

# Proteomics of Wheat Bran

(*Triticum aestivum* var. Babbler)

A thesis submitted in fulfilment of the requirements  
for the Honours Degree of Master of Science

By

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## **Declaration**

The work presented in this thesis was carried out between March 2005 and October 2006 on a full-time basis. This work represents original research which has not been submitted for any other degree. All work was carried out by the author unless otherwise acknowledged.

Candidates Signature

Ante Jerkovic

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## **Abstract**

Wheat is a major crop in Australia with around 25 million tonnes of grain harvested in an average year. Improved wheat grain cultivars and wheat grain milling can result in higher biological yields and flour quality. The introduction covers the general aspects of the wheat grain from bran development and structure through to milling and the importance of flour quality in flour-based products. It also highlights the problem with bran contamination in flour during milling and other factors that may have an effect on flour quality. Proteomics was used to identify proteins in three separate bran tissue fractions: the inner fraction (aleurone), intermediate fraction (nucellar tissue, testa, tube cells and cross cells) and the outer fraction (hypodermis and epidermis). The aim of the project was to identify proteins in bran tissue fractions which may potentially be useful in improvements in wheat quality for farmers and consumers and flour yield for millers. The results show that more than 80% of the identified proteins in the outer and intermediate tissue fractions are defence-and stress-related proteins (chitinase, xylanase, thaumatin-like protein, wheatwin 1, lipid-transfer protein, oxalatae oxidase (OXO), polyphenol oxidase (PPO), peroxidase (POX). Almost 60% of the proteins identified in the inner tissue fraction are 7S Globulin storage proteins and around 15% are protein synthesis-and energy-related. Water-soluble proteins were also identified and it was found that endochitinase, OXO, PPO and POX all leach out from the grain during imbibition. This study has added to the knowledge of bran tissue-specific proteins and has broad implications for improving crop yield and flour quality.

## Abbreviations

|                     |  |
|---------------------|--|
| <b>1-DE</b>         | One-dimensional gel electrophoresis  |
| <b>2-DE</b>         | Two-dimensional gel electrophoresis  |
| <b>ABA</b>          | Absciscic acid   |
| <b>ABI</b>          | Applied Biosystems International   |
| <b>ACTH</b>         | Adrenocorticotrophic hormone   |
| <b>APS</b>          | Ammonium persulphate   |
| <b>AR</b>           | Acquired resistance  |
| <b>ATP</b>          | Adenosine triphosphate   |
| <b>AX</b>           | Arabinoxylan   |
| <b>BSA</b>          | Bovine serum albumin   |
| <b>BLAST</b>        | Basic Local Alignment Tool (from NCBI)                                       |
| <b>BTH</b>          | Benzothiadiazole   |
| <b>CHAPS</b>        | 3-[-1-propanesulfonate   |
| <b>DPA</b>          | Days post anthesis   |
| <b>DTT</b>          | 1,4-Dithio-DL-threitol   |
| <b>ESI</b>          | Electrospray ionization  |
| <b>EST</b>          | Expressed sequence tag   |
| <b>GA</b>           | Gibberellic acid   |
| <b>IAA</b>          | Indole-3-acetic acid   |
| <b>IDA</b>          | Information dependent acquisition  |
| <b>IEF</b>          | Iso-electric focussing   |
| <b>IPG</b>          | Immobilised pH gradient  |
| <b>kDa</b>          | kilo Da  |
| <b>LC</b>           | Liquid chromatography  |
| <b>LOX</b>          | Lipoxygenase   |
| <b>LTP</b>          | Lipid transfer protein   |
| <b>MALDI-TOF MS</b> | Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry |
| <b>MS</b>           | Mass spectrometry  |
| <b>MS/MS</b>        | Tandem mass spectrometry   |

|                     |   |
|---------------------|---|
| <b>MudPIT</b>       | Multi dimensional protein identification technology |
| <b>NCBI</b>         | National Center for Biotechnology Information       |
| <b>OXO</b>          | Oxalate oxidase                                     |
| <b>PAGE</b>         | Polyacrylamide gel electrophoresis                  |
| <b>PCD</b>          | Programmed cell death                               |
| <b>PCT</b>          | Patent Cooperation Treaty                           |
| <b>PDI</b>          | Protein disulfide isomerase                         |
| <b>PHYLIP</b>       | Phylogeny Inference Package                         |
| <b>PMF</b>          | Peptide mass fingerprinting                         |
| <b>POX</b>          | Peroxidase  |
| <b>PPO</b>          | Polyphenol oxidase                                  |
| <b>PR</b>           | Pathogenesis related                                |
| <b>SDS</b>          | Sodium dodecyl sulfate                              |
| <b>TBP</b>          | Tributyl phosphine                                  |
| <b>TCA</b>          | Trichloroacetic acid                                |
| <b>TEMED</b>        | N,N,N',N'-Tetramethylethylenediamine                |
| <b>TFA</b>          | Trifluoroacetic acid                                |
| <b>TL</b>           | Thaumatococcus                                      |
| <b>TOF</b>          | Time of flight                                      |
| <b>VDAC</b>         | Voltage dependant anion channel                     |
| <i>A. fatua</i>     | <i>Avena fatua</i>                                  |
| <i>A. oryzae</i>    | <i>Aspergillus oryzae</i>                           |
| <i>H. vulgare</i>   | <i>Hordeum vulgare</i>                              |
| <i>M. viride</i>    | <i>Mesostigma viride</i>                            |
| <i>O. sativa</i>    | <i>Oryza sativa</i>                                 |
| <i>P. glaucum</i>   | <i>Pennisetum glaucum</i>                           |
| <i>P. miliaceum</i> | <i>Panicum miliaceum</i>                            |
| <i>S. cereale</i>   | <i>Secale cereale</i>                               |
| <i>T. aestivum</i>  | <i>Triticum aestivum</i>                            |
| <i>X. oryzae</i>    | <i>Xanthomonas oryzae</i>                           |
| <i>Z. mays</i>      | <i>Zea mays</i>                                     |

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- Protein ID Tables (Excel format) with links to mass spectra
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# **1. Introduction**

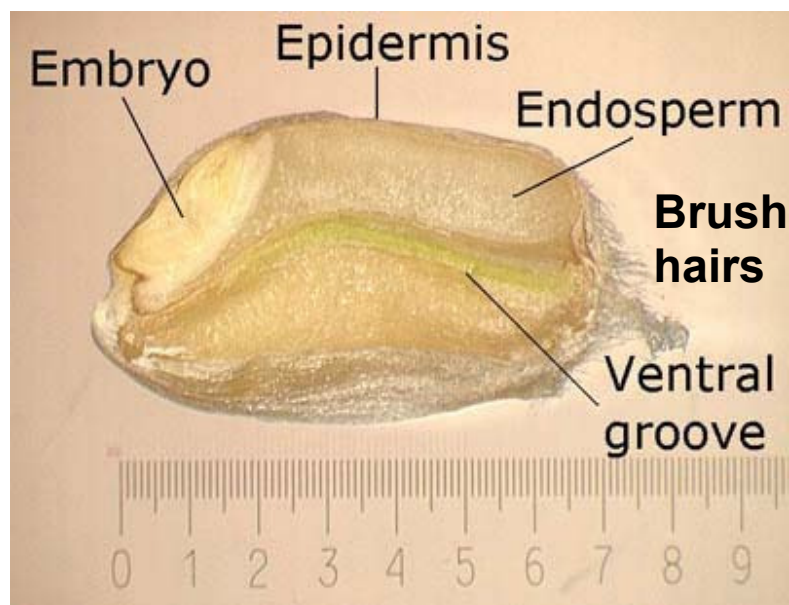
## ***1.1 Wheat in Australia***

Wheat is a major food crop in Australia. Wheat growers produced around 25 million tonnes of grain in 2004, worth around five billion dollars for that year (Bread Research Institute (BRI) Australia, [www.bri.com.au](http://www.bri.com.au); Commonwealth Scientific and Industrial Research Organisation (CSIRO), [www.csiro.au](http://www.csiro.au); Grain Foods Cooperative Research Centre (GFCRC), [www.grainfoodscrc.com.au](http://www.grainfoodscrc.com.au)). The major economic and mass component of wheat grain is the starchy endosperm which is extracted in the milling process to produce flour and used in turn to make breads, pastas, cakes, biscuits and other baking products. Other parts of the mill stream, such as bran, germ and pollard are used in making products such as cereals, biscuits and animal feed (Every et al., 2002). To reduce the costs of maintenance and to increase profitability of crops, farmers choose to grow wheat based on yield and a number of factors such as resistance to disease, climate stress and watering requirements, whereas millers choose wheat based on flour quality and milling properties (Dupont and Altenbach, 2003). Thus there is great importance in understanding the physical and biochemical characteristics of wheat grain which make it suitable for all of these purposes. This information can potentially be used to provide improvements in yield and management of crops for farmers and increased flour yield and quality for millers.

### ***1.1.1 Wheat grain structure***

Wheat grain is generally ready for harvesting at around 30 days post anthesis (DPA) depending on the cultivar. The basic structural composition of the grain at this stage is

shown in Figure 1.1. The three main components of whole grain are bran tissue, germ (which contains the embryo and scutellum), and endosperm. The distinct external feature of the grain is its ‘blimp’-like shape. A cleft/crease runs longitudinally along the grain and indents approximately half way inside the grain. The embryo is indented at one end of the grain and the brush hairs are located at the opposite end.



**Figure 1.1** Longitudinal section showing wheat grain structure (modified from Home-Grown Cereals Authority (HGCA), [www.wheatbp.net](http://www.wheatbp.net)).

When grain is being milled, the ideal outcome is to separate the bran and embryo from the endosperm. The quality of flour in terms of bran contamination will largely depend on how well the bran separates from the endosperm and how much the bran fractures into smaller fragments. Conditioning grain (addition of small quantities of water) prior to



milling has the effect of increasing bran strength and improving bran separation from the endosperm, resulting in a reduction of bran contamination in the flour (Butcher and Stenvert, 1973; Moss et al., 1980).

### ***1.1.2 Milling process***

After wheat is harvested, it is cleaned from rocks, dirt and unwanted vegetation matter. The grains are then stored in silos where they are kept dry until later distribution. When the grain reaches the flour mill, it is firstly conditioned by addition of water for around 16 h to increase moisture content of the grain from around 10% to around 15%, depending on the wheat cultivar. As mentioned previously, the accepted wisdom is that the conditioning process toughens the bran by making it more elastic, giving it more integrity when passing through the break rollers. The result is better endosperm separation from bran during milling and reduction in bran contamination in the flour (Butcher and Stenvert, 1973; Moss et al., 1980; Every et al., 2002). After conditioning, the grains are passed through the first break rollers. The first break mostly removes semolina (flour and germ with minimal bran contamination). At this stage the cleanest fractions are collected, however the yield is quite low. Semolina and bran are then separated by fluting. The heavier bran/endosperm pieces are recycled back through to the break rollers and are passed through twice more to remove as much endosperm from the bran as possible. The remaining bran/endosperm pieces are then passed through reduction rollers, which have a smooth surface unlike the break rollers, and their purpose is to crush the starch granules into very fine flour (Moss et al., 1980; Every et al., 2002; Fang and Campbell, 2002). The

main products collected after milling are bran, semolina, flour and pollard (Every et al., 2002).

‘Ash content’ in flour is a name used to indicate bran contamination. When there is a high ash content, the flour is considered to have high levels of tiny fragments of bran (Peyron et al., 2002). This gives flour a slight brownish colour and can cause a reduction in dough elasticity resulting in poor baking qualities. The aim of the flour miller is to minimise the ash content as much as possible. This can mostly be achieved by conditioning the wheat prior to milling as discussed earlier. However, most of the cleanest flour with low ash content is collected after the first break rollers with relatively low flour yield. Developing a method to keep the bran intact and reduce bran/endosperm adhesiveness is thus economically important.

### ***1.1.3 Hard and soft wheat***

During milling the grains are crushed and sheared between two break rollers rotating towards each other at a differential speed. The hardness of the wheat cultivar will determine how the starch granules break during this process. Soft and hard wheat have different fracturing patterns when they are passed through the break rollers. Soft wheats tend to produce less mechanically damaged starch granules than hard wheats (Oliver, 1998; Fang and Campbell, 2002). This form of starch damage generally occurs when the starch granules break into smaller fragments caused by the shearing forces of the break rollers. In this case, fracturing tends to occur along the starch ‘cell wall’, and is termed ‘intra-cellular’ fracturing. In hard wheat, the shearing force from the break rollers causes

‘inter-cellular’ fracturing, a fracturing that occurs through the starch granule (Moss et al., 1980).

The two main properties of mechanical starch damage are increased water absorption and a reduction in dough viscosity (Evers et al., 1999). The consequence of starch damage for bakers is that more water will be required when forming dough with flour that has high starch damage when compared to flour with low starch damage (Oliver, 1998).

In bread baking, the baker needs to be certain that when making dough, the flour will require a set volume of water which will create a reasonable dough texture every time. This is important in getting a consistent final baked bread of predictable loaf volume, air pocket size, wall thickness and crumb texture. To achieve a standard flour quality, grains are blended prior to milling and/or flour is blended after milling (Bread Research Institute (BRI) Australia, [www.bri.com.au](http://www.bri.com.au); Kuakpetoon et al., 2001). Grains are first tested for quality, protein content, and hardness. Grains with high protein content and hardness are blended to produce flour for bread baking, whereas soft wheats with low protein content are blended for flour used in baking cakes and biscuits. Blending of wheat cultivars and/or flour is thus important in providing a standard flour for each specific baking product and also to eliminate uncertainty when adding water to get the required dough texture (Personal Communication M. Southen, BRI; Kuakpetoon et al., 2001).

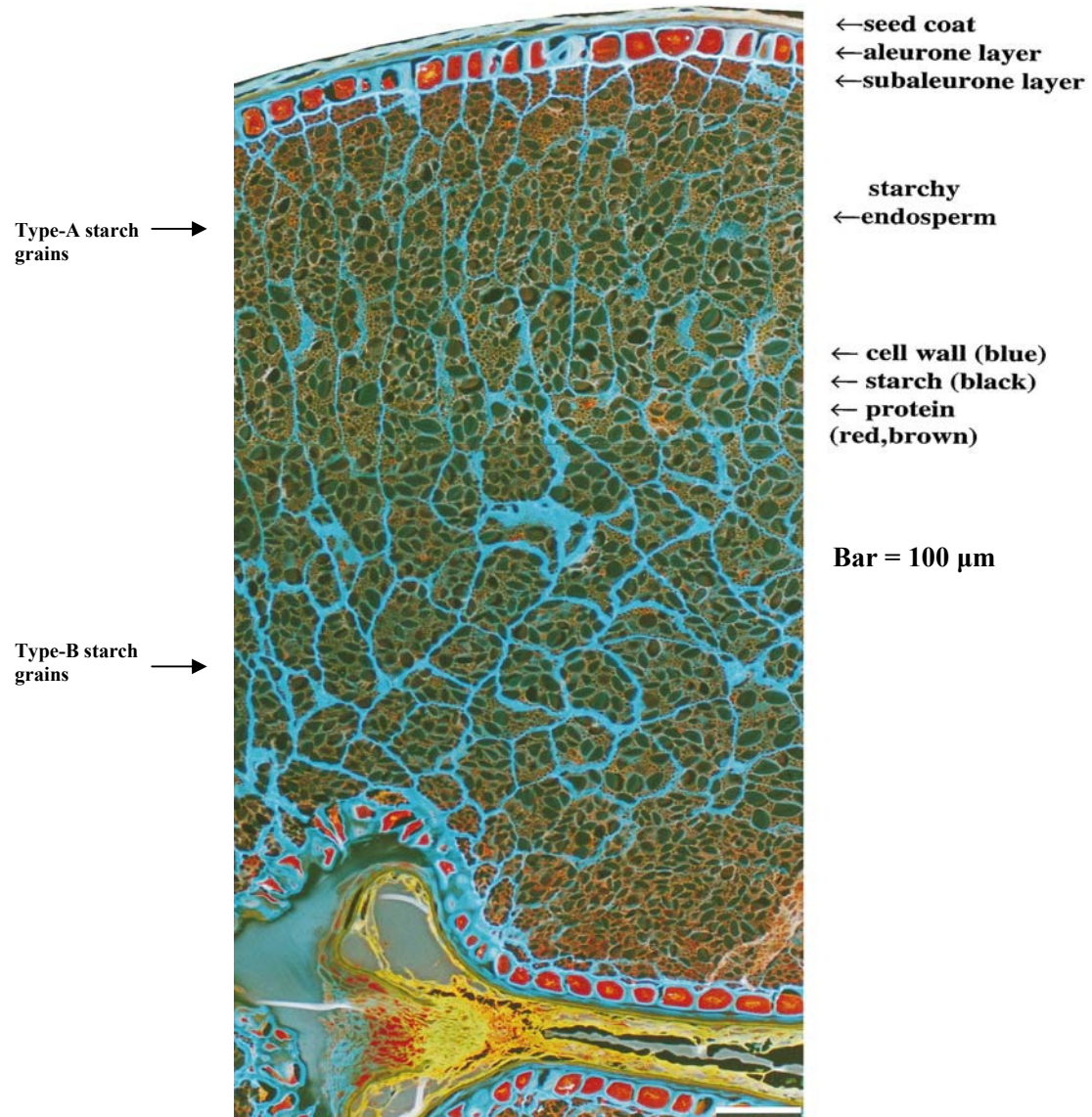
## ***1.2 Protein distribution***

Starch grain size in the endosperm is generally larger around the centre of the grain (type-A) and tends to be smaller (type-B) towards the edges adjacent to the aleurone cells (Fig. 1.2). The endosperm cell walls (Fig. 1.2) surrounding the starch granules are primarily comprised of arabinoxylans (AX) and (1→3, and 1→4)-β-glucans. Protein concentration is low around the centre of the endosperm, increasing as the starch granule size decreases nearer to the aleurone cell wall (Tervilä-Wilo et al., 1996; Evers et al., 1999). For whole grains, most of the total protein pool is found in the large endosperm fraction; however, when based on milligrams of protein per gram of tissue fraction, the protein content in the embryo, scutellum and aleurone tissue fraction is much higher than the endosperm and pericarp/testa (see Table 1.1).

**Table 1.1** Distribution of proteins, fraction weight and starch in the wheat grain  
(Spurway, 1998)

| Grain Fraction                                    | % of grain Weight | % Total protein | mg protein per gram of tissue fraction* | % of Total starch |
|---|-------------------|-----------------|---|-------------------|
| Outer and intermediate fraction (Pericarp, Testa) | 8                 | 4.5             | 56                                      | 0                 |
| Inner fraction (aleurone cells)                   | 7                 | 15.5            | 221                                     | 0                 |
| Endosperm   | 82.5              | 72              | 87                                      | 100               |
| Scutellum   | 1.5               | 4.5             | 300                                     | 0                 |
| Embryo  | 1                 | 3.5             | 350                                     | 0                 |

\* Based on milligrams of protein per gram of grain fraction



**Figure 1.2** Cross section of wheat grain (Tervilä-Wilo et al., 1996).

### ***1.2.1 Major hydrolytic enzymes***

$\alpha$ -amylase and  $\beta$ -amylase are hydrolytic enzymes found in wheat. They are both classified as  $\alpha$ -(1 $\rightarrow$ 4)-D-glucanases and are involved in hydrolysis of the starchy endosperm into oligo- and monosaccharides to feed the developing plant during germination (Evers et al., 1999; Every et al., 2002).  $\alpha$ -amylase is an endo-acting enzyme, in that it hydrolyses starch at random points along the polysaccharide chain.  $\beta$ -amylase however, can hydrolyse starch only at the reducing end of the polysaccharide chain.  $\alpha$ -amylase is mainly found in the scutellum of the embryo. At the beginning of germination,  $\alpha$ -amylase is secreted into the endosperm and begins starch hydrolysis. The aleurone cells then start to express and secrete  $\alpha$ -amylase, as this whole cascade is triggered by hormones that are released from the scutellum upon hydration.  $\beta$ -amylase is contained in the endosperm and it is inactive when the grain is dry. Upon hydration, hydrolysis of starch by  $\beta$ -amylase is quite slow, however it is greatly increased when  $\alpha$ -amylase is secreted by the scutellum and aleurone cells into the endosperm.  $\alpha$ -amylase hydrolyses starch into smaller fragments to expose more reduced ends for  $\beta$ -amylase to hydrolyse it further into maltose (Cejudo et al., 1995; Every et al., 2002).

Apart from starch, arabinoxylans (AX) and (1 $\rightarrow$ 3, and 1 $\rightarrow$ 4)- $\beta$ -glucans are the most abundant carbohydrates in the remnant cell walls of the endosperm (Evers et al., 1999). Water-extractable AX's give flour its viscous property when adding water to make the dough. AX's which cannot be extracted with water give flour its water absorptive properties (Courtin and Delcour, 2002). Endoxylanase is found in wheat endosperm at very low levels. During germination, the AX cell walls are hydrolysed by endoxylanase,

providing the amylases greater access to in turn hydrolyse starch (Courtin and Delcour, 2002).

### ***1.2.2 $\alpha$ -amylase and its effect on flour quality***

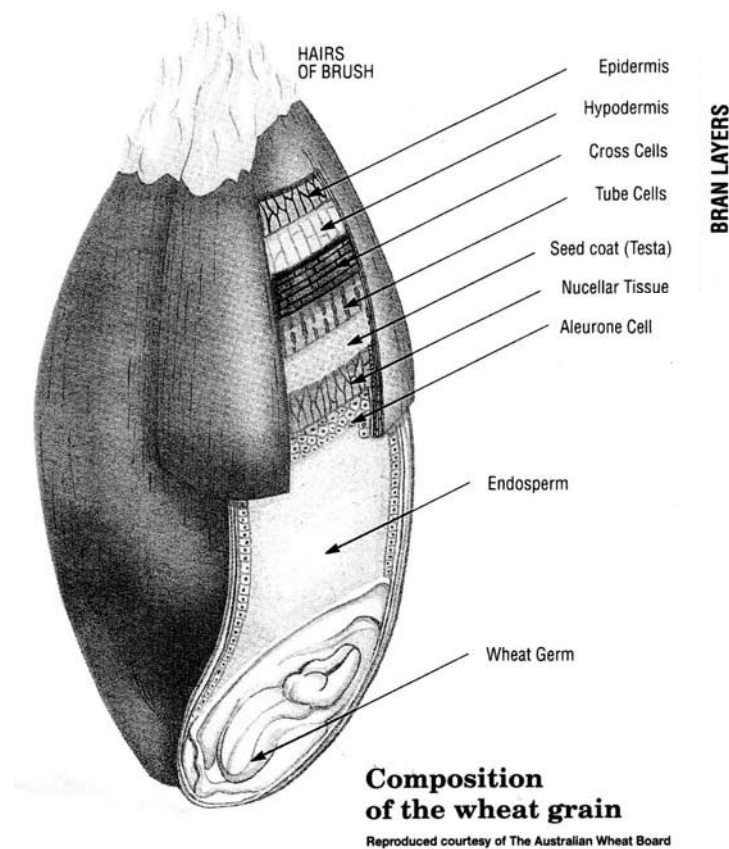
The presence of high levels of  $\alpha$ -amylase in flour is an indicator of potentially poor quality flour.  $\alpha$ -amylase greatly increases the breakdown of starch into smaller fragments, resulting in flour losing dough viscosity (Evers et al., 1999). The presence of high levels of  $\alpha$ -amylase indicates that germination has begun. This may be a result of late harvesting and/or water damage in the field or exposure to moisture during storage. A standard measure of grain quality, called the “falling number” is related to the level of  $\alpha$ -amylase present in grain. The number is the time in seconds it takes a plunger device to fall to the bottom of a mixture of water and ground-up whole grain. High falling number indicates better quality grain, as the mixture will be more viscous, thus decreasing the speed at which the plunger is falling. For good quality flour, the falling number should not be below 250 (Grain Foods Cooperative Research Centre (GFCRC), [www.grainfoodscrc.com.au](http://www.grainfoodscrc.com.au)). Other factors may also affect the falling number, such as protein and ash content and starch damage (Evers et al., 1999; Every et al., 2002).

### ***1.3 Bran layers in grain development***

#### ***1.3.1 Bran layers***

The outermost component of the grain is the bran, which is composed of about seven distinct tissue layers as mentioned previously (Fig. 1.3). The outermost layers of the bran are collectively called the pericarp. The pericarp consists of the epidermis (being the outermost layer), hypodermis, cross cells and finally the tube cells. The next three tissue layers are the testa, nucellar and aleurone. The aleurone tissue is in contact with the endosperm and it is the only bran tissue layer that is still alive and functional at the cellular level in mature grain and is critical during germination. Bran has an important function as a protective barrier for the grain. The grain is a potential food for insects, fungi, and bacteria, and it is also exposed to many environmental stresses, thus the bran must have properties which protect it from all these factors. Much work has been done on the extraction and isolation of defence proteins in wheat. These proteins are categorized into major classes depending on their mode of action and structural similarities. Some of these major classes of defence proteins are termed pathogenesis-related proteins (PR), and include PR-1, PR-2 (1, 3- $\beta$ -glucanases), PR-3 (chitinases), PR-4 (wheatwin), and PR-5 (thaumatin-like proteins) (Selitrennikoff 2001; Desmond et al. 2006).





**Figure 1.3** Bran tissue layers (modified from The Australian Wheat Board (AWB), [www.awb.com.au](http://www.awb.com.au)).

### ***1.3.2 Formation of bran layers during grain development***

Wheat is a self-pollinating plant, where the pollen is released from the anther (at anthesis) and attaches to the stigma. The gamete is then delivered to the ovum which then becomes fertilized. The triploid gamete and ovum fuse together to form a hexaploid zygote. The zygote begins cell division and forms into an embryo, which contains the primordial plant organs. During grain development, the embryo develops together with food and nutrient

storage compartments, and protective outer tissue layers. Development is complete at around 30 to 40 DPA. The grain becomes dormant at the end of development until the right conditions allow it to germinate (Home-Grown Cereals Authority (HGCA), [www.wheatbp.net](http://www.wheatbp.net)). There are many physiological changes that occur during grain development, however for the purpose of this paper only bran tissue layers will be discussed.

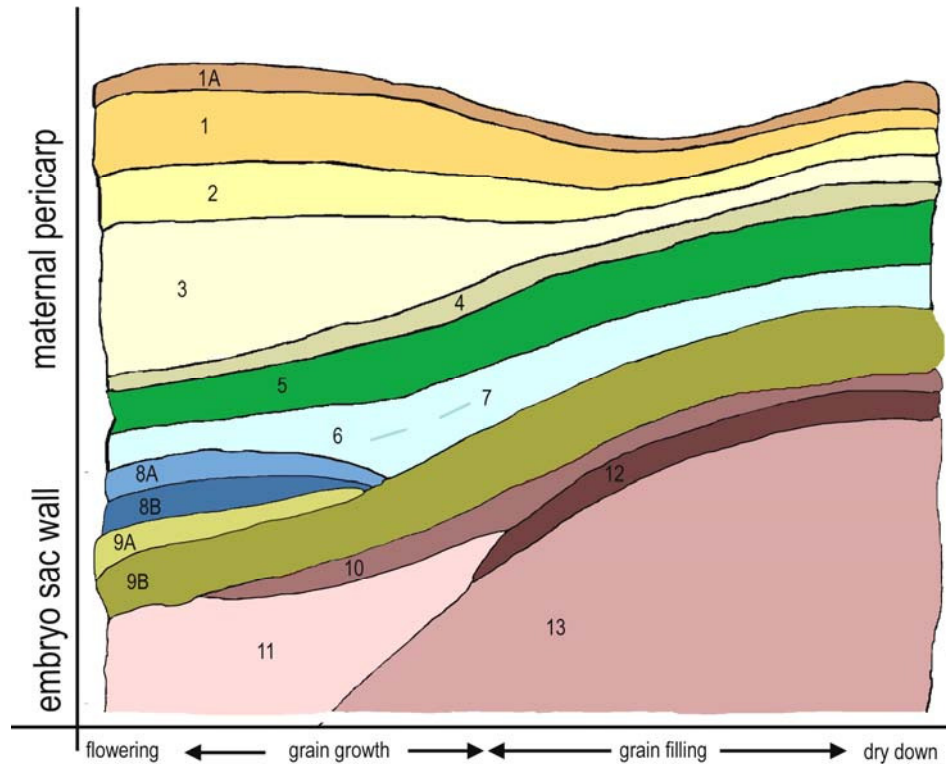
During grain development, there are around 16 different cell types that ultimately form the outer bran tissue. Once grain development is complete at around 30 DPA, only seven distinct layers can be seen (Fig. 1.4). The early tissue layers are present up to around 11 DPA, and are involved in providing food and nutrients for the developing grain. The green colour of the developing grain is due to chloroplasts inside the cross cells. They gradually collapse towards the end of grain development and give the grain a brown colour. At around 11 DPA grain filling begins, accumulating starch to form the endosperm. The outermost cells of the endosperm differentiate to become aleurone cells (Drea et al., 2005). At this stage, all of the tissue layers present will become part of the bran. The changes that occur are due to compression caused by grain filling. The inner epidermis is compressed as the endosperm fills out and is torn and crushed, forming the tube cell layer. The inner integuments collapse and form the seed coat (testa), whereas the outer integuments are absorbed and disappear. Finally, the nucellus and nucellar epidermis compact together to form the nucellar tissue that surrounds the aleurone layer (Fig. 1.4) (Home-Grown Cereals Authority (HGCA), [www.wheatbp.net](http://www.wheatbp.net)).

**Pericarp(outside)**

1A - cuticle of Outer Epidermis  
 1 - Outer Epidermis  
 2 - Hypodermis  
 3 - Parenchyma (thin-walled)  
 4 - Intermediate cells  
 5 - Cross cells  
 6 becomes 7 - Inner Epidermis/Tube cells

**Closer to the Embryo sac(inside)**

8A and 8B - Outer Integument  
 9A and 9B -Inner Integument  
 10 - Nucellar epidermis  
 11 - Nucellus  
 12 - Aleurone  
 13 - Starchy endosperm



**Figure 1.4** Bran tissue layer formation during wheat grain development (from Home-Grown Cereals Authority (HGCA), [www.wheatbp.net](http://www.wheatbp.net)).

**1.3.3 Effects on bran layers during germination**

Grain is harvested at around 30 DPA when it is in the dormant stage. Given that the moisture content of the grain remains low at around 10%, the grain should not germinate. However, once the moisture content increases, many biochemical processes begin and the embryo begins to grow.

