anastomoses emergence dikaryotic cells – aeciospores are formed infecting wheat. At the same time on leaves, more rarely on leaf sheaths pustules (pads) appear in diameter of 5 – 20 μm. To make sure that this is the pustule of brown rust, and not the color of a leaf spot, a place of destruction should be examined under a magnifying glass or under a binocular microscope. Urediniospores are spherical, 19 – 22 microns in diameter, with yellow-orange contents [4]. An important mean of control is breeding of wheat with application of new, non traditional techniques, combining the efforts of classical breeders, geneticists, biochemists, physiologists, immunologists and biotechnologists, as only the complex approach may increase the breeding performance. This trend implies, first of all, identifying the biological signs that provide the best possible adaptation to the natural conditions of the arid zone in order to obtain initial material for breeding of new productive varieties [5].

Model plant *Arabidopsis thaliana* provided unique opportunities for the study of key biological aspects of plant biology, including resistance to disease. However, the fungi of the genus *Puccinia* are not able to infect *Arabidopsis* what provided further prospects for *Brachypodium distachyon* application in rust research [6-10].

Seeds of *Brachypodium distachyon* L. (Bd21 line) were obtained from the RIKEN BioResource Center (Japan). The reason for the choice of wheat varieties of local breeding for the planned stages of the experimental work was the degree of resistance or susceptibility to leaf rust. According to the results of evaluations by the laboratory of plant immunity and protection of Kazakh Research Institute of Agriculture and Crop Production and Research Institute for Biological Safety variety Kazakhstanskaya 19 shows resistance to brown (14%) and yellow rust. According to the laboratory assessment of the quality of grain by RK state commission for variety testing both varieties meet the requirements of the state standard. 14-day old seedlings of *Brachypodium distachyon* and chosen wheat varieties served the material of the study.

A change in the activity of enzymes of metabolism is one of the significant criteria changes the genetic apparatus exposed to mutagens. In this regard, of particular interest is the study of the activity of several key enzymes of nitrogen and energy metabolism in mutant genotypes as compared to the initial variety. The study of enzymatic activity makes it possible to judge the intensity of the metabolism of the plant body, and more reliably estimate the vitality of these mutant genotypes. Xanthine dehydrogenase (XDH) is a key enzyme of purine degradation, oxidizing hypoxanthine to xanthine and then to uric acid and ureides, which catalyzes the oxidative hydroxylation of a wide range of aromatic and heterocyclic aldehydes. XDH is not strictly specific to hypoxanthine and xanthine and may catalyze the oxidation of about thirty aliphatic and aromatic aldehydes. It is assumed that the process of complete degradation of purines occurs in peroxisomes, which play a role in the circuit of nucleic acids in a plant cell. Despite this undeniable fact, the main biological role of XDH is still not fully clarified, because in the leaves and roots of leguminous plants, as well as in all vegetative organs of non-leguminous plants, this enzyme is constitutively synthesized in significant quantities. It should be noted that plant hormones – cytokines are the 6-substituted-purine derivatives. So it does not exclude the possibility of participation in the XDH cytokine degradation in plants. Thus, with aging in the leaves of pea XDH activity abruptly increased and with it in parallel the activity of superoxide dismutase and other enzymes associated with oxygen. However, the role of the XDH in this process remains unclear at the moment, as well as the subcellular localization of the XDH in a plant cell [11].

The increased interest to the composition of storage proteins in wheat is associated with functional significance of specific proteins in the determination of the baking properties. Refinement of genetic control and identification of new and rare protein subunits, detected in the course of studying the collection and breeding material necessary for a reliable assessment of samples, as well as for the expansion of the genetic basis of cultivars created by examining the value of genotypes with specific variants of alleles and their inclusion in the selection process.

STUDY OF STORAGE PROTEINS IN ENDOSPERM AND ANTIOXIDANT ENZYME ACTIVITY OF SOFT WHEAT AND *BRACHYPODIUM DISTACHYON* INFECTED BY *PUCCINIA RECONDITA*

Prof. Nargul Omirbekova,

Dr. Aizhan Zhussupova,

Ms. Balzhan Askanbayeva,

Prof. Saule Kenzhebaeva,

Dr. Zhazira Zhunusbayeva

School of Biology and Biotechnology, Al-Farabi Kazakh National University, **Kazakhstan**

