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Quantitative Determination of Signaling Molecules in the Spring Wheat Varieties Differing in Resistance to Infection after Puccinia Recondita Inoculation

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Abstract: It was studied the quantitative content of salicylic (SA), jasmonic (JA), arachidonic (AA) acids, during Puccinia recondita infection of the spring wheat cultivars differing in resistance to leaf rust. Method for determining the concentration of signaling molecules by a liquid chromatograph was developed.The trend of change in the content of signaling molecules during the leaf rust infection of wheat, subject to the different resistance of cultivars was shown.The results showed that the studied signaling molecules are notifies plant about the threat of infection by leaf rust as well as stimulate the functioning of certain parts of metabolism in normal and stress conditions.

Key words: Arachidonic acid · Jasmonic acid · Salicylic acid · Puccinia recondita · Signalling · Wheat

INTRODUCTION

Infectious plant diseases cause significant damage to productivity of crops.Yield losses of grain and leguminous crops from fungal diseases can be up to 30% [1]. Plant resistance to different infections is determined by a complex physiological and biochemical reactions, each contributing to the protection of the pathogen. A set of signaling molecules that trigger a cascade of response biochemical reactions in the plant body under contact with the pathogen was revealed in plant tissues. This functional role may be performed by phytohormones, oligosaccharides, jasmonate, salicylate, nitric oxide and some other compound [2-5]. Signalling systems of cells - a cascade of biochemical reactions involved in the recognition and reception of extracellular signal, their transformation, enhancement and transmission in the genome. As a result of these processes the program of gene expression is changed and plant adaptation to varying environmental conditions is changed, too. It is assumed that the signaling molecules assist in the formation of primary responses as well as determine the nature of next biochemical and physiological processes that set conditions for the adaptation and resistance of plant to pathogen attack [2].

The basis of the effective development of the wheat defense response to infection by fungal pathogens is alteration in the hormonal system associated with increased levels of cytokines in the background of a relatively stable balance of IAA and ABA.Molecular mechanisms of plant resistance to fungal pathogens are still far from the final answer.The aim of our study was the quantitative content of signaling molecules during Puccinia recondita infection of the spring wheat cultivars differing in resistance to leaf rust.

MATERIALS AND METHODS

Plant Materials and Inoculation: 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) was studied in pot experiments.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}. After installation, the plants were left untreated for 2 days for stabilisation. Plant was inoculated (pathotype TKT/Y) in the phase of two leaves. The

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inoculation was carried out manually by treatment the leaves of water-20% TWEEN solution containing urediniospores.Before infecting spores kept in water at 36°C, for 2 hours. Samples for analysis were taken at 6th day after infection.

Isolation of Low-Molecular Organic Compounds (LMWOC) from the Leaves: Extraction 1 hour in Ultrasonic bath at 50°C, mix 30 min, centrifuged. Take 1 ml of ethanol extraction, add 9 ml H_2O and 5 ml tert-butyl-methyl ether (TBME), mix 15 min. Take TBME and evaporated, dilute in 0.1 ml acetonitrile.

Liquid Chromatography (LC) Method: Pump: Thermo Scientific 600 pump; column: Hypersil Gold C-18, 50x2.1mm, 1.9mcm; injection volume 10mcl.

Mass Spectrometry (MS) Method: Mass-spectrometr: Thermo Scientific TSQ Vantage; ion source: HESI in negative mode; capillary temperature:300°C; vapporizer temperature:400°C; sheath gas pressure:50Arb; aux gas pressure:15Arb; ion sweep gas pressure:1Arb; spray voltage: 3000V; collision gas pressure: 1,2 mTorr.

KOH Hydrolysis: Take 1ml of ethanol extraction, add 0.1ml 3M KOH in H_2O, keep 1 hour at 80°C, after cooling add 4ml H_2O + 0.1ml 5M HCl, mix. Add 5 ml TBME, mix 15 min, centrifuged. Take TBME and evaporate, dilute in 0.1 ml acetonitrile.

