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Effect of Leaf Rust on the Molybdenum-Containing Enzymes Activity in Spring Wheat Varieties Differing in Resistance to Infection

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Abstract: The effect leaf rust infection on the activity of molybdenum-containing enzymes - nitrate reductase (NR), aldehyde oxidase (AO) and xanthine dehydrogenase (XDH) - of the spring wheat varieties differing in stability to fungus infection was studied. It was shown that the NR activity decreases in the beginning of the infection process and then increases during progression of leaf rust infection process, then to the time occurrence of necrotic spots in the leaves it decreases again. AO activity in roots and leaves had a general tendency to increase during leaf rust infection process. XDH activity and the content of hydrogen peroxide (H_2O_2) in leaves of the sensitive wheat cv. Akmola-2 after the infection by the fungus rapidly increased, whereas these indicators in leaves of the medium resistant wheat cv. Astana not changed. The problem of oxidative burst and XDH activity was discussed.

Key words: Aldehyde oxidase · Leaf rust (*Puccinia recondita*) · Nitrate reductase · Xanthine dehydrogenase and wheat

INTRODUCTION

Wheat in Kazakhstan is the main food crop. However, its productivity in environmental conditions of the North Kazakhstan is not high and ranging from 4 to 15 c/ha when the potential productivity of spring wheat 70 c/ha. The rust fungus no small role-played in the low yields of spring wheat. In certain years, productivity of spring wheat is reduced by 30-60% thanks to leaf rust infection [1]. Therefore, the study of mechanisms of stability and protection responses against leaf rust infection play an important role in the selection of varieties, selection process and development of measures to reduce its spread.

The mechanism of protective reactions of the plants against leaf rust and other fungal infections is insufficiently studied. Molybdenum containing enzymes play an important role in plant resistance to stress. They are involved in redox reactions of energy metabolism and detoxification of xenobiotics. It is now well-understood three of the five molybdenum-containing proteins - nitrate reductase (NR; EC 1.7.1.1), xanthine dehydrogenase (XDH; EC 1.2.1.37) and aldehyde oxidase (AO, EC 1.2.3.1) - in plants. Xanthine oxidase (XO; EC 1.2.3.2) catalyzes the transformation of hypoxanthine to xanthine and then to uric acid and the oxidation number of the pteridine, aldehydes and imidazoles [2]. Under oxygen-deficient XO functions as a NAD+-dependent XDH, but the mechanisms of action of these two functional forms are fundamentally different [3, 4]. Therefore, this enzyme is sometimes referred to as xanthine oxidoreductase (XOR). In the late 1980s, the study of the XOR had become increasingly important in connection with the discovery of powerful generator of superoxide and apoptotic activity of this enzyme and possible participation of it in carcinogenesis [5]. When it

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was revealed that XO is the main system generating reactive oxygen species in living organisms began the "second wave" of studies on the role of XO in the biochemical processes. At present, the mechanisms of carcinogenesis induction and also apoptosis by of XO-generated reactive oxygen species are still poorly understood [6]. However, there is no doubt that the XO is one of the most important enzymes of living organisms and the main system generating active oxygen species.

The aim of our study was to investigate the effect of rust fungi infection of spring wheat cultivars differing in the resistance to leaf rust on the activity of molybdenum-containing enzymes, which involved in plant defense reactions.

MATERIALS AND METHODS

Plant Materials and Inoculation: In pot experiments studied the effect of leaf rust (Puccinia recondita) on the regionalized in the Akmola region spring wheat cultivars - Akmola-2 (very susceptible to the leaf rust) and Astana (relatively resistant the leaf rust). The seeds were sown in pots (V=250-300 ml) with soil substratum. 5-7 plants were grown in each pot. The light regime followed a 12-h daylight regime, with photosynthetic active radiation ?uxes ranging from 200 to 1300 μmol m^{-2} s^{-1}. In the phase of the two leaves of the plant was inoculated with urediniospores of leaf rust *Puccinia recondita* pathotype *TKT/Y*. The inoculation was carried out manually by treatment the leaves of water-20%TWEEN solution containing urediniospores. The samples of leaves and roots were fixed in liquid nitrogen in dynamics within 8 days for assays. Collected samples were stored at -70°C. Also, the samples of leaves in 8 days after inoculation were extracted with hexane in a ratio of 1:5, centrifuged at 5000 rpm and the supernatant was examined.