ABSTRACT

Production of high-quality grain in Kazakhstan is an important strategic direction. Currently glutenin and gliadin spectra are regarded as reliable genetic characteristics of any variety, indicators of flour quality depend on these proteins. Two local breeding wheat varieties and *Brachypodium distachyon* infected by the Kazakhstani strain of *Puccinia recondita* served the material of the study. Analysis of seed storage proteins was carried out in alkaline and acidic systems. According to our results, high molecular weight (HMW) subunits of Kazakhstanskaya early variety comprise subunit 2* encoded by locus Glu1A, 7+9 subunits encoded by locus Glu1B and 5+12 subunits encoded by locus Glu1D. HMW subunits composition of other variety: 2*; 7 + 9; 5 + 10. These subunits contribute to the baking quality and are highly ranked. Overall quality rating for wheat glutenin complies 9 points. The wheat prolamin spectrum (gliadins) shows clearly marked changes in the accumulation of the individual components under the influence of the pathogen. The intensity of ω 9 component appearance is significantly weaker in comparison with control. Weakening of components intensity in test samples is also noted in gliadin regions α, β, γ. *Puccinia* effect is largely not expressed; however the weakening of bands intensity is observed in zones corresponding to gliadin and HMW subunits. Data shows that fungus might cause changes in the accumulation of the individual components of storage proteins. Spectrum of storage proteins in *Brachypodium* shows the absence of slow-moving HMW subunits similar to cereal (wheat, barley, corn) glutenins. Components seen in the middle part of the gel, apparently, are not prolamins, as by fractionation in acidic system, those proteins are not observed or are present in trace amounts. Fast-moving proteins related to globulin fraction can also be seen on spectrum corresponding to the wheat. Spectrum of storage proteins in *Brachypodium* shows consistent intensity of protein components of the spectrum in the experimental samples in comparison to control. Estimation of xanthine dehydrogenase (XDH) activity was performed by the native gel electrophoresis. It is found that the infection of the plant resulted in a slight increase in XDH activity from 5 to 10% in wheat, while enzymatic activity in *Brachypodium* decreased by 36% in comparison to control.

Keywords: plant, storage proteins, antioxidant enzymes.

Analysis of seed storage proteins was carried out in an alkaline (sodium dodecyl sulphate electrophoresis) and acidic systems according to Galili G. [12]. In particular, gliadin extraction was performed with 70% ethanol. Electrophoresis was performed in polyacrylamide gel in glycine acetate buffer pH 3.1. Gels were fixed in 10% trichloroacetic acid, stained with 0.2% Coomassie R-250. Gliadin components were recorded by their electrophoretic mobility in the gel within α, β, γ and ω subfractions.

Estimation of XDH activity was performed by the native gel electrophoresis. Leaves were extracted (1:4) with extraction buffer containing 250 mM Tris-HCl (pH 8.48), 1 mM EDTA, 14 mM L-glutathione, 4 mM dithiothreitol, 5 mM L-cysteine, 0.05 mM solution $Na_2MoO_4 \cdot 2H_2O$, 0.1 mM phenylmethylsulfonyl fluoride, pepstatin 0.001 mM and 250 mM sucrose solution. The extracts were centrifuged at 14,000 g at 4°C for 20 minutes. Supernatants obtained from the leaves were heated at 60°C for 2 minutes, centrifuged for 5 minutes, in same conditions, and used for analysis. XDH enzymatic activity was estimated in 7.5% polyacrylamide gel after fractionation of native proteins by gel electrophoresis according to standard procedures [13]. Upon completion of electrophoresis, gels were removed from the glass and treated with the reaction mixture containing 50 mM Tris-HCl (pH 8.48), 3.4 mM 3(4,5-dimethylthiazolyl-2) 2,5-diphenyltetrazolium bromide and 0.1 mM phenazine methosulfate. 1.5 mM hypoxanthine and 0.5 mM xanthine were used as substrates for XDH. In order to visualize the XDH activity gels were incubated for 20-30 minutes in the above mixture in the dark on a shaker at 37°C. XDH activity was assessed by relative color intensity of formazan bands using ImageJ processing of digital images of gels obtained on the Epson Perfection scanner.

Comparative analysis of storage proteins in healthy plants (control – c) and after infection with *Puccinia recondita* (experiment – e) showed the following (Figure 1).

