Review

Proteome Analysis in Plant Stress Research

Pavel VÍTÁMVÁS, Klára KOSOVÁ and Ilja T. PRÁŠIL

Department of Genetics and Plant Breeding, Crop Research Institute, Prague-Ruzyně, Czech Republic

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

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

Supported by the Ministry of Agriculture of the Czech Republic, Project No. MZE 0002700602.

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 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 (Thiellement *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. Vagujfalvi *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 Ndimba *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 (Ndimba *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.

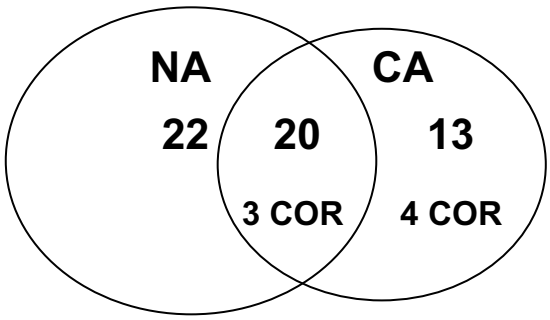

Figure 1. Results of protein identification by LC-MS/MS on a total protein sample of non-acclimated (NA) and cold-acclimated (CA) leaves of cultivar Mironovskaya 808; the numbers represent total identified proteins including COR (cold-regulated) proteins which are presented separately by the numbers of identified COR proteins; from Vítámvás *et al.* (2007)

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. Marra *et al.* (2006) used bean (*Phaseolus vulgaris* L.) plants, fungal pathogens (*Botrytis cinerea*, *Rhizoctonia solani*) and the antagonistic fungus *Trichoderma atroviride* 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

2-DE – two-dimensional gel electrophoresis
BN-PAGE – blue native-polyacrylamide gel electrophoresis
COFRADIC – combined fractional diagonal chromatography
COR – cold-regulated
DIGE – two-dimensional difference gel electrophoresis
HSP – heat-shock protein
ICAT – isotope coded affinity tags
iTRAQ™ – Applied Biosystems trademark name for multiplexed isobaric tagging technology for relative and absolute quantitation
LC – liquid chromatography
MALDI – matrix-assisted laser desorption/ionization
MS – mass spectrometry
MS/MS – tandem mass spectrometry
MudPIT – multidimensional protein identification
PAGE – polyacrylamide gel electrophoresis
PR – pathogenesis-related
SELDI – surface-enhanced laser desorption/ ionization
SILAC – stable isotope labelling by amino acids in cell culture
TOF – time-of-flight

References

Amme S., Matros A., Schlesier B., Mock H.P. (2006): Proteome analysis of cold stress response in *Arabidopsis thaliana* using DIGE-technology. Journal of Experimental Botany, **57**: 1537–1546.

Bae M.S., Cho E.J., Choi E.Y., Park O.K. (2003) Analysis of the *Arabidopsis* nuclear proteome and its response to cold stress. Plant Journal, **36**: 652–663.

Canovas F.M., Dumas-Gaudot E., Recorbet G., Jorrin J., Mock H.P., Rossignol M. (2004): Plant proteome analysis. Proteomics, **4**: 285–298.

Caputo E., Moharram R., Martin B.M. (2003): Methods for on-chip protein analysis. Analytical Biochemistry, **321**: 116–124.

Choi D.W., Zhu B., Close T.J. (1999): The barley (*Hordeum vulgare* L.) dehydrin multigene family: sequences, allele types, chromosome assignments, and expression characteristics of 11 Dhn genes of cv Dicktoo. Theoretical and Applied Genetics, **98**: 1234–1247.

Close T.J. (1997): Dehydrins: A commonality in the response of plants to dehydration and low temperature. Physiologia Plantarum, **100**: 291–296.

Close T.J., Meyer N.C., Radik J. (1995): Nucleotide--sequence of a gene encoding a 58.5-kilodalton barley dehydrin that lacks a serine tract. Plant Physiology, **107**: 289–290.

Cooper B., Eckert D., Andon N.L., Yates J.R., Haynes P.A. (2003): Investigative proteomics: Identification of an unknown plant virus from infected plants using mass spectrometry. Journal of American Society of Mass Spectrometry, **14**: 736–741.

Deng Z.X., Pang Y.Z., Kong W.W., Chen Z.H., Wang X.L., Liu X.J., Pi Y., Sun X.F., Tang K.X. (2005): A novel

ABA-dependent dehydrin ERD10 gene from *Brassica napus*. DNA Sequence, **16**: 28–35.

Elek G., Lapis K. (2006): A path or a new road in laboratory diagnostics? Biological mass spectrometry: Facts and perspectives. Pathology and Oncology Research, **12**: 179–183.