The determination of molybdenum-containing enzymes activity: Activity of molybdenum-containing enzymes - NR, AO, XDH - was determined in the native gel. To detect XDH activity used 1.0 ml Tris-HCl (pH 8.0), 1.5 ml NAD, 20 granules hypoxanthine, 5 granules MTT, 5 granules PMS, 2 ml agar. To detect AO activity used 0.6 ml Tris-HCl (pH 8.0), 1 granules benzaldehyde, 5 granules MTT, 5 granules PMS, 4 ml agar. The NR activity was determined by authors [7-10]. The buffer for extraction consisted of 100mM phosphate buffer (pH 7.5), 5mM $(CH_3COO)_2Mg$, 10% (v/v) glycerol, 10% (w/v) polyvinylpyrollidone, 0.1% (v/v) Triton X-100, 1mM EDTA, 1mM DTT, 1mM PMSF, 1mM Benzamidine (freshly prepared), 1mM 6-aminocaproic acid. Leaves were extracted in an appropriate buffer (0.02g/1.5ml), using a mortar, pestle and shaker Vortexing. Extracts were centrifugated at 14000 rpm for 15 minutes. An extracts was kept on ice during the operation to determine the activity. NR activity was determined immediately after centrifugation. Bradford with BSA as standard determined the protein content.

The Determination of Total Content of Hydrogen Peroxide (H_2O_2): The total content of H_2O_2 in plant samples was determined spectrophotometrically at SPEKOL-1300 (Jena analityk, Germany) at 500 nm for 5, 10, 15 minutes after the reaction initiation. The samples were extracted in 50 mM phosphate buffer (pH = 7.5) in ratio 1:8. These extracts were centrifuged at 14000 rpm in a refrigerated centrifuge (5804R, Eppendorf) at 4°C for 10 minutes. To the reaction mixture containing 50 mM phosphate buffer (pH 7.5), 8.5 mM aminoantipurin, 34 mM 3.5-dichloro-2-hydroxybenzenesulfonate sodium, 45 units/ml horseradish peroxidase were added supernatants in the ratio 4:1 and determined the total content of hydrogen peroxide in the samples, which are expressed in μmoles H_2O_2 g^{-1} FW. Determinations were performed in triplicate for each variant.

RESULTS AND DISCUSSIONS

Visual and microscopic observations showed sufficient activity of spores of the leaf rust fungi Puccinia recondita (Fig. 1-4). It may be noted a high percentage of germination of spores and infection of leaves, i.e. our model of the leaf rust fungus inoculation was rather effective. Activity of NR, AO and XDH was determined in the leaves and roots of wheat cv. Astana and Akmola-2, which differ in their resistance to leaf rust fungus. We used various methods of determination of enzymes activity - NR (spectrophotometric method), XDH and AO (electrophoresis method).

Figure 5 shows the daily dynamics of NR activity of wheat leaves after inoculation with leaf rust fungus. The enzyme activity decreases in the beginning of process of the leaf rust infection (3-rd day of vegetation) as against control, then it increases (4-5-st days of vegetation) and then it decreases again (6-7-st days of vegetation). Moreover, on 5-th day after inoculation, when the indicators of infection visually begin to show

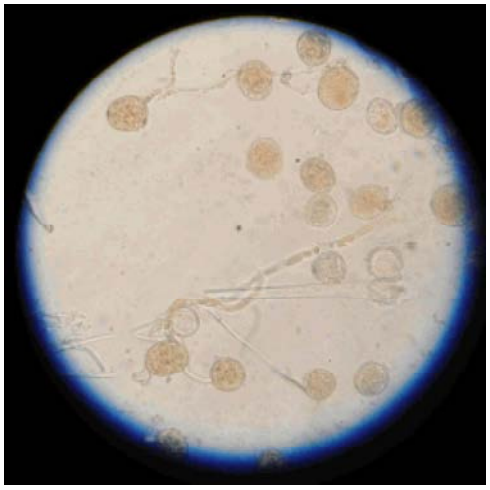

Fig. 1: Germinated spores of leaf rust fungi *Puccinia recondita*.

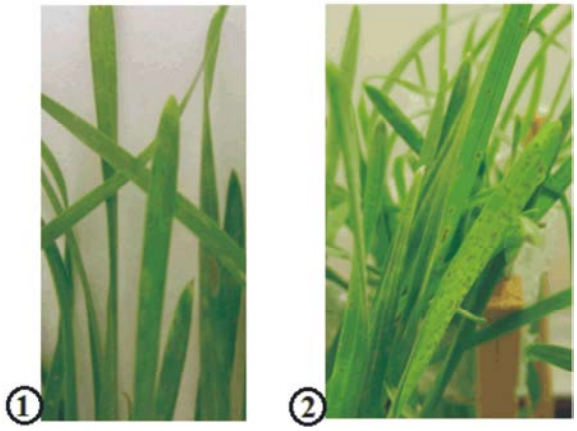

Fig. 2: The leaves of wheat cv. Astana (1) and Akmola-2 (2) infected by leaf rust fungus Puccinia recondita.

Fig. 3: The leaves of wheat cv. Astana after infection by leaf rust fungus: 1 – control leaves, 2 – infected leaves.

