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THESES OF THE DOCTORAL DISSERTATION

STUDY OF THE EFFECT OF ENVIRONMENTAL FACTORS ON THE BARLEY WITH PROTEOMIC METHODS

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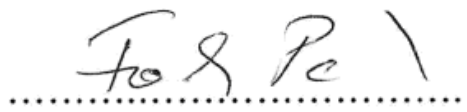

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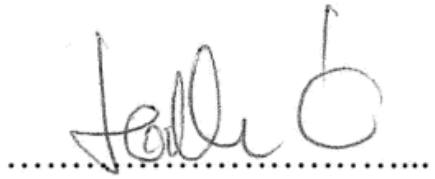

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1. BACKGROUND

In the 21th century, cereals with their two milliard-ton-yield are the most important crops in the world. The amount of barley grown ranks as the fourth largest among cereals. Barley became widely used about 10000 years ago in the the Middle East and from that time it has been used for feeding animals and making beer, and in smaller quantities for making foods.

Barley, like other grown plants, is continuously exposed to biotic and abiotic stresses during its life cycle, which can significantly influence its development, growth and productivity. Environmental conditions, which fall outside the optimum values for a plant species (too low or too high temperature, not adequate supply of minerals, not adequate light and lack of water, etc.) represent stressful situations for the plant. The yield of cereals in Hungary is half of what would be expected in an ideal environment, which is the result of different environmental, abiotic effects (mainly drought), leading to considerable economical loss. Our goal is to breed plants that are resistant to abiotic and biotic stresses, which will help us to decrease the economical losses.

Based on this evidence, in my Ph.D. work I conducted research using proteomics (2-DE, MS) in order to study the change of the expressed protein pattern in Hungarian conditions grown and abiotic stress-susceptible ‘Jubilant’ and abiotic stress-tolerant ‘Mandolina’ barley cultivars in response to different abiotic stress effects, like high temperature, cadmium contamination and different growing conditions. To study the influence of different stresses on the proteom level it is highly important because the function of 20% of expressed sequence tags (EST), which were detected in different stress responses on the transcriptom level, are still unknown. With the help of proteomics it is possible to study on the protein level the signal transduction, regulation, enzymatic activity and the structural functions.

In the defence mechanism against different stresses among stress-induced molecules stress proteins have a very important role, because they contribute to the increase of resistance. The identification of proteins, which are involved in stress responses, is essential because of the risk of their intake of our nutrition chain. In recent years there

were isolated and characterized some allergens from foods, which contained defense mechanism involved proteins too.

Beside the study of defense mechanism involved proteins I wanted to make a detailed insight into how abiotic stress factors can influence the protein composition of the basic material of our foods.

2. AIMS OF THE RESEARCH

In my Ph.D. work I conducted research using proteomics methods (2-DE, MS) in order to study:

1. and to compare the carbamid soluble two dimensional protein patterns of the seedlings of three different barley cultivars (’Jubilant’, ’Bivoy’, ’Mandolina’) and to investigate the influence of short term heat stress influence on the two-dimensional protein pattern of seedlings from an abiotic stress-tolerant ‘Mandolina’ and an abiotic stress-susceptible ‘Jubilant’ barley cultivar.

2. the influence of different growing conditions on the carbamid soluble protein composition of Jubilant spring barley cultivar.

3. the influence of different cadmium concentrations (200 μM és 1 mM $CdCl_2$) on the carbamid soluble two dimensional protein patterns of the seedlings of ’Jubilant’ barley cultivar.

4. the influence of two different years on the water and salt soluble two dimensional protein patterns of grains of three barley cultivars (’Jubilant’, ’Bivoy’, ’Mandolina’).

3. MATERIALS AND METHODS

Samples:

Two-rows spring *Hordeum vulgare* barley cultivars were from a field crops research station in Táplánszentkereszt of Szegedi Gabonatermesztési Közhasznú Társaság.

'Jubilant' is a Slovakian barley, which received state acknowledgement in Hungary in 1993. The productivity of the cultivar is good, but it is sensitive to environmental anomalies. The quality of the brew is excellent. In Hungary this cultivar is the most grown barley.