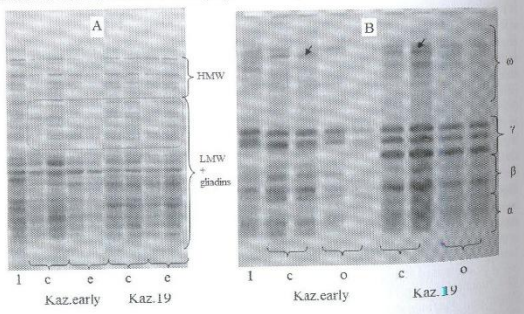

Figure 1 – Spectrum of storage proteins of soft wheat in alkaline (A) and acidic (B) electrophoretic systems; where: 1– marker protein of Bogarnaya 56 winter wheat.

According to HMW subunits composition analysis Kazakhstanskaya early variety has a subunit 2* encoded locus Glu1A, 7 + 9 subunit encoded by locus Glu1B and subunit 5 + 12 locus controlled Glu1D. HMW in Kazakhstanskaya 19 is composed of: 2*; 7 + 9; 5 + 10. These subunits contribute to the potential baking quality; highly ranked and overall assessment of the quality of these varieties for glutenin is 9 points. Effect of *Puccinia recondita* pathogen on high molecular weight glutenin subunit is not expressed to a large extent, but the attenuation band intensity is observed in gliadins (Figure 1).

The wheat prolamin spectrum (gliadins), fractionated in an acidic system, shows more clearly marked changes in the accumulation of the individual components under the influence of leaf rust pathogen. The spectrum of prolamins of wheat is usually divided into α, β, γ- and ω- zones. It should be noted that in both cases the experienced intensity of wheat ω 9 component (Figure 1, B) is much weaker than in control samples. It is known that the slow-moving components of ω zone (8 and 9) are controlled by soft wheat D genome and significantly contribute to the baking quality indicators. It can be assumed that plant infection with leaf rust adversely affects its quality. Weakening of components intensity in test samples is also noted in gliadin regions α, β, γ. Data shows that leaf rust causes changes in the accumulation of the individual components of storage proteins in wheat grain.

The response of *Brachypodium* plants to infection with rust spores was also studied in the spectrum of storage proteins fractionated in both systems (Figure 2). The range of storage proteins *Brachypodium distachyon*, resulting in alkaline system (Figure 2, A) has slow-moving high-molecular subunits, similar to glutenin in cereals – wheat, barley, corn. In acidic system components in the middle part of the gel, apparently, are not prolamins; these proteins are not detected or are present in trace amounts (Figure 2, B). Fast moving proteins related to wheat albumin-globulin fraction are observed as well.

According to Larre C. et al. (2010) [14], who studied the protein composition of *Brachypodium* grain, salt-soluble proteins as well as salt-insoluble proteins separated by two-dimensional gel electrophoresis were revealed as 284 and 120 spots, respectively. Proteins from the major spots were sequenced by mass spectrometry and identified by searching against a *Brachypodium* putative protein database. The authors found prolamins and globulins, no albumins were found. Globulins were represented mainly by the 11S type and their solubility properties corresponded to the glutelin found in rice. Microscopic examination of endosperm cells revealed scarce small-size starch granules surrounded by protein bodies containing 11S globulins. According to the authors, the presence of protein bodies containing glutelins makes *Brachypodium distachyon* closer to rice or oat than to wheat endosperm.

In another study by Laudencia-Chingcuanco D.L. and Vensel W.H. (2008) seven major protein groups, six of which have been identified as globulins, were found using sodium dodecyl sulfate polyacrylamide gel electrophoresis and mass spectrometry. A subset of the major storage proteins extracted from three hexaploid accessions, Bd4, Bd14 and Bd17 has also been identified as globulins. Several clones of *Brachypodium*, encoding globulins were completely sequenced. Two types of globulin genes were identified, Bd.glo1 and Bd.glo2, which are similar to maize 7S and oat 12S globulins,

respectively. The derived polypeptide sequences of the globulins contain a typical signal peptide sequence in their polypeptide N-termini and two cupin domains. Bd.glo1 is encoded by a single copy gene, whereas, Bd.glo2 belongs to a gene family [15].

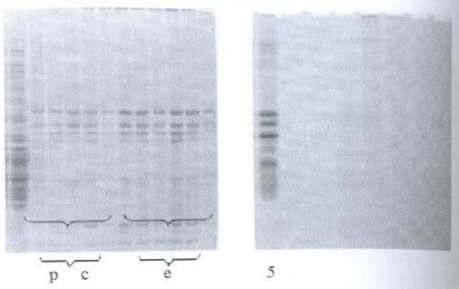

Figure 2 – Electrophoregrams of storage proteins of *Brachypodium distachyon* in alkaline (A) and acidic (B) systems

The reduction of the intensity of expression in the spectrum of storage proteins of *Brachypodium distachyon* is not observed in the experimental samples in comparison to control.

