During their life cycles, plants have to handle a range of biotic and abiotic stresses. Under these stresses, numerous physiological, biochemical and metabolic functions are modified in plants. The research of plant responses to stress on the DNA or Rna level provided an important insight into defence processes (e.g. Seki et al. 2001; Fowler & Thomashow 2002; Gulick et al. 2005), but it is known that the levels of transcripts and proteins are not strictly correlated as shown in yeast (Gygi et al. 1999; Ideker et al. 2001). In addition, many proteins are modified by posttranslational modifications such as phosphorylation, glucosylation, ubiquitinylation, sumoylation, and many others (Mann & Jensen 2003; Schweppe et al. 2003; Canovas et al. 2004) which significantly influence protein functions. Knowledge of the full complement of proteins expressed by the genome of a cell, tissue or organism at a specific time point (i.e. proteome) is necessary to understand the biology of a cell or an organism. The proteome reflects the actual state of the cell or the organism and is an essential bridge between the transcriptome and the metabolome.

Proteins act directly on biochemical processes, and thus must be closer to the phenotype, compared to DNA-based markers. In this context, proteins certainly represent more informative markers compared to DNA markers (Thiellement et al. 2002).

Compared to the analysis of the transcriptome, the analysis of the plant proteome in response to abiotic and biotic stresses is still limited, but technical progress has been achieved in the separation of proteins and their identification by mass spectrometry (e.g. Canovas et al. 2004; Marra et al. 2006). The methods of proteomics were intimately described in many other reviews (e.g. Renaut et al. 2006), so these methods will be recapitulated only shortly. Two-dimensional gel electrophoresis (2-DE) developed about 30 years ago is still the most frequently used method to

**Review**

**Proteome Analysis in Plant Stress Research**

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**Abstract:** Proteomic techniques that allow the identification and quantification of stress-related proteins, mapping of dynamics of their expression and posttranslational modifications represent an important approach in the research of plant stresses. In this review, we show an outline of proteomics methods and their applications in the research of plant resistance to various types of stresses.

**Keywords:** plant proteomics; mass spectrometry; two-dimensional gel electrophoresis

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investigate differential protein abundance in large-scale proteomics experiments on crude protein mixtures (O’Farrell 1975). 2D-DIGE (two-dimensional difference gel electrophoresis) is one of the recent improvements of 2-DE. The basis of this technique is the labelling of an internal standard and two different samples by fluorescent dyes (Cy2, Cy3, Cy5), which are then mixed and separated on one 2-DE gel. Among its multiple advantages, DIGE provides quantitative results and increases the dynamic range of the technique (Tonge et al. 2001; Renaut et al. 2006). Non-denaturing 2-DE, referred to as blue-native PAGE (BN-PAGE) (Schagger & von Jagow 1991), has been developed to study protein complexes. One of the limitations of 2-DE is the low representation of one 2-DE gel. Among its multiple advantages, DIGE provides quantitative results and increases the dynamic range of the technique (Tonge et al. 2001; Renaut et al. 2006). Non-denaturing 2-DE, referred to as blue-native PAGE (BN-PAGE) (Schagger & von Jagow 1991), has been developed to study protein complexes. One of the limitations of 2-DE is the low representation of the genome expression, with 2 000 to 3 000 gene products revealed in the best 2-DE gels instead of 10 000 to 15 000 genes expressed in the same tissue and at the same developmental stage. Thus, fractionation of the crude extract is becoming a necessity (Thielemann et al. 2002).

After the separation of proteins by 2-DE, selection of spots of interest and their isolation, proteins are digested by trypsin and identified using mass spectrometry (MS) while searching in public databases using computer software programs. If the genome of the plant has already been sequenced, it is usually sufficient to identify a protein of interest by peptide mass fingerprinting. When no such genomic data exist, sequencing of the peptide by tandem mass spectrometry (MS/MS) may be required to identify the protein of interest (Hirano et al. 2004).