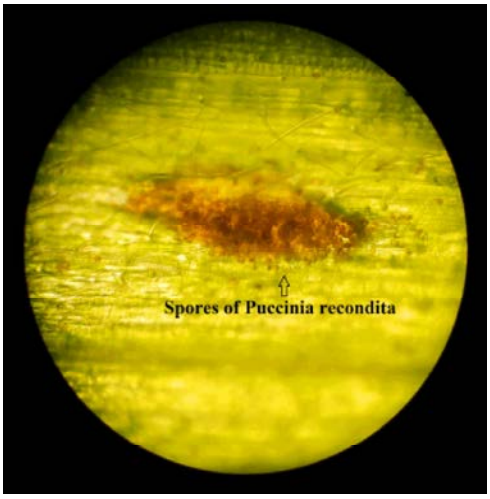

Fig. 4: The spores of leaf rust fungus Puccinia recondita germinated in the epithelium of leaf of the wheat cv. Akmola-2.

on the leaves, NR activity is increases significantly. This indicates to the participation of the enzyme in the defense reactions of cells and changes the direction of metabolism in the infected leaf.

The activity of other enzymes also changed. Thus, in the spectrum of the enzyme AO after native polyacrylamide gel electrophoresis in the leaves identified 1 band, in roots - 2 bands. Moreover, 2-nd band of roots has a higher mobility than the 1-st band (Fig. 6). After infection by leaf rust fungus AO activity in roots and leaves had a general tendency to increase. Thus, AO activity of leaves of very susceptible to the leaf rust wheat cv. Akmola-2 and medium resistant wheat cv. Astana increased by 40%. However, the distribution of roots AO activity was observed some differences. The intensity of AO 1-st and 2-nd band in the roots of infected wheat cv. Akmola-2 increased by 19% and 118 % respectively, whereas the intensity of AO 1-st band in the roots of infected wheat cv. Astana was increased by 17%, while the intensity AO 2-nd band reduced by 14% (Fig. 6). The total enzymatic activity of AO was not determined.

After native polyacrylamide gel electrophoresis in the spectrum of the enzyme XDH in leaves and roots of wheat were identified one band (Fig. 7). XDH activity in the leaves of wheat cv. Akmola-2 after infection by leaf rust sharply increased (by 123%), while the enzyme activity in the leaves of wheat cv. Astana didn't change. In the roots we observed the opposite trend: XDH activity in the roots of wheat cv. Akmola-2 decreased by 9% and it in the

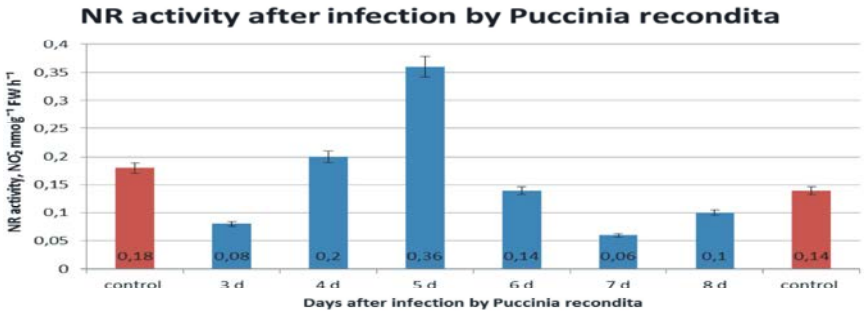

Fig. 5: Effect of leaf rust infection on the NR activity of leaves of the wheat cv. Astana. Data is shown after 1-8 days inoculation of leaf rust fungus. On the ordinate is the relative enzyme activity, expressed in NO_2-nmolg-1FWh-1

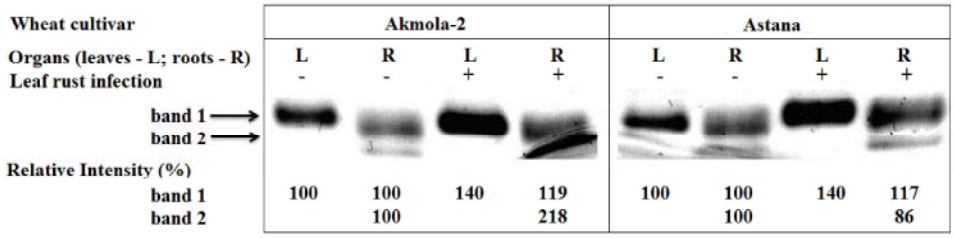

Fig. 6: Effect of leaf rust infection (*Puccinia triticina*) on AO activity in leaves and roots of wheat cv. Akmola-2 and Astana