RESULTS AND DISCUSSIONS

The level of signaling molecules was determine quantitatively in the spring wheat cultivars differing in the resistance to infection in a model system after Puccinia recondita inoculation (Figs. 1-6).It was found that amount of Salicylic acid in the leaves of wheat cv. Astana after leaf rust infection (experiment) and without KOH hydrolysis of extract increases by 50%, while its content in the leaves of wheat cv. Akmola-2 - only by 8% (Table 1). Amount of JA in the leaves of wheat cv. Astana increased by 10% and its amount in the leaves of wheat cv. Akmola-2 reduced by almost 30%. As opposed to JA, amount of AA in the leaves of wheat cv. Astana is reduced by 50%, while its amount in the leaves of wheat cv. Akmola-2 is increased by two times.

After KOH hydrolysis of the extract, amount of Salicylic acid in the leaves of wheat cv. Astana increased more than 50% after leaf rust infection and its content in the leaves of wheat cv. Akmola-2 not changed (Table 2). Amount of JA in the leaves of wheat cv. Astana is reduced by 20% and its content in the leaves of wheat cv. Akmola-2 - by 60%. The availability of Arachidonic acid in the extract after hydrolysis doesn't found, probably it was to result from its saponification.

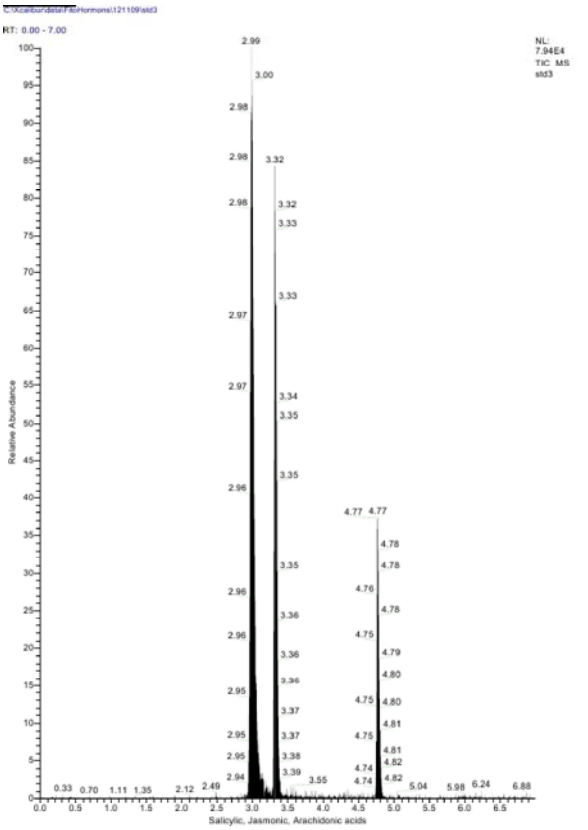

Fig. 1: The separation of organic compounds using liquid chromatography.The calibration curve for the content of SA, JA, AA.

Table 1: The content of signaling molecules in the leaves of wheat after leaf rust infection.Without KOH hydrolysis, in $ng_{*}g^{-1}$ ($mcg_{*}kg^{-1}$).

Variant	SA	JA	AA
Control Astana	46.0±1.5	100.9±2.3	26.8±0.6
Experiment Astana	62.3±2.1	110.7±2.3	12.7±0.2
Control Akmola-2	54.9±1.5	160.8±3.2	21.7±0.3
Experiment Akmola-2	58.9±2.1	105.0±3.0	35.8±0.7

Table 2: The content of signaling molecules in the leaves of wheat after leaf rust infection.After KOH hydrolysis, in $ng_{*}g^{-1}$ ($mcg_{*}kg^{-1}$).