Study of XDH activity as an indicator of the degree of oxidative stress and resistance of the test plants to biotic stress conditions showed the following results (Figure 3). The relative intensities correspond to the appropriate XDH formazan bands.

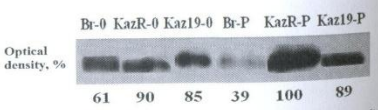

Figure 3 – XDH activity in leaves of soft wheat and *Brachypodium distachyon*; where: 0 – control, P – pathogen

Data on XDH activity as an indicator of the degree of oxidative stress and of resistance on the test plants under biotic stress showed that its activity in the leaves of Kazakhstanskaya early and Kazakhstanskaya 19 after infection with pathogen increased slightly (at 10 and 5% in comparison to control), whereas in *Brachypodium* it decreased by 36% in comparison to control. Based on these data it can be concluded that the inhibition of XDH activity in *Brachypodium distachyon* may be associated with the

increased plant resistance to pathogen action. In accordance with our findings we can conclude that this enzyme plays an important role in the process of plant adaptation to the fungus *Puccinia recondita*.

CONCLUSION

Comparative study of molecular genetic and biochemical features of the model of wild cereal *Brachypodium distachyon* with related cereal grains enables us to understand mechanisms of resistance and increase of resistance of wheat plants to both abiotic and biotic factors. A change in the activity of enzymes of metabolism is one of the significant criteria changes the genetic apparatus exposed to mutagens. In this regard, of particular interest is the study of the activity of several key enzymes of nitrogen and energy metabolism in mutant genotypes as compared to the initial variety, which makes it possible to judge the intensity of the metabolism in plant, and more reliably estimate the vitality of these mutant genotypes. The increased interest to the composition of storage proteins in wheat is associated with functional significance of specific proteins in determination of the baking properties. Refinement of genetic control and the identification of new and rare protein subunits, detected in the course of studying the collection and breeding material is necessary for a reliable assessment of samples, as well as for the expansion of the genetic basis of cultivars created by examining the value of genotypes with specific variants of alleles and their inclusion in the selection process. Some results of this study are presented in the current paper.

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STUDY ON THE DETECTION AND IDENTIFICATION OF GENETICALLY MODIFIED SOYA FOOD OR FEED MARKETED IN ROMANIA

Assoc. Prof. Dr. Elena Roşculete[1]

Dr. Cătălin Aurelian Roşculete[1]

Dr. Elena Teleanu[2]

[1]University of Craiova, **Romania**
[2]D.S.V.S.A. Prahova,11 Corlăteşti Street, Ploiesti, **Romania**

ABSTRACT

Genetically modified organisms and foods derived from GMOs placed on the market must meet the requirements of European legislation regarding labeling and traceability.

As European Union law assess, the labeling of foods containing genetically modified organisms is obligatory, the imposed threshold being of 0.9% for each ingredient. In Romania, until 2007 (the year of EU admission) Roundup Ready soybean was no longer cultivated in Romania, but cross-border exchanges can lead to its introduction on the market of food, so closer monitoring of food products derived there from is required, using appropriate testing techniques.

The following work presents the results of a study concerning food and feed containing soya and soya beans existing on the market in Romania, in order to detect the presence or absence of GM soya and its quantification in the samples found to be positive.

Regarding this matter, we analyzed 15 samples using the PCR technique in real time (using analyzer Light Cycler), of which, five food samples, 4 soybeans' samples and 5 samples of feed were found positive in identifying the specific gene of the plant (lectin) and the genetically modified insert (RRS-DNA). Only for one sample (soybeans, aboriginal production), no DNA was detected specific to the line GTS 40-3-2 (Roundup Ready).

The results obtained in real time PCR quantification, of samples that were tested positive, showed percentage values situated between 0.25% and 100%.

Keywords: soya, GMO, PCR quantification

INTRODUCTION

The term *Genetically Modified Organism* (GMO) or *Transgenic* is used to define organisms whose genetic material has been altered through genetic engineering techniques (transfer of genes from other species) in order to obtain organisms with new traits or properties. The initial aim was to protect crops by building resistance against plant diseases and pests (viruses and insects) or by enhancing tolerance to herbicides in agriculture.

The genetic modification of living organisms is a way to solve food security problems. This does not mean that the worldwide issues regarding food can be solved solely in one way, but that genetic engineering can significantly contribute to remedy them [1].