The separation of a complex protein mixture before protein identification by mass spectrometry (MS) could also be done by gel-free methods. Examples of gel-free methods include multidimensional liquid chromatography (LC) in multidimensional protein identification (MudPIT) (e.g. McDonald et al. 2002), combined fractional diagonal chromatography (COFRADIC) for the sorting and identification of methionyl, cysteinyl amino terminal, phosphorylated and glycosylated peptides (Gevaert et al. 2003, 2005; Martens et al. 2005; Ghesquiere et al. 2006) or proteome chips (protein-microarrays) for the analysis of protein-protein interactions and protein activities (Zhu et al. 2001). Using the technology of the protein-chip coupled with bioinformatics, it is becoming possible to screen any protein sample for putative disease biomarkers from a small sample volume by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) (Caputo et al. 2003; Elek & Lapis 2006; Kuramitsu & Nakamura 2006). Recently, the quantification of proteins in the complex protein sample could be solved by stable isotope methods like SILAC (stable isotope labelling by amino acids in cell culture) where the cells are metabolically labelled by non-radioactive amino acids, which makes their proteomes distinguishable from cells labelled by ‘wild type’ amino acids (Ong et al. 2002). Some other quantitative methods, like isotope coded affinity tags (ICAT) (Pan et al. 2005; Stroher & Dietz 2006) or novel mass tagging strategy (iTRAQ™ Applied Biosystems) (Jones et al. 2006), label peptides by stable isotopes through the chemical modification. Gel-free techniques are more automated and are not so time consuming as gel-based techniques. However, they are not widely used in the plant biology yet. This is due to the fact that most of the gel-free techniques require the sequence of the entire genome of the organism to be available (Renaut et al. 2006).

Abiotic and biotic stress

About 200 original articles focusing on plant proteomics have been published during the last two years, i.e. less than 1% of the global proteomics output (Rossignol et al. 2006). These papers concern the proteome of at least 35 plant species but have concentrated mainly on Arabidopsis thaliana and Oryza sativa. In these papers, the authors used a proteomic analysis of organs, tissues, cell suspensions, or subcellular fractions for the study of plant development and response to various stresses (Rossignol et al. 2006).

Plants responding to environmental stresses (e.g. pathogens, cold, frost, drought, and heat) produce several protective compounds and proteins such as PR proteins (pathogenesis related), HSPs (heat-shock proteins), AFPs (antifreeze proteins), dehydrins and other proteins.

Here we describe only some examples of using proteomics in the research of abiotic and biotic stress.

Abiotic stress

Primary stresses, such as cold, drought, salinity, heat and chemical pollution, and secondary stresses, such as osmotic and oxidative stress cause
cellular damage (Wang et al. 2003). During these stresses, analogous sets of genes are activated whose products lead to the re-establishment of homeostasis and participate in the protection and restoration of damaged proteins and membranes. For instance dehydrins, also known as Lea D-11 or Lea II proteins, are proteins whose expression is induced by various environmental factors which cause the dehydration of cells (Close 1997; Kosová et al. 2007). Among these factors, cold, frost, drought, enhanced salinity of the substrate and enhanced evaporation are the most notable (e.g. Close et al. 1995; Kirch et al. 1997; Choi et al. 1999; Welling et al. 2004). The expression of many dehydrins is also induced by an increased level of ABA (Lang & Palva 1992; Deng et al. 2005).

Using an image analysis of 2-DE gels of heat-stable proteins, Vítámvás et al. (2007) distinguished two highly frost tolerant winter wheat cultivars differing in the levels of accumulation of wheat dehydrins (WCS120 proteins). Recent DNA markers for frost tolerance could distinguish only spring and winter cultivars (e.g. Vágcafalvi et al. 2000). Differences in protein patterns were also determined by a gel-free technique (LC-MS/MS) in the heat-stable protein extracts of cold-acclimated and non-acclimated plants of winter wheat Mironovskaya 808 (Figure 1) (Vítámvás et al. 2007).

The 2-DE analysis of cold stress response has also been used for the study of the subproteome of organelles or specific tissues, for instance the nucleus of Arabidopsis (Bae et al. 2003), Arabidopsis leaves (Kawamura & Uemura 2003) and poplar leaves (Renaut et al. 2004), rice anthers (Imin et al. 2004) and mitochondria of Pisum sativum (Taylor et al. 2005). Moreover, Amme et al. (2006) used a DIGE technique for analysing the cold response in Arabidopsis leaves. In experiments using 6°C as cold treatment, 22 spots with at least 2-fold altered expression were found compared to the control (20°C); among them 18 were increased and four were decreased. Three of the 18 proteins were identified as dehydrins. Spot identification was performed by MALDI-TOF and ESI-MS/MS (Amme et al. 2006).