Variant	SA	JA	AA
Control Astana	196.7±4.1	65.1±1.6	0.0
Experiment Astana	319.2±5.6	48.3±1.3	0.0
Control Akmola-2	201.7±4.1	86.8±2.1	0.0
Experiment Akmola-2	202.3±5.1	32.0±0.7	0.0

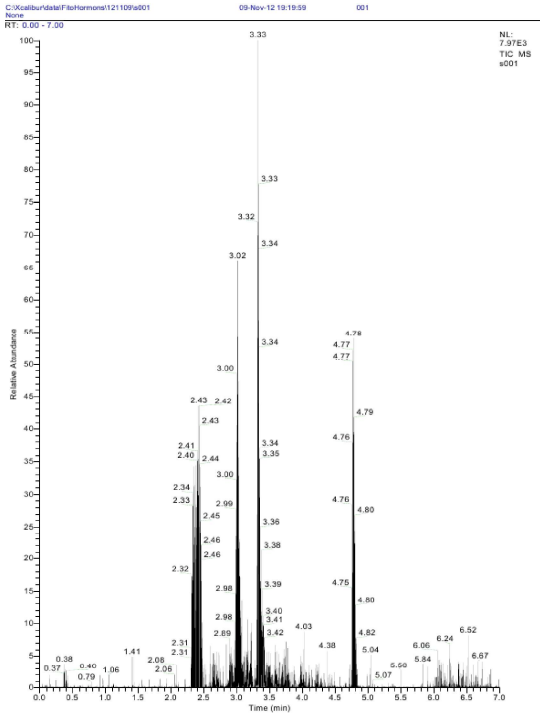

Fig. 2: Separation of LMWOC of the uninfected leaf rust wheat leaf cultivar Astana by liquid chromatography.

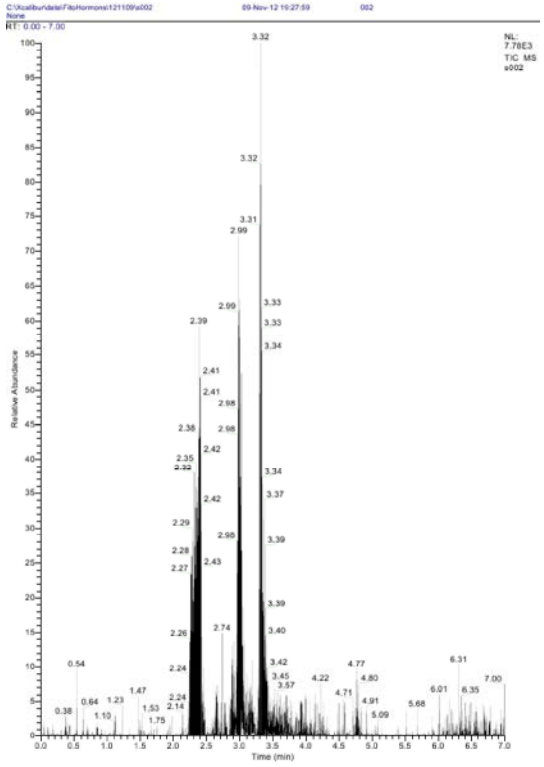

Fig. 3: Separation of LMWOC of the infected leaf rust wheat leaf cultivar Astana by liquid chromatography.

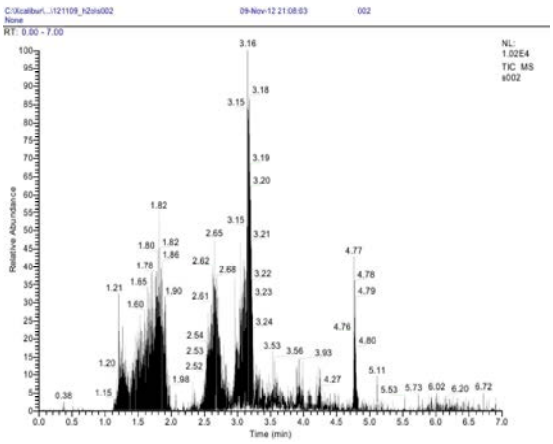

Fig. 4: Separation of LMWOC of the infected leaf rust wheat leaf cultivar Astana by liquid chromatography. Without adding formic acid into eluent.