There are many hormones involved at the early stages of development. The hormones abscisic acid (ABA) and gibberellic acid (GA) are important in determining if the grain remains dormant. ABA acts as a germination suppressant and GA has the opposite effect. The embryo of the grain is the initiator of germination and is where growth-promoting hormones are produced and or released. Experiments have shown that when the embryo was dissected from a grain and then imbibed in a solution containing GA, germination mechanisms are activated in the aleurone cells (Bethke et al., 1998). When ABA levels decrease and GA levels increase, the aleurone cells begin to breakdown and release  $\alpha$ -amylases into the endosperm. As discussed earlier,  $\alpha$ -amylase works together with  $\beta$ -amylase to break down the starchy endosperm into simpler sugars. This dual process in starch hydrolysis is important in supplying the embryo with a carbon source. Another hormone that is involved in germination is auxin, or indole-3-acetic acid (IAA). It is involved in the mobilization and breakdown of phytin (potassium, magnesium, calcium salt of inositol hexaphosphate), which is a macronutrient storage compound found mainly in the aleurone cells (Eastwood and Laidman, 1971; Fincher, 1989; Evers et al., 1999).

## ***1.4 Analysis of protein composition in wheat grain***

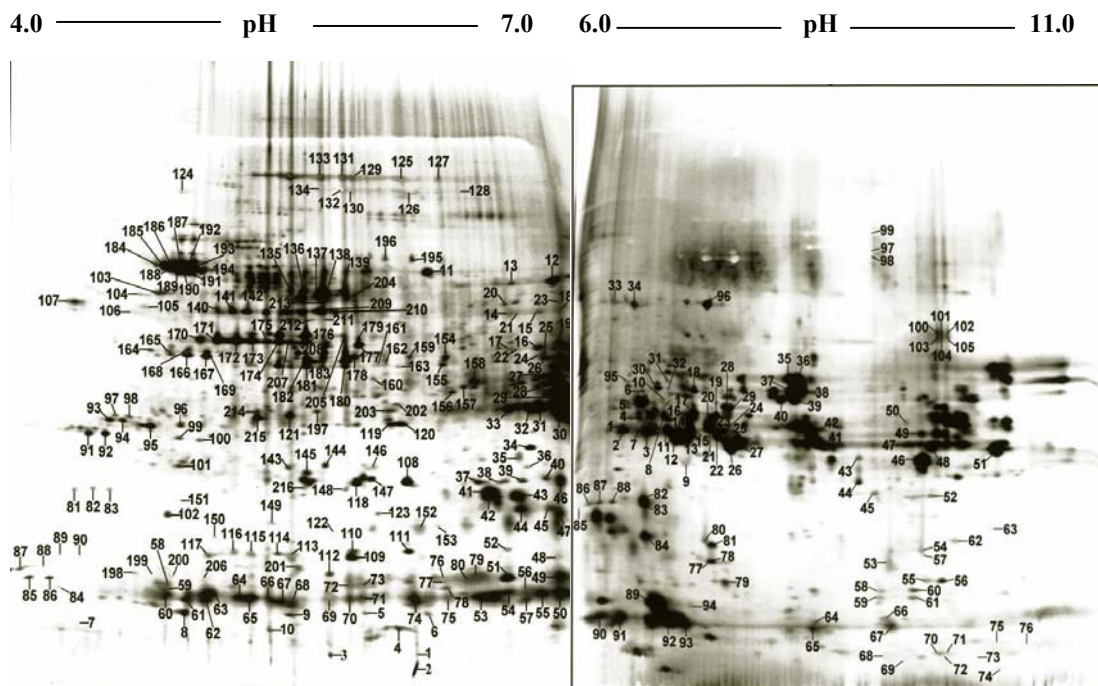
### ***1.4.1 High resolution 2-dimensional electrophoresis (2-DE)***

The first step in proteomic work is to extract proteins from the tissue sample. The extracted proteins are then solubilised into appropriate buffers for first-dimension separation.

The first-dimension separation in 2-DE separates proteins based on their intrinsic pI by isoelectric focusing (IEF). The pI of each protein represents the pH required for it to have a neutral charge. Immobilised pH gradient (IPG) strips can range from pH 1 to pH 13. An electric current is applied across the strip which mobilizes the proteins. Each different protein will stop migrating when it reaches its corresponding pI on the IPG strip. In situations where there is an accumulation of many proteins around a particular pI, focusing over a narrow pH range can improve separation and resolution (Fig. 1.5). The second dimension of 2-DE is the separation of proteins based on their molecular weight.

Once proteins have been separated by their pI, the IPG strip is equilibrated with sodium dodecyl sulfate (SDS) to coat the proteins with an overall negative charge. The strip is then transferred onto an SDS polyacrylamide gel for electrophoresis (PAGE). As the proteins travel electrophoretically through the polyacrylamide gel matrix, they begin to separate according to molecular weight. Low-molecular-weight proteins travel faster through the gel matrix and higher-molecular-weight proteins travel slower. After electrophoresis the gel is stained and ready for further analysis. Figure 1.5 shows the

complex mixture of proteins separated by 2-DE, using two narrow pH bands to improve resolution.



**Figure 1.5** Protein spots on 2-DE gel of wheat grain endosperm at 17 DPA.

Approximately 200 ug of protein extract was loaded per gel and was stained with diamine silver (Skylas et al., 2000).

#### ***1.4.2 Identification of protein spots in 2-D gels***

Protein spots are excised from a 2-D gel and residual protein stain is washed out. Gel pieces are then dehydrated and digested with trypsin which fragments proteins at arginine and lysine residues (except those that are followed by a proline). After trypsin digestion, the peptides are extracted from the gel pieces for further analysis. Peptides can then be analysed using matrix assisted laser desorption/ionization time-of-flight mass spectrometry combined with tandem mass spectrometry (MALDI-TOF MS/MS) or electrospray ionization triple-quadrupole tandem mass spectrometry (ESI MS/MS).

MALDI-TOF MS can be used for peptide mass fingerprinting (PMF) and selected peptide masses can be further analysed by (MS/MS). The sample is co-crystallised with a matrix on a metal plate and a laser is used to ionize and desorb the peptides into the TOF analyzer. Resolution of a large mass range can be achieved using TOF as it measures the flight time of an ion between the source and the detector to give a calculated charge-to-mass ratio. The effective length of the flight tube in TOF mass spectrometers can be increased by ion reflectors, which increase the resolution and mass range (Yates III, 1998; Chernushevich et al., 2001).

ESI triple-quadrupole MS/MS is different to MALDI TOF MS in that the sample is in solution prior to analysis and the ion masses are detected by selecting ions using a frequency-to-current ratio. The sample is sprayed into an electric field using an inert carrier gas. This causes the particles to desolvate and ionize without fragmenting. The ionized particles accelerate into a constant direct current electric field generated by two

longitudinal poles. Perpendicular to this electric field plane are two poles which have an alternating current. A frequency-to-current ratio is used to stabilize an ion with particular mass-to-charge ratio as it travels through the electric field. Stabilized ions which pass through the electric field are then detected, and ions that are not transmitted collide into the poles. A scan of different frequencies allows for different mass-to-charge ratio ions to pass through and hit the detector. The resolution of this type of MS is dependent on the electric field current and frequency. High frequency and current increases an ion's oscillation in its trajectory through the quadrupole and thus increases mass resolution (Yates III, 1998; Chernushevich et al., 2001; Lay Jr, 2001; Mak et al., 2006b).

Peptide masses and MS/MS peptide ion masses can then be used together to search against cereal and wheat expressed sequence tag (EST) databases through Mascot (Mascot, [www.matrixscience.com](http://www.matrixscience.com)). MS/MS ion masses can also be used to search against proteins in the wheat EST database, using The Grand Proteome Machine (thegpm) (thegpm, [http://137.111.103.157/tandem/thegpm\\_tandem.html](http://137.111.103.157/tandem/thegpm_tandem.html)).

Peptides can be sequenced by Edman degradation, also called *N*-terminal sequencing. The sequences can then be used for protein identification. Some of the limitations in using this method are that it takes more time to identify each protein and there is potential for the *N*-terminal of proteins to be blocked. Although this method is still in use, high-throughput techniques like peptide fragment sequencing using multi-dimensional protein identification technology (MudPIT), and the types of MS mentioned previously are more commonly used (Skylas et al., 2005).



### ***1.4.3 Wheat grain proteomics***

Proteomics is essentially the separation and identification of proteins from any part of an organism. The total number of genes in an organism's genome is generally larger than the number of expressed proteins. Proteomics reveals those genes that are currently, or recently, expressed in different tissues and cell types (Skylas et al., 2000; Wong et al., 2004; Skylas et al., 2005).

As mentioned previously, it is important to understand the biochemical processes of wheat to determine favorable characteristics which can improve crop yield and management for farmers, and flour quality and yield for millers. Some of the recent studies on wheat using a proteomic approach have identified proteins in wheat grain endosperm, enzyme-modifying proteins in the endosperm, and proteins in the germ (Skylas et al., 2000; Wong et al., 2004; Mak et al., 2006b).

Proteomic studies on wheat grain endosperm by Skylas et al., (2000) were made at two stages of grain development, at 17 DPA and 45 DPA. More protein spots were observed at 17 DPA (around 1298 protein spots) than to at 45 DPA (1125 protein spots). Protein disulfide isomerase (PDI) isoforms and 60S ribosomal proteins are abundant during grain development and less abundant when the grain matures, thus possibly explaining the reduction in protein spots at 45 DPA. Because proteins were sequenced by *N*-terminal Edman degradation, *N*-terminal blocking prevented 28% of proteins in the endosperm at 17 DPA being sequenced. This problem may be overcome by using the mass spectrometry protein identification procedures mentioned previously. Nonetheless, this

work highlights the dynamic changes in gene expression that occur during wheat grain development together with a large number of identified endosperm proteins. The identification of these proteins may be useful in flour trait correlation with protein quantity and type.

Proteomics has also been used to identify thioredoxin target proteins in wheat grain endosperm by Wong et al. (2004). Thioredoxin is present in plants and acts as an enzyme regulator, a process which is called redox regulation. It does this by activating enzymes or altering the enzyme activity via disulfide bond reshuffling. This work has identified a large number of thioredoxin target proteins, thus increasing knowledge of which proteins are modified during the development of the endosperm.

Proteomics of germ and whole grain (Mak et al., 2006b) has recently identified many major proteins in the germ, and has shown major protein class differences in the germ compared to proteins in the endosperm identified by Skylas et al. (2000). This work has added to knowledge concerning major proteins in the germ and is potentially useful in breeding programs where nutritional value and wheat quality can be improved.

Further proteomics work has compared the germ of wheat affected by Black Point with non-affected wheat (Mak et al., 2006a). Black Point disease affects many wheat growing countries. The disease causes an undesirable discoloration of the germ and the bran in severe cases, and as a result the value of the crop is down-graded. The study has shown that cultivars not affected by Black Point had higher levels of stress related proteins

compared to non affected cultivars (Mak et al., 2006a). This could lead to better understanding of the function of these proteins in the germ and their role in Black Point resistance.

#### ***1.4.4 Proteomics of bran components***

Proteomic analysis of bran and bran tissue fractions have not been reported. The difficulty with collecting clean bran from dry grain is that there is a strong bond between the endosperm and aleurone cells. This makes it difficult to cleanly separate the aleurone cells from the endosperm, to make the bran free from contaminants and suitable for protein analysis. Another problem is separating the bran tissue fractions from each other. It is difficult to separate the bran tissue layers from dry grain as the layers are brittle and firmly bonded together.

There are methods for collecting aleurone cells and other tissue fractions by various techniques such as floating, differential centrifugation and electrostatics. However, they mostly result only in tissue enrichment, which still may contain contaminants from other tissue fractions (Bacic and Stone 1981; Stone et al. 1988). This makes these methods unsuitable for proteomics studies.

Imbibing whole grain in water will soften the bran, making it more flexible and increasing bran/endosperm separation. Manual bran tissue separation in this way has been reported by Antoine et al. (2004). The idea is to initiate germination and begin the endogenous breakdown of endosperm at the aleurone endosperm interface by  $\alpha$ - and  $\beta$ -

amylases. This then softens the endosperm around the aleurone surface and allows the bran to be removed easily and washed further with water. Softening of the bran tissue allows it to be more easily separated manually into three distinct tissue layers, the outer layer (epidermis and hypodermis), intermediate layer (cross cells, tube cells, testa and nucellar tissue), and inner layer (aleurone cells). Any water-soluble proteins that may leach out during grain imbibition can be collected and analysed.

The bran tissue collected using this method will be representative of bran at the early stages of germination. For the purpose of this study, the focus is on protein composition of bran at this early stage, as it will be similar to bran from conditioned grain. Collecting bran tissues from dry (dormant) grain and comparing them to bran at the early stages of germination will give more information of the changes in gene expression. Improvements in bran tissue separation from dry grain would be required to do further work in this area.

As the conditioning step in milling is about stabilizing bran and improving the separation of endosperm from bran, knowledge of unique proteins which may aid in this process could potentially provide insights into how the conditioning and milling process could be optimised. A very optimistic goal in milling would be to achieve complete endosperm and bran separation. However, even a slight increase in flour yield and quality after milling would have a large impact, as millions of tonnes of wheat grain are milled annually.

## **2. Materials and Methods**

### ***Materials 2.1***

#### ***2.1.1 Wheat sample***

Wheat grain *Triticum aestivum* cultivar Babbler, was supplied by the Bread Research Institute Australia Limited (BRI).

#### ***2.1.2 Chemicals***

##### ***2.1.2.1 Protein extraction***

Acetone (AJAX Finechem), ammonium acetate (BDH Chemicals), glycerol (AJAX Finechem), 2-mercaptoethanol (Sigma-Aldrich), methanol (AJAX Finechem), SDS (Amresco), sucrose (AJAX Chemicals), trichloroacetic acid (TCA) (BDH Chemicals), Tris (Amresco), Tris-buffered phenol at pH 8.0 (Sigma-Aldrich).

##### ***2.1.2.2 Protein quantification***

Bovine serum albumin (BSA) (Sigma-Aldrich), Bradford reagent (Bio-Rad), nitrocellulose paper (0.2 µm pore size, Whatman, Schleicher and Schuell).

##### ***2.1.2.3 SDS-PAGE***

Bromophenol blue (Edward Gurr), glycerol (AJAX Finechem), Pre-cast SDS 4-20% gradient polyacrylamide gels (0.1 cm x 10 cm x 8 cm) (Life Therapeutics, Long Life Gels), 2-Mercaptoethanol (Sigma-Aldrich), SDS (Amresco), Tricine (Sigma-Aldrich), Tris (Amresco).

#### ***2.1.2.4 IEF and SDS-PAGE***

Acrylamide (Bio-Rad), agarose (Bio-Rad), ammonium persulphate (APS) (Bio-Rad), bis-acrylamide (Bio-Rad), bromophenol blue (Bio-Rad), carrier ampholytes (Amersham Biosciences, GE), 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) (Bio-Rad), 1,4-dithio-DL-threitol (DTT) (Bio-Rad), glycerol (Merck), glycine (Bio-Rad), IPG strips pH 4-7 (Bio-Rad, Catalogue number 163-2008), IPG strips pH 6-11 (Amersham Biosciences, Lot number 309990), SDS (Bio-Rad), sulfobetaine 3-10 (Sigma-Aldrich), N,N,N',N'-tetramethylethylenediamine (TEMED) (Bio-Rad), thiourea (Sigma-Aldrich), tributyl phosphine (TBP) (Sigma-Aldrich), Tris (Sigma), urea (Bio-Rad).

#### ***2.1.2.5 Gel staining***

Acetic acid (Merck), Deep Purple (Amersham GE Health Care), G-250 Coomassie (Bio-Rad), methanol (Merck), phosphoric acid (BDH Chemicals), sodium carbonate (Sigma-Aldrich).

#### ***2.1.2.6 Sample preparation for MS***

Acetonitrile (Merck), ammonium bicarbonate (Sigma-Aldrich), C-18 Zip Tips (Eppendorf), formic acid (AJAX Finechem), N-terminal sequencing grade 25% trifluoroacetic acid (TFA) (Applied Biosystems), Prep mix (bradykinin, angiotensin I, neurotensin and adrenocorticotrophic hormone (ACTH) fragment) (Sigma-Aldrich), recrystallised  $\alpha$ -cyano-4-hydroxycinnamic acid (Sigma-Aldrich), sequencing grade trypsin (Promega, Catalogue number V5111).

## ***2.2 Tissue fraction collection***

### ***2.2.1 Grain treatment prior to tissue separation***

Whole grains were imbibed in water for two days at room temperature. Once the endosperm had softened, each end of the grain was cut off with a fine pair of scissors, removing the embryo and brush hairs. The cleft running along the grain was also cut out using scissors. Finally, the endosperm was removed and the bran was washed with water. The washed bran was kept in fresh milli-Q water and stored at approximately 4°C.

### ***2.2.2 Bran cleaning and tissue separation of inner and intermediate fractions***

Bran tissue was further washed under a dissecting microscope using forceps to scrape off any remaining endosperm. During dissection, the bran was kept in a volume of water (approximately 200 µl) on the surface of the dissecting microscope (Kyowa, Tokyo). This was needed to collect the inner fraction (aleurone cells) and to stop the bran from drying out. The inner fraction was scraped off into water on the glass dissecting surface of the microscope with forceps and collected using a pipette. The cells were then placed into a 1.5-ml plastic tube and centrifuged for 2 min at 14000 rpm. Excess water was removed and the cells were left to dry inside the tube. The outer layer of the remaining bran was removed and collected into a plastic tube. Finally, the remaining intermediate tissue fraction was further washed with water under the dissecting microscope and was also collected into a plastic tube. All tissue fractions were left to dry overnight. Once the tissue fractions were dry, they were stored at -20°C. A light microscope was used to visualize each bran fraction to confirm its purity. The method used to separate the tissue fractions was successful in that all the tissue fractions had unique cell structure,

indicating the tissue types present. Micrographs of the tissue fractions were all captured at 200x magnification using a light microscope (Olympus BX50) and digital microscope camera (Sony DFW-SX700) and were edited using Image J software. The same digital camera was used with a stereo zoom microscope (Olympus SZH) to capture larger whole images of the bran fractions (Figs. 3.1, 3.2, and 3.3a). An Olympus BH2 epifluorescence microscope equipped with a mercury lamp and a filter set for UV excitation at 395 nm and emission at 420 nm was used to image the autofluorescent inner fraction. The UV image was captured using a Nikon DXM 1200F (Fig. 3.3b).

The separation and collection of inner and intermediate bran tissue fractions was successful, and enough of each tissue sample was collected for protein extraction. However, much more of the outer tissue fraction was required because of its low protein content.

### ***2.2.3 Improved method for collecting outer fraction***

Grains were imbibed in water for 5 min, followed by freezing with dry ice. Once frozen, the grains were left to thaw out at room temperature. After thawing, the outer fraction was easily removed using forceps with the aid of a dissecting microscope (Fig. 2.1). The brush hairs were cut off with scissors and the remaining tissue was stored in a plastic tube to dry overnight at room temperature. The dry tissue was then stored at -20°C.

Amount of tissue collected for protein extraction – inner fraction (Aleurone) (45 mg), intermediate fraction (42 mg), and outer fraction (200 mg).





**Figure 2.1** Removal of the outer fraction (Hypodermis and epidermis) using the ‘freeze thaw’ method.

## ***2.3 Protein extraction and quantification***

### ***2.3.1 Protein extraction from bran fractions***

#### ***2.3.1.1 Tissue preparation and washing***

Samples were kept on ice during all steps in the protein extraction. All centrifugation was done using a 1.5-ml plastic tube centrifuge (Sigma).

The method used for protein extraction from plant tissue was followed as described by Wang et al. (2003).

The tissue sample was placed into a 2-ml screw-cap plastic tube and washed with 1 ml cold acetone. The sample was vortexed for 30 s and then centrifuged at 14000 rpm for 3 min. Acetone was removed and this step was repeated. The tissue was then left to dry inside the plastic tube in a fume hood. Once dry, the tissue was placed into a mortar, together with a small amount of acid-washed sand, and was ground down to a fine powder. The fine powder was collected back into the 2-ml plastic tube.

One millilitre of cold 10% TCA in acetone was added to the ground-up tissue. The sample was then vortexed for 30 s and then centrifuged at 11000 rpm for 3 min. The solution was removed and this step was repeated twice. The sample was then washed twice with cold 10% TCA in water and then finally washed twice with cold 80% acetone. Again for each step, the sample was vortexed for 30 s and then centrifuged at 11000 rpm for 3 min. The tissue was left to dry at room temperature overnight.

#### ***2.3.1.2 Protein extraction***

To the washed and dried tissue was added 800 µl of Tris-buffered phenol at pH 8.0, followed by 800 µl of SDS buffer (30% sucrose, 2% SDS, 0.1M Tris-HCl at pH 8.0, and 5% 2-mercaptoethanol). The sample was then vortexed for 30 s and centrifuged at 11000 rpm for 3 min. The top phenol layer was removed and placed into a new 2-ml plastic tube.

#### ***2.3.1.3 Protein precipitation***

To the phenol was added five volumes of cold methanol, 0.1 M ammonium acetate. The tube was then stored at -20°C for 1 hr to precipitate the protein. The precipitated protein was centrifuged at 11000 rpm for 5 min and the supernatant was removed. The protein pellet was washed twice with cold methanol, 0.1 M ammonium acetate and then twice with cold 80% acetone. Each time, the pellet was vortexed for 30 s and centrifuged for 5 min at 11000 rpm. After final wash, the pellet was left to air dry to evaporate any acetone.

#### ***2.3.1.4 Solubilising protein pellet in rehydration solution***

All protein pellet samples were solubilised in 500 µl rehydration buffer (5 M urea, 2 M thiourea, 65 mM DTT, 2% CHAPS, 2% sulfobetaine 3-10, 1% carrier ampholytes, 40 mM tris, 0.002% bromophenol blue dye, milli-Q water).

### ***2.3.2 Protein collection from grain and outer fraction supernatant***

Approximately 35 to 40 whole grains, and 37 mg of isolated outer fraction collected from 50 grains, were placed into separate 1.5-ml plastic tubes and filled with Milli-Q water. The tubes were stored at 4°C overnight to allow for any water-soluble proteins to leach out. The samples were then stored at -80°C prior to freeze drying. 50 µl 2x loading buffer was added to the freeze-dried samples to solubilise the proteins. The samples were then centrifuged for 5 min at 14000 rpm to remove any tissue material. The supernatant was collected into fresh 1.5-ml plastic tubes.

### ***2.3.3 Protein quantification***

#### ***2.3.3.1 Protein quantification of tissue fractions***

Nitrocellulose paper was pre-wet with milli-Q water for 15 min. The paper was then placed onto a 96-well Bio-Dot micro filtration apparatus. An aliquot of water (100 µl) was loaded into each well and was drawn through the paper using a low vacuum. This was done to check if the apparatus is sealed properly.

Bovine serum albumin (BSA) was diluted in water for one dilution series and rehydration buffer for a comparison as a standard curve. The dilutions were 200 µg.ml<sup>-1</sup>, 50 µg.ml<sup>-1</sup>, 12.5 µg.ml<sup>-1</sup>, 3.125 µg.ml<sup>-1</sup>, 781 ng.ml<sup>-1</sup>, 195 ng.ml<sup>-1</sup>, 48.8 ng.ml<sup>-1</sup> and a blank. An aliquot (100 ul) of each dilution was loaded onto the 96-well Bio Dot apparatus in triplicate. Samples were diluted 1:10, 1:100, and 1:1000. Each well was then washed with 150 µl water four times, each time allowing the vacuum to draw the water through the membrane. The membrane was removed from the apparatus and rinsed several times with

water. It was then placed in fresh water on rocker for 10 min and rinsed again. The membranes were placed in between two filter papers wrapped in foil and stored in the fridge overnight.

The Deep Purple staining method was followed as described in the manufacturer's instructions for nitrocellulose blot staining (Amersham Biosciences). Membrane was washed with 200 mM sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) for 15 min. The wash solution was replaced with 25 ml 1:200 dilution of Deep Purple stain in water. The membrane was left on a rocker to stain for 1 h and kept covered from light. The membrane was left to dry in the dark and then scanned using a Typhoon variable mode imager (Amersham Biosciences). The membrane image was analysed using Image J software to measure the relative intensities of the protein spots. From the relative intensities, a standard curve was calculated for BSA dilutions in water and the same for BSA dilution in rehydration buffer. The blanks were used to subtract background intensities and then protein concentrations were determined.

#### *2.3.3.2 Protein quantification of water-soluble proteins*

The water-soluble proteins in the supernatant were quantified using Bio-Rad protein assay dye reagent (Catalogue number 500-0006). Proteins from the supernatant were extracted as described earlier. After freeze drying, the samples were solubilised in 250  $\mu\text{l}$  of Milli-Q water. BSA dilutions ( $160\text{ }\mu\text{g.ml}^{-1}$ ,  $80\text{ }\mu\text{g.ml}^{-1}$ ,  $40\text{ }\mu\text{g.ml}^{-1}$ ,  $20\text{ }\mu\text{g.ml}^{-1}$ ,  $10\text{ }\mu\text{g.ml}^{-1}$ ,  $5\text{ }\mu\text{g.ml}^{-1}$ ) for a standard curve were prepared. Protein assay dye was diluted, one part to four parts Milli-Q water. An aliquot (200  $\mu\text{l}$ ) of this dilution was added to wells in

a 96-well microplate (Greiner, Catalogue number 655101). Ten microlitres of each BSA standard and sample was added to the protein assay dye in duplicate. The plate was left to incubate at room temperature for 5 min prior to reading absorbance at a wavelength of 620 nm (Labsystems Multiskan).

## ***2.4 Iso-electric focusing (IEF) (First dimension in 2-DE)***

### ***2.4.1 Sample preparation***

Prior to IEF, samples in rehydration buffer were reduced by adding 5 mM tributyl phosphine (TBP) and alkylated by adding 10 mM acrylamide. The sample was then left on the bench to incubate for 1 h. After incubation, the samples were centrifuged (Eppendorf centrifuge 5417R) at 14000 rpm for 10 min at 4°C. The required total volume of sample in buffer was dependent on the type of immobilized pH gradient (IPG) strip used. For the 17 cm pH 4-7 IPG strip, the sample volume per strip was 300 µl and for the 18 cm pH 6-11 IPG strip, the sample volume was 120 µl, as it is recommended to use cup loading (see below) in the alkaline pH range.

### ***2.4.2 IPG strip rehydration***

Sample loading onto IPG strips was different for acidic pH ranges and alkaline pH ranges. For the 4-7 pH range samples could be loaded onto the IPG strip during rehydration. On the other hand, the manufacturer (Amersham Biosciences, GE) recommends cup loading for the 6-11 pH range. Firstly, the IPG strip was rehydrated with the rehydration solution. When placing the IPG strip onto the IEF machine, a cup

attachment was placed onto the strip at the acidic end. The sample was then loaded into the cup and the sample was electrophoresed out from an opening at the bottom of the cup and into the strip. This means that only proteins with an alkaline pI migrated onto the IPG strip and not the acidic pI proteins. The result of using this method was better protein focusing in the alkaline pH range.

The 4-7 pH IPG strips were rehydrated for 4 h with 300 µl of the prepared sample. The 6-11 pH IPG strips were rehydrated for 4 h with 300 µl of rehydration buffer alone. An aliquot (120 µl) of the prepared sample in buffer was cup loaded during IEF.

#### **2.4.3 IEF**

IPG strips were focused overnight at 8000 V for 11.5 h, on an IEF machine (Ettan IPGphor3, GE Healthcare) (method: 300 V for 4 h, ramp to 8000 V over 8 h, finally remained at 8000 V for 11.5 h).

Total V hr for each IEF run:

|  |                        |
|--|------------------------|
| Inner fraction                           | pH range 4-7 – 129000  |
| Inner fraction                           | pH range 6-11 – 118649 |
| Intermediate fraction and outer fraction | pH range 4-7 – 127820  |
| Intermediate fraction and outer fraction | pH range 6-11 – 107818 |

After focusing, the IPG strips were placed into a tray and sealed with cling wrap to be stored at -80°C overnight or when ready for SDS-PAGE. It is recommended to store the IPG strips at -80°C at least overnight for better second-dimension resolution.

#### ***2.4.4 IPG strip equilibration***

The IPG strips were removed from the -80°C freezer and were thawed at room temperature. The tray was placed onto a rocker and equilibration solution (6 M urea, 2% SDS, 0.375 M Tris/HCl pH 8.8, 20% glycerol, 5 mM TBP, 2.5% acrylamide) was poured over the strips and left to equilibrate for 15 min. This was repeated twice, and after each time, the equilibration solution was poured off and replaced with fresh solution.

### ***2.5 SDS-PAGE (Second dimension in 2-DE)***

#### ***2.5.1 SDS-PAGE of IPG strips from tissue fractions***

##### ***2.5.1.1 Casting 8-18% polyacrylamide gradient gels***

Glass plates, spacers and gel cast chambers (Protean II systems chamber) were used to cast six gels (17 x 17 cm) at a time. An 8% acrylamide buffer solution was prepared containing 44 ml 5x Tris/HCl buffer, 44 ml 40% bis-acrylamide, and 132 ml Milli-Q water. An 18% acrylamide buffer solution was prepared containing, 44 ml 5x Tris/HCl buffer, 99 ml 40% bis-acrylamide, and 77 ml 50% glycerol. To polymerise the acrylamide, 36 µl TEMED followed by 363 µl 10% APS was added to each buffer solution. The solutions were then stirred and immediately poured into two separate gradient pouring chambers (Bio-Rad, Gradient Former Model 395). The 18% acrylamide solution was poured into the reservoir chamber and the 8% acrylamide solution into the mixing chamber which contained a magnetic stir bar rotated gently by a magnetic stirrer (Selby Australia). A cartridge pump (Masterflex L/S) was used to draw the acrylamide solution out of the mixing chamber and into the gel casts. The 8% acrylamide solution



was first pumped into the gel casts at a rate of 60-75 ml.min<sup>-1</sup> until there was an even layer of about 1 cm on the bottom of the casting chamber. The 18% acrylamide solution in the reservoir chamber was released into the mixing chamber. The gels were poured until the acrylamide solution reached 2 cm from the top of the glass casts. The pump was then stopped and the inlet hose was clamped. Isobutanol was poured on top of the gels to create an even layer. Once the acrylamide had polymerized, the isobutanol was washed off with water and the gels were ready to be used.

#### ***2.5.1.2 Electrophoresis***

IPG strips were imbedded on top of the 8-18% gradient polyacrylamide gels (17 cm x 17 cm) using hot agarose (0.5% agarose, 0.001% bromophenol blue, 192 mM glycine, 0.1% SDS, 24.8 mM Tris base pH 8.3). The gels were then electrophoresed in Protean II multi-cell tanks (Bio-Rad) using a power box (Bio-Rad Power Pac 3000), set at 5 mA per gel for 30 min, and then at 40 mA per gel for approximately 4.5 h, or until the dye front had run off the gel. Once the dye front had run off, gels were removed from their casts and placed into fixing solution (30% methanol, 7.5% acetic acid) for at least 1 h to prepare for Deep Purple staining.