Fowler S., Thomashow M.F. (2002): *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell, **14**: 1675–1690.

Gevaert K., Goethals M., Martens L., Van Damme J., Staes A., Thomas G.R., Vandekerckhove J. (2003): Exploring proteomes and analyzing protein processing by mass spectrometric identification of sorted N-terminal peptides. Nature Biotechnology, **21**: 566–569.

Gevaert K., Van Damme P., Martens L., Vandekerckhove J. (2005): Diagonal reverse-phase chromatography applications in peptide-centric proteomics: Ahead of catalogue-omics? Analytical Biochemistry, **345**: 18–29.

Ghesquiere B., Van Damme J., Martens L., Vandekerckhove J., Gevaert K. (2006): Proteome-wide characterization of N-glycosylation events by diagonal chromatography. Journal of Proteome Research, **5**: 2438–2447.

Gulick P.J., Drouin S., Yu Z.H., Danyluk J., Poisson G., Monroy A.F., Sarhan F. (2005): Transcriptome comparison of winter and spring wheat responding to low temperature. Genome, **48**: 913–923.

Gygi S.P., Rist B., Gerber S.A., Turecek F., Gelb M.H., Aebersold R. (1999): Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nature Biotechnology, **17**: 994–999.

Hajós G., Szabo E., Janaky T. (2004): Two-dimensional electrophoresis, MALDI-TOF MS and blotting for identification and characterisation of pathogenesis-related proteins in wheat cultivar. Chromatographia, **60**: S257–S259.

Hirano H., Islam N., Kawasaki H. (2004): Technical aspects of functional proteomics in plants. Phytochemistry, **65**: 1487–1498.

Hoffmann-Sommergruber K. (2002): Pathogenesis-related (PR)-proteins identified as allergens. Biochemical Society Transactions, **30**: 930–935.

Ideker T., Thorsson V., Ranish J.A., Christmas R., Buhler J., Eng J.K., Bumgarner R., Goodlett D.R., Aebersold R., Hood L. (2001): Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. Science, **292**: 929–934.

Imin N., Kerim T., Rolfe B.G., Weinman J.J. (2004): Effect of early cold stress on the maturation of rice anthers. Proteomics, **4**: 1873–1882.

Jones A.M.E., Bennett M.H., Mansfield J.W., Grant M. (2006): Analysis of the defence phosphoproteome of *Arabidopsis thaliana* using differential mass tagging. Proteomics, **6**: 4155–4165.

Kawamura Y., Uemura M. (2003): Mass spectrometric approach for identifying putative plasma membrane proteins of *Arabidopsis* leaves associated with cold acclimation. Plant Journal, **36**: 141–154.

Kirch H.H., van Berkel J., Glaczinski H., Salamini F., Gebhardt C. (1997): Structural organization, expression and promoter activity of a cold-stress-inducible gene of potato (*Solanum tuberosum* L). Plant Molecular Biology, **33**: 897–909.

Kosová K., Vítámvás P., Prášil I.T. (2007): The role of dehydrins in plant response to cold. Biologia Plantarum. (accepted)

Kuramitsu Y., Nakamura K. (2006): Proteomic analysis of cancer tissues: Shedding light on carcinogenesis and possible biomarkers. Proteomics, **6**: 5650–5661.

Lang V., Palva E.T. (1992): The expression of a *rab*-related gene, *rab18*, is induced by abscisic-acid during the cold-acclimation process of *Arabidopsis thaliana* (L) Heynh. Plant Molecular Biology, **20**: 951–962.

Mann M., Jensen O.N. (2003): Proteomic analysis of post-translational modifications. Natural Biotechnology, **21**: 255–261.

Marra R., Ambrosino P., Carbone V., Vinale F., Woo S.L., Ruocco M., Ciliento R., Lanzuise S., Ferraioli S., Soriente I., Gigante S., Turra D., Fogliano V., Scala F., Lorito M. (2006): Study of the three-way interaction between *Trichoderma atroviride*, plant and fungal pathogens by using a proteomic approach. Current Genetics, **50**: 307–321.

Martens L., Van Damme P., Van Damme J., Staes A., Timmerman E., Ghesquiere B., Thomas G.R., Vandekerckhove J., Gevaert K. (2005): The human platelet proteome mapped by peptide-centric proteomics: A functional protein profile. Proteomics, **5**: 3193–3204.

McDonald W.H., Ohi R., Miyamoto D.T., Mitchison T.J., Yates J.R. (2002): Comparison of three directly coupled HPLC MS/MS strategies for identification of proteins from complex mixtures: single-dimension LC-MS/MS, 2-phase MudPIT, and 3-phase MudPIT. International Journal of Mass Spectrometry, **219**: 245–251.