'Bivoy' is a German barley cultivar which has the best productivity and excellent adaptability and is drought tolerant. In Hungary this cultivar got state acknowledgement in 2002.

'Mandolina' is a Dutch barley cultivar which has excellent productivity and low protein content. It is drought tolerant. In Hungary this cultivar received state acknowledgement in 1998. It has the best adaptability among spring barley cultivars.

Extraction of barley proteins:

The protein samples were taken from two different parts of the barley plants: from seedlings and from grains.

In order to extract the proteins of seedlings I first used TCA to precipitate proteins. Afterwards I used acetone to remove components which can be disturbing for isoelectric focusing (eg poliphenols, salts, plant pigments). This is one of the most widely used protocols to extract proteins from plants for the purpose of two dimensional gel electrophoresis. The TCA precipitated proteins were resolved using carbamid-based lysis buffer. I used the aforementioned extraction protocol for the investigation of the effect of cadmium and heat stress on the barley seedings and also to study the different growing conditions on the grains of barley.

To investigate the effect of different years on the barley grains I first selected the 2,5 mm larger barley grains which are used for malting barley and then I extracted proteins with 0,1M NaCl solution. In this way retained the metabolically active albumin and globulin proteins in the solution.

Protein separation

One dimensional gel electrophoresis

Proteins were separated according to molecular weight in sodium dodecil sulphate (SDS) polyacrilamide gel in a Mini Protean II (Bio-Rad, Hercules, Ca, USA) system. Gels were stained with Coomassie Brillant Blue G-250 dye.

Two dimensional gel electrophoresis

Proteins were separated according to their isoelectirc point in a Multiphor II (Amersham Biosciences, Uppsala, Sweden) or a PROTEAN IEF cell (Bio-Rad) system with the use of pH 3-10 strips. For the molecular weight separation SDS polyacrilamide gels were used in Protean Plus Dodeca Cell (Bio-Rad) or PROTEAN II XL multi-cell (Bio-Rad) systems. Gels were stained with Coomassie Brillant Blue G-250 dye.

Gel evaluation

The gel images were digitized with a 12-bit GS-710 calibrated densitometer (Bio-Rad)and analyzed with the PDQuest 7.1 software (Bio-Rad). After spot detection, the 2-D maps were automatically aligned followed by manual spot editing to increase the correlation between the different 2-D maps. Statistical analysis of the relative abundance of each matched protein spot was accomplished by using two-tailed t test. Quantitative differences with a p-value of at least <0.05 were considered significant.

Protein identification with mass spectrometry

Proteins were identified in the Laboratory of Protein Biochemistry and Protein Engineering of Ghent University and the Department of Medical Chemistry of University

of Szeged with the following instruments: Bruker Reflex III MALDI-TOF MS (Bruker-Daltonics, Bremen, Germany), MALDI TOF/TOF MS (Applied Biosystems, Framingham, CA, USA) and ESI-Q-TRAP MS (Applied Biosystems).

The genome of *Hordeum vulgare* has not been fully sequenced, but several *Hordeum vulgare* EST databases (www.ncbi.nlm.nih.gov) were downloaded and formatted to make it accessible via the database searching program MASCOT (www.matrixscience.com). If mass spectral data fitted a translated EST sequence, this sequence was loaded into a BLASTquery for annotation. In some cases proteins were identified according to peptid mass fingerprint (PMF).

4. SUMMARY

In my Ph.D. work I conducted research using proteomics (2-DE, MS) in order to study the change of the expressed protein pattern in barley in response to different abiotic stress effects, such as high temperature, different growing conditions, high concentration of cadmium and drought. I demonstrated the expression of numerous proteins, which are involved in the defense system against abiotic stressors and I also demonstrated the consequence of these kind of stressors to the protein components of our raw food materials. My proteomic approach demonstrated significant changes of the components of different type of barley cultivars upon abiotic stresses. The high-resolution 2-D gel electrophoresis combined with mass spectrometry proved to be a successful strategy despite the fact that the genome sequence of barley is not yet available. I was able to identify most of the proteins displaying differential protein synthesis upon stresses, which was made possible by the MS and MS/MS capabilities in the mass spectrometric analyses.