#### ***2.5.2 SDS-PAGE and protein analysis of water-soluble proteins from supernatant***

An aliquot (50 µl) of 2x 1D SDS loading buffer (100 mM Tris-HCl pH 6.8, 0.2% bromophenol blue, 20% glycerol, 4% SDS, 200 mM 2-mercaptoethanol) was added to the tube to solubilise the protein. The tube was vortexed for 1 min and then centrifuged for 5 min at 14000 rpm. The supernatant was collected and placed into a fresh plastic tube. The

sample was then reduced by adding 1  $\mu$ l 2-mercaptoethanol, incubated in a boiling water bath for 2 min to denature the proteins, and centrifuged for 2 min at 14000 rpm. An aliquot (30  $\mu$ l) of this sample was then loaded onto a 4-20% gradient pre-cast SDS polyacrylamide gel (0.1 cm x 10 cm x 8 cm). The sample was run using a Bio-Rad Power Pac 200 at constant 150 V for approximately 1 h. The gel was then stained with Coomassie and selected bands were excised for MS identification.

## ***2.6 Staining, imaging and protein spot excision***

### ***2.6.1 Deep Purple staining***

After fixing, the solution was poured off and replaced with 200 mM Na<sub>2</sub>CO<sub>3</sub> for 1 h to basify the gels. Sodium carbonate was then poured off and replaced with water, approximately 10x the gel volume. Deep Purple stain was added to the water to make a final dilution of 1 in 200. The gels were covered with foil and left to stain overnight on a rocker. After staining, the stain was poured off and destained twice with 1% acetic acid. The gels were immediately scanned using a Typhoon variable mode imager (Amersham Biosciences). The gel image was scanned in fluorescence mode, 610 BP Deep Purple emission filter, green (532) laser, and with 100 micron pixel resolution.

### ***2.6.2 Protein spot selection***

The scanned images were transferred and uploaded into image analysis software Progenesis Discovery version 2005 (Nonlinear Dynamics LTD). The uploaded images were transferred to, and edited using, Progenesis PG240 version 2006 (Nonlinear

Dynamics LTD) software to match and number protein spots in each of the triplicate gels. This software was used to match spots only in the intermediate and outer fraction samples to identify as many spots as possible. The protein spots in the inner fraction gel images were selected on the basis of proteins visible with Coomassie stain. The images were scanned on the spot cutter (Bio-Rad, EXQuest) and only spots that could be clearly seen with Coomassie stain were manually selected for excision.

### ***2.6.3 Coomassie staining***

After Deep Purple staining and scanning, the gels were counter stained with colloidal Coomassie Blue G-250 stain (17% ammonium sulfate, 3% phosphoric acid, 0.1% Coomassie G-250, 34% methanol, Milli-Q water to make up 1 L total) (Neuhoff et al. 1988) and left on a rocker overnight. The gels were then destained with 1% acetic acid and bagged in seal tight plastic bags provided in APAF lab. All gels were stored at 4°C until needed.

### ***2.6.4 Spot cutting***

Prior to spot cutting, all gels were placed in Milli-Q water for at least 1 h. This was important to minimise any swelling of the gel during spot cutting. An image was taken of the gel and all spots visible with Coomassie stain were numbered and selected for excision. A spot cutter (Bio-Rad, EXQuest) was used to excise 96 spots at a time. It was found that the cutting becomes less accurate over time if more than 96 spots at a time were selected. Spots were placed into 96 well plates for further processing.

## ***2.7 Peptide extraction for MS***

### ***2.7.1 Gel plug destaining***

Gel plugs were manually destained 3x with 120  $\mu$ l wash solution (50% (v/v) acetonitrile, 25 mM ammonium bicarbonate). For each wash, the gel plug-in wash solution was placed onto an orbital shaker and incubated at 37°C for 10 min. The solution was removed and replaced with fresh wash solution each time. After destaining, the gel plugs were dried using a Savant Speed Vac Plus SC210A. For large gel samples, automated destaining was performed on Xcise - Automated gel processing platform (Shimadzu Biotech) using standard "Wash and Destain" program. The same destaining solutions were used as described previously; however, the final drying step used 100% acetonitrile to dehydrate the gel plugs instead of the Speed Vac.

### ***2.7.2 Passive trypsin digest***

To each dry gel plug was added 8  $\mu$ l of 15 ng. $\mu$ l<sup>-1</sup> sequencing grade trypsin in 25 mM ammonium bicarbonate, pH 7.8. The plugs were then placed in the refrigerator for 1 h to allow the trypsin to be absorbed into the gel. After incubation, any excess trypsin was removed. The gel plugs were then sealed in the 96-well plate and incubated over night at 37°C.

### ***2.7.3 Peptide extraction from gel plugs (for Zip Tip clean up)***

To each gel plug was added 10  $\mu$ l of extraction solution (10% acetonitrile, 0.1% TFA). The wells were sealed and the plate was placed in a water bath sonicator (Transsonic

700/H, Elma) for 20 min. After sonication, the seal on the well plate was removed to allow any excess acetonitrile to evaporate off for 30 min.

#### ***2.7.4 C18 column Zip Tip clean up***

Zip Tips were first washed with 10 µl of 70% acetonitrile, 0.1% TFA by pipetting 10 µl up and down three times. The tips were then further washed in the same manner using 0.1% TFA. The peptide extraction solution was taken up into the Zip Tip, drawing up and down 8µl. This was repeated ten times to concentrate the peptides onto the column. Once the peptides were loaded onto the Zip Tip, the tip was further washed three times with 10 µl of 0.1% TFA.

#### ***2.7.5 Loading peptides onto ABI plate for MS***

Four microlitres of extraction solution (4 mg.ml<sup>-1</sup> Matrix,  $\alpha$ -cyano-4-hydroxycinnamic acid, 70% acetonitrile, 0.1% TFA) was drawn up into the Zip Tip. The extraction solution in the tip was drawn up and down forming a drop at the end of the tip at least five times to elute the peptides from the column. Finally, 2 µl of this solution was spotted onto a designated circle marked on the ABI plate. A standard (prep mix with matrix) was also spotted after each sample on the ABI plate to externally calibrate using near point calibration with four peptide standards (bradykinin, angiotensin I, neurotensin and adrenocorticotrophic hormone (ACTH) fragment). Samples on the plate were allowed to dry/crystallise, ready to be analysed using an Applied Biosystems 4700 MALDI TOF MS MS/MS (Foster City, CA).

## ***2.8 Protein identification***

### ***2.8.1 MALDI TOF MS***

Protein peptide samples were analysed using an Applied Biosystems 4700 MALDI MS/MS with TOF/TOF optics (Foster City, CA) in reflector mode for positive ion detection. A Nd:YAG laser with wavelength and repetition rate of 355 nm and 200 Hz, respectively, was used. All MS spectra resulted from accumulation of 4000 laser shots (20 sub-spectrum were accumulated with 200 shots per sub-spectrum). Laser intensity varied between 3000 and 4000. Data was collected over a mass range of 750 to 3500 Da. Mass spectral data was analysed using Mascot (Matrixscience). Peak detection criteria for mass lists were MS: mass range 500-4000 Da, maximum 30 peaks per 200 Da, minimum signal to noise ratio (S/N) 20, minimum area 200, maximum peak/spot 200, and for MS/MS: mass range 60 Da to precursor -15, maximum 20 peaks per 200 Da, minimum S/N 18, minimum area 300, maximum peak/spot 60. This converts the mass lists into Mascot and The Global Proteome Machine (GPM) compatible text files.

MS searches were done on cereal and wheat EST data bases on Mascot (Matrix Science, London, UK), which uses both MS and MS/MS data for protein identification. Searches using The Global Proteome Machine (GPM) wheat EST database uses MS/MS data for protein identifications. Identification of proteins with high scores and low e-values with good peptide matches and coverage were then tabulated with corresponding spot number and location on the 2D gel. Spots that showed no significant protein identification matches were further analysed using electro-spray ionization (ESI) MS/MS.

### **2.8.2 ESI MS/MS**

Digested peptides were separated by nano-LC using a CapLC system (Agilent 1100 Series, Agilent Technologies, Germany). Sample (39  $\mu$ l) was injected onto a peptide trap (Michrome peptide Captrap) for preconcentration and desalted with 0.1% formic acid at 10  $\mu$ l.min<sup>-1</sup>. The peptide trap was then switched into line with the analytical column containing C18 RP silica (SGE ProteCol C18, 300A, 3  $\mu$ m, 150  $\mu$ m x 10 cm). Peptides were eluted from the column using a linear solvent gradient, with steps, from H<sub>2</sub>O:CH<sub>3</sub>CN (95:5; + 0.1% formic acid) to H<sub>2</sub>O:CH<sub>3</sub>CN (20:80 + 0.1% formic acid) at 600 nl.min<sup>-1</sup> over 45 min. The LC eluent was subject to positive ion nanoflow electrospray analysis on an Applied Biosystems QSTAR XL mass spectrometer (ABI, CA, USA). The QSTAR was operated in an information dependant acquisition mode (IDA).

In IDA mode a TOF/MS survey scan was acquired (m/z 400-2000, 1.0 s), with the four largest multiply charged ions (counts >25) in the survey scan sequentially subjected to MS/MS analysis. MS/MS spectra were accumulated for 1 s (m/z 50-2000).

The LC/MS/MS data was searched using Mascot (Matrix Science, London, UK). Mascot was used to search cereal entries in the NCBI non-redundant protein database. High scores in the database searches indicated a likely match, confirmed or qualified by inspection of the spectra and search results.

### ***2.8.3 Construction of Wheat EST Database for searching using XTandem and Mascot***

Approximately 620,000 wheat EST sequences were available through GenBank in February 2006. These sequences were assembled into contigs at the plant genome database

([www.plantgdb.org/search/misc/plantlistconstruction.php?mySpecies=Triticum%20aestivum](http://www.plantgdb.org/search/misc/plantlistconstruction.php?mySpecies=Triticum%20aestivum)) which reduced the dataset to approximately 200,000 contig sequences. These contig sequences were downloaded as a single file in FASTA format. A biopython program was written to translate these EST-contigs in all six reading frames and also to give each translated product a unique name while maintaining the FASTA format (see Appendix-3). These translated EST-contigs were assembled into a single file which was searchable using Mascot and XTandem (thegpm, [http://137.111.103.157/tandem/thegpm\\_tandem.html](http://137.111.103.157/tandem/thegpm_tandem.html)) (Dong et al., 2004; Dong et al., 2005).

### ***2.8.4 Wheat EST alignments***

Wheat EST sequences were aligned using Clustal W (1.81) to create multiple sequence alignments. Phylogenetic relationships were created using PHYLIP and alignments were viewed using SEAVIEW (Galtier et al., 1996) software. NJplot was used to draw an unrooted phylogenetic tree diagram from the PHYLIP output (Perriere and Gouy, 1996).



## 3. Results

### *3.1 Collection of tissue fractions and microscopy*

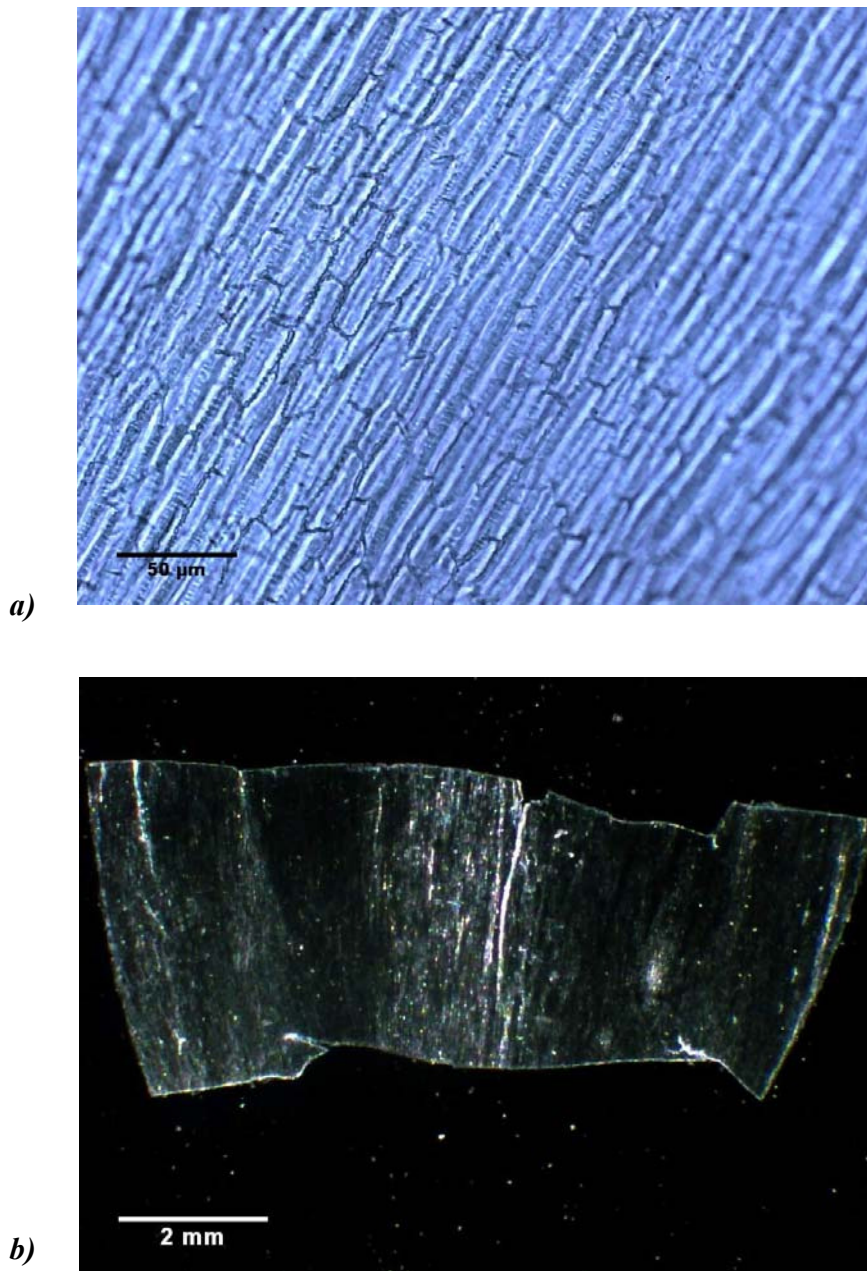
Bran was separated into three separate tissue fractions based on ease of separation.

Firstly, the outer fraction was removed, which comprised the epidermis and hypodermis (Fig. 3.1). This fraction was the easiest to remove using the ‘freeze-thaw’ technique.

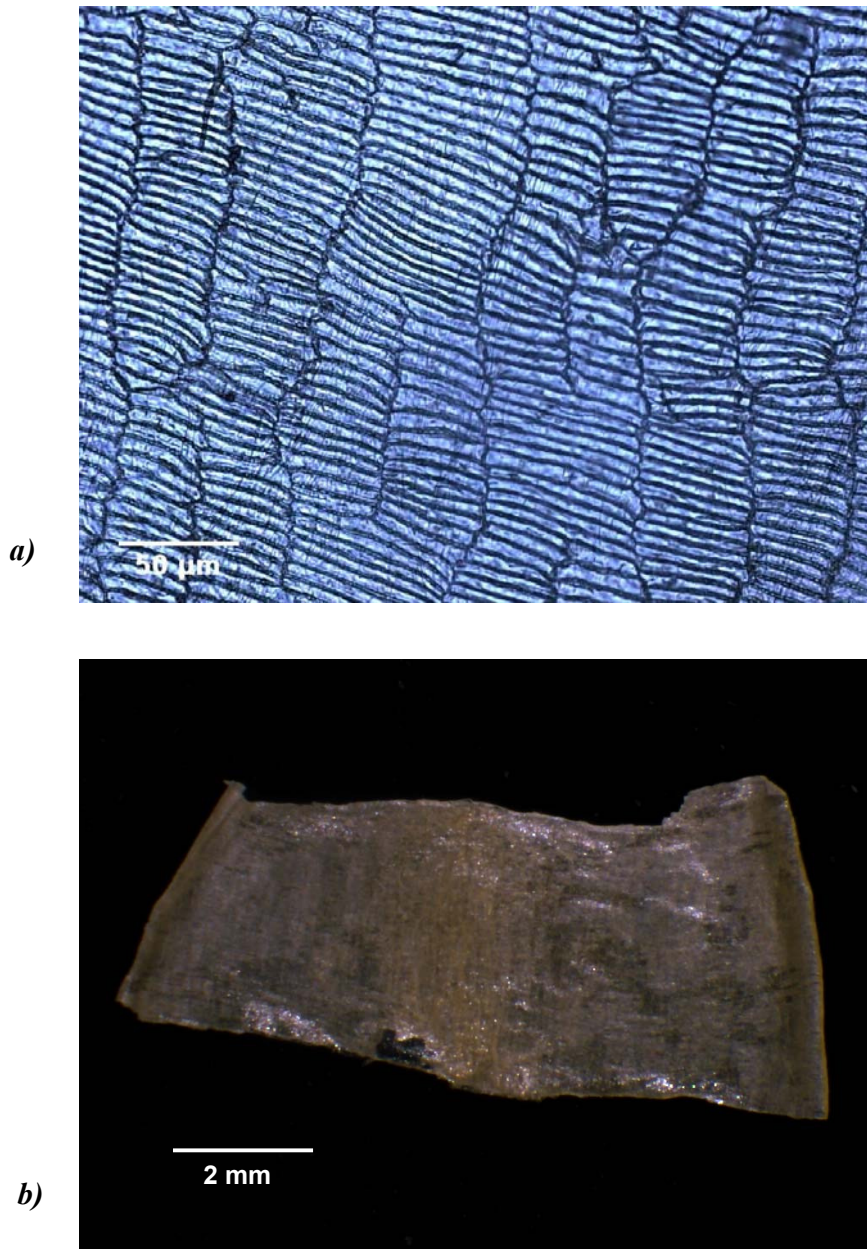
Next, the inner fraction was scraped off and collected in water. This fraction contained aleurone cells and possibly some connecting tissue between the endosperm and the nucellar tissue (Fig. 3.4). Lastly, the remaining tissue after removing the inner fraction was the intermediate fraction. This fraction is comprised of nucellar tissue, seed coat (testa), tube cells and cross cells, together forming a thin compact multilayer tissue fraction (Fig. 3.2).

All of the tissue fractions were checked for purity using light microscopy.

Photomicrographs showed the distinctive cell patterns of the outer fraction epidermis and hypodermis (Fig. 3.1) and the intermediate fraction cross cells (Fig. 3.2). The individual tissue layers of the intermediate fraction could be seen (Fig. 3.3). The majority of the inner-fraction cells (aleurone cells) were still relatively intact, showing cell walls and cell contents (Fig. 3.4).

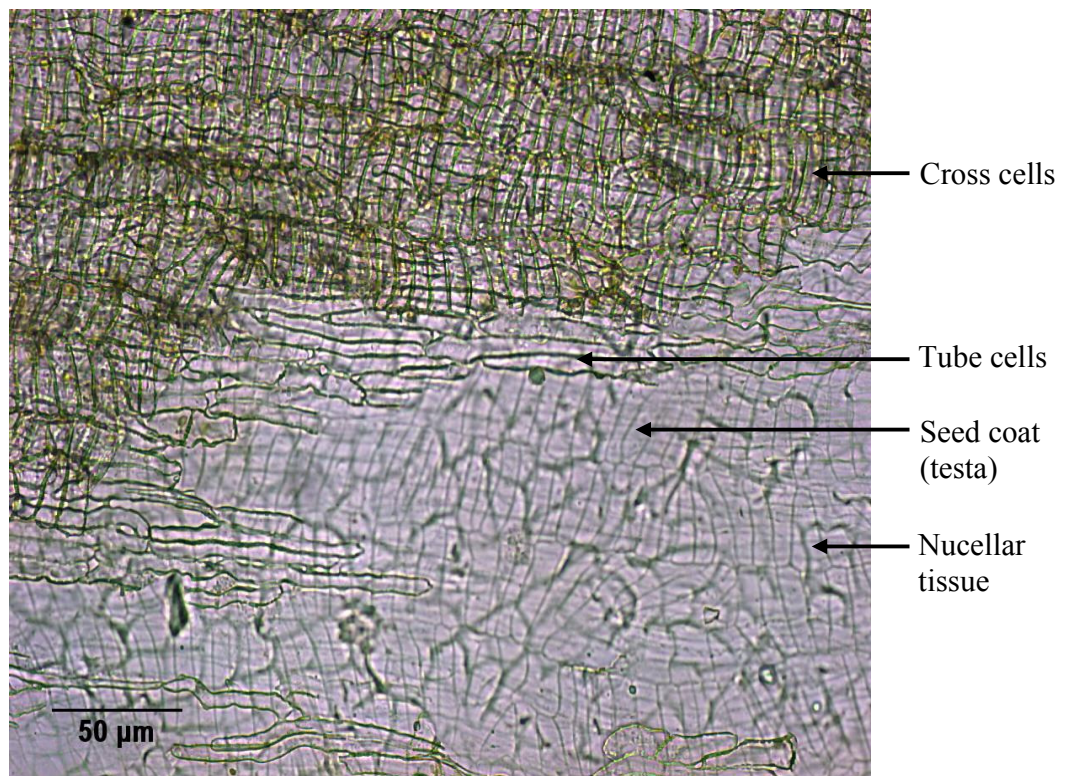


**Figure 3.1** Mechanically stripped-off outer fraction (epidermis and hypodermis): a) viewed using a light microscope, b) whole outer fraction viewed under a dissecting microscope with variable zoom.

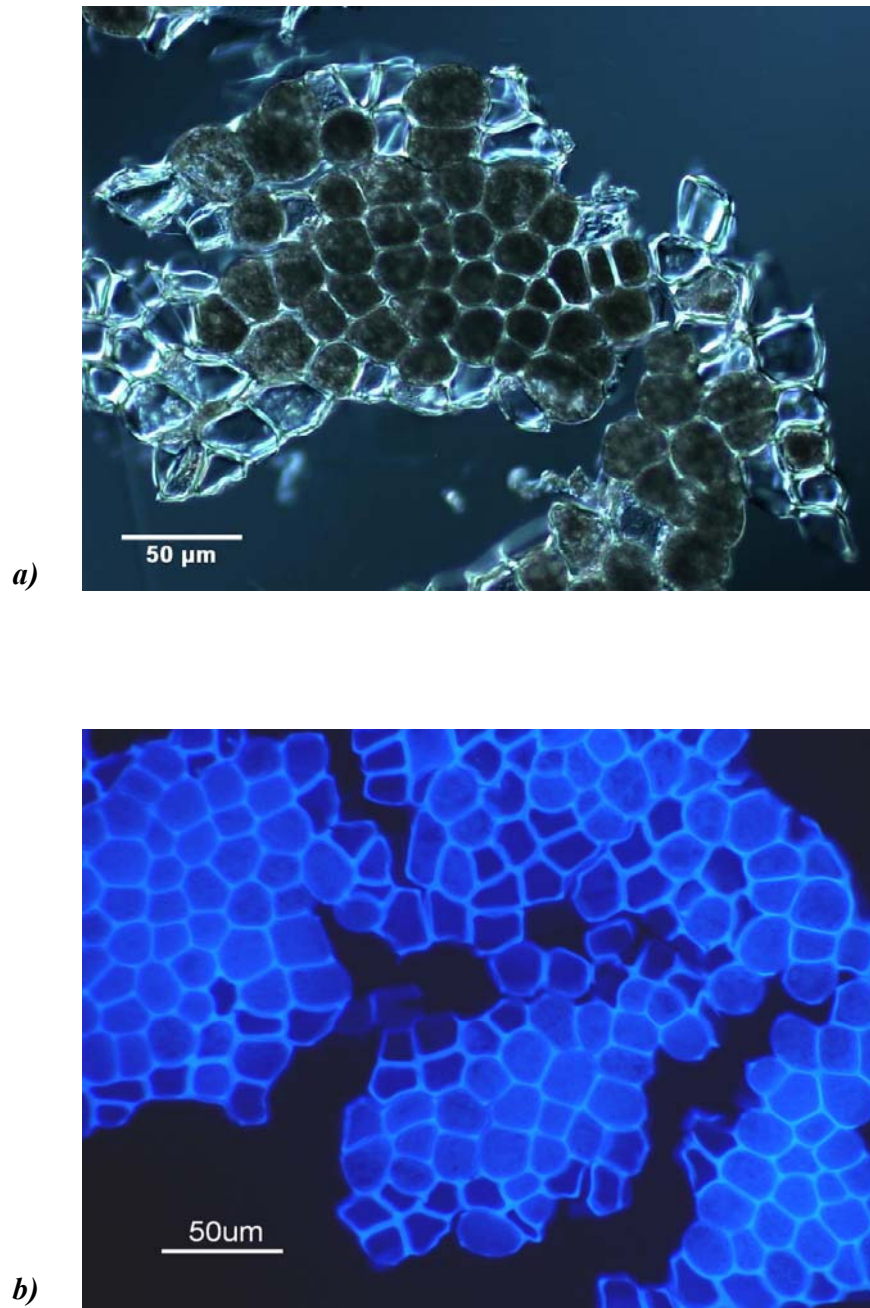


**Figure 3.2** Mechanically stripped-off intermediate fraction (cross cells, tube cells, testa, and nucellar tissue): a) viewed using a light microscope, b) whole intermediate fraction viewed under a dissecting microscope with variable zoom.





**Figure 3.3** Photomicrograph of the intermediate fraction (cross cells, tube cells, testa, and nucellar tissue), viewed using a light microscope.



**Figure 3.4** Mechanically stripped-off inner fraction (aleurone cells): a) viewed under dark field microscopy, b) viewed under fluorescence during excitation with UV (395 nm).

### ***3.2 Extraction and quantification of proteins from bran tissue fractions and supernatant from imbibed grain***

Proteins extracted from the intermediate and outer tissue fractions (see Table 3.1) represented less than 10% yield predicted from the data of Spurway (1998). In contrast, the inner fraction showed a much higher concentration of protein using the same extraction method used by Wang et al. (2003). The amount of protein extracted was 70% of the expected protein content in the inner fraction shown in Table 3.1; thus, there is a large difference in the percentage of proteins extracted between the intermediate and outer tissue fractions and the inner fraction.

To account for any loss of water-soluble protein during tissue collection, the supernatant was collected from the imbibed grain and also from the imbibed isolated outer tissue fraction. The amount of protein that leached out from the whole grain into the supernatant was higher ( $1.3 \mu\text{g}\cdot\text{grain}^{-1}$ ) than the amount protein that leached out from the isolated outer fraction ( $0.5 \mu\text{g}\cdot\text{grain}^{-1}$  equivalent) (see Table 3.1). This difference in protein amounts can be seen in the 1D gels where a number of protein bands seen in the whole-grain supernatant were missing from extracts of isolated outer fraction (Fig. 3.11). The extra protein bands seen in the whole-grain supernatant are likely to be due to proteins leaching out from inner bran tissue fractions, or more likely from the germ.

It was estimated that 0.6 mg of protein per gram of outer tissue fraction had leached out into the supernatant. This is a relatively large amount of protein per gram of tissue material when compared to the  $0.4 \text{ mg}\cdot\text{gram}^{-1}$  of protein detected in the water-insoluble

component of the outer tissue fraction (see Table 3.1). This probably indicates that supernatant proteins were highly water-soluble, or that the protein extraction method did not accurately reflect actual protein extracted from these fractions.

**Table 3.1** Estimated protein content in tissue fractions (Spurway, 1998): a) protein extracted from bran tissue fractions using the method from Wang et al., (2003) (see Appendix A-1.1); b) water-soluble protein extracted from supernatant of imbibed whole grain and isolated outer fraction (see Appendix A-1.2)

| <i>Fraction and protein extraction method</i>           | <i>Protein content mg.g<sup>-1</sup></i> | <i>Protein content µg.grain<sup>-1</sup></i> | <i>Reference</i> |
|---|--|--|------------------|
| <b>Reference</b>  |  |  |                  |
| Outer and intermediate (pericarp and testa)             | 56.0                                     | NA   | Spurway, 1998    |
| Inner (aleurone)  | 221.0                                    | NA   | Spurway, 1998    |
| <b>Protein extraction by Wang et al., (2003) method</b> |  |  |                  |
| a) Outer  | 0.4                                      | NA   | This study       |
| Intermediate  | 3.6                                      | NA   | This study       |
| Inner (aleurone)  | 156.0                                    | NA   | This study       |
| <b>Water extraction</b>                                 |  |  |                  |
| b) Isolated outer fraction (pericarp)                   | 0.6*                                     | 0.5*   | This study       |
| Whole grain   | NA                                       | 1.3*   | This study       |

NA – not applicable

\* Water-soluble proteins

### ***3.3 Protein spot distribution in 1D and 2D gels***

#### ***3.3.1 Water-soluble proteins***

Two methods were used to discriminate the location of water-soluble proteins that leached out from the grain during imbibition. The first was imbibing the whole grain in water and the second was to imbibe isolated outer tissue fraction alone. The proteins were collected from each method and were separated by SDS-PAGE. The protein bands that were present in both gels were assumed to originate from the outer fraction alone. Other proteins that were not present in the isolated outer fraction gel were assumed to have either leached out from the inner bran tissue fractions or the germ (Fig. 3.11).

#### ***3.3.2 Outer fraction***

The results of 2D electrophoresis analysis of the outer fraction using both the 4 to 7 pH (Fig. 3.5) and the 6 to 11 pH (Fig. 3.6) range gels showed very few protein spots. The majority of proteins were in the acidic pH range of 4 to 5, and around a molecular weight of 25 kDa (Fig. 3.5). The only major proteins in the alkaline range were around pH 10, with a low molecular weight of around 5 kDa (Fig. 3.6). There were also very faint protein spots around pH 7 with molecular weights around 20 kDa.