Ndimba B.K., Chivasa S., Simon W.J., Slabas A.R. (2005): Identification of *Arabidopsis* salt and osmotic stress responsive proteins using two-dimensional difference gel electrophoresis and mass spectrometry. Proteomics, **5**: 4185–4196.

O´Farrell P.H. (1975): High resolution two-dimensional electrophoresis of proteins. Journal of Biological Chemistry, **250**: 4007–4021.

Ong S.E., Blagoev B., Kratchmarova I., Kristensen D.B., Steen H., Pandey A., Mann M. (2002): Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Molecular and Cellular Proteomics, **1**: 376–386.

Pan S.Q., Carter C.J., Raikhel N.V. (2005): Understanding protein trafficking in plant cells through proteomics. Expert Review of Proteomics, **2**: 781–792.

Renaut J., Lutts S., Hoffmann L., Hausman J.F. (2004): Responses of poplar to chilling temperatures: Proteomic and physiological aspects. Plant Biology, **6**: 81–90.

Renaut J., Hausman J.F., Wisniewski M.E. (2006): Proteomics and low-temperature studies: bridging the gap between gene expression and metabolism. Physiologia Plantarum, **126**: 97–109.

Rossignol M., Peltier J.B., Mock H.P., Matros A., Maldonado A.M., Jorrin J.V. (2006): Plant proteome analysis: A 2004-2006 update. Proteomics, **6**: 5529–5548.

Schagger H., von Jagow G. (1991): Blue native electrophoresis for isolation of membrane-protein complexes in enzymatically active form. Analytical Biochemistry, **199**: 223–231.

Schweppe R.E., Haydon C.E., Lewis T.S., Resing K.A., Ahn N.G. (2003): The characterization of protein post-translational modifications by mass spectrometry. Accounts of Chemical Research, **36**: 453–461.

Seki M., Narusaka M., Yamaguchi-Shinozaki K., Carninci P., Kawai J., Hayashizaki Y., Shinozaki K. (2001): *Arabidopsis* encyclopaedia using full-length cDNAs and its application. Plant Physiology and Biochemistry, **39**: 211–220.

Stroher E., Dietz K.J. (2006): Concepts and approaches towards understanding the cellular redox proteome. Plant Biology, **8**: 407–418.

Taylor N.L., Heazlewood J.L., Day D.A., Millar A.H. (2005): Differential impact of environmental stresses on the pea mitochondrial proteome. Molecular and Cellular Proteomics, **4**: 1122–1133.

Thiellement H., Zivy M., Plomion C. (2002): Combining proteomic and genetic studies in plants. Journal of Chromatography B, **782**: 137–149.

Tonge R., Shaw J., Middleton B., Rowlinson R., Rayner S., Young J., Pognan F., Hawkins E., Currie I., Davison M. (2001): Validation and development of fluorescence two-dimensional differential gel electrophoresis proteomics technology. Proteomics, **1**: 377–396.

Vagujfalvi A., Crosatti C., Galiba G., Dubcovsky J., Cattivelli L. (2000): Two loci on wheat chromosome 5A regulate the differential cold-dependent expression of the cor14b gene in frost-tolerant and frost-sensitive genotypes. Molecular and General Genetics, **263**: 194–200.

Vítámvás P., Saalbach G., Prášil I. T., Čapková V., Opatrná J., Jahoor A. (2007): WCS120 protein family and proteins soluble upon boiling in cold-acclimated winter wheat. Journal of Plant Physiology. (in press)

Wang Y.J., Zhang Z.G., He X.J., Zhou H.L., Wen Y.X., Dai J.X., Zhang J.S., Chen S.Y. (2003): A rice transcription factor OsbHLH1 is involved in cold stress response. Theoretical and Applied Genetics, **107**: 1402–1409.

Welling A., Rinne P., Vihera-Aarnio A., Kontunen-Soppela S., Heino P., Palva E.T. (2004): Photoperiod and temperature differentially regulate the expression of two dehydrin genes during overwintering of birch (*Betula pubescens* Ehrh.). Journal of Experimental Botany, **55**: 507–516.

Zhu H., Bilgin M., Bangham R., Hall D., Casamayor A., Bertone P., Lan N., Jansen R., Bidlingmaier S., Houfek T., Mitchell T., Miller P., Dean R.A., Gerstein M., Snyder M. (2001): Global analysis of protein activities using proteome chips. Science, **293**: 2101–2105.

Received for publication January 2, 2007
Accepted after corrections February 16, 2007

Corresponding author:

Mgr. Pavel Vítámvás, Výzkumný ústav rostlinné výroby, v.v.i., oddělení genetiky a šlechtitelských metod, Drnovská 507, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 443, fax: + 420 233 310 636, e-mail: vitamvas@vurv.cz