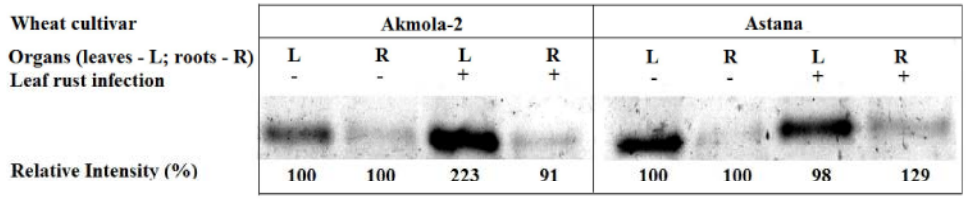

Fig. 7: Effect of leaf rust infection (*Puccinia triticina*) on XDH activity in leaves and roots of wheat cv. Akmola-2 and Astana

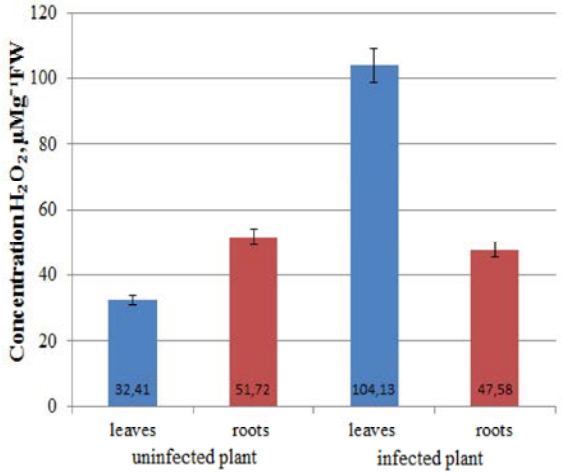

Fig. 8: Effect of leaf rust infection (*Puccinia recondita*) on the total content of H_2O_2 in leaves and roots of wheat cv. Akmola-2.

roots of wheat cv. Astana - increased by 29% (Fig. 8). Changes of XDH activity are accompanied by increasing of the total content of H_2O_2 in leaves by 3.2 times. The total amount of H_2O_2 in the roots didn't change.

Plants have a wide range of protective mechanisms against pathogen attack. They have physical barriers such as the cuticle and cell wall and biochemical protection (antimicrobial toxins). Moreover, quickly inducible defense mechanisms may be activated by the attack of the pathogen. One particularly important among them is the hypersensitive response (HSR), when death of plant cells stops further spread of the pathogen infection due to the incompatibility between host plant and pathogen. HSR includes induction of oxidative burst on plasma membrane of cells, as a result formation of reactive oxygen species (ROS) such as superoxide, which is converted to H_2O_2. The ROS formation is a very early response of plant cells to pathogen infection. The earliest

response of the plant to pathogen inculcation is the local generation of ROS - oxidative burst, which is activating a chain of following protective reactions [11]. A significant role in the generation and regulation of ROS content in plant tissues play redox enzymes, primarily oxidoreductases [12]. Data showed that the formation of ROS during oxidative burst occurs with the participation of enzymes located on the surface of cells: NADPH-oxidase, XO, superoxide dismutase, oxalate oxidase, peroxidase [13-16].

We were determined the activity of the three enzymes - NR, AO, XDH. These enzymes are actively involved in the defense reactions of plants under the effect of many kinds of stress. We have shown changes in these enzymes activity under fungal infection. And changes in the enzyme activity not depend on the action of leaf rust infection: activity as increases and decreases or little change. Thus, there are different directions of change of nitrogen compounds under leaf rust infection. In this regard, changes in the activity of NR are interesting: initially it decreases and then increases by almost 2 times, then decrease again. What is behind this change in enzyme activity? It is not clear. We supposed that the active cell damage of leaves was affected on the enzyme activity.

Observed by us increasing of the total amount of H_2O_2 in the leaves can be attributed to the effects of oxidative burst. The plant is struggling with the pathogen through intensive synthesis of ROS, which inhibit the activity of the pathogen. This is accompanied by increased activity of XDH in leaves of very susceptible to the leaf rust wheat cv. Akmola-2. The activity of this enzyme in leaves of medium resistant wheat cv. Astana didn't change, which indirectly indicates the absence of an active oxidative burst.

CONCLUSIONS

Research was carried out in the intervarietal context. Cultivars differing in resistance to leaf rust fungus were taken. Unidirectional or multidirectional changes in the activity of enzymes on the studied wheat cultivars infected by leaf rust fungus were revealed: increase or decrease. We can only state the fact of a change in the activity of enzymes in medium resistant or very susceptible to the leaf rust wheat cultivars. Further study of the metabolism of cells exposed to the pathogen, may explain the features of changes in the enzymes activity of wheat cultivars differing in resistance to leaf rust infection. Changes in the activity of enzymes are indirect evidence of fighting against the pathogen. However, it is necessary further study of the enzymes during infection of spring wheat by leaf rust fungus.

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