#### ***3.3.3 Intermediate fraction***

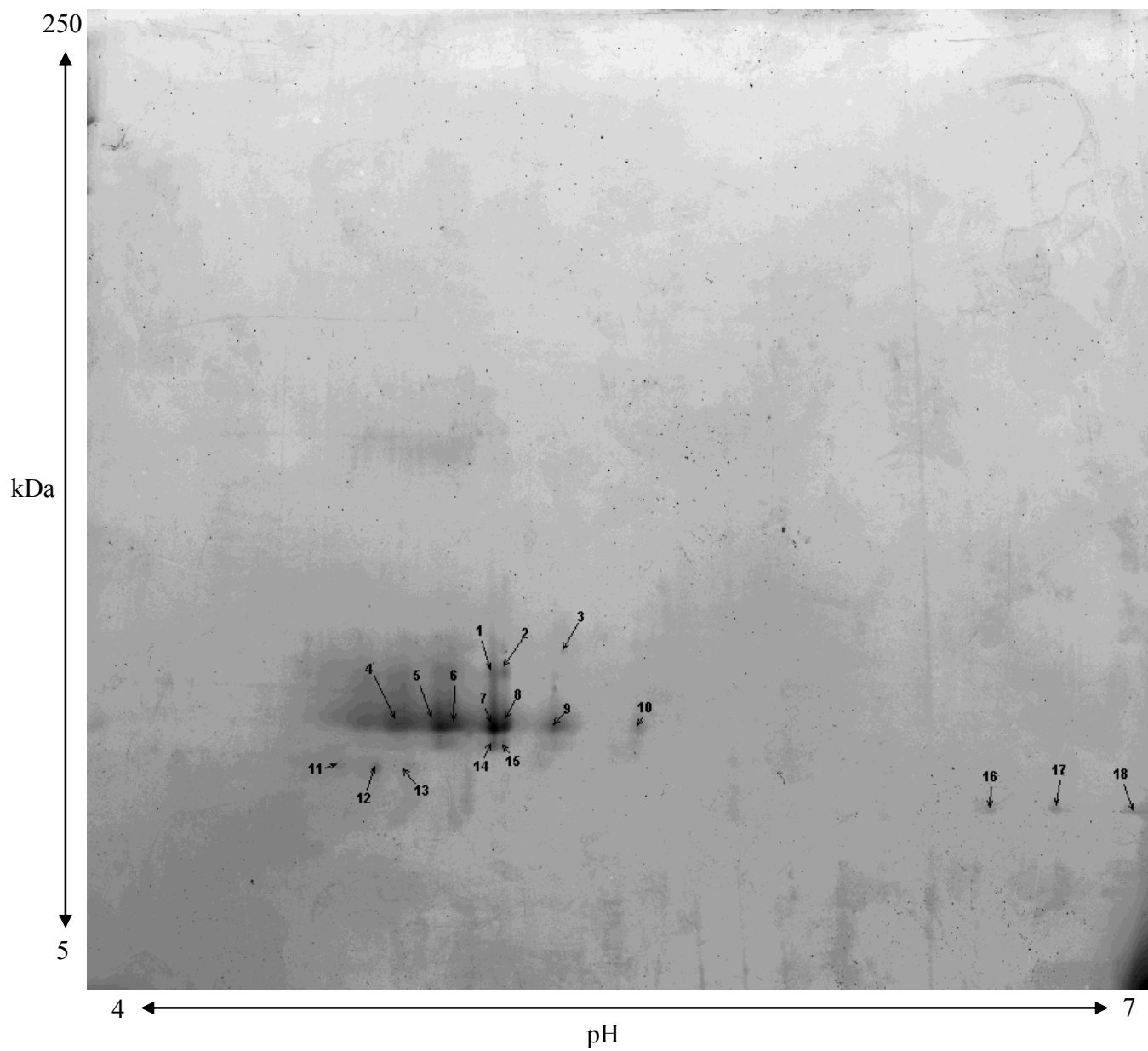
In contrast to the outer fraction, almost all of the protein spots in the 2D gels of the intermediate fraction (Figs. 3.7 and 3.8) were in the alkaline pH range 6 to 11 with molecular weights ranging from 20 to 40 kDa (Fig. 3.8). There were only a few protein spots in the 4 to 7 pH range, mainly between 5 and 10 kDa (Fig. 3.7).



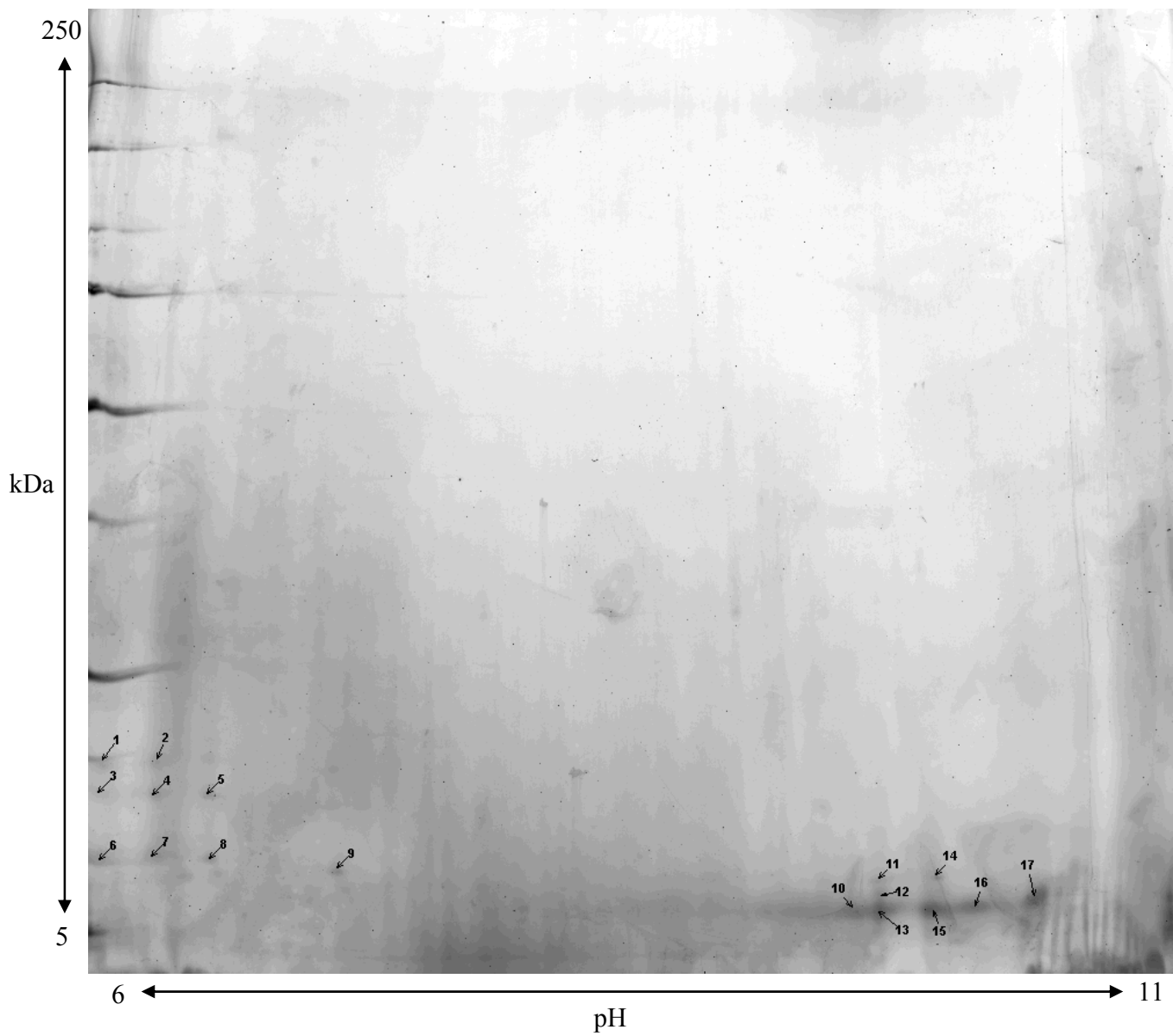
#### ***3.3.4 Inner fraction***

Two-dimensional electrophoresis analysis of the inner fraction, show a clear pattern of protein distribution across the pH range from 4 to 11 (Figs. 3.9 and 3.10). In the acidic pH range between pH 4 and pH 7, the majority of protein spots were of low molecular weight (Fig. 3.9). In the alkaline pH range between pH 6 and pH 11, the majority of proteins were of high molecular weight (Fig. 3.10). As expected based on the quantity of protein extracted, the 2D gels of the inner tissue fraction yielded more proteins than in the outer and intermediate fractions.

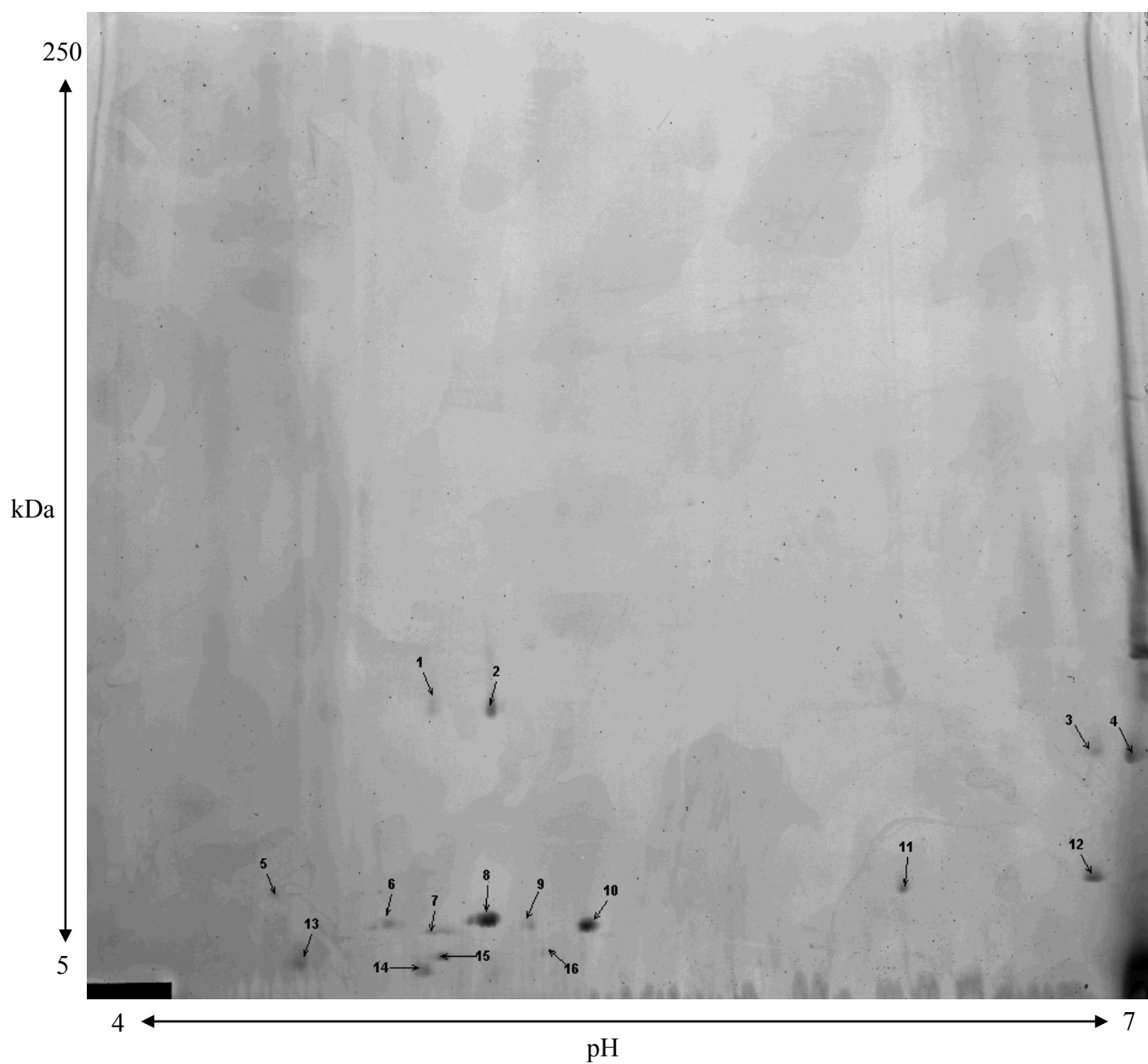
The purity of the inner tissue fraction was a concern due to the difficulty of separating it from the endosperm with minimal endosperm protein contamination. The distribution patterns in these gels (Figs. 3.9 and 3.10) differed significantly from patterns in endosperm 2D gels across a similar pH range (Skylas et al., 2000). Proteins in the 2D gels of endosperm were of relatively high molecular weight in the acidic pH range and clustered around pH 5, while in the alkaline pH range the majority of proteins were of medium molecular weight and located around pH 8 and pH 10. Comparison of displays between the endosperm and inner fraction 2D gels showed major differences. Thus the inner tissue fraction collection technique was successful as there were no obvious similarities between the 'endosperm' and 'inner fraction' 2D gels.



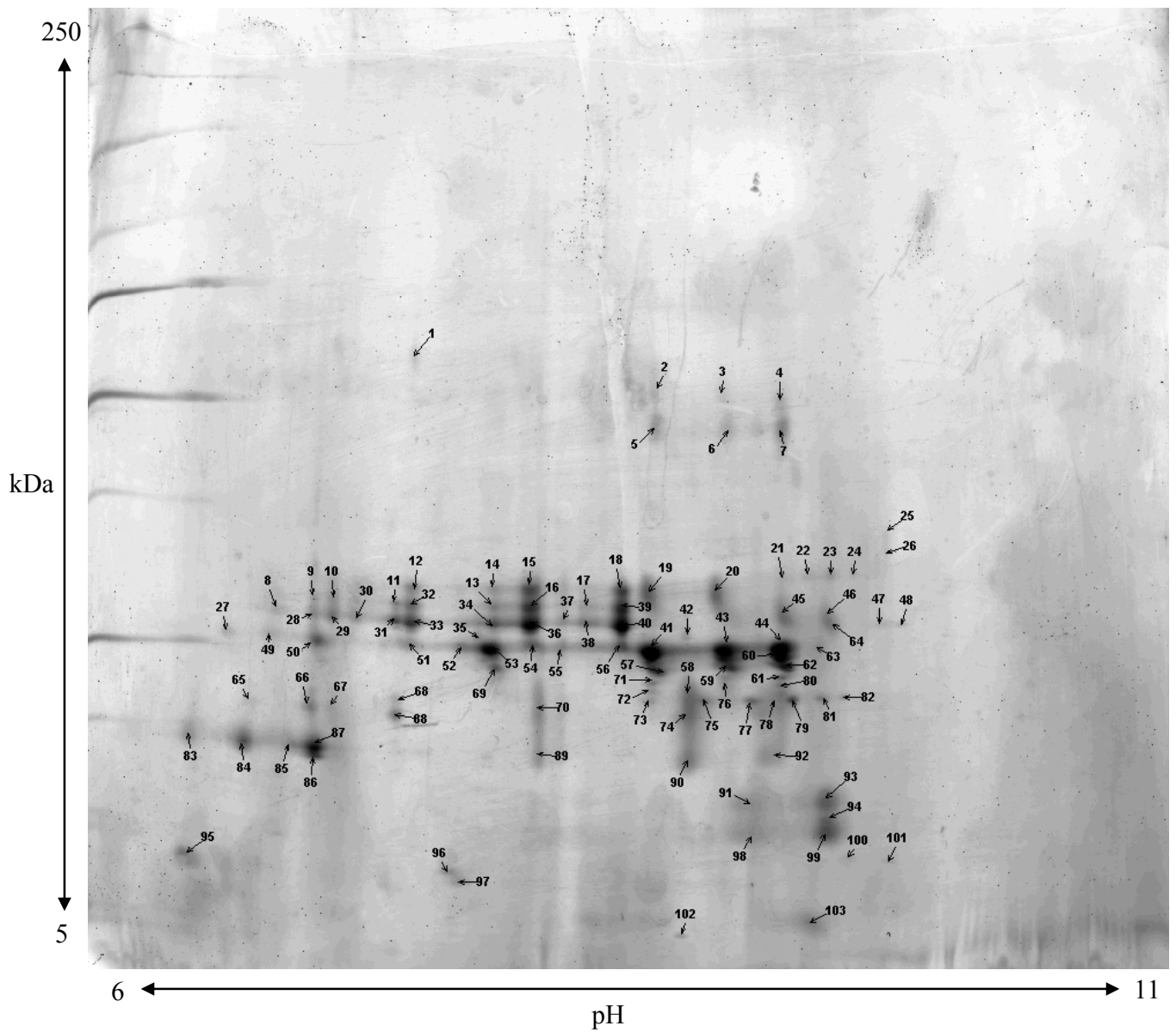
**Figure 3.5** Two-dimensional gel of the outer tissue fraction (epidermis and hypodermis) between the pH range 4 to 7 stained with Deep Purple. Protein spot numbers refer to Appendix-2, Table A-2.1.



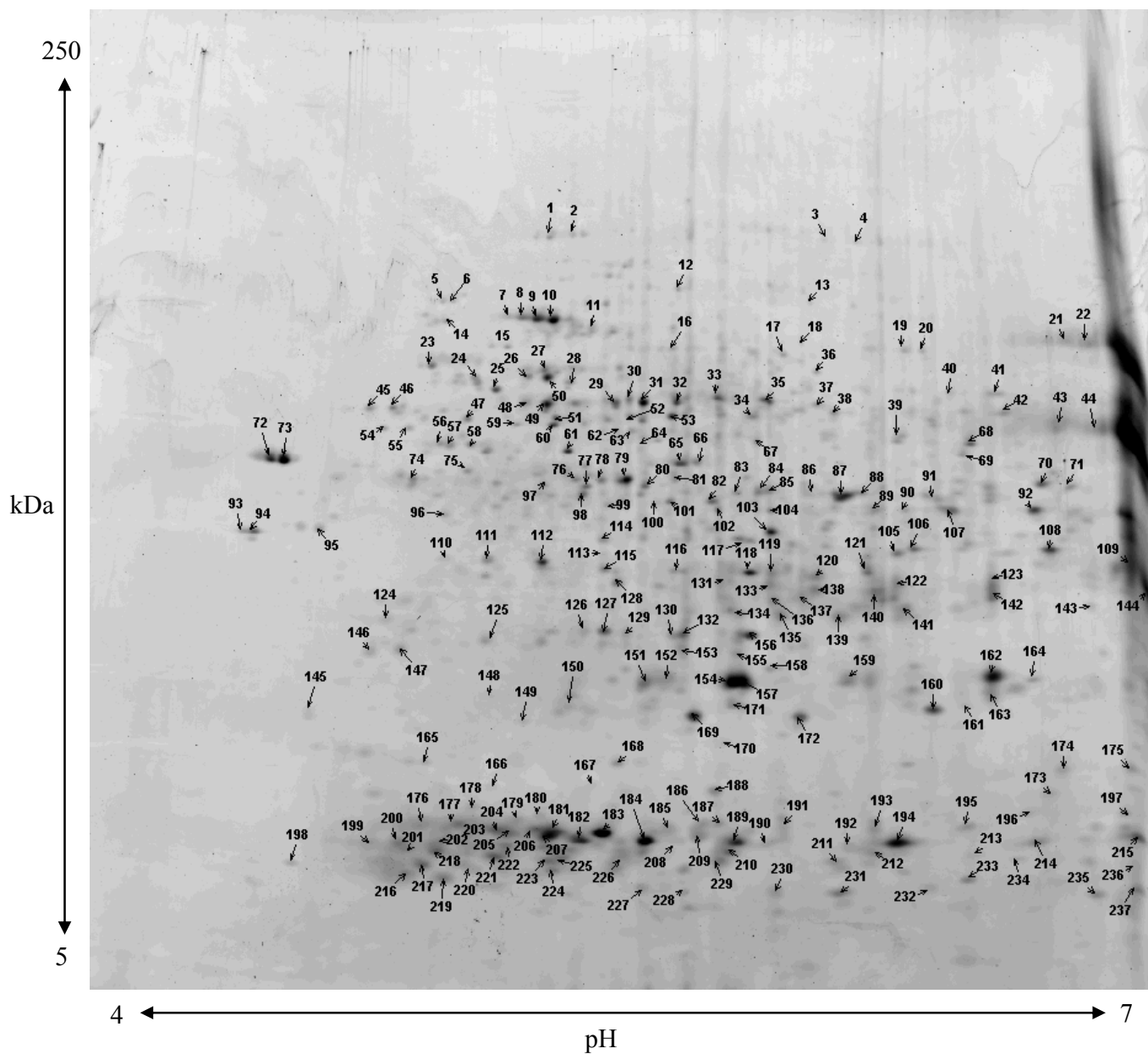
**Figure 3.6** Two-dimensional gel of the outer tissue fraction (epidermis and hypodermis) between the pH range 6 to 11 stained with Deep Purple. Protein spot numbers refer to Appendix-2, Table A-2.2.



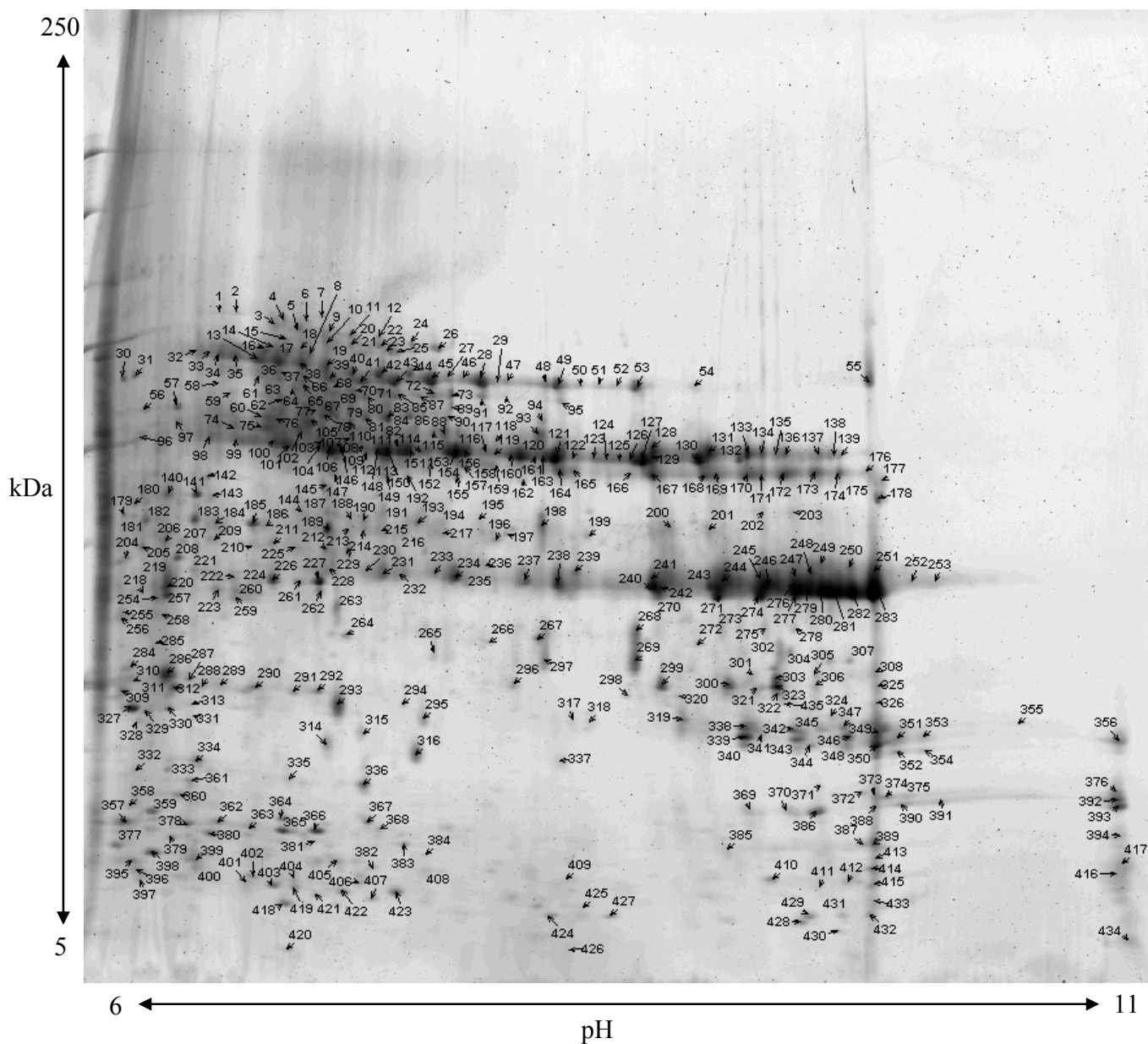
**Figure 3.7** Two-dimensional gel of the intermediate tissue fraction (nucellar, testa, tube cells and cross cells) between the pH range 4 to 7 stained with Deep Purple. Protein spot numbers refer to Appendix-2, Table A-2.3.



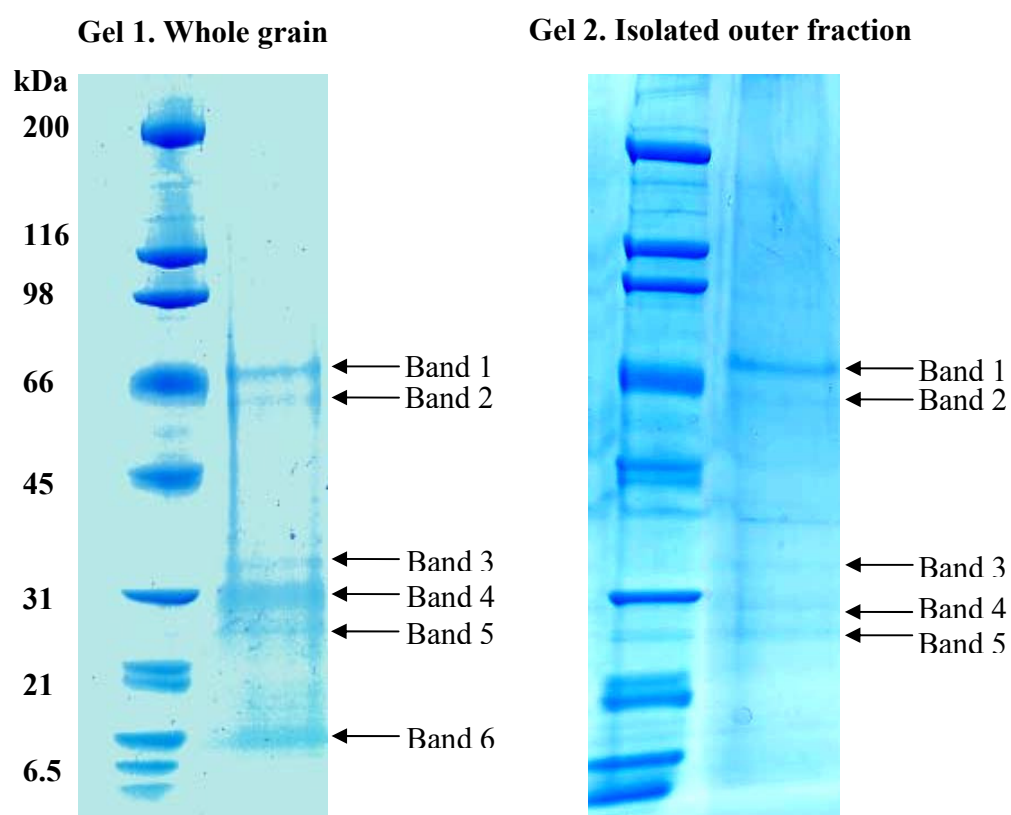
**Figure 3.8** Two-dimensional gel of the intermediate tissue fraction (nucellar, testa, tube cells and cross cells) between the pH range 6 to 11 stained with Deep Purple. Protein spot numbers refer to Appendix-2, Table A-2.4.



**Figure 3.9** Two-dimensional gel of the inner tissue fraction (aleurone cells) between the pH range 4 to 7 stained with Deep Purple. Protein spot numbers refer to Appendix-2, Table A-2.5.



**Figure 3.10** Two-dimensional gel of the inner tissue fraction (aleurone cells) between the pH range 6 to 11 stained with Deep Purple. Protein spot numbers refer to Appendix-2, Table A-2.6.



**Figure 3.11** Gel 1, proteins extracted from supernatant of whole grains; Gel 2, proteins extracted from supernatant of the outer fraction only. Gels were stained with Coomassie Blue G-250. Protein band numbers refer to Appendix-2, Table A-2.7.

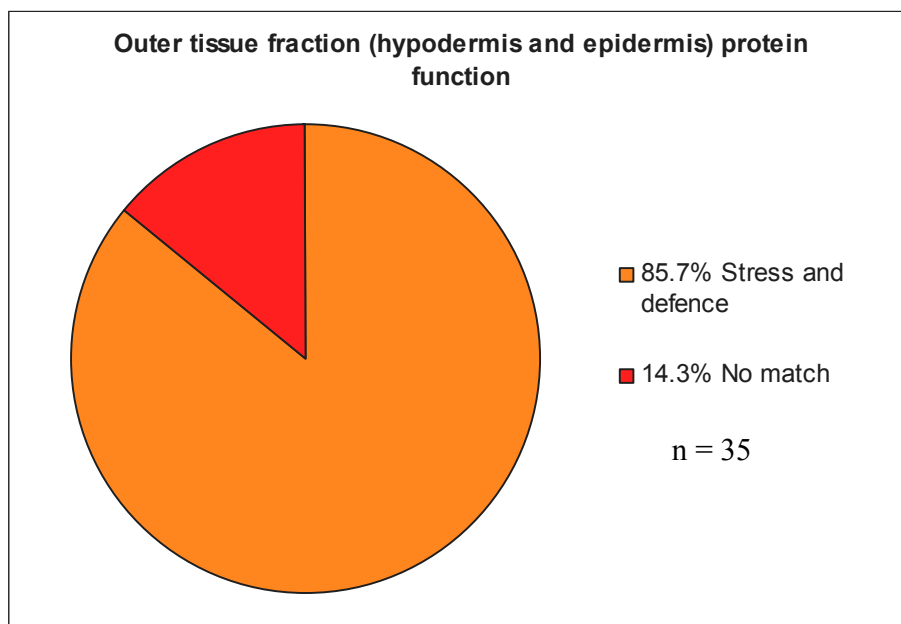


### ***3.4 Proteins identified in bran tissue fractions and supernatant from imbibed grain***

Criteria for all the protein identifications (matches) were based on high scores (generally above 70) from Mascot searches, however low Mascot scores below 70 were still considered based on good peptide-to-protein coverage and low log (e) values (below -1) from 'thegpm' searches together with a combination of the number of peptide matches and percent peptide coverage of the protein. Wheat EST's identified were searched against NCBI BLAST and matched to proteins in GenBank with e-values less than 0.0001. All of the protein summary tables refer to the complete protein identification tables in Appendix-2. The complete protein identification tables in Appendix-2 show further information such as search scores as described above, the pI and MW of each protein and its position on the 2D gels, the protein homology to species, matching peptides and percent coverage of the identified protein and accession numbers for each protein. The 'protein summary tables' show a summary of all the identified proteins in Appendix-2 grouped based on their major function and with a more specific sub-class function together with spot number references to 1D and 2D gels.

### 3.4.1 Proteins identified in the outer fraction

A summary of the proteins identified in the outer fraction from Appendix-2, Tables A-2.1 and A-2.2 are shown in Table 3.2. In total 35 spots were selected for identification. The major proteins identified in this fraction were oxidative-stress and defence-related proteins (30 spots or 85.7%) (Fig. 3.12). All of the identified proteins were matched to wheat species with two spots matching to wheat EST's. Of these, there were no matches to five spots. The mass spectra data of the unmatched proteins were poor, with very few peptide masses and MS/MS ion peaks together with low peak intensities. The poor spectral data could be a result of low amount of protein as the spots excised for analysis were very faint.