However, DIGE experiments were also used not only for cold research. For example Ndímba et al. (2005) studied the effects of salinity and hyperosmotic stress on plant cellular proteins extracted from Arabidopsis thaliana cell suspension cultures by DIGE. Of a total of 2 949 protein spots detected on the gels, 266 showed significant changes in abundance across five independent experiments after NaCl and sorbitol treatments. Using MALDI-TOF MS, they identified 75 salt and sorbitol responsive spots including H+ transporting ATPases, signal transduction related proteins, transcription/translation related proteins, detoxifying enzymes, amino acid and purine biosynthesis related proteins, proteolytic enzymes, heat-shock proteins, carbohydrate metabolism-associated proteins and proteins with unknown biological functions (Ndímba et al. 2005).

Most of these proteomic studies have confirmed the previously published data on cold-inducible proteins obtained from one-dimensional SDS-PAGE or gene expression studies.

**Biotic stress**

The research of biotic stresses also took advantage of proteomic techniques in previous years. The most frequented technique (and one of the cheapest ones) is the identification of differentially expressed spots on 2-DE gels by mass spectrometry like in the other fields of plant biology. Marr et al. (2006) used bean (Phaseolus vulgaris L.) plants, fungal pathogens (Botrytis cinerea, Rhizoctonia solani) and the antagonistic fungus Trichoderma atrovire on to analyze the changes in the proteomes caused by multiple-player interactions by this technique. In the bean proteome, some of the spots analyzed by peptide mass fingerprint (MALDI-TOF
MS) corresponded to PR-proteins (pathogenesis-related) and were less up-regulated by the pathogen alone than by both Trichoderma and the pathogen interacting with the plant (MARRA et al. 2006). COOPER et al. (2003) demonstrated the identification of a previously unknown virus by the analogous technique coupled with LC-MS/MS.

The study of PR-proteins is also important for food research due to the fact that many plant-derived allergens have been identified as members of PR-protein families 2, 3, 4, 5, 8, 10 and 14 (HOFFMANN-SOMMERGRUBER 2002). For instance HAJÓS et al. (2004) identified allergens in wheat flour by a proteomic approach. Having used the separation of proteins on 2-DE gels and their further identification by MALDI-TOF MS, one chitinase, one inhibitor and one heat-shock protein were identified in the water and salt-soluble extract of a wheat cultivar (HAJÓS et al. 2004).

The first description of the novel mass tagging strategy (iTRAQ™ Applied Biosystems) applied to plant pathogen interactions was done by JONES et al. (2006), who described changes in the phosphoproteome of Arabidopsis during Pseudomonas syringae infection. They identified five proteins (e.g. dehydrin, HSP 81, putative p23 co-chaperone) being potentially phosphorylated as a part of the plant basal defence response.

**CONCLUSION**

The proteomic analysis is a very useful tool for providing complex information about differences in the plant proteome during abiotic and biotic stresses. This information can show us the complexity of the plant response to various environmental stress factors and can enable us to find the biomarkers of plant tolerance to stresses which would be usable by breeders. Moreover, it is becoming possible to identify unknown pathogens, quantify the biomarkers in different cultivars or evaluate the quality of plant products using modern proteomic techniques.

**List of symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>DIGE</td>
<td>two-dimensional difference gel electrophoresis</td>
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<tr>
<td>HSP</td>
<td>heat-shock protein</td>
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<tr>
<td>ICAT</td>
<td>isotope coded affinity tags</td>
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<tr>
<td>iTRAQ™</td>
<td>Applied Biosystems trademark name for multiplexed isobaric tagging technology for relative and absolute quantitation</td>
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<tr>
<td>LC</td>
<td>liquid chromatography</td>
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<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption/ionization</td>
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<td>MS</td>
<td>mass spectrometry</td>
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<tr>
<td>MS/MS</td>
<td>tandem mass spectrometry</td>
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<tr>
<td>MudPIT</td>
<td>multidimensional protein identification</td>
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<tr>
<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
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<tr>
<td>PR</td>
<td>pathogenesis-related</td>
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<tr>
<td>SELDI</td>
<td>surface-enhanced laser desorption/ ionization</td>
</tr>
<tr>
<td>SILAC</td>
<td>stable isotope labelling by amino acids in cell culture</td>
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<tr>
<td>TOF</td>
<td>time-of-flight</td>
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