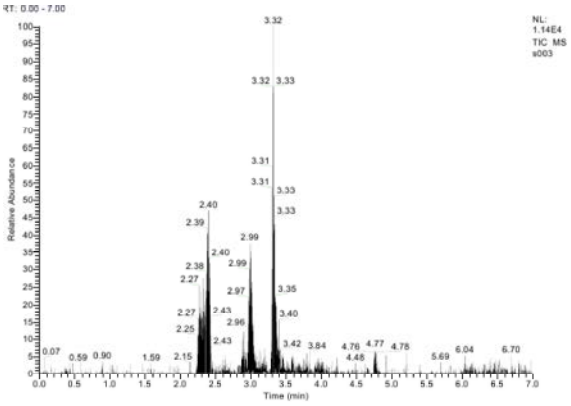

Fig. 5: Separation of LMWOC of the uninfected leaf rust wheat leaf cultivar Akmola-2 by liquid chromatography.

The amount of LMWOC in very susceptible wheat cv. Akmola-2 increased after inoculation with leaf rust (Figs. 5 and 6). Leaf rust infection of relatively resistant wheat cv. Astana does not lead to complicate the spectrum (Figs. 2-4). This results correlate with data by Delaney *et al*. [6]. He *et al*. discovered the induction of the phenolic compounds synthesis after treatment of plant with JA.We also have shown that the adding of formic acid in eluent and extract treatment by TBME improves the chromatographic separation (Figs. 3 and 4).

Our data show lower concentrations of LMWOC in wheat leaf tissue. They are comparable with the concentration of phytohormones in plants. Therefore LMWOC are called the plant growth regulators. We have identified changes in the content of these compounds

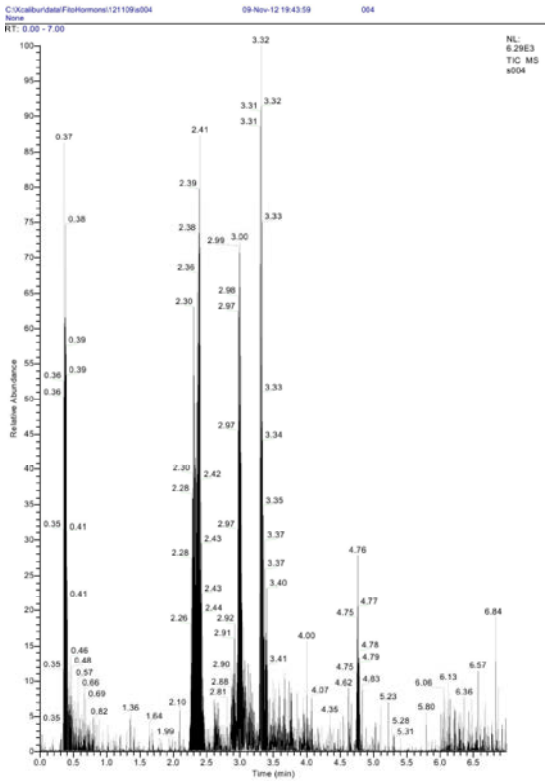

Fig. 6: Separation of LMWOC of the infected leaf rust wheat leaf cultivar Akmola-2 by liquid chromatography.

(Tables 1 and 2, Figs. 1-6). It is indicate on the continued involvement of LMWOC in the metabolism of cells. Thus, in some cases,the amount of LMWOC decreases after leaf rust infection.The function of LMWOC is a warning of leaf rust infection danger, as well as maintenance of the functioning of certain parts of metabolism in normal and stress conditions, including plant wound healing, tuberization, fruit ripening, roles in biotic/abiotic s tress responses, defense and senescence. It is known, that exogenous SA between 10 and 500 µMcould induce thermotolerance in mustard seedlings [7]. Authors [8]considers thatthe endogenous SA levels are about 15 to 120 µM, which is within the range of concentrations used to induce thermotolerance. 10 to 50 µMSA potentiates the response of soybean cells to an avirulent*Pseudomonas syringae*pv*glycinea*strain [7]. The mechanism of JAaction has been documented to be hormonal by regulating the translation of varying genes. SA is involved inendogenoussignaling, mediating in plant defense againstpathogens.It plays a role in the resistance to pathogens by inducing the production ofpathogenesis-related proteins. This confirms the view [2] on the signaling and metabolic role of examined molecules in vital activity of plant.

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