**Figure 3.12** Functional distribution of protein spots on the 2D gel of the outer tissue fraction.

**Table 3.2** Summary of proteins identified in the outer tissue fraction from Appendix-2  
(see Tables A-2.1 and A-2.2)

| Defence proteins  | Spot number*  | Sub-class                        |
|---|---|----------------------------------|
| Oxalate oxidase precursor   | 1a, 1b, 2a, 2b, 3a, 4a, 5a, 6a, 7a, 7b, 8a, 9a, 10a, 11a, 12a, 13a, 14a, 15a, 16a, 17a, 18a | Cupin domain-containing proteins |
| Oxalate oxidase GF-3.8 precursor (EC 1.2.3.4) (Germin GF-3.8)                                 | 4b  |                                  |
| Oxalate oxidase GF-2.8 precursor (EC 1.2.3.4) (Germin GF-2.8)                                 | 3b, 5b, 6b, 8b, 9b  |                                  |
| PLAT (Polycystin-1, Lipoxygenase, Alpha-Toxin) domain or LH2 (Lipoxygenase homology 2) domain | 11b   | Oxidative stress and defence     |
| Type 1 non-specific lipid transfer protein precursor  | 12b, 17b  | Transport and defence            |
| No match  | 10b, 13b, 14b, 15b, 16b   |                                  |

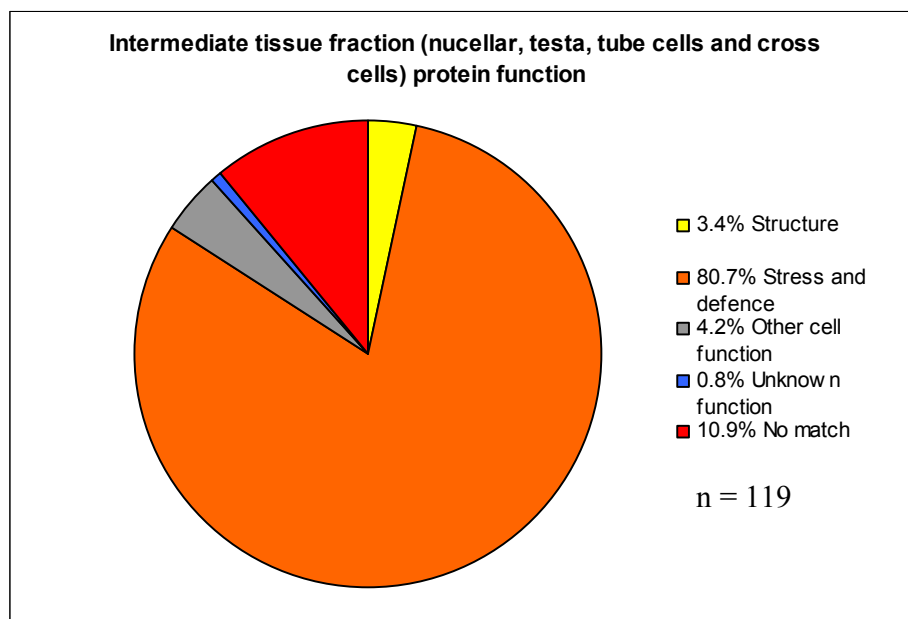
\* Spot numbers with suffix 'a' and 'b' correspond to pH range 4-7 and 6-11 gels respectively displayed in Figures 3.5 and 3.6

### ***3.4.2 Proteins identified in the intermediate fraction***

A summary of the proteins identified in the intermediate fraction from Appendix-2, Tables A-2.3 and A-2.4 is shown in Table 3.3. There were 119 spots selected for identification. The major proteins identified in this fraction are also oxidative-stress and defence-related proteins (106 spots or 89.1%; Fig. 3.13). The identified proteins (84 spots or 79.2%) were matched to wheat species with 22 (20.8%) of these matched to wheat EST's. Proteins that were not identified to wheat species were identified to cereal or plant homologues (22 spots or 20.8%). Of the selected spots, 13 were unable to be identified. The mass spectral data of the unmatched proteins were poor, with very few peptide masses and few MS/MS ion peaks together with low peak intensities.

Oxalate oxidase (OXO) was the only protein identified in the intermediate fraction that was also identified in the outer fraction and supernatant. Thus OXO may be located in the intermediate fraction or it might have leached across from the outer fraction into the intermediate fraction during grain imbibition.

The major defence-related proteins xylanase inhibitor, chitinase and endochitinase,  $\alpha$ -amylase/subtilisin inhibitor, wheatwin1, thaumatin like protein (TL) and benzothiadiazole (BTH)- clone of a wheat chemically-induced protein (cWCI-5) were all identified in this fraction (see Table 3.3).



**Figure 3.13** Functional distribution of protein spots on the 2D gel of the intermediate tissue fraction.

**Table 3.3** Summary of identified proteins in the intermediate tissue fraction from Appendix-2  
(see Tables A-2.3 and A-2.4)

| Defence proteins  | Spot number*  | Sub-class                |
|---|---|--------------------------|
| 26 kDa endochitinase 1 precursor  | 20b, 41b, 43b, 50b, 52b, 61b, 62b, 76b, 78b, 80b, 91b                                 | Chitinases               |
| 26 kDa endochitinase 2 precursor (EC 3.2.1.14) (CHI-26)   | 9b, 19b, 26b  |                          |
| Basic endochitinase A precursor (EC 3.2.1.14) (Rye seed chitinase-a) (RSC-a)  | 25b   |                          |
| Chitinase a   | 63b   |                          |
| Class II chitinase (EC 3.2.1.14)  | 27b, 28b, 29b, 34b, 42b, 44b, 45b, 48b, 49b, 56b, 59b, 72b, 73b, 79b, 86b, 100b, 101b |                          |
| Chain B, Crystal Structure of Chitinase At 1.91a Resolution   | 5b, 55b   |                          |
| Xylanase inhibitor protein 1 precursor (Class III chitinase homolog) (XIP-I protein)                                  | 3b, 4b, 8b, 10b, 13b, 14b, 15b, 18b, 22b, 51b, 54b, 66b, 67b                          | Xylanase inhibitors      |
| Xylanase inhibitor protein I  | 16b, 30b, 32b, 37b, 38b, 39b, 40b, 71b, 102b  |                          |
| Xylanase inhibitor XIP-III  | 69b   |                          |
| Chain A, Crystal Structure of Family 11 Xylanase in Complex with Inhibitor (Xip-I)                                    | 12b, 65b, 85b, 87b, 89b, 90b, 92b, 94b, 103b  |                          |
| Chain A, Crystal Structure Of Xylanase Inhibitor Protein (Xip-I)  | 36b, 46b, 53b, 57b  |                          |
| Xylanase inhibitor precursor (Xylanase inhibitor TAXI-I)  | 64b   |                          |
| Chain B, Crystal Structure Of The Triticum aestivum Xylanase Inhibitor-I In Complex With Aspergillus Niger Xylanase-I | 6a, 8a  |                          |
| Xylanase inhibitor  | 7a, 10a   |                          |
| Alpha amylase inhibitor   | 3a, 4a, 33b   | Alpha amylase inhibitors |
| Endogenous alpha-amylase/subtilisin inhibitor (WASI)  | 23b, 35b, 60b, 68b, 83b, 84b  |                          |
| Benzothiadiazole-induced protein (clone WCI-5)  | 75b, 82b  | Other defence proteins   |
| Oxalate oxidase precursor   | 1a, 2a  |                          |
| Pathogenesis-related protein 4 (Fragment)   | 5a, 12a, 95b, 96b, 97b  |                          |
| Thaumatococcus-like protein   | 17b   |                          |
| Wheatwin1   | 11a   |                          |



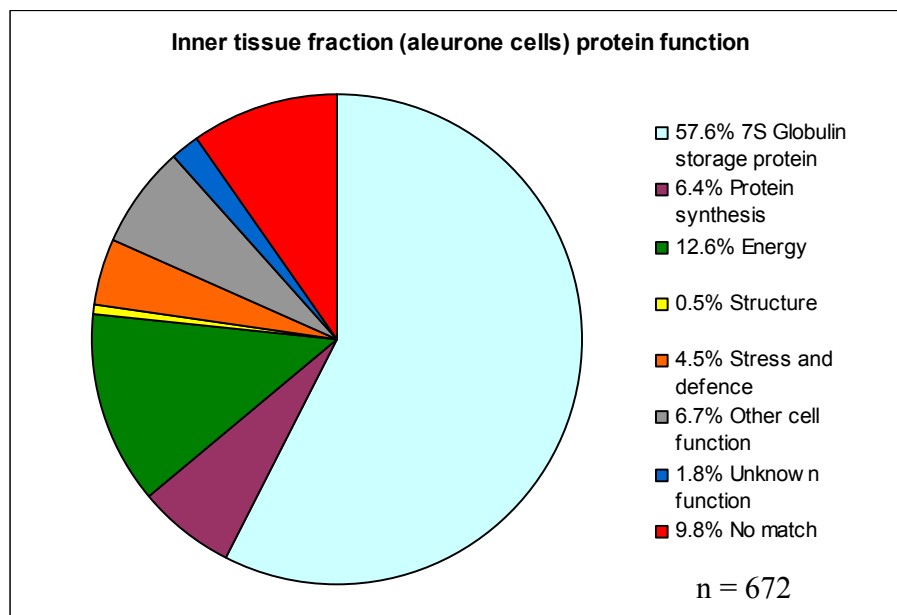
**Table 3.3** Summary of identified proteins in the intermediate tissue fraction (continued)

| Other                         | Spot number*                         | Sub-class           |
|-------------------------------|--------------------------------------|---------------------|
| Plant Basic Secretory Protein | 31b                                  | Transport           |
| Secretory protein             | 11b, 77b, 88b                        |                     |
| Actin (065316)                | 2b, 6b, 7b, 21b                      | Structural proteins |
| Uncoupling protein            | 13a                                  | Other               |
| Unknown                       | 81b                                  |                     |
| No match                      | 1b, 9a, 14a, 15a, 16a, 24b,          |                     |
|                               | 47b, 58b, 70b, 74b, 93b,<br>98b, 99b |                     |

\* Spot numbers with suffix 'a' and 'b' correspond to pH range 4-7 and 6-11 gels respectively displayed in Figures 3.7 and 3.8

### 3.4.3 Proteins identified in the inner fraction (*Aleurone cells*)

A summary of the proteins identified in the inner fraction from Appendix-2, Tables A-2.5 and A-2.6 is shown in Table 3.4. There were 672 spots selected for identification. The major proteins identified in this fraction were 7S globulin storage proteins (387 spots or 57.6%) and cell function proteins (128 spots or 19%) involved in protein synthesis and carbohydrate metabolism (Fig. 3.14). Of the identified proteins, 480 spots (79.2%) were matched to wheat species with 223 spots (36.8%) of these matched to wheat EST's. Proteins that were not identified to wheat species were identified to cereal or plant homologues (126 spots or 20.8%). Of the selected spots, 66 were unable to be identified. The mass spectral data of the unmatched proteins were good, showing many MS peaks and MS/MS ion peaks together with high peak intensities similar to spectral data of matched to proteins.



**Figure 3.14** Functional distribution of protein spots on the 2D gel of the inner tissue fraction.

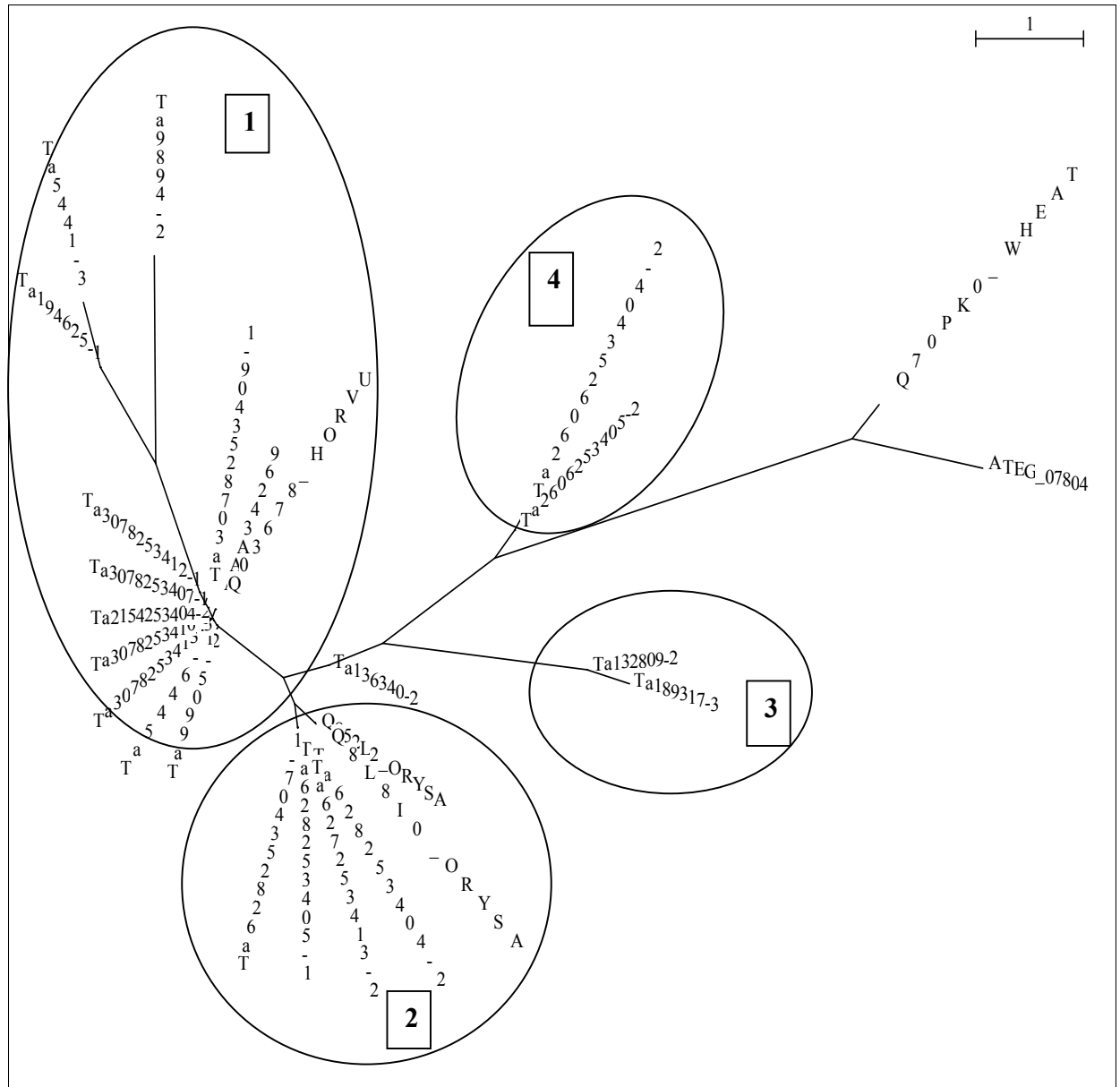
### ***3.4.4 Further analysis of major protein classes in inner fraction***

#### ***3.4.4.1 Cupin domain-containing proteins***

The majority of proteins identified from the inner tissue fraction 2D gel images (Figs. 3.9 and 3.10) were found to be cupin domain-containing proteins from the cupin super family (387 spots or 58%). Mascot search against the cereal database identified 207 spots (53%) that were wheat 7S globulin storage proteins, four spots (1%) matched to rice globulin-like protein, one spot (0.3%) matched to a rice putative globulin and finally one spot (0.3%) matched to a barley embryo globulin. Wheat EST sequences identified were searched against NCBI BLAST; 163 spots (42%) were matched to the wheat 7S globulin storage protein and 11 spots (3%) were matched to a cupin family expressed protein (Table 3.4).

Alignments were performed of all the translated wheat EST amino acid sequences that matched to cupin family proteins, together with amino acid sequences from wheat 7S globulin storage protein, rice globulin-like protein, barley-embryo globulin protein and also cupin family oxalate-metabolizing proteins, oxalate oxidase (identified in the outer tissue fraction) and an oxalate decarboxylase sequence (Appendix A-3). An unrooted phylogenetic tree diagram was drawn showing the relationship of the alignments (Fig. 3.15). There were three main clusters of wheat EST sequence orthologous to three different groups of cupin family proteins. Cluster (1) represents all orthologs of the wheat 7S globulin storage protein together with the barley embryo globulin protein. Cluster (2) represents all orthologs of the rice globulin-like and putative globulin proteins. Finally, clusters (3 and 4) represent orthologs of the oxalate-metabolizing proteins oxalate oxidase and oxalate decarboxylase (Table 3.4).





**Figure 3.15** Unrooted phylogenetic tree showing clusters of wheat EST sequence alignments to all cupin containing protein showing closest ortholog.

**Table 3.4** Clusters of wheat EST sequence alignments to cupin containing protein showing closest ortholog

| Cluster        | Protein  | Wheat EST orthologues   |
|----------------|--|---|
| <b>1</b>       | 7S globulin storage protein (Q7DMU0_WHEAT)<br>Embryo globulin (Q03678_HORVU)     | Ta3078253413-3<br>Ta136340-2<br>Ta194625-1<br>Ta2154253404-2<br>Ta2606253405-2<br>Ta3078253409-1<br>Ta3078253410-1<br>Ta3078253411-3<br>Ta3078253412-1<br>Ta3078253413-3<br>Ta3079253404-3<br>Ta5441-3<br>Ta5446-1<br>Ta628253407-1<br>Ta9894-2<br>Ta9905-2 |
| <b>2</b>       | Globulin-like protein (Q8L8I0_ORYSA)<br>Putative globulin (Q852L2_ORYSA)         | Ta3078253407-1<br>Ta628253405-1<br>Ta627253413-2<br>Ta628253404-2   |
| <b>3 and 4</b> | Oxalate oxidase precursor (Q70PK0_WHEAT)<br>Oxalate decarboxylase (gi:114190366) | Ta132809-2<br>Ta189317-3<br>Ta2606253405-2<br>Ta2606253404-2  |

#### 3.4.4.2 Cell function proteins

The other major proteins identified in the inner fraction 2D gels are involved in protein synthesis, folding and stability, carbohydrate metabolism (128 spots or 19%) as mentioned previously and the stress-and defence-related proteins (31 spots or 4.5%) that were similarly identified in the intermediate tissue fraction (Fig. 3.14).

**Table 3.5** Summary of identified proteins in the inner tissue fraction from Appendix-2  
(see Tables A-2.5 and A-2.6)

| Storage   | Spot number*   | Sub-class                               |
|---|--|---|
| 7S Globulin storage protein   | (370 spots)  | Cupin domain containing storage protein |
| Cupin family protein, expressed   | 152a, 154a, 157a, 171a, 256b, 286b, 293b, 295b, 314b, 316b, 337b |   |
| Embryo globulin   | 410b   |   |
| Putative globulin   | 73b  |   |
| Globulin-like protein   | 93b, 105b, 109b, 215b  |   |
| <b>Protein synthesis</b>  |  |   |
| Elongation factor 1-alpha (EF-1-alpha)  | 137b, 138b, 139b, 431b   | Gene expression                         |
| Elongation factor 1-alpha, putative, expressed                                | 432b   |   |
| Elongation factor Tu  | 76a  |   |
| Putative ribophorin I homologue   | 198a   |   |
| R2R3MYB-domain protein (Fragment)   | 381b   |   |
| RNA recognition motif family protein, expressed                               | 193b   |   |
| 16.9 kDa class I heat shock protein (Low molecular weight heat shock protein) | 192a, 209a, 380b   | Protein synthesis and folding           |
| 30S ribosomal protein S2  | 272b   |   |
| 40S subunit ribosomal protein   | 387b   |   |
| 50S ribosomal protein L31   | 418b   |   |
| 60 kDa chaperonin   | 25a  |   |
| Chaperonin CPN60-1, mitochondrial precursor (HSP60-1                          | 27a  |   |
| Heat shock protein 16.9B  | 186a, 359b   |   |
| Heat shock protein 17.9.  | 195a   |   |
| Heat shock protein HSP26  | 148a, 150a, 170a, 167a   |   |
| HSP70   | 7a, 8a, 9a, 10a, 84a, 85a, 86a, 87a, 124a                        |   |
| Putative 40S ribosomal protein S3   | 308b   |   |
| Putative 60S ribosomal protein L12  | 386b   |   |
| Putative 60S ribosomal protein L36  | 111a   |   |
| Putative alpha 1 subunit of 20S proteasome                                    | 164a, 287b   |   |
| Putative dnaK-type molecular chaperone  | 11a  |   |

**Table 3.5** Summary of identified proteins in the inner tissue fraction (continued)

| <b>Protein synthesis</b>   | <b>Spot number*</b>                                    | <b>Sub-class</b>                   |
|--|--|------------------------------------|
| Small heat shock protein Hsp23.5 precursor                           | 166a   |                                    |
| Small heat shock protein Hsp23.6 precursor                           | 149a   |                                    |
| Protein disulfide isomerase (EC 5.3.4.1) (Fragment)                  | 23a  |                                    |
| <br>Histone H3   | <br>434b   | <br>DNA binding                    |
| <b>Enzymes</b>   |  |                                    |
| Citrate synthase, eukaryotic   | 142b   | Citric acid cycle                  |
| Malate dehydrogenase   | 204b   |                                    |
| Putative malate dehydrogenase  | 105a, 208b   |                                    |
| Cytosolic malate dehydrogenase (EC 1.1.1.37) (Fragment)              | 103a   |                                    |
| Aconitate hydratase, cytoplasmic, putative, expressed                | 4a   |                                    |
| <br>NADP-specific isocitrate dehydrogenase (EC 1.1.1.42)             | <br>88a  |                                    |
| Putative dihydrolipoamide dehydrogenase                              | 40a, 41a, 57b  |                                    |
| Triosephosphate-isomerase  | 151a   |                                    |
| <br>Aldose reductase-related protein (EC 1.1.1.21)                   | <br>144a, 226b   | <br>Glycolysis and gluconeogenesis |
| Cytoplasmic aldolase   | 179b   |                                    |
| Cytosolic glyceraldehyde-3-phosphate dehydrogenase GAPDH (Fragment)  | 89a, 90a, 91a, 92a, 107a, 121a, 189b, 207b, 212b, 216b |                                    |
| Enolase (EC 4.2.1.11)  | 29a, 31a, 32a, 180b                                    |                                    |
| Glyceraldehyde-3-phosphate dehydrogenase, cytosolic (EC 1.2.1.12)    | 145b, 182b, 183b, 185b                                 |                                    |
| 2,3-bisphosphoglycerate-independent phosphoglycerate mutase          | 16a  |                                    |
| Putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase | 17a, 36a   |                                    |
| Putative aldose reductase  | 222b, 227b, 261b, 262b, 288b, 290b, 291b               |                                    |
| Phosphoglycerate kinase, cytosolic (EC 2.7.2.3)                      | 65a, 82a, 101a   |                                    |
| Putative glyceraldehyde-3-phosphate dehydrogenase                    | 211b   |                                    |

**Table 3.5** Summary of identified proteins in the inner tissue fraction (continued)

| Enzymes   | Spot number*                                   | Sub-class                 |
|---|--|---------------------------|
| Glucose and ribitol dehydrogenase homolog - barley          | 123a, 142a, 219b, 220b, 256b, 257b, 260b, 263b |                           |
| Phosphoglucomutase (EC 5.4.2.2) (Fragment)                  | 18a  |                           |
| Pyruvate orthophosphate dikinase (Fragment)                 | 2a   |                           |
| Putative S-formylglutathione hydrolase                      | 218b   | Oxidative stress          |
| Glutathione transferase (EC 2.5.1.18)                       | 160a, 161a, 327b                               |                           |
| Putative cytosolic 6-phosphogluconate dehydrogenase         | 67a  | Pentose phosphate pathway |
| Putative fructose 1-,6-bisphosphate aldolase (Fragment)     | 184b, 206b                                     |                           |
| UTP--glucose-1-phosphate uridylyltransferase (EC 2.7.7.9)   | 51a  | Carbohydrate metabolism   |
| Cytosolic NADP malic enzyme                                 | 19a, 20a, 29a, 30b, 31b                        | C4 pathway                |
| Aspartate aminotransferase precursor (EC 2.6.1.1)           | 181b   |                           |
| Alanine aminotransferase                                    | 96b  |                           |
| ATP synthase beta subunit                                   | 48a, 49a                                       | ATP synthesis             |
| Atp1 protein  | 33a, 35a, 37a                                  |                           |
| Nucleoside diphosphate kinase (EC 2.7.4.6) (Fragment)       | 357b   |                           |
| Nucleoside diphosphate kinase 1, putative, expressed        | 233a   |                           |
| Putative nucleoside diphosphate kinase                      | 377b, 383b, 396b, 398b                         |                           |
| Formate dehydrogenase (Fragment)                            | 70a, 141b                                      | C1 Metabolism             |
| Formate dehydrogenase, mitochondrial precursor (EC 1.2.1.2) | 140b   |                           |

**Table 3.5** Summary of identified proteins in the inner tissue fraction (continued)

| Enzymes  | Spot number*                       | Sub-class    |
|--|------------------------------------|--------------|
| Subtilisin-like protease   | 66a                                | Protease     |
| Aspartic proteinase  | 146a, 147a                         |              |
|  |                                    |              |
| 12-oxo-phytodienoic acid reductase                                   | 61a                                | Other        |
| Glutamine synthetase isoform GS1c                                    | 99a                                |              |
| Manganese superoxide dismutase (EC 1.15.1.1)                         | 329b, 330b                         |              |
| O-methyltransferase  | 205b                               |              |
| Putative beta-N-acetylhexosaminidase (Fragment)                      | 45a                                |              |
| Putative proteasome subunit alpha type 3                             | 158a                               |              |
| Putative serine/threonine protein kinase                             | 264b                               |              |
| Transposase  | 161b, 236a, 433b                   |              |
| Xylose isomerase (EC 5.3.1.5)  | 30a                                |              |
| Putative glyoxalase I  | 128a                               |              |
| Glyoxalase family protein, expressed                                 | 227a, 232a                         |              |
| Putative rubisco subunit binding-protein alpha subunit precursor     | 24a                                |              |
|  |                                    |              |
| <b>Structural proteins</b>   |                                    |              |
| Actin and related proteins   | 302b, 303b, 304b, 339b             | Cytoskeletal |
|  |                                    |              |
| <b>Defence and stress related proteins</b>                           |                                    |              |
| 1-Cys-peroxiredoxine   | 122a, 309b, 311b, 312b             | Stress       |
| Stress responsive protein  | 221b                               |              |
| Stress-inducible membrane pore protein                               | 364b                               |              |
| Translationally controlled tumor protein                             | 145a                               |              |
|  |                                    |              |
| 26 kDa endochitinase 1 precursor (EC 3.2.1.14)                       | 301b, 306b, 435b                   | Defence      |
| Basic endochitinase C precursor (EC 3.2.1.14) (Rye seed chitinase-c) | 323b                               |              |
| Class II chitinase (EC 3.2.1.14)                                     | 273b, 296b, 299b, 300b, 305b, 322b |              |
| Disease resistance protein (Fragment)                                | 106a, 108a                         |              |
| Disease-resistant-related protein                                    | 416b                               |              |
| Endogenous alpha-amylase/subtilisin inhibitor (WASI)                 | 175a, 315b, 335b, 336b             |              |
| PR-4 (Fragment)  | 235a                               |              |
| Xylanase inhibitor (Fragment)  | 198b                               |              |
| Xylanase inhibitor precursor (Xylanase inhibitor TAXI-I)             | 201b                               |              |
| Xylanase inhibitor protein 1 precursor (Class III chitinase homolog) | 268b, 269b, 297b                   |              |
| Xylanase inhibitor XIP-III   | 265b                               |              |

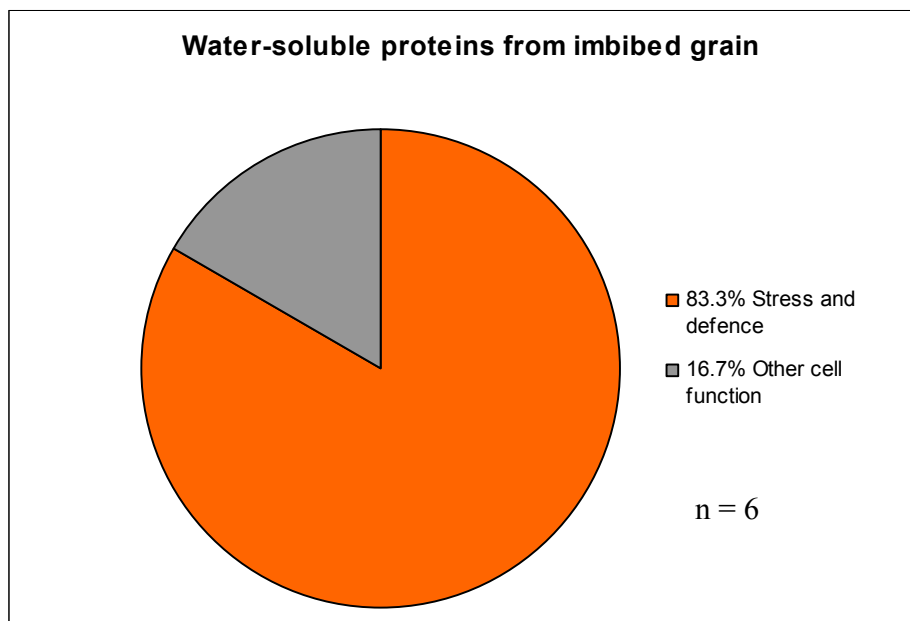
**Table 3.5** Summary of identified proteins in the inner tissue fraction (continued)

| Other  | Spot number*           | Sub-class                   |
|--|------------------------|-----------------------------|
| ABA inducible protein  | 230a                   | Programmed cell death (PCD) |
| Putative cell death associated protein                             | 90b                    |                             |
| Voltage dependent anion channel (VDAC)<br>(Fragment)               | 278b                   |                             |
|  |                        |                             |
| Amylogenin   | 104a                   | Other cell function         |
| Annexin  | 59a, 294a              |                             |
| Caleosin 1   | 223b, 284b             |                             |
| Cyclophilin A-2 (EC 5.2.1.8) (Cyclophilin A)                       | 13a                    |                             |
| DNA-binding protein (Fragment)                                     | 110a                   |                             |
| Em protein H5  | 39a                    |                             |
| Globosa  | 42a                    |                             |
| Lipoprotein-like   | 267b                   |                             |
| MLo protein  | 50a                    |                             |
| OJ991113_30.23 protein   | 197b                   |                             |
| Oleosin (Fragment)   | 389b                   |                             |
| OSJNBa0027P08.9  | 191a, 384b             |                             |
| OSJNBa0093F12.16 protein   | 370b                   |                             |
| P0481E12.28  | 320b                   |                             |
| Prohibitin protein Wph   | 313b, 369b             |                             |
| Reversibly glycosylated polypeptide                                | 100b                   |                             |
| Secretory protein  | 342b, 345b             |                             |
| Squamosa promoter binding protein 2-like                           | 292b                   |                             |
|  |                        |                             |
| Hypothetical protein OJ1124_E11.8.                                 | 405b                   | Unknown                     |
| Hypothetical protein OSJNBb0094P23.22                              | 367b                   |                             |
| Hypothetical protein P0431E05.13                                   | 47b                    |                             |
| Hypothetical protein P0686H11.1 (Hypothetical protein P0605H02.41) | 209b, 285b             |                             |
| Unknown protein  | 59b, 412b, 414b        |                             |
| Unnamed protein product  | 169a, 172a, 174a, 332b |                             |

\* Spot numbers with suffix 'a' and 'b' correspond to pH range 4-7 and 6-11 gels respectively displayed in Figures 3.9 and 3.10

### ***3.4.5 Proteins identified in supernatant from imbibed grain***

A summary of the water-soluble proteins identified in the supernatant from imbibed grain from Appendix-2, Table A-2.7 are shown in Table 3.6. There were six protein bands selected from the 1D gel for identification (Fig. 3.11). The major group of proteins identified were oxidative-stress and defence-related proteins (five bands or 83.3%); the other band was a histone (Fig. 3.16). All of the identified proteins were homologous to wheat proteins.