I first compared the two-dimensional protein pattern of seedlings from an abiotic stress-tolerant ‘Mandolina’ and an abiotic stress-susceptible ‘Jubilant’ barley cultivar. One isoform of the protein SAM-S displayed increased expression in the stress-tolerant (Mandolina) cultivar. This isoform was not detectable in the stress-susceptible cultivar, but it was present in the drought tolerant ‘Bivoy’ cultivar. In studying short-term temperature stress on ‘Jubilant’ and ‘Mandolina’ barley shoots, we detected increased protein abundance following stress for several low molecular weight HSPs and isoforms of these proteins.

I next studied the effect of different growing conditions on the protein patterns in the grain of ’Jubilant’ cultivar. I identified proteins whose expression changed upon abiotic stresses, which included 22,0 kDa (IV class) heat shock protein precursor, GroEL heat shock protein, peroxidase enzyme and glyceraldehyde 3-phosphate dehydrogenases. I demonstrated that the different growing conditions can induce or inhibit the expression of some proteins, and can also cause post-translational modifications. This was evidenced by the presence of a shift in the position of GAPDH upon stress. Finally, we investigated changes of protein patterns in the seedlings of ‘Jubilant’ barley cultivar after the application of different 200 μM and 1 mM cadmium-chloride concentrations There was a

decrease of the expression of the RuBisCo large subunit in response to cadmium-chloride. In addition, the oxygen evolving enhancer 2 protein was found to be reduced upon cadmium chloride treatments.

An in-depth analysis of proteins which show altered expression upon abiotic stresses can help in devising ways to more effective resistance breeding. This kind of approach can help to find functions for stress inducible genes whose functions are still unknown and also can help us to better understand the defense system of plants. It will, therefore, be necessary to investigate in the future the effect of abiotic stress on the proteome of different barley cultivars and to extend the research to more abiotic stress susceptible and abiotic stress tolerant barley cultivars in order to better understand the role of the detected SAM-S isoform.

4.1 NEW SCIENTIFIC RESULTS

1. I found one isoform of the protein SAM-S in the abiotic stress-tolerant ‘Mandolina’ and ‘Bivoy’ cultivars, which was not detectable in the ‘Jubilant’ cultivar that is sensitive to environmental anomalies. I propose that the lack of this isoform may play a role in the sensitivity of ‘Jubilant’ cultivar to environmental anomalies.

2. In studying short-term temperature stress on ‘Jubilant’ and ‘Mandolina’ barley shoots, I detected increased protein abundance following stress for several low molecular weight HSPs (16,9 kDa, 17,8 kDa, sHSP), which showed high homology with the HSPs from rice and wheat. The HSPs were present in more isoforms and they showed expression differences between the two cultivars of these proteins.

3. During the study of the effect of different growing conditions and drought on the protein patterns in the grain of ’Jubilant’ cultivar I identified carbamid soluble proteins whose expression changed upon abiotic stresses. I demonstrated that the different growing conditions can induce or inhibit the expression of some proteins.

4. I identified the glyceraldehyde 3-phosphate dehydrogenase homolog proteins, whose expression positions changed upon different growing condition. This observation can indicate the different role of these isoforms, to the different isoform regulation of different compartments of the grain and to post-translational modifications upon different growing conditions.

5. During the study of different growing conditions I found the increased expression of several defense related proteins homolog proteins, which included 22,0 kDa (IV class) heat shock protein precursor, GroEL heat shock protein and one isoform of peroxidase enzymes.

6. Comparison of carbamid soluble protein patterns of normal and difficult growing conditions revealed the decreased intensity of serin protease inhibitor Z7 upon

difficult growing condition. With the present study I identified a specific growing condition whose influence is negative for the quantity of beer foam stability forming protein and can cause the increased synthesis of hordein proteins.

7. Treating the barley seedlings with different cadmium-chloride (200 μM and 1 mM) decreased expression of the RuBisCo large subunit in response. In addition, the oxygen evolving enhancer 2 protein was found to be reduced upon cadmium chloride treatment. These results represents the first identification in barley of protein expression changes using proteomic methods.

5. PUBLICATIONS:

Publications in scientific journals:

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