Aspects of the barley seed proteome during development and germination

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Abstract
Analysis of the water-soluble barley seed proteome has led to the identification of proteins by MS in the major spots on two-dimensional gels covering the pI ranges 4–7 and 6–11. This provides the basis for in-depth studies of proteome changes during seed development and germination, tissue-specific proteomes, cultivar differences related to quality parameters, analysis of the genetic basis for spot variations and targeted investigations of specific proteins.

Introduction
Barley is an important cereal crop grown both for the feed and malting industries. Proteins contained in barley seeds are determinants of quality and have as such been studied for decades. More recently, developments in high-resolution two-dimensional gel electrophoresis for separation of proteins in complex mixtures and MS for protein identification, have enabled new approaches to study cereal seed proteins. Here we describe our efforts to map the barley seed proteome and identify proteins involved in the development and germination.

Barley seed proteomics
In barley seeds, approx. 80% of the protein present is in the form of storage proteins synthesized during seed development and broken down during germination to fuel seedling growth. Earlier studies of cereal seeds [1,2] showed that storage proteins dominated two-dimensional gels with total protein extracts. To identify new proteins involved in seed development and germination, which may influence malting quality, it was desirable to avoid extraction of storage proteins, and therefore a low-salt extraction buffer (5 mM Tris/HCl, pH 7.5/1 mM CaCl2) was used. Proteins in seed extracts were subjected to isoelectric focusing on immobilized pH gradient strips covering the pI ranges 4–7 and 6–11, followed by SDS/PAGE in the second dimension.

Proteins in two-dimensional-gel spots were identified by matrix-assisted laser-desorption ionization–time-of-flight MS analysis of peptide mixtures after digestion by trypsin. Since barley protein sequence data are limited, information from expressed sequence tags was frequently used to match peptide mass maps. Thus 30% of more than 250 identifications from mature seed gels were based on barley expressed sequence tags.

Changes in the seed proteome during seed development and germination
Changes in two-dimensional-gel spot patterns were monitored during seed development [3] and germination [4–6] (Figure 1). The proteome changed significantly during these processes. Individual protein spots were traced through the time-course and different appearance patterns were characterized. A spot containing thioredoxin peroxidase was present at a constant level in developing, mature and germinated seeds (Figure 1). A spot containing the cold-regulated protein Cor14b appeared during seed desiccation, was abundant in the mature seed and was degraded during germination (Figure 1), supporting a role for this protein in desiccation tolerance. Many α-amylase/trypsin inhibitors increased in amount throughout grain filling, whereas chymotrypsin inhibitor spots appeared at a later stage of development (Figure 1). The inhibitors are supposed to protect the seed reserves against insects [7] and were abundant in mature seeds, decreasing slightly in amount during germination.

Two isoforms of thioredoxin h, HvTrxh1 and HvTrxh2 were identified in the barley seed proteome (Figure 1) [6]. Thioredoxin h is a protein disulphide reductase that probably regulates proteins important in seed germination [8]. Both isoforms were present during development and in the mature seed. During germination, HvTrxh1 remained whereas HvTrx2 decreased slightly in abundance [6] (Figure 1).

Embryo, starchy endosperm and aleurone layer-specific proteomes
The embryo, aleurone layer and starchy endosperm have differing functions in the seed during development and...
germination, and their protein profiles differ accordingly [9]. By analysing these seed components separately, it is possible to determine the distribution of proteins among the seed tissues and to enrich for proteins that are abundant in a particular tissue.

The endosperm is metabolically inactive after seed desiccation during development and contains defence-related proteinaceous inhibitors and other abundant proteins that dominate the two-dimensional-gel pattern of mature seeds (Figure 1), obscuring less-abundant proteins that migrate to the same region of the two-dimensional gels. During germination, many protein spots on two-dimensional gels are masked by the vast amount of serpins released from the starchy endosperm (results not shown) [4]. Analysis of dissected seed tissues enables these proteins to be visualized.

The appearance of thioredoxin h isoforms was analysed in dissected seed tissues. Both isoforms were present in the embryo (Figure 1) and HvTrxh1 was also present in the endosperm and aleurone layer of mature barley seeds (results not shown) [6]. In germinated embryo extracts (Figure 1), HvTrxh1 was abundant whereas HvTrxh2 was strongly decreased in amount. Thioredoxin peroxidase was also abundant in the embryo, as was Cor14b (Figure 1) again in agreement with a role in desiccation tolerance since the embryo must survive in a desiccated state until the onset of germination.

**Cultivar comparisons and genetic determination of the proteome**

A series of barley cultivars with differing malting properties was analysed and their protein patterns compared. During seed development, prominent differences were observed in a group of spots of which several were identified as the serpin Z4 (Figure 2). Seeds of cultivar Mentor collected 4 weeks before harvest contained several additional abundant serpin spots compared with cultivars Meltan, Barke and Morex. Some of the spots were present in Meltan at a lower intensity. Barke contained fewer serpin spots. Later in development
(Figure 2) and in mature seeds, the additional spots in Mentor decreased in intensity resulting in a spot pattern similar to Meltan and Barke. Serpin Z4 spots were not at all detected in Morex (Figure 2). Serpins are inhibitors of chymotrypsin-like serine proteases. The function of serpins in cereal seeds is unknown but they may have a role as storage proteins and in defence [10].

Additional cultivar differences were observed in α-amylase spots, α-amylase/trypsin inhibitor spots [4] and in β-amylase spots [3]. By MS, the different β-amylase spots were shown to be due to allelic forms of the protein, highlighting the genetic basis for proteome differences [3]. Analysis of a doubled-haploid mapping population is in progress to link spot pattern differences to chromosome locations and malting parameters.

**Targeted analysis of the role of thioredoxin h in barley seeds**

MS data from identified spots (Figure 1) enabled the genes encoding HvTrxh1 and HvTrxh2 to be cloned. Recombinant proteins were produced and characterized [6] and are currently used in a sensitive fluorescent-labelling assay combined with two-dimensional-gel electrophoresis and MS to identify new thioredoxin h target proteins in barley seed extracts [11]. Several targets have been identified, including various α-amylase/trypsin inhibitors, barley α-amylase/subtilisin inhibitor and a number of proteins not previously recognized as thioredoxin targets.

**Conclusion**

Despite limited available sequence information, proteomics techniques generate new knowledge about appearance patterns, tissue distribution and cultivar variation of proteins in barley seeds. By combining proteomics with malting quality analysis and genetics, two-dimensional-gel patterns can be related to cultivar characteristics. Targeted proteome analysis of thioredoxin h and the proteins it regulates will provide new insight into its role in cereal seeds.

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**References**


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