**Figure 3.16** Functional distribution of protein bands on the 1D gel of the water-soluble proteins



**Table 3.6** Summary of identified proteins in supernatant from imbibed grain  
(see Appendix-2 Table A-2.7)

| Enzyme                              | Band number | Sub-class                    |
|-------------------------------------|-------------|------------------------------|
| Endochitinase precursor             | 4           | Defence                      |
| Oxalate oxidase precursor           | 1           | Oxidative stress and defence |
| PSBGer3 protein                     | 5           |                              |
| Polyphenol oxidase (Fragment)       | 2           |                              |
| Peroxidase (EC 1.11.1.7) (Fragment) | 3           |                              |
| Other                               |             |                              |
| Histone H4 variant TH011            | 6           | DNA folding                  |

#### ***3.4.6 Supernatant from isolated outer fraction***

To further investigate the location of the proteins identified in the supernatant from imbibed whole grain, 1D-PAGE was used to investigate proteins that leach out from the supernatant of the outer tissue fraction alone. The results showed that only polyphenol oxidase (PPO), peroxidase (POX), and oxalate oxidase (OXO) were present in the supernatant (Fig. 3.11 and Table 3.6). This suggests that PPO, POX and OXO are mainly contained in the outer tissue fraction and that the other proteins, endochitinase and histone, leach out from the inner and intermediate fractions or the germ during imbibition.

## 4. Discussion

The bran layer of the grain is essentially a barrier that protects the germ and endosperm from bacterial, fungal, viral and insect attack (Selitrennikoff, 2001; Desmond et al., 2006). It was thus not surprising to find that the majority of proteins identified in the outer and intermediate bran fractions were oxidative stress- and defence-related proteins. However, the aleurone cells, which are part of isolated bran and in our study form the major component of the 'inner fraction', are live cells and are involved in the early stages of germination. This layer should thus contain proteins related to active cellular processes as the aleurone cells are expected to satisfy their function within the germination process, which is to help breakdown the endosperm to provide energy for the growing plant (Eastwood and Laidman, 1971; Fincher, 1989). Although antifungal proteins were detected in this layer, proteins related to metabolic processes expected of living cells, such as energy metabolism and protein synthesis, were some of the more common types of proteins. Surprisingly though, the major protein identified in the inner fraction was a 7S globulin storage protein, which has not been reported as a major aleurone protein. All of these major proteins with respect to their location in each tissue fraction will be further discussed in detail later in the Discussion.

#### ***4.1 Bran fractionation and properties of bran fractions***

The major hurdle of this study was to collect bran tissue suitable for proteomic analysis and to separate it into distinct tissue fractions. This was overcome, allowing an efficient separation into three fractions – outer, intermediate and inner – as described earlier. The reason for separating bran into fractions was to look at the distribution of proteins across the bran tissue and to identify the location of proteins with respect to their function. For instance, if only whole bran were analysed, the results would be less informative in that the abundance of proteins in the inner fraction would have overshadowed the less abundant proteins in the outer and intermediate fractions. Furthermore, identifying the location of proteins unique to different tissue fractions is informative in that the proteins can be linked to gene expression during the early stages of bran development. The method used to remove and separate the bran into fractions has been reported (Antoine et al., 2004), however the ‘freeze thaw’ technique developed in this study was novel and very effective in removing the outer (epidermis and hypodermis) fraction (Antoine et al., 2004). It allowed for relatively quick and easy collection of large amounts of the outer fraction, which was required for protein extraction.

The difficulty in collecting bran and then separating it into distinct bran tissue fractions is that it is tightly bound to the endosperm and is also compact and brittle in its dry state. Achieving this separation requires the grain to be wet, in turn making the bran flexible and tougher. As a result, the grain used for the collection of tissue fractions had to be imbibed in water for two days at room temperature to allow for its removal, and also to further manually separate the bran into three distinct tissue fractions. As a result of doing

this, the proteins identified in this study were necessarily representative of bran at the germination stage, especially in the inner tissue fraction which is composed of living cells. Any water-soluble proteins that may leach out during grain imbibition were collected and analysed.

Once the bran was removed from the endosperm, the manual dissection of bran into three distinct tissue fractions was essentially based on the easiest separating points of adjoining tissue. Microscopy of the dissected bran fractions showed that they were virtually free from adjoining tissue contamination (Figs. 3.1, 3.2, and 3.4). Interestingly, for the purpose of bran fractionation in milling, these points of mechanical failure might also be areas to target by using enzymes to further degrade and weaken the tissue bonds and thus improve bran fractionation (i.e., separating bran from endosperm and/or removing particular bran layers all to improve flour quality and yield) and will be discussed later in the discussion.

The analysis of proteins in the tissue fractions required a suitable protein extraction method for two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). A protein extraction method developed especially for the purpose of extracting protein from olive leaf and that was suitable for 2D-PAGE was used (Wang et al., 2003). This method was effective in extracting protein from the inner fraction, however it was apparently less effective in extracting proteins from the intermediate and outer fractions.

One of the possible reasons for this may be that the proteins in the intermediate and outer tissue fractions are difficult to extract from the tissue itself because of the compacted cross-linked tissue. Protein extraction from compacted tissue fractions, such as the outer and intermediate fraction, may require a better method for grinding up tissue, to expose and release as much protein as possible into the extraction buffer.

Another possible reason for the low apparent protein levels in the bran tissue fractions could be due to the Deep Purple protein stain used to quantify proteins. The fluorescent compound in Deep Purple stain covalently binds to lysine and N-terminal residues of proteins (Coghlan et al., 2005). There is a three-to four-fold difference in the lysine content in proteins between grain components. The lysine content in the flour fraction is four times lower than the germ fraction and three times lower than the aleurone fraction (Spurway, 1998). Thus, this kind of variation in lysine content may explain the low apparent concentration of proteins in the intermediate and outer fractions as it will affect staining intensity.

Furthermore, lysine is 9.9% of the amino acid residues in bovine serum albumin (BSA). BSA serial dilutions were used to develop standard curves of stain intensity, thus underestimating quantities of lysine-poor proteins that are typical of cereal grains (Spurway, 1998; van der Meer et al., 2001).

The extracted proteins from each of the tissue fractions were separated by two-dimensional electrophoresis (2-DE) and stained with Deep Purple to visualise the protein

spot array (Figs. 3.5, 3.6, 3.7, 3.8, 3.9 and 3.10). The first observation from these gel images is that there was a protein array/concentration gradient going from the outer fraction toward the inner fraction, there being less protein in the outer fraction and increasing moving toward the inner fraction. Although protein concentration estimation of bran fractions has been reported by Spurway (1998), this study has shown for the first time the protein composition and distribution across the bran.

The protein spots from the 2DE gels (Figs. 3.5, 3.6, 3.7, 3.8, 3.9 and 3.10) and the protein bands from the 1D gels (Fig. 3.11) were selected and processed for identification as described in the methods. The identified proteins in each of these tissue fractions and the water-soluble proteins that leached out during grain imbibition will briefly be discussed highlighting their functions and purpose in the bran.

#### ***4.2 Proteins identified in the outer fraction***

As mentioned previously, the function of the bran is to act as a physical barrier to protect the nutrient-rich endosperm. The outer-most tissue fraction is thus the first line of defence in biotic stress such as fungal, microbial and insect attack on the grain. The following proteins that were identified in this fraction will be briefly discussed as to their possible roles in this protective function for wheat grain.

#### ***4.2.1 Oxalate oxidase (OXO) and peroxidase (POX)***

OXO is a known defence protein in plants involved in resistance to oxalate-secreting fungi (Lane et al., 1993). OXO has been reported to be commonly found in the outer surface tissues of plants (Lane et al., 1993; Lane, 2000). This study has shown that OXO is a major enzyme located in the outer bran fraction. Oxalate secreted by fungi is oxidized by OXO, thus producing  $H_2O_2$  which in turn is toxic to living cells. This phenomenon is termed the hypersensitive response (Jabs, 1999). The plant overcomes damage by the presence of peroxidase (POX) which in turn breaks down  $H_2O_2$ . POX is a stress-related enzyme, which protects against abiotic stress, mechanical damage or pathogen responses, all of which could result in the production of  $H_2O_2$  (Lane, 2000). Furthermore, the role of OXO and POX in producing  $H_2O_2$  is involved in cross linking of lignin to create a tougher impenetrable barrier in this outer layer (Gane et al., 1998; Peyron et al., 2001; Antoine et al., 2003). This is consistent with the intactness of this whole layer in our isolation method.

#### ***4.2.2 Lipoxygenase (LOX)***

LOX is a pathogen-induced acquired resistance (AR) protein found in plants. This protein also becomes expressed when there is mechanical damage to the plant caused by insects (Kolomiets et al., 2000). LOX has previously been reported to be mainly located in the bran and germ fractions (Rani et al., 2001). This study shows that LOX is located only in the outer fraction of the bran. LOX is a desirable protein in flour in that it causes flour bleaching and strengthening of dough in bread making (Rani et al., 2001). Moreover, its

location in the outer fraction means it is unlikely to end up in white flour where these properties will be of most use commercially.

#### ***4.2.3 Lipid transfer protein (LTP)***

LTP protein is involved in transferring phospholipids between membranes. It is also believed to be involved in defence against fungal and bacterial pathogens (Drea et al., 2005). The LTP protein gene is expressed in the outer epidermis during bran development and its expression is greatly increased after six days post anthesis (DPA) (Selitrennikoff, 2001; Drea et al., 2005). In this study, LTP was located only in the outer fraction of the bran. It is possible that the LTP is still active in the outer fraction of the mature grain, and might contribute to pathogen defence.

### ***4.3 Proteins identified in the intermediate fraction***

The next line of defence in the bran is the intermediate tissue fraction. As described in the introduction, this tissue fraction is comprised of a multilayer of compacted remnant tissues that were present during bran development (Fig. 1.4). This tissue fraction is the last barrier that protects the next tissue fraction (inner fraction) which contains live cells that are important in germination. The following proteins (discussed in more detail below) were identified in this fraction and are more specifically targeted defence type proteins than those defence proteins in the outer fraction.

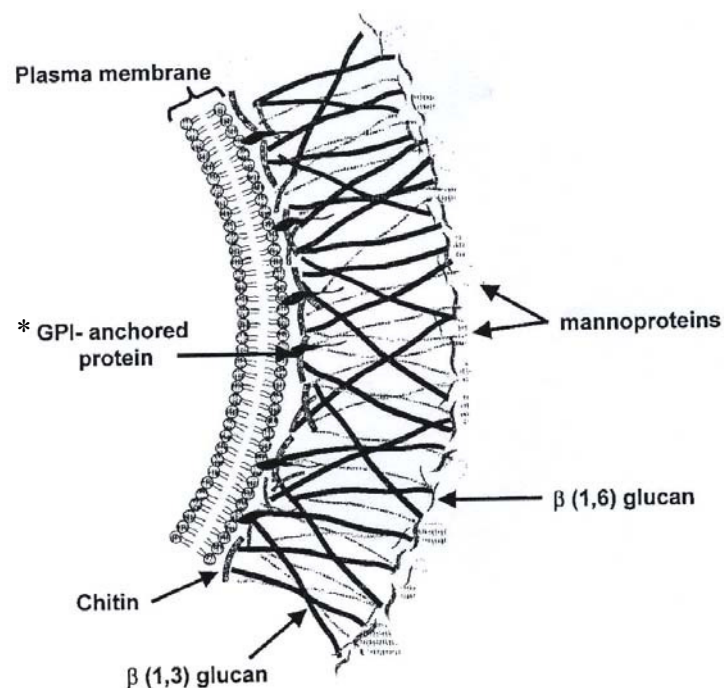


#### ***4.3.1 Xylanase inhibitor proteins***

Fungi are common pathogens of plants. They secrete xylanase in order to break down plant cell walls for food, in turn damaging the plant (Flatman et al., 2002). Xylanase inhibitor proteins have previously been isolated from wheat (Debyser et al., 1999). This study has shown that xylanase inhibitor proteins were mostly identified in the intermediate and to a lesser extent in the inner tissue fractions. This suggests that the first point of xylanase inhibition occurs at this intermediate fraction as no xylanase inhibitor proteins were identified in the outer fraction.

#### ***4.3.2 Chitinase and endochitinase***

Chitinases are anti-fungal proteins found in many different plants. Their function is to break down the chitin in fungal cell walls (Fig. 4.1). Once the cell walls of the fungi are broken down, turgor pressure will cause cell lysis and death (Molano et al., 1979; Selitrennikoff, 2001; Desmond et al., 2006). This study shows that these enzymes were mainly located in the inner and intermediate tissue fractions, and not in the outer fraction. Surprisingly, chitinase was also found in the supernatant from the imbibed grain and not in the supernatant from isolated outer tissue fraction. This suggests that the chitinase possibly leaches out from the inner tissue fractions when the grain is exposed to moisture. As damp conditions are favorable to fungi, the water-soluble property of chitinase possibly allows it to diffuse out from the bran and into the fungal cell wall. These enzymes may thus prevent fungi establishing colonies on the surface of grains in damp conditions.



**Figure 4.1** Schematic structure of a fungal cell wall (Selitrennikoff, 2001)

\* Glycophosphatidylinositol (GPI)

#### **4.3.3 *$\alpha$ -amylase/subtilisin inhibitor proteins***

$\alpha$ -amylase and  $\alpha$ -amylase/subtilisin inhibitors were identified in the inner and intermediate tissue fractions. These proteins were also identified in the endosperm by Skylas et al. (2000), and in the germ by Mak et al. (2006b).  $\alpha$ -amylase/subtilisin inhibitor is reported to be involved in defence against microbial and insect damage. It inhibits amylases and subtilisin secreted by microbes and insects used to breakdown plant proteins and starch for food (Ohtsubo and Richardson, 1992).

#### ***4.3.4 Benzothiadiazole (BTH) – clone of wheat chemically induced protein (cWCI-5)***

Acquired resistance (AR) in plants is where a pathogen induces the expression of defence genes which give the plant protection from future infection. BTH induces the expression of several WCI genes, one of which is WCI-5; however, the function of this protein in defence is not yet known and it has not been reported in grain before (Gorlach et al., 1996; Schaffrath et al., 1997).

#### ***4.3.5 Wheatwin1***

The classing of pathogenesis-related (PR) antifungal proteins is based on their mode of action and their structural similarity (Selitrennikoff, 2001). Wheatwin1 proteins are class four pathogenesis-related (PR-4) proteins, which contain chitin-binding sites. They are active against a broad range of plant and human fungal pathogens. Their mode of action is not clearly understood, however Wheatwin1 is believed to bind to chitin in the fungal cell wall causing disruption of cell wall formation (Selitrennikoff, 2001).

#### ***4.3.6 Thaumatin-like protein (TL)***

Thaumatin-like protein is a class five pathogenesis-related (PR-5) antifungal protein. The mechanism of PR-5 defence proteins is not yet known, however some hypotheses are that these proteins may cause changes in fungal wall cell permeability, and some were found to have 1,3- $\beta$ -glucanase activity (Selitrennikoff, 2001).

#### ***4.4 Proteins identified in the inner fraction***

As described earlier, this tissue fraction contains live cells that are required for germination. It was also interesting to find that the major protein identified in this fraction was a 7S globulin storage protein not previously reported in wheat aleurone.

##### ***4.4.1 7S globulin storage proteins***

Storage protein is an important source of carbon and nitrogen for the developing plant during germination. 7S globulin storage proteins are a major carbon and nitrogen source in plants such as peas, nuts and legumes (Dunwell et al., 2000). This protein has previously been isolated from wheat, however its localisation has not yet been reported (Robert et al., 1985). Interestingly, the majority of protein spots in the inner fraction (around 58%) were 7S globulin storage proteins. However, this type of 7S globulin storage protein is uniquely different to the major endosperm storage proteins (gliadins) identified in the work done by Skylas et al. (2000). Since the inner fraction is only a small percentage of the whole grain, it is thus not likely to be a major contributor as a carbon and nitrogen source during germination since the gliadins in the endosperm are the major storage proteins. This suggests that these 7S globulin storage proteins may be involved in some other function and not primarily as a carbon and nitrogen source.

During grain development, there is a large accumulation of oxalate and it has been speculated that the 7S storage proteins may have a role in oxalate metabolism; for example, in binding oxalate or detoxifying oxalate (Dunwell et al., 2000). Evidence for this is in the reporting of the structural similarity of the conserved  $\beta$ -barrel structures

found in cupins and is suggested that this may be the active site of OXO activity (Gane et al., 1998).

The 7S globulin proteins are trimers in the 50 kDa to 70 kDa molecular weight range. They form a compact structure that protects them from degradation by proteases. 7S globulins are major storage proteins found of legumes and are also a major allergen (Breiteneder and Mills, 2005). The stable structure of the 7S globulins allows them to bypass the intestinal proteases (when ingested by humans) and to be absorbed into the small intestines causing an allergic reaction (Dunwell et al., 2000).

During the two-day imbibition of the grain, the storage protein seems to have been degraded gradually, likely due to its stable compact structure mentioned previously (Dunwell et al., 2000). Degradation products can be seen in the inner tissue fraction 2D gel images (Figs. 3.9 and 3.10). In the acidic pH range (Fig. 3.9) the majority of 7S globulin storage protein fragments were in the low-molecular-weight range, thus showing degradation products. On the other hand, in the alkaline pH range (Fig. 3.10) the majority of these proteins were in the medium to high-molecular-weight range.

#### ***4.4.2 Protein synthesis and metabolism***

The next major groups of proteins that were identified are involved in protein synthesis, gene expression and energy production. These groups of proteins are essential for the growing plant during germination (Eastwood and Laidman, 1971; Fincher, 1989). The sub-classes of these main groups are gene expression, protein synthesis and folding, citric

acid cycle, glycolysis and gluconeogenesis, oxidative stress, pentose phosphate pathway, carbohydrate metabolism, adenosine triphosphate (ATP) synthesis and C1 metabolism.

#### ***4.4.3 Voltage dependent anion channel (VDAC)***

Another sub-class of protein that was identified is involved in programmed cell death (PCD). PCD occurs in the inner fraction (aleurone cells) at the beginning of germination (Eastwood and Laidman, 1971; Fincher, 1989). The voltage dependent anion channel (VDAC) protein is a mitochondrial membrane porin protein that regulates the flow of cell metabolites between the cytosol and mitochondria. PCD signaling is activated by various stimuli like hormones or a pathogen response, thus causing the VDAC protein to change its permeability to release intermembrane proteins such as cytochrome *c* and proteases, which are involved in cell-death pathways (Godbole et al., 2003).

#### ***4.4.4 $\alpha$ -amylase***

A major hydrolytic enzyme that was not identified in the inner tissue fraction (aleurone cells) was  $\alpha$ -amylase. This is a major hydrolytic enzyme known to be expressed in aleurone cells and secreted into the endosperm during germination (Eastwood and Laidman, 1971; Fincher, 1989; Evers et al., 1999). It is likely that after two days of grain imbibition, all of the  $\alpha$ -amylase had been secreted into the endosperm. Another possibility could be that the  $\alpha$ -amylase was washed out during bran preparation.

#### ***4.5 Proteins identified in supernatant from imbibed grain***

Endochitinase, histone, OXO, POX, and PPO were all identified in the supernatant from imbibed grain which had leached out during the tissue extraction process. PPO and POX were the only proteins which were not identified in the other tissue fractions. OXO was identified in the outer and intermediate tissue fractions and is likely that it had leached out into the supernatant from these fractions; however, endochitinase and histone were identified in the intermediate and inner fractions respectively. This suggests that these proteins may also have leached out from the inner and intermediate tissue fractions.

##### ***4.5.1 Polyphenol oxidase (PPO)***

PPO has previously been reported to be found in bran and especially in the aleurone cells (Rani et al., 2001; Demeke and Morris, 2002). However, this study shows that no PPO was identified in the inner fraction (aleurone cells) or any other bran fraction and was identified only in the supernatant collected from grain and isolated outer tissue fraction imbibition. The results suggest that PPO is likely to be located in the outer tissue fraction and is easily leached out in water.

##### ***4.5.2 Peroxidase (POX)***

POX is a H<sub>2</sub>O<sub>2</sub> scavenger. It is a protective enzyme against factors such as pathogen, environmental stress and mechanical tissue damage that may initiate a response that results in oxidation reactions that produce H<sub>2</sub>O<sub>2</sub> (Lane, 2000; Almeselmani et al., 2006).

#### ***4.6 Potential applications***

Much of this study had focused on identifying the composition and functional distribution of proteins in the bran with respect to its tissue fractions, thus giving a new insight into the function of bran as a whole. Another objective that was hoped to be achieved from this study was the application of this new knowledge to biotechnology, investigating the possibility of new methods and/or processes that will improve flour quality and yield in the milling industry. A few applications potentially exist and will briefly be discussed.

PPO and POX are oxidizing enzymes that were identified in the supernatant from imbibed grain as mentioned previously. PPO and POX are also reported to be involved in the undesirable browning of flat breads, pastas and Asian noodles (Rani et al., 2001; Demeke and Morris, 2002; Aalami et al., 2007). These proteins were identified in the outer bran fraction and in the supernatant. This indicates that these proteins leach out of the grain quite easily when exposed to water. It may be possible that these proteins diffuse and spread out from bran particle contamination in flour when water is added. Washing these proteins out from grain prior to milling may reduce browning of dough caused by these proteins.

Another possible application of the information generated from this study is the potential to use enzymes to hydrolyse bran tissue bonds in order to improve bran separation from endosperm during conditioning, as was mentioned earlier in the discussion. Treating grain with hydrolytic enzymes during the conditioning process to improve bran separation from endosperm has previously been attempted by Novozymes



(PCT/DK98/00460, 1998). Their approach was to condition grain with a broad and non-specific assortment of enzymes to degrade bran tissue bonds in the hope to improve flour yield and to minimise bran contamination in flour during milling. A Patent Cooperation Treaty (PCT) patent application was submitted by Novozymes claiming that their enzyme treatment improved flour yield and shortened conditioning time; however, the patent was not granted and further work was abandoned.

As mentioned earlier in the discussion, many of the proteins identified in the bran layers were involved in pathogen defence. Some of these proteins are hydrolytic enzyme inhibitors such as xylanase and  $\alpha$ -amylase inhibitors. These inhibitor proteins could be a problem when treating grain with enzymes during conditioning as was attempted by Novozymes. A possible way to overcome this problem is to increase the concentration of enzymes in the conditioning solution; however, this approach is not cost effective when millions of tonnes of wheat are milled annually. Studies on the wheat xylanase inhibitor XIP-I has shown that recombinant xylanases derived from fungi were inhibited, whereas xylanases derived from bacteria were not inhibited (Flatman et al., 2002). These bacterially derived xylanases will potentially be more specific and effective in hydrolyzing arabinoxylans (AX's) in bran tissue. This kind of approach will require enzymes that are more specific to the target tissue material and also to be not inhibited by the enzyme inhibitors identified in this study.

Further work will be required to analyse the composition of the tissue material at the junction between bran layers to find suitable enzymes for hydrolysis of cell wall

components. This kind of bran fractionation approach will be of great value to the milling industry as one of their primary aims is to separate cleanly bran or bran fractions from endosperm during milling (Butcher and Stenvert, 1973; Moss et al., 1980; Every et al., 2002).

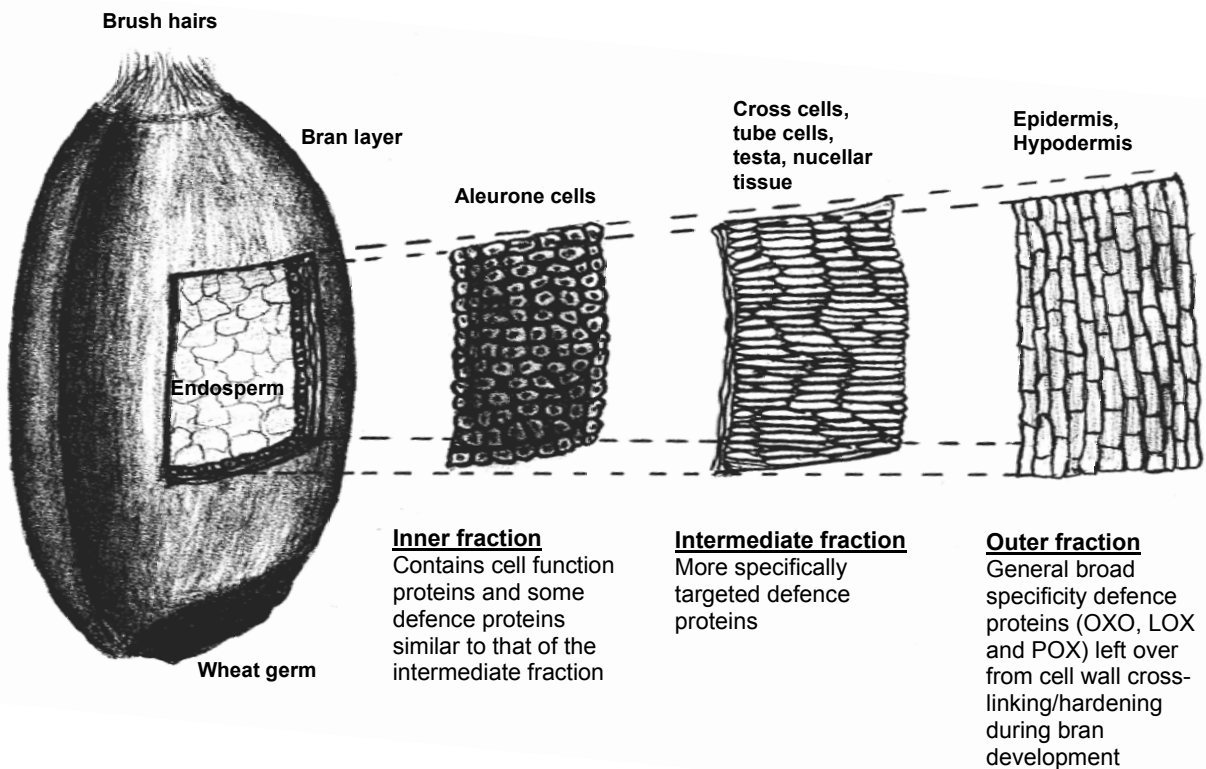
The amino acids arginine and lysine have great nutritional value in foods containing these amino acids (van der Meer et al., 2001; Flynn et al., 2002). Since the 7S globulin storage proteins are abundant in the inner fraction, the arginine and lysine content was calculated to assess the nutritional value of this protein. The arginine content in the 7S globulin storage protein is 14.9%, and thus contributes to the high arginine content previously reported in the inner (aleurone) fraction (12.3%). The inner fraction is also reported to have approximately 2.5 times higher arginine content when compared to the arginine content in the whole grain (4.6%). The lysine content however, is only 2.2% of the amino acid composition of the 7S globulin protein and thus is not likely to be a major contributor to the major lysine containing proteins in the inner fraction previously reported to be 5.9% lysine. The lysine-content in the inner fraction is also two times higher than in the whole grain (Spurway, 1998); thus, the inner fraction is important with respect to the arginine and lysine content, and introducing the inner (aleurone) fraction into flour will be nutritionally beneficial. One potential way to do this is to specifically degrade the bran tissue bonds between the inner fraction (aleurone cells) and the outer bran fractions by using enzymes as described earlier.

## 5. Conclusion and future directions

An overall summary of the broad functional groups of proteins identified in this study were the defence-and stress-related proteins found in the outer and intermediate tissue fractions and storage and metabolic proteins in the inner layers. Around 80% of proteins in the outer layers were associated with various defence-and stress-related functions. Defence-and stress-related proteins were also identified in the inner fraction, however they comprised only around 5% of the protein array. Interestingly, the major protein in the inner fraction was 7S globulin storage protein that was 57.6% of the total protein spots. Water-soluble proteins that have leached out during grain imbibition were also mostly involved in oxidative-stress response and/or have anti-fungal activity. The location of the defence-and stress-related proteins in the outer and intermediate tissues fractions is thus ideal in acting as a barrier, protecting the grain from environmental and pathogenic damage.

Consolidating this summary, a model for bran development and its role in biotic-and abiotic-defence is shown below in Fig. 5.1. This model illustrates the difference in specificity of the identified defence-related proteins contained in each tissue fraction outlined in the Discussion. Starting with the outer fraction, there were general defence-related proteins that are likely to be left over from the formation of this tissue fraction during bran development as they are associated with lignin cross-linking to form a tough outer tissue barrier (Gane et al., 1998; Peyron et al., 2001; Antoine et al., 2003). The intermediate fraction contains a much broader and more specific set of defence-related proteins. They are distributed throughout this compact multilayer tissue fraction which

also acts as a physical barrier. Finally, the inner fraction also contains some specific defence-related proteins and cell function proteins. However, the major protein (7S globulin storage protein) in this fraction is likely to be involved in defence against oxalate-secreting fungi and also to serve in protecting itself during grain development when there is potentially high oxalate levels (Dunwell et al., 2000). The model also



**Figure 5.1** Schematic diagram of a model for the development and defence protein distribution in bran showing the three tissue fractions analysed in this study and their overall role as a protective barrier for wheat grain.

shows the structural difference of each bran tissue fraction (as mentioned previously) that collectively forms a physical barrier to protect the endosperm. This physical property difference of each tissue fraction allowed it to separate from each other during tissue fraction isolation, thus suggesting that the proteins identified in this study are likely to be representative of each tissue fraction.

In conclusion, this proteomic study of bran (never before attempted to our knowledge) has given an insight into the location of protein complements with respect to the different tissue fractions. The location and functions of these proteins illustrates the function of bran as a whole. This was shown in terms of the stress- and defence-related proteins forming an outer protective barrier and the inner layer mostly containing cupin family storage proteins and the biochemical machinery necessary for germination. Lastly, protein maps generated in this study may be useful in comparing protein compositions of different wheat cultivars with respect to their farming and milling properties for the purpose of selective breeding programs.

## 6. Appendix

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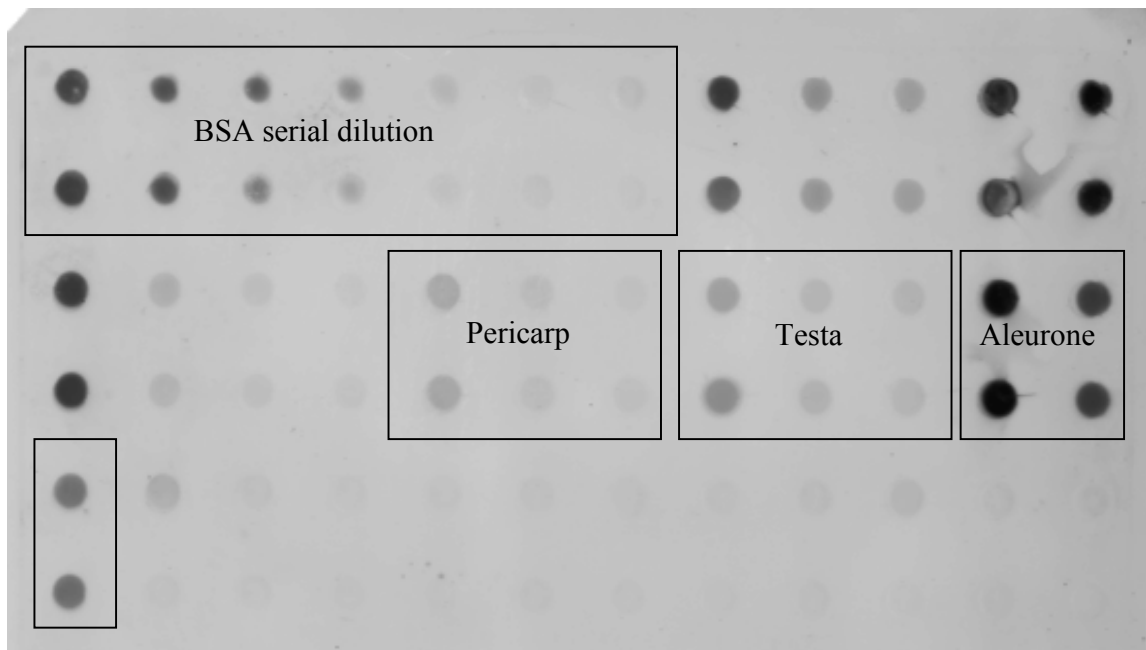
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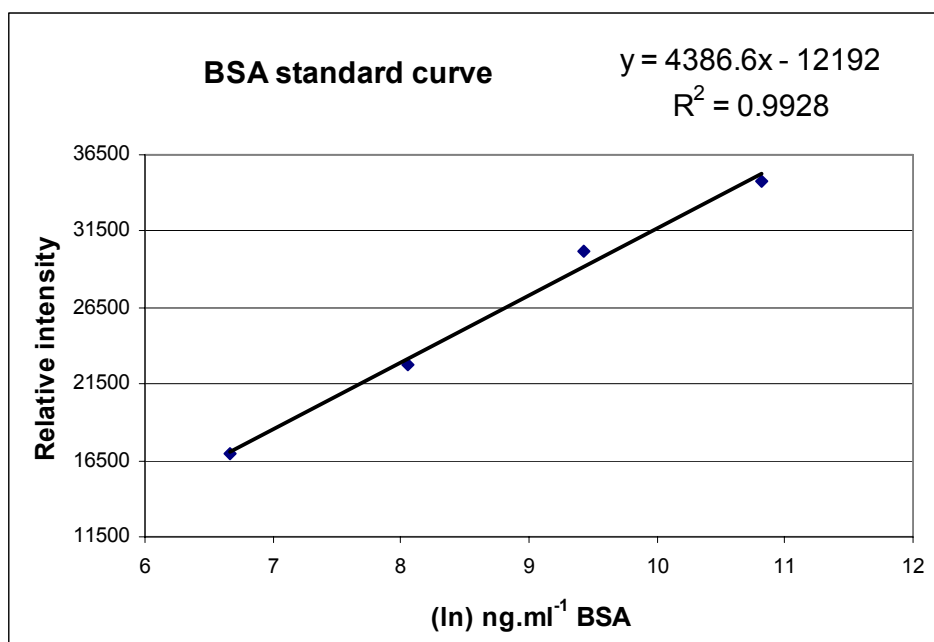
## ***A-1 Protein quantification***

## A-1.1 Protein quantification of tissue fractions



**Figure A-1.1** Proteins spotted onto nitrocellulose membrane and stained with Deep Purple



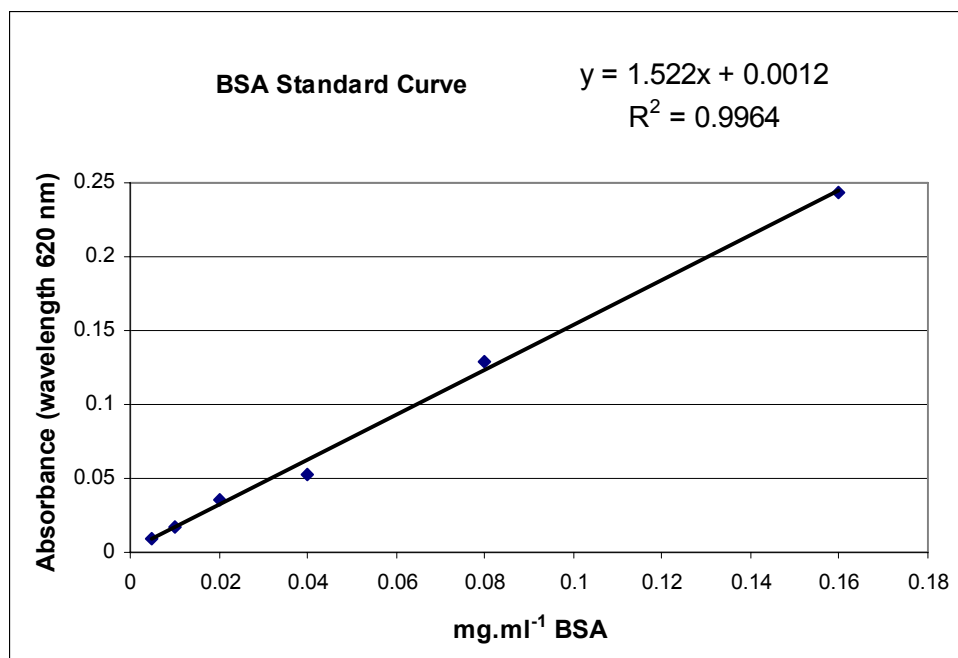


**Figure A-1.2** BSA standard curve equation

**Table A-1.1** BSA standard curve calculations

| <i>BSA</i><br><i>ng.ml<sup>-1</sup></i> | <i>BSA (ln)</i><br><i>ng.ml<sup>-1</sup></i> | <i>Intensity</i> | <i>Intensity</i> | <i>Ave.</i><br><i>intensity</i> |
|---|--|------------------|------------------|---------------------------------|
| 50000                                   | 10.81977828                                  | 34398            | 35099            | <b>34748.5</b>                  |
| 12500                                   | 9.43348392                                   | 32868            | 27439            | <b>30153.5</b>                  |
| 3125                                    | 8.04718956                                   | 24912            | 20570            | <b>22741.0</b>                  |
| 781                                     | 6.66057515                                   | 17772            | 16123            | <b>16947.5</b>                  |

## A-1.2 Protein quantification of supernatant



**Figure A-1.3** BSA standard curve equation

**Table A-1.2** BSA standard curve calculations

| <i><b>BSA<br/>mg.ml<sup>-1</sup></b></i> |       |       | <i><b>Average<br/>Absorbance</b></i> | <i><b>Blank<br/>Subtracted</b></i> |
|--|-------|-------|--------------------------------------|------------------------------------|
| <b>0.160</b>                             | 0.618 | 0.653 | <b>0.635</b>                         | 0.2435                             |
| <b>0.080</b>                             | 0.503 | 0.539 | <b>0.521</b>                         | 0.1290                             |
| <b>0.040</b>                             | 0.429 | 0.460 | <b>0.444</b>                         | 0.0525                             |
| <b>0.020</b>                             | 0.425 | 0.431 | <b>0.428</b>                         | 0.0360                             |
| <b>0.010</b>                             | 0.409 | 0.408 | <b>0.408</b>                         | 0.0165                             |
| <b>0.005</b>                             | 0.399 | 0.403 | <b>0.401</b>                         | 0.0090                             |
| <b>Blank</b>                             | 0.399 | 0.385 | <b>0.392</b>                         |                                    |

**Table A-1.3** Protein concentrations determined for whole grain and isolated outer fraction supernatant

| <i>Sample</i>         |       |       | <i>Average Absorbance</i> | <i>Blank Subtracted</i> | <i>mg.mL<sup>-1</sup></i> | <i>mg.g<sup>-1</sup></i> |
|-----------------------|-------|-------|---------------------------|-------------------------|---------------------------|--------------------------|
| <i>Whole grain</i>    | 0.473 | 0.465 | <b>0.469</b>              | 0.077                   | 0.04980                   | NA                       |
| <i>Outer fraction</i> | 0.408 | 0.433 | <b>0.421</b>              | 0.029                   | 0.01794                   | 0.6                      |

## ***A-2 Protein identification tables***

**Table A-2.1** Outer fraction (epidermis and hypodermis) proteins identified from 2D gel pH 4-7

| Spot No. | Protein name (Mascot cereal search) | Mascot or GPM <sup>1</sup> score | Protein name (Wheat EST BLAST search) <sup>2</sup> | Observed MW (KDa)/pI | Matching peptides / %Coverage | Theoretical <sup>3</sup> MW (Da)/pI | Species with homologous protein | Swiss-Prot accession number <sup>4</sup> | GenBank accession number |
|----------|-------------------------------------|----------------------------------|--|----------------------|-------------------------------|-------------------------------------|---------------------------------|--|--------------------------|
| 1        | Oxalate oxidase precursor           | 70                               |  | 29 / 5.2             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 2        | Oxalate oxidase precursor           | 77                               |  | 29 / 5.22            | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 3        | Oxalate oxidase precursor           | 20                               |  | 30 / 5.26            | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 4        | Oxalate oxidase precursor           | 85                               |  | 25 / 4.9             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 5        | Oxalate oxidase precursor           | 84                               |  | 25 / 5.0             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 6        | Oxalate oxidase precursor           | 72                               |  | 25 / 5.1             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 7        | Oxalate oxidase precursor           | 74                               |  | 25 / 5.2             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 8        | Oxalate oxidase precursor           | 99                               |  | 25 / 5.22            | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 9        | Oxalate oxidase precursor           | 70                               |  | 25 / 5.25            | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 10       | Oxalate oxidase precursor           | 56                               |  | 25 / 5.5             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 11       | Oxalate oxidase precursor           | 64                               |  | 22 / 4.75            | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 12       | Oxalate oxidase precursor           | 73                               |  | 22 / 4.8             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 13       | Oxalate oxidase precursor           | 43                               |  | 22 / 4.9             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 14       | Oxalate oxidase precursor           | 81                               |  | 24 / 5.2             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 15       | Oxalate oxidase precursor           | 68                               |  | 24 / 5.22            | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 16       | Oxalate oxidase precursor           | 53                               |  | 20 / 6.4             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 17       | Oxalate oxidase precursor           | 57                               |  | 20 / 6.6             | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |
| 18       | Oxalate oxidase precursor           | 79                               |  | 20 / 6.76            | 1 / 7                         | 23.5 / 6.9                          | <i>T. aestivum</i>              | Q70PK0_WHEAT                             |                          |

**Table A-2.2** Outer fraction (epidermis and hypodermis) proteins identified from 2D gel pH 6-11

| Spot No. | Protein name<br>(Mascot cereal search)  | Mascot or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed MW<br>(kDa)/pI | Matching peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|---|-------------------------------------|---|-------------------------|----------------------------------|--|---------------------------------------|---|--------------------------------|
| 1        | Oxalate oxidase precursor   | 77                                  |   | 14 / 6.1                | 3 / 12                           | 23.5 / 6.9                             | <i>T. aestivum</i>                    | Q70PK0_WHEAT                                |                                |
| 2        | Oxalate oxidase precursor   | 45                                  |   | 14 / 6.3                | 3 / 12                           | 23.5 / 6.9                             | <i>T. aestivum</i>                    | Q70PK0_WHEAT                                |                                |
| 3        | Oxalate oxidase GF-2.8 precursor (EC 1.2.3.4) (Germin GF-2.8)                               | 92                                  |   | 12 / 6.1                | 5 / 12                           | 23.6 / 6.41                            | <i>T. aestivum</i>                    | GER2_WHEAT;<br>P15290                       |                                |
| 4        | Oxalate oxidase GF-3.8 precursor (EC 1.2.3.4) (Germin GF-3.8)                               | 98                                  |   | 12 / 6.3                | 3 / 12                           | 23.5 / 6.9                             | <i>T. aestivum</i>                    | GER3_WHEAT;<br>P26759                       |                                |
| 5        | Oxalate oxidase GF-2.8 precursor (EC 1.2.3.4) (Germin GF-2.8)                               | 72                                  |   | 12 / 6.5                | 4 / 11                           | 23.6 / 6.41                            | <i>T. aestivum</i>                    | GER2_WHEAT;<br>P15290                       |                                |
| 6        | Oxalate oxidase GF-2.8 precursor (EC 1.2.3.4) (Germin GF-2.8)                               | 71                                  |   | 6.5 / 6.1               | 4 / 16                           | 23.6 / 6.41                            | <i>T. aestivum</i>                    | GER2_WHEAT;<br>P15290                       |                                |
| 7        | Oxalate oxidase precursor   | -4.2 <sup>1</sup>                   |   | 6.5 / 6.4               | 1 / 6.7                          | 23.5 / 6.9                             | <i>T. aestivum</i>                    | gi 46408929                                 |                                |
| 8        | Oxalate oxidase GF-2.8 precursor (EC 1.2.3.4) (Germin GF-2.8)                               | 73                                  |   | 6.5 / 6.5               | 7 / 21                           | 23.6 / 6.41                            | <i>T. aestivum</i>                    | GER2_WHEAT;<br>P15290                       |                                |
| 9        | Oxalate oxidase GF-2.8 precursor (EC 1.2.3.4) (Germin GF-2.8)                               | 52                                  |   | 6.5 / 7.2               | 3 / 11                           | 23.6 / 6.41                            | <i>T. aestivum</i>                    | GER2_WHEAT;<br>P15290                       |                                |
| 10       | No match  |                                     |   |                         |                                  |  |                                       |   |                                |
| 11       | PLAT (Polycystin-1, Lipoygenase, Alpha-Toxin) domain or LH2 (Lipoygenase homology 2) domain | -7.5                                |   | 6 / 9.6                 | 1 / 5.2                          | 26.5 / 9.3                             | <i>T. aestivum</i>                    | gnl CDD 28747                               |                                |
| 12       | Type 1 non-specific lipid transfer protein precursor  | 57                                  |   | 5 / 9.6                 | 1 / 9                            | 12.4 / 9.46                            | <i>T. aestivum</i>                    | Q5NE29_WHEAT                                |                                |
| 13       | No match  |                                     |   |                         |                                  |  |                                       |   |                                |
| 14       | No match  |                                     |   |                         |                                  |  |                                       |   |                                |
| 15       | No match  |                                     |   |                         |                                  |  |                                       |   |                                |
| 16       | No match  |                                     |   |                         |                                  |  |                                       |   |                                |
| 17       | Type 1 non-specific lipid transfer protein precursor  | 97                                  |   | 4 / 10.3                | 2 / 14                           | 12.4 / 9.46                            | <i>T. aestivum</i>                    | Q5NE29_WHEAT                                |                                |

**Table A-2.3** Intermediate fraction (nucellar tissue, testa, tube cells and cross cells) proteins identified from 2D gel pH 4-7

| Spot No. | Protein name<br>(Mascot cereal search)       | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup>  | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|--|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 1        | Oxalate oxidase precursor                    | 70                                     |  | 32 / 5.1                   | 1 / 7                               | 23.5 / 6.9                             | <i>T. aestivum</i>                    | Q70PK0_WHEAT                                |                                |
| 2        | Oxalate oxidase precursor                    | 89                                     |  | 31 / 5.2                   | 1 / 7                               | 23.5 / 6.9                             | <i>T. aestivum</i>                    | Q70PK0_WHEAT                                |                                |
| 3        | Alpha-amylase inhibitor                      | 141                                    |  | 27 / 6.8                   | 4 / 22                              | 19.6 / 6.77                            | <i>T. aestivum</i>                    | S38955                                      |                                |
| 4        | Alpha-amylase inhibitor                      | 183                                    |  | 27 / 6.9                   | 4 / 22                              | 19.6 / 6.77                            | <i>T. aestivum</i>                    | S38955                                      |                                |
| 5        | Pathogenesis-related protein 4<br>(Fragment) | 30                                     |  | 16 / 4.6                   | 1 / 6                               | 13.1 / 7.0                             | <i>T. aestivum</i>                    | Q9SQG8_WHEAT                                |                                |
| 6        | Wheat EST match                              | 73                                     | Chain B, Crystal Structure Of The T.<br><i>aestivum</i> Xylanase Inhibitor-I In<br>Complex With <i>Aspergillus Niger</i><br>Xylanase-I | 14 / 4.9                   | 8 / 10                              | 33.1 / 9.45                            | <i>T. aestivum</i>                    | Ta78954-3 <sup>4</sup>                      | GI:55669878                    |
| 7        | Wheat EST match                              | 63                                     | Xylanase inhibitor   | 14 / 5.1                   | 10 / 9                              | 47.2 / 8.8                             | <i>T. aestivum</i>                    | Ta745253405-2                               | GI:23954367                    |
| 8        | Wheat EST match                              | 90                                     | Chain B, Crystal Structure Of The T.<br><i>aestivum</i> Xylanase Inhibitor-I In<br>Complex With <i>Aspergillus Niger</i><br>Xylanase-I | 15 / 5.2                   | 9 / 17                              | 33.1 / 9.45                            | <i>T. aestivum</i>                    | Ta78954-3                                   | GI:55669878                    |
| 9        | No match                                     |  |  |                            |                                     |  |                                       |   |                                |
| 10       | Wheat EST match                              | 145                                    | Xylanase inhibitor   | 14 / 5.5                   | 14 / 22                             | 47.2 / 8.8                             | <i>T. aestivum</i>                    | Ta745253405-2                               | GI:23954367                    |
| 11       | Wheat EST match                              | 105                                    | Wheatwin1  | 16 / 6.3                   | 4 / 25                              | 25.6 / 9.53                            | <i>T. aestivum</i>                    | Ta7944-1                                    | GI:3135957                     |
| 12       | Pathogenesis-related protein 4<br>(Fragment) | 25                                     |  | 17 / 6.8                   | 1 / 10                              | 13.1 / 7.0                             | <i>T. aestivum</i>                    | Q9SQG8_WHEAT                                |                                |
| 13       | Wheat EST match                              | 33                                     | Uncoupling protein   | 10 / 4.7                   | 11 / 44                             | 18.7 / 11.83                           | <i>T. aestivum</i>                    | Ta192131-2                                  | GI:10716672                    |
| 14       | No match                                     |  |  |                            |                                     |  |                                       |   |                                |
| 15       | No match                                     |  |  |                            |                                     |  |                                       |   |                                |
| 16       | No match                                     |  |  |                            |                                     |  |                                       |   |                                |

**Table A-2.4** Intermediate fraction (nucellar tissue, testa, tube cells and cross cells) proteins identified from 2D gel pH 6-11

| Spot No. | Protein name<br>(Mascot cereal search)   | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 1        | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 2        | Actin (O65316)   | 265                                    |   | 50 / 8.5                   | 15 / 35                             | 41.6 / 5.3                             | <i>M. viride</i>                      | ACT_MESVI                                   |                                |
| 3        | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) (XIP-I<br>protein) | 144                                    |   | 50 / 8.8                   | 14 / 33                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 4        | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) (XIP-I<br>protein) | 63                                     |   | 50 / 9.1                   | 11 / 40                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 5        | Chain B, Crystal Structure Of Chitinase<br>At 1.91a Resolution                             | 128                                    |   | 49 / 8.5                   | 6 / 27                              | 26 / 8.45                              | <i>H. vulgare</i>                     | gi 1310889                                  |                                |
| 6        | Actin (O65316)   | 200                                    |   | 49 / 9.0                   | 16 / 34                             | 41.6 / 5.3                             | <i>M. viride</i>                      | ACT_MESVI                                   |                                |
| 7        | Actin (O65316)   | 281                                    |   | 47 / 9.1                   | 14 / 32                             | 41.6 / 5.3                             | <i>M. viride</i>                      | ACT_MESVI                                   |                                |
| 8        | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) (XIP-I<br>protein) | 117                                    |   | 29 / 6.9                   | 4 / 14                              | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 9        | 26 kDa endochitinase 2 precursor (EC<br>3.2.1.14) (CHI-26)                                 | 76                                     |   | 28 / 7.1                   | 6 / 27                              | 28.1 / 8.54                            | <i>H. vulgare</i>                     | CHI2_HORVU;<br>P23951                       |                                |
| 10       | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) (XIP-I<br>protein) | 339                                    |   | 28 / 7.2                   | 23 / 51                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 11       | Secretory protein  | 76                                     |   | 27 / 7.4                   | 7 / 32.6                            | 24.2 / 9.32                            | <i>T. aestivum</i>                    | Q9SWZ5_WHEAT                                |                                |
| 12       | Chain A, Crystal Structure Of Family<br>11 Xylanase In Complex With Inhibitor<br>(Xip-I)   | 206                                    |   | 30 / 7.5                   | 11 / 33                             | 30.3 / 8.27                            | <i>T. aestivum</i>                    | gi 51247633                                 |                                |
| 13       | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) (XIP-I<br>protein) | 167                                    |   | 31 / 7.9                   | 4 / 14                              | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 14       | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) (XIP-I<br>protein) | 413                                    |   | 28 / 7.8                   | 22 / 53                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 15       | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) (XIP-I<br>protein) | 217                                    |   | 28 / 8.0                   | 17 / 48                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |



**Table A-2.4** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)   | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 16       | Xylanase inhibitor protein I   | 201                                    |   | 26 / 8.0                   | 11 / 29                             | 33.2 / 8.66                            | <i>T. aestivum</i>                    | gi 20804336                                 |                                |
| 17       | Thaumatin-like protein   | 85                                     |   | 27 / 8.3                   | 3 / 13                              | 23.6 / 7.85                            | <i>T. aestivum</i>                    | Q8S4P7_WHEAT                                |                                |
| 18       | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) (XIP-I<br>protein) | 137                                    |   | 28 / 8.4                   | 14 / 40                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 19       | 26 kDa endochitinase 2 precursor (EC<br>3.2.1.14) (CHI-26)                                 | 100                                    |   | 28 / 8.5                   | 7 / 29                              | 28.1 / 8.54                            | <i>H. vulgare</i>                     | CHI2_HORVU;<br>P23951                       |                                |
| 20       | 26 kDa endochitinase 1 precursor   | 118                                    |   | 27 / 8.9                   | 5 / 20                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 21       | Actin (O65316)   | 309                                    |   | 29 / 9.1                   | 19 / 32                             | 41.6 / 5.3                             | <i>M. viride</i>                      | ACT_MESVI                                   |                                |
| 22       | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) (XIP-I<br>protein) | 287                                    |   | 29 / 9.3                   | 12 / 30                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 23       | Endogenous alpha-amylase/subtilisin<br>inhibitor (WASI)                                    | 305                                    |   | 29 / 9.4                   | 19 / 76                             | 19.6 / 6.77                            | <i>T. aestivum</i>                    | IAAS_WHEAT;<br>P16347                       |                                |
| 24       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 25       | Basic endochitinase A precursor (EC<br>3.2.1.14) (Rye seed chitinase-a) (RSC-<br>a)        | 97                                     |   | 35 / 9.7                   | 9 / 38                              | 33.4 / 8.28                            | <i>S. cereale</i>                     | CHIA_SECCE;<br>Q9FRV1                       |                                |
| 26       | 26 kDa endochitinase 2 precursor (EC<br>3.2.1.14) (CHI-26)                                 | 80                                     |   | 33 / 9.7                   | 5 / 18                              | 28.1 / 8.83                            | <i>H. vulgare</i>                     | CHI2_HORVU;<br>P23951                       |                                |
| 27       | Class II chitinase   | -3.7                                   |   | 26 / 6.6                   | 1 / 5.6                             | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465516                                 |                                |
| 28       | Class II chitinase   | -10.5                                  |   | 26 / 7.1                   | 1 / 5.6                             | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465516                                 |                                |
| 29       | Class II chitinase   | -21.6                                  |   | 26 / 7.2                   | 1 / 5.6                             | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465516                                 |                                |
| 30       | Xylanase inhibitor protein I   | 164                                    |   | 26 / 7.3                   | 11 / 30                             | 33.2 / 8.66                            | <i>T. aestivum</i>                    | gi 20804336                                 |                                |
| 31       | Plant Basic Secretory Protein  | -3.1                                   |   | 26 / 7.4                   | 1 / 5.6                             | 25.4 / 4.7                             | <i>T. aestivum</i>                    | gi 1323750                                  |                                |
| 32       | Xylanase inhibitor protein I   | 202                                    |   | 26 / 7.5                   | 11 / 30                             | 33.2 / 8.66                            | <i>T. aestivum</i>                    | gi 20804336                                 |                                |
| 33       | Alpha amylase inhibitor  | -1.2                                   |   | 26 / 7.5                   | 1 / 3.9                             | 19.6 / 6.7                             | <i>T. aestivum</i>                    | gi 225042                                   |                                |
| 34       | Class II chitinase   | -13.6                                  |   | 26 / 7.8                   | 2 / 11                              | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465516                                 |                                |
| 35       | Endogenous alpha-amylase/subtilisin<br>inhibitor (WASI)                                    | 362                                    |   | 25 / 7.8                   | 17 / 72                             | 19.6 / 6.77                            | <i>T. aestivum</i>                    | IAAS_WHEAT;<br>P16347                       |                                |

**Table A-2.4** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)   | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 36       | Chain A, Crystal Structure Of Xylanase Inhibitor Protein (Xip-I)                     | -23.9                                  |   | 26 / 8.0                   | 4 / 14                              | 30.3 / 8.2                             | <i>T. aestivum</i>                    | gi 31615809                                 |                                |
| 37       | Xylanase inhibitor protein I   | 124                                    |   | 26 / 8.2                   | 9 / 21                              | 33.2 / 8.66                            | <i>T. aestivum</i>                    | gi 20804336                                 |                                |
| 38       | Xylanase inhibitor protein I   | 201                                    |   | 26 / 8.4                   | 10 / 26                             | 33.2 / 8.66                            | <i>T. aestivum</i>                    | gi 20804336                                 |                                |
| 39       | Xylanase inhibitor protein I   | 224                                    |   | 27 / 8.5                   | 12 / 37                             | 33.2 / 8.66                            | <i>T. aestivum</i>                    | gi 20804336                                 |                                |
| 40       | Xylanase inhibitor protein I   | 319                                    |   | 26 / 8.5                   | 16 / 49                             | 33.2 / 8.66                            | <i>T. aestivum</i>                    | gi 20804336                                 |                                |
| 41       | 26 kDa endochitinase 1 precursor   | 118                                    |   | 25 / 8.6                   | 5 / 20                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 42       | Class II chitinase   | -19.7                                  |   | 25 / 8.7                   | 1 / 5.6                             | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465516                                 |                                |
| 43       | 26 kDa endochitinase 1 precursor   | 130                                    |   | 25 / 9.0                   | 6 / 27                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 44       | Class II chitinase   | -23.4                                  |   | 25 / 9.3                   | 2 / 11                              | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465516                                 |                                |
| 45       | Class II chitinase   | -37.1                                  |   | 26 / 9.3                   | 1 / 5.6                             | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465516                                 |                                |
| 46       | Chain A, Crystal Structure Of Xylanase Inhibitor Protein (Xip-I)                     | -60.7                                  |   | 27 / 9.4                   | 5 / 21                              | 30.3 / 8.2                             | <i>T. aestivum</i>                    | gi 31615809                                 |                                |
| 47       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 48       | Class II chitinase   | -33.4                                  |   | 26 / 9.7                   | 2 / 11                              | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465516                                 |                                |
| 49       | Class II chitinase (EC 3.2.1.14)   | 72                                     |   | 26 / 6.9                   | 1 / 6                               | 28.2 / 8.66                            | <i>T. aestivum</i>                    | Q4Z8L7_WHEAT                                |                                |
| 50       | 26 kDa endochitinase 1 precursor   | 106                                    |   | 25 / 7.1                   | 4 / 16                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 51       | Xylanase inhibitor protein 1 precursor (Class III chitinase homolog) (XIP-I protein) | 155                                    |   | 25 / 7.5                   | 4 / 16                              | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 52       | 26 kDa endochitinase 1 precursor   | 105                                    |   | 25 / 7.7                   | 4 / 16                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 53       | Chain A, Crystal Structure Of Xylanase Inhibitor Protein (Xip-I)                     | -9.6                                   |   | 25 / 7.8                   | 2 / 7.7                             | 30.3 / 8.2                             | <i>T. aestivum</i>                    | gi 31615809                                 |                                |
| 54       | Xylanase inhibitor protein 1 precursor (Class III chitinase homolog) (XIP-I protein) | 470                                    |   | 25 / 8.0                   | 21 / 51                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 55       | Chain B, Crystal Structure Of Chitinase At 1.91a Resolution                          | 58                                     |   | 25 / 8.1                   | 6 / 17                              | 26 / 8.45                              | <i>H. vulgare</i>                     | gi 1310889                                  |                                |

**Table A-2.4** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)   | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 56       | Class II chitinase   | -26.6                                  |   | 25 / 8.4                   | 2 / 5.6                             | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465516                                 |                                |
| 57       | Chain A, Crystal Structure Of Xylanase Inhibitor Protein (Xip-I)                     | -46.9                                  |   | 24 / 8.6                   | 6 / 18                              | 30.3 / 8.2                             | <i>T. aestivum</i>                    | gi 31615809                                 |                                |
| 58       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 59       | Class II chitinase   | -9                                     |   | 24 / 8.9                   | 1 / 5.6                             | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465516                                 |                                |
| 60       | Endogenous alpha-amylase/subtilisin inhibitor (WASI)                                 | 158                                    |   | 24 / 9.4                   | 15 / 70                             | 19.6 / 6.77                            | <i>T. aestivum</i>                    | IAAS_WHEAT;<br>P16347                       |                                |
| 61       | 26 kDa endochitinase 1 precursor   | 177                                    |   | 25 / 9.2                   | 6 / 20                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 62       | 26 kDa endochitinase 1 precursor   | 196                                    |   | 25 / 9.2                   | 6 / 20                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 63       | Chitinase a  | 81                                     |   | 25 / 9.4                   | 5 / 24                              | 31.7 / 8.13                            | <i>S. cereale</i>                     | gi 741317                                   |                                |
| 64       | Xylanase inhibitor precursor (Xylanase inhibitor TAXI-I)                             | 140                                    |   | 27 / 9.4                   | 2 / 8                               | 40.9 / 8.18                            | <i>T. aestivum</i>                    | Q8H0K8_WHEAT                                |                                |
| 65       | Chain A, Crystal Structure Of Family 11 Xylanase In Complex With Inhibitor (Xip-I)   | 325                                    |   | 22 / 6.7                   | 14 / 37                             | 30.3 / 8.3                             | <i>T. aestivum</i>                    | gi 51247633                                 |                                |
| 66       | Xylanase inhibitor protein 1 precursor (Class III chitinase homolog) (XIP-I protein) | 130                                    |   | 21 / 7.0                   | 3 / 10                              | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 67       | Xylanase inhibitor protein 1 precursor (Class III chitinase homolog) (XIP-I protein) | 59                                     |   | 21 / 7.1                   | 2 / 6                               | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 68       | Endogenous alpha-amylase/subtilisin inhibitor (WASI)                                 | 167                                    |   | 22 / 7.4                   | 11 / 32                             | 19.6 / 6.8                             | <i>T. aestivum</i>                    | gi 123975                                   |                                |
| 69       | Xylanase inhibitor XIP-III   | 151                                    |   | 24 / 7.9                   | 13 / 24                             | 33.3 / 7.1                             | <i>T. aestivum</i>                    | gi 66766322                                 |                                |
| 70       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 71       | Xylanase inhibitor protein I   | 184                                    |   | 24 / 8.6                   | 11 / 26                             | 33.2 / 8.7                             | <i>T. aestivum</i>                    | gi 20804336                                 |                                |
| 72       | Class II chitinase   | 120                                    |   | 23 / 8.6                   | 5 / 19                              | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465514                                 |                                |
| 73       | Class II chitinase   | 135                                    |   | 22 / 8.5                   | 5 / 24                              | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465514                                 |                                |

**Table A-2.4** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)   | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 74       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 75       | Benzothiadiazole-induced protein<br>(clone WCI-5)  | 104                                    |   | 22 / 8.7                   | 1 / 6                               | 25.4 / 4.65                            | <i>T. aestivum</i>                    | T06278                                      |                                |
| 76       | 26 kDa endochitinase 1 precursor   | 53                                     |   | 24 / 8.9                   | 4 / 16                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 77       | Secretory protein  | 68                                     |   | 23 / 9.1                   | 4 / 13                              | 24.2 / 9.3                             | <i>T. aestivum</i>                    | gi 5669008                                  |                                |
| 78       | 26 kDa endochitinase 1 precursor   | 76                                     |   | 24 / 9.1                   | 4 / 16                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 79       | Class II chitinase   | 97                                     |   | 22 / 9.2                   | 4 / 14                              | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465514                                 |                                |
| 80       | 26 kDa endochitinase 1 precursor   | 108                                    |   | 24 / 9.2                   | 4 / 16                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 81       | Unknown  | 93                                     |   | 24 / 9.4                   | 3 / 14                              | 25.4 / 4.65                            | <i>T. aestivum</i>                    | gi 1323750                                  |                                |
| 82       | Benzothiadiazole-induced protein<br>(clone WCI-5)  | 93                                     |   | 22 / 9.4                   | 1 / 6                               | 25.4 / 4.65                            | <i>T. aestivum</i>                    | T06278                                      |                                |
| 83       | Endogenous alpha-amylase/subtilisin<br>inhibitor (WASI)                                  | 110                                    |   | 19 / 6.5                   | 7 / 32                              | 19.6 / 6.77                            | <i>T. aestivum</i>                    | gi 123975                                   |                                |
| 84       | Endogenous alpha-amylase/subtilisin<br>inhibitor (WASI)                                  | 251                                    |   | 19 / 6.7                   | 15 / 62                             | 19.6 / 6.77                            | <i>T. aestivum</i>                    | gi 123975                                   |                                |
| 85       | Chain A, Crystal Structure Of Family<br>11 Xylanase In Complex With Inhibitor<br>(Xip-I) | 289                                    |   | 19 / 6.9                   | 14 / 33                             | 30.3 / 8.3                             | <i>T. aestivum</i>                    | gi 51247633                                 |                                |
| 86       | Class II chitinase   | 104                                    |   | 17 / 7.1                   | 4 / 14                              | 28.2 / 8.7                             | <i>T. aestivum</i>                    | gi 62465514                                 |                                |
| 87       | Chain A, Crystal Structure Of Family<br>11 Xylanase In Complex With Inhibitor<br>(Xip-I) | 353                                    |   | 19 / 7.1                   | 15 / 37                             | 30.3 / 8.3                             | <i>T. aestivum</i>                    | gi 51247633                                 |                                |
| 88       | Secretory protein  | 99                                     |   | 21 / 7.4                   | 5 / 13                              | 24.2 / 9.3                             | <i>T. aestivum</i>                    | gi 5669008                                  |                                |
| 89       | Chain A, Crystal Structure Of Family<br>11 Xylanase In Complex With Inhibitor<br>(Xip-I) | 409                                    |   | 19 / 8.0                   | 15 / 37                             | 30.3 / 8.3                             | <i>T. aestivum</i>                    | gi 51247633                                 |                                |
| 90       | Chain A, Crystal Structure Of Family<br>11 Xylanase In Complex With Inhibitor<br>(Xip-I) | 138                                    |   | 16 / 8.7                   | 9 / 30                              | 30.3 / 8.3                             | <i>T. aestivum</i>                    | gi 51247633                                 |                                |

**Table A-2.4** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)   | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 91       | 26 kDa endochitinase 1 precursor   | 80                                     |   | 15 / 9.0                   | 4 / 16                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | gi 2506281                                  |                                |
| 92       | Chain A, Crystal Structure Of Family<br>11 Xylanase In Complex With Inhibitor<br>(Xip-I) | 236                                    |   | 19 / 9.1                   | 13 / 33                             | 30.3 / 8.3                             | <i>T. aestivum</i>                    | gi 51247633                                 |                                |
| 93       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 94       | Chain A, Crystal Structure Of Family<br>11 Xylanase In Complex With Inhibitor<br>(Xip-I) | 141                                    |   | 11 / 9.4                   | 9 / 22                              | 30.3 / 8.3                             | <i>T. aestivum</i>                    | gi 51247633                                 |                                |
| 95       | Pathogenesis-related protein 4<br>(Fragment)   | 67                                     |   | 7 / 6.5                    | 1 / 10                              | 13.1 / 7.0                             | <i>T. aestivum</i>                    | Q9SQG8_WHEAT                                |                                |
| 96       | Pathogenesis-related protein 4<br>(Fragment)   | 74                                     |   | 6 / 7.6                    | 1 / 10                              | 13.1 / 7.0                             | <i>T. aestivum</i>                    | Q9SQG8_WHEAT                                |                                |
| 97       | Pathogenesis-related protein 4<br>(Fragment)   | 62                                     |   | 6 / 7.7                    | 1 / 10                              | 13.1 / 7.0                             | <i>T. aestivum</i>                    | Q9SQG8_WHEAT                                |                                |
| 98       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 99       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 100      | Class II chitinase (EC 3.2.1.14)   | 82                                     |   | 8 / 9.5                    | 1 / 6                               | 28.2 / 8.66                            | <i>T. aestivum</i>                    | Q4Z8L7_WHEAT                                |                                |
| 101      | Class II chitinase (EC 3.2.1.14)   | 46                                     |   | 8 / 9.7                    | 1 / 6                               | 28.2 / 8.66                            | <i>T. aestivum</i>                    | Q4Z8L7_WHEAT                                |                                |
| 102      | Xylanase inhibitor protein I   | 91                                     |   | 2 / 8.7                    | 4 / 11                              | 33.2 / 8.7                             | <i>T. aestivum</i>                    | gi 20804336                                 |                                |
| 103      | Chain A, Crystal Structure Of Family<br>11 Xylanase In Complex With Inhibitor<br>(Xip-I) | 146                                    |   | 4 / 9.3                    | 5 / 12                              | 30.3 / 8.3                             | <i>T. aestivum</i>                    | gi 51247633                                 |                                |

**Table A-2.5** Inner bran fraction (aleurone cells) proteins identified from 2D gel pH 4-7

| Spot No. | Protein name<br>(Mascot cereal search)                               | Mascot or GPM <sup>1</sup> score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed MW (kDa)/pI | Matching peptides / %Coverage | Theoretical <sup>3</sup> MW (Da)/pI | Species with homologous protein | Swiss-Prot accession number <sup>4</sup> | GenBank accession number |
|----------|--|----------------------------------|---|----------------------|-------------------------------|-------------------------------------|---------------------------------|--|--------------------------|
| 1        | No match   |                                  |   |                      |                               |                                     |                                 |  |                          |
| 2        | Pyruvate orthophosphate dikinase (Fragment)                          | 42                               |   | 110 / 5.4            | 20 / 38                       | 32.6 / 4.97                         | <i>T. aestivum</i>              | Q7XYB5_WHEAT                             |                          |
| 3        | No match   |                                  |   |                      |                               |                                     |                                 |  |                          |
| 4        | Wheat EST match  | 80                               | Aconitate hydratase, cytoplasmic, putative, expressed | 100 / 6.2            | 25 / 30                       | 83.5 / 7.66                         | <i>O. sativa</i>                | Ta18566-2 <sup>4</sup>                   | GI:108706066             |
| 5        | No match   |                                  |   |                      |                               |                                     |                                 |  |                          |
| 6        | No match   |                                  |   |                      |                               |                                     |                                 |  |                          |
| 7        | HSP70  | 255                              |   | 75 / 5.2             | 24 / 32                       | 70.99 / 5.14                        | <i>T. aestivum</i>              | Q9SAU8_WHEAT                             |                          |
| 8        | HSP70  | 294                              |   | 75 / 5.25            | 29 / 32                       | 70.99 / 5.14                        | <i>T. aestivum</i>              | Q9SAU8_WHEAT                             |                          |
| 9        | HSP70  | 229                              |   | 75 / 5.3             | 37 / 38                       | 70.99 / 5.14                        | <i>T. aestivum</i>              | Q9SAU8_WHEAT                             |                          |
| 10       | HSP70  | 150                              |   | 75 / 5.35            | 29 / 29                       | 70.99 / 5.14                        | <i>T. aestivum</i>              | Q9SAU8_WHEAT                             |                          |
| 11       | Putative dnaK-type molecular chaperone                               | 183                              |   | 74 / 5.45            | 18 / 22                       | 72.9 / 5.49                         | <i>O. sativa</i>                | Q6Z7L1_ORYSA                             |                          |
| 12       | No match   |                                  |   |                      |                               |                                     |                                 |  |                          |
| 13       | Cyclophilin A-2 (EC 5.2.1.8) (Cyclophilin A)                         | 21                               |   | 77 / 6.15            | 6 / 28                        | 18.4 / 8.52                         | <i>T. aestivum</i>              | Q93XQ6_WHEAT                             |                          |
| 14       | No match   |                                  |   |                      |                               |                                     |                                 |  |                          |
| 15       | No match   |                                  |   |                      |                               |                                     |                                 |  |                          |
| 16       | 2,3-bisphosphoglycerate-independent phosphoglycerate mutase          | 121                              |   | 72 / 5.8             | 10 / 11                       | 60.6 / 5.29                         | <i>Z. mays</i>                  | PMGI_MAIZE, P30792                       |                          |
| 17       | Putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase | 94                               |   | 70 / 5.9             | 14 / 21                       | 60.8 / 5.25                         | <i>O. sativa</i>                | Q5KQH5_ORYSA                             |                          |
| 18       | Phosphoglucomutase (EC 5.4.2.2) (Fragment)                           | 221                              |   | 74 / 5.95            | 17 / 23                       | 62.8 / 5.66                         | <i>T. aestivum</i>              | Q8VX48_WHEAT                             |                          |
| 19       | Wheat EST match  | 54                               | Cytosolic NADP malic enzyme                           | 72 / 6.3             | 12 / 42                       | 27.4 / 8.8                          | <i>O. sativa</i>                | Ta963253410-3                            | GI:38261493              |
| 20       | Wheat EST match  | 122                              | Cytosolic NADP malic enzyme                           | 72 / 6.35            | 13 / 43                       | 27.4 / 8.8                          | <i>O. sativa</i>                | Ta965253413-3                            | GI:38261493              |
| 21       | 7S Globulin storage protein  | 55                               |   | 74 / 6.75            | 11 / 11                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 22       | 7S Globulin storage protein  | 50                               |   | 74 / 6.8             | 14 / 16                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 23       | Protein disulfide isomerase (EC 5.3.4.1) (Fragment)                  | 118                              |   | 65 / 4.95            | 12 / 88                       | 10.2 / 5.43                         | <i>T. aestivum</i>              | Q6JAB7_WHEAT                             |                          |

**Table A-2.5** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)                               | Mascot or GPM <sup>1</sup> score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup>            | Observed MW (kDa)/pI | Matching peptides / %Coverage | Theoretical <sup>3</sup> MW (Da)/pI | Species with homologous protein | Swiss-Prot accession number <sup>4</sup> | GenBank accession number |
|----------|--|----------------------------------|--|----------------------|-------------------------------|-------------------------------------|---------------------------------|--|--------------------------|
| 24       | Wheat EST match  | 100                              | Putative rubisco subunit binding-protein alpha subunit precursor | 57 / 5.15            | 4 / 67                        | 6.1 / 4.08                          | <i>O. sativa</i>                | Ta228991-3                               | GI:50920285              |
| 25       | 60 kDa chaperonin  | 129                              |  | 56 / 5.15            | 8 / 9                         | 57.1 / 5.05                         | <i>X. oryzae</i>                | Q2NY29_XANOR                             |                          |
| 26       | No match   |                                  |  |                      |                               |                                     |                                 |  |                          |
| 27       | Chaperonin CPN60-1, (HSP60-1) mitochondrial precursor                | 40                               |  | 57 / 5.25            | 18 / 30                       | 61.2 / 5.68                         | <i>Z. mays</i>                  | CH61_MAIZE                               |                          |
| 28       | No match   |                                  |  |                      |                               |                                     |                                 |  |                          |
| 29       | Enolase (EC 4.2.1.11)  | 512                              |  | 53 / 5.6             | 26 / 46                       | 47.9 / 5.41                         | <i>O. sativa</i>                | ENO_ORYSA                                |                          |
| 30       | Xylose isomerase (EC 5.3.1.5)  | 181                              |  | 52 / 5.55            | 14 / 17                       | 53.6 / 5.31                         | <i>H. vulgare</i>               | XYLA_HORVU                               |                          |
| 31       | Enolase (EC 4.2.1.11)  | 469                              |  | 52 / 5.6             | 28 / 46                       | 47.9 / 5.41                         | <i>O. sativa</i>                | ENO_ORYSA                                |                          |
| 32       | Enolase (EC 4.2.1.11)  | 648                              |  | 52 / 5.7             | 32 / 52                       | 47.9 / 5.41                         | <i>O. sativa</i>                | ENO_ORYSA                                |                          |
| 33       | Atp1 protein   | 305                              |  | 52 / 5.75            | 27 / 41                       | 55.3 / 5.7                          | <i>T. aestivum</i>              | Q332R4_WHEAT                             |                          |
| 34       | No match   |                                  |  |                      |                               |                                     |                                 |  |                          |
| 35       | Atp1 protein   | 85                               |  | 52 / 5.9             | 19 / 33                       | 55.3 / 5.7                          | <i>T. aestivum</i>              | Q332R4_WHEAT                             |                          |
| 36       | Putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase | 61                               |  | 67 / 6.0             | 19 / 21                       | 60.8 / 5.25                         | <i>O. sativa</i>                | Q5KQH5_ORYSA                             |                          |
| 37       | Atp1 protein   | 51                               |  | 52 / 6.05            | 27 / 41                       | 55.3 / 5.7                          | <i>T. aestivum</i>              | Q332R4_WHEAT                             |                          |
| 38       | Wheat EST match  | 165                              | 7S Globulin storage protein                                      | 51 / 6.05            | 42 / 47                       | 67.9 / 8.86                         | <i>T. aestivum</i>              | Ta628253404-2                            | GI:170696                |
| 39       | Em protein H5  | 20                               |  | 49 / 6.25            | 7 / 60                        | 10.05 / 5.14                        | <i>T. aestivum</i>              | EM4_WHEAT                                |                          |
| 40       | Putative dihydrolipoamide dehydrogenase                              | 42                               |  | 53 / 6.4             | 7 / 10                        | 52.6 / 7.21                         | <i>O. sativa</i>                | Q9ASP4_ORYSA                             |                          |
| 41       | Putative dihydrolipoamide dehydrogenase                              | 186                              |  | 52 / 6.6             | 11 / 17                       | 52.6 / 7.21                         | <i>O. sativa</i>                | Q9ASP4_ORYSA                             |                          |
| 42       | Globosa  | 23                               |  | 51 / 6.6             | 5 / 16                        | 24.1 / 7.14                         | <i>T. aestivum</i>              | Q6QPJ1_WHEAT                             |                          |
| 43       | 7S Globulin storage protein  | 74                               |  | 50 / 6.7             | 14 / 17                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 44       | 7S Globulin storage protein  | 80                               |  | 50 / 6.8             | 13 / 14                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 45       | Putative beta-N-acetylhexosaminidase (Fragment)                      | 23                               |  | 51 / 4.75            | 4 / 66                        | 5.98 / 10.06                        | <i>T. aestivum</i>              | Q2L3V7_WHEAT                             |                          |
| 46       | No match   |                                  |  |                      |                               |                                     |                                 |  |                          |
| 47       | No match   |                                  |  |                      |                               |                                     |                                 |  |                          |
| 48       | ATP synthase beta subunit  | 475                              |  | 52 / 5.25            | 28 / 39                       | 59.2 / 5.56                         | <i>T. aestivum</i>              | Q41534_WHEAT                             |                          |

**Table A-2.5** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)                       | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 49       | ATP synthase beta subunit                                    | 598                                    |   | 53 / 5.3                   | 35 / 49                             | 59.2 / 5.56                            | <i>T. aestivum</i>                    | Q41534_WHEAT                                |                                |
| 50       | Wheat EST match  | 43                                     | MLo protein   | 52 / 5.3                   | 3 / 10                              | 40.5 / 8.88                            | <i>T. aestivum</i>                    | Ta224200-1                                  | GI:14334167                    |
| 51       | UTP--glucose-1-phosphate<br>uridylyltransferase (EC 2.7.7.9) | 418                                    |   | 51 / 5.3                   | 23 / 37                             | 51.6 / 5.2                             | <i>H. vulgare</i>                     | UGPA_HORVU                                  |                                |
| 52       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 53       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 54       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 55       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 56       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 57       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 58       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 59       | Annexin  | 29                                     |   | 50 / 5.25                  | 10 / 27                             | 35.4 / 9.01                            | <i>T. aestivum</i>                    | Q6S9D8_WHEAT                                |                                |
| 60       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 61       | Wheat EST match  | 38                                     | 12-oxo-phytodienoic acid reductase                    | 47 / 5.35                  | 5 / 11                              | 38.9 / 6.33                            | <i>Z. mays</i>                        | Ta139439-3                                  | GI:63021725                    |
| 62       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 63       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 64       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 65       | Phosphoglycerate kinase, cytosolic (EC 2.7.2.3)              | 84                                     |   | 42 / 5.7                   | 11 / 33                             | 42.1 / 5.64                            | <i>T. aestivum</i>                    | PGKY_WHEAT                                  |                                |
| 66       | Wheat EST match  | 43                                     | Subtilisin-like protease                              | 45 / 5.7                   | 4 / 11                              | 39.9 / 9.32                            | <i>T. aestivum</i>                    | Ta250319-2                                  | GI:86439745                    |
| 67       | Putative cytosolic 6-phosphogluconate<br>dehydrogenase       | 160                                    |   | 48 / 5.8                   | 17 / 18                             | 53.0 / 5.92                            | <i>Z. mays</i>                        | O81237_MAIZE                                |                                |
| 68       | Wheat EST match  | 358                                    | 7S Globulin storage protein                           | 48 / 6.5                   | 49 / 51                             | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 69       | 7S Globulin storage protein                                  | 64                                     |   | 47 / 6.45                  | 26 / 28                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 70       | Formate dehydrogenase (Fragment)                             | 181                                    |   | 36 / 6.7                   | 13 / 40                             | 28.99 / 8.61                           | <i>T. aestivum</i>                    | Q7X9L3_WHEAT                                |                                |
| 71       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 72       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 73       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 74       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 75       | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 76       | Elongation factor Tu   | 47                                     |   | 43 / 5.4                   | 16 / 23                             | 43.1 / 5.45                            | <i>X. oryzae</i>                      | Q2NZX1_XANOR                                |                                |



**Table A-2.5** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)                              | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|---|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 77       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 78       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 79       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 80       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 81       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 82       | Phosphoglycerate kinase, cytosolic (EC 2.7.2.3)                     | 82                                     |   | 38 / 5.75                  | 14 / 40                             | 42.1 / 5.64                            | <i>T. aestivum</i>                    | PGKY_WHEAT                                  |                                |
| 83       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 84       | HSP70   | 276                                    |   | 39 / 5.8                   | 19 / 16                             | 70.99 / 5.14                           | <i>T. aestivum</i>                    | Q9SAU8_WHEAT                                |                                |
| 85       | HSP70   | 198                                    |   | 39 / 5.8                   | 16 / 16                             | 70.99 / 5.14                           | <i>T. aestivum</i>                    | Q9SAU8_WHEAT                                |                                |
| 86       | HSP70   | 43                                     |   | 39 / 6.0                   | 12 / 16                             | 70.99 / 5.14                           | <i>T. aestivum</i>                    | Q9SAU8_WHEAT                                |                                |
| 87       | HSP70   | 304                                    |   | 39 / 6.1                   | 23 / 17                             | 70.99 / 5.14                           | <i>T. aestivum</i>                    | Q9SAU8_WHEAT                                |                                |
| 88       | NADP-specific isocitrate dehydrogenase (EC 1.1.1.42)                | 71                                     |   | 39 / 6.2                   | 12 / 21                             | 46.1 / 6.29                            | <i>O. sativa</i>                      | Q9XGU7_ORYSA                                |                                |
| 89       | Cytosolic glyceraldehyde-3-phosphate dehydrogenase GAPDH (Fragment) | 63                                     |   | 38 / 6.2                   | 5 / 23                              | 25.3 / 7.83                            | <i>T. aestivum</i>                    | Q9M4V4_WHEAT                                |                                |
| 90       | Cytosolic glyceraldehyde-3-phosphate dehydrogenase GAPDH (Fragment) | 68                                     |   | 38 / 6.3                   | 11 / 39                             | 25.3 / 7.83                            | <i>T. aestivum</i>                    | Q9M4V4_WHEAT                                |                                |
| 91       | Cytosolic glyceraldehyde-3-phosphate dehydrogenase GAPDH (Fragment) | 56                                     |   | 39 / 6.3                   | 10 / 32                             | 25.3 / 7.83                            | <i>T. aestivum</i>                    | Q9M4V4_WHEAT                                |                                |
| 92       | Cytosolic glyceraldehyde-3-phosphate dehydrogenase GAPDH (Fragment) | 135                                    |   | 37 / 6.65                  | 9 / 32                              | 25.3 / 7.83                            | <i>T. aestivum</i>                    | Q9M4V4_WHEAT                                |                                |
| 93       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 94       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 95       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 96       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 97       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 98       | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 99       | Glutamine synthetase isoform GS1c                                   | 181                                    |   | 38 / 5.45                  | 10 / 12                             | 39.2 / 5.41                            | <i>T. aestivum</i>                    | Q45NB5_WHEAT                                |                                |

**Table A-2.5** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)                              | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|---|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 100      | Reversibly glycosylated polypeptide                                 | 156                                    |   | 38 / 5.5                   | 18 / 29                             | 41.5 / 5.82                            | <i>T. aestivum</i>                    | Q9ZR33_WHEAT                                |                                |
| 101      | Phosphoglycerate kinase, cytosolic (EC 2.7.2.3)                     | 245                                    |   | 38 / 5.6                   | 14 / 27                             | 42.1 / 5.64                            | <i>T. aestivum</i>                    | PGKY_WHEAT                                  |                                |
| 102      | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 103      | Cytosolic malate dehydrogenase (EC 1.1.1.37) (Fragment)             | 528                                    |   | 36 / 5.9                   | 21 / 54                             | 24.3 / 6.6                             | <i>T. aestivum</i>                    | Q6XEB8_WHEAT                                |                                |
| 104      | Wheat EST match   | 36                                     | Amylogenin  | 38 / 5.85                  | 4 / 13                              | 28 / 9.79                              | <i>T. aestivum</i>                    | Ta2337253412-1                              | GI:4158230                     |
| 105      | Wheat EST match   | 67                                     | Putative malate dehydrogenase                         | 34 / 6.25                  | 6 / 8                               | 52.4 / 9.39                            | <i>O. sativa</i>                      | Ta2794-3                                    | GI:50932771                    |
| 106      | Disease resistance protein (Fragment)                               | 27                                     |   | 36 / 6.3                   | 7 / 30                              | 20.6 / 9.2                             | <i>T. aestivum</i>                    | Q3HNP9_WHEAT                                |                                |
| 107      | Cytosolic glyceraldehyde-3-phosphate dehydrogenase GAPDH (Fragment) | 87                                     |   | 37 / 6.4                   | 8 / 40                              | 25.3 / 7.83                            | <i>T. aestivum</i>                    | Q9M4V4_WHEAT                                |                                |
| 108      | Disease resistance protein (Fragment)                               | 35                                     |   | 35 / 6.7                   | 8 / 35                              | 20.6 / 9.2                             | <i>T. aestivum</i>                    | Q3HNP9_WHEAT                                |                                |
| 109      | 7S Globulin storage protein   | 47                                     |   | 32 / 6.8                   | 13 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 110      | DNA-binding protein (Fragment)                                      | 24                                     |   | 35 / 5.1                   | 5 / 19                              | 20.4 / 9.15                            | <i>T. aestivum</i>                    | Q41541_WHEAT                                |                                |
| 111      | Putative 60S ribosomal protein L36                                  | 32                                     |   | 34 / 5.15                  | 9 / 47                              | 12.4 / 11.43                           | <i>O. sativa</i>                      | Q8L5X0_ORYSA                                |                                |
| 112      | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 113      | Wheat EST match   | 123                                    | 7S Globulin storage protein                           | 33 / 5.45                  | 24 / 27                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 114      | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 115      | Wheat EST match   | 79                                     | 7S Globulin storage protein                           | 32 / 5.45                  | 30 / 35                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 116      | Wheat EST match   | 47                                     | 7S Globulin storage protein                           | 32 / 5.65                  | 11 / 15                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 117      | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 118      | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 119      | 7S Globulin storage protein   | 125                                    |   | 33 / 5.85                  | 7 / 5                               | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 120      | 7S Globulin storage protein   | 46                                     |   | 32 / 6.0                   | 6 / 5                               | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 121      | Cytosolic glyceraldehyde-3-phosphate dehydrogenase (Fragment)       | 50                                     |   | 33 / 6.15                  | 5 / 15                              | 18.2 / 6.34                            | <i>T. aestivum</i>                    | Q7XJJ1_WHEAT                                |                                |
| 122      | 1-Cys-peroxiredoxine  | 22                                     |   | 32 / 6.25                  | 7 / 28                              | 23.95 / 6.08                           | <i>T. aestivum</i>                    | Q6W8Q2_WHEAT                                |                                |

**Table A-2.5** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)         | Mascot or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed MW<br>(kDa)/pI | Matching peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|-------------------------------------|---|-------------------------|----------------------------------|--|---------------------------------------|---|--------------------------------|
| 123      | Wheat EST match                                | 318                                 | Glucose and ribitol dehydrogenase homolog - barley    | 31 / 6.55               | 25 / 37                          | 48.3 / 8.17                            | <i>H. vulgare</i>                     | Ta631253406-3                               | GI:7431022                     |
| 124      | Wheat EST match                                | 80                                  | HSP70   | 28 / 4.8                | 11 / 21                          | 40.4 / 4.92                            | <i>T. aestivum</i>                    | Ta236253405-2                               | GI:2827002                     |
| 125      | No match                                       |                                     |   |                         |                                  |  |                                       |   |                                |
| 126      | Wheat EST match                                | 117                                 | 7S Globulin storage protein                           | 29 / 5.3                | 11 / 15                          | 60.7 / 8.24                            | <i>T. aestivum</i>                    | Ta2606253405-2                              | GI:170696                      |
| 127      | No match                                       |                                     |   |                         |                                  |  |                                       |   |                                |
| 128      | Wheat EST match                                | 278                                 | Putative glyoxalase I                                 | 31 / 5.5                | 22 / 30                          | 46.6 / 8.19                            | <i>T. aestivum</i>                    | Ta52588-3                                   | GI:7619802                     |
| 129      | Wheat EST match                                | 203                                 | 7S Globulin storage protein                           | 31 / 5.5                | 14 / 16                          | 60.7 / 8.24                            | <i>T. aestivum</i>                    | Ta2606253405-2                              | GI:170696                      |
| 130      | No match                                       |                                     |   |                         |                                  |  |                                       |   |                                |
| 131      | Wheat EST match                                | 390                                 | 7S Globulin storage protein                           | 32 / 5.7                | 18 / 26                          | 61.7 / 8.08                            | <i>T. aestivum</i>                    | Ta3078253410-1                              | GI:170696                      |
| 132      | Wheat EST match                                | 184                                 | 7S Globulin storage protein                           | 29 / 5.65               | 22 / 25                          | 66.9 / 7.64                            | <i>T. aestivum</i>                    | Ta2606253404-2                              | GI:170696                      |
| 133      | Wheat EST match                                | 579                                 | 7S Globulin storage protein                           | 30 / 5.8                | 25 / 31                          | 61.7 / 8.08                            | <i>T. aestivum</i>                    | Ta3078253410-1                              | GI:170696                      |
| 134      | Wheat EST match                                | 117                                 | 7S Globulin storage protein                           | 29 / 5.75               | 15 / 22                          | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 135      | Wheat EST match                                | 456                                 | 7S Globulin storage protein                           | 29 / 5.9                | 19 / 19                          | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 136      | 7S Globulin storage protein                    | 74                                  |   | 30 / 5.9                | 5 / 6                            | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 137      | 7S Globulin storage protein                    | 47                                  |   | 30 / 5.95               | 6 / 6                            | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 138      | Wheat EST match                                | 641                                 | 7S Globulin storage protein                           | 32 / 6.0                | 24 / 31                          | 61.7 / 8.08                            | <i>T. aestivum</i>                    | Ta3078253410-1                              | GI:170696                      |
| 139      | 7S Globulin storage protein                    | 86                                  |   | 29 / 6.1                | 7 / 10                           | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 140      | Wheat EST match                                | 572                                 | 7S Globulin storage protein                           | 28 / 6.1                | 23 / 30                          | 61.7 / 8.08                            | <i>T. aestivum</i>                    | Ta3078253410-1                              | GI:170696                      |
| 141      | 7S Globulin storage protein                    | 65                                  |   | 29 / 6.25               | 6 / 5                            | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 142      | Wheat EST match                                | 448                                 | Glucose and ribitol dehydrogenase homolog - barley    | 32 / 6.55               | 26 / 34                          | 48.3 / 8.17                            | <i>H. vulgare</i>                     | Ta631253406-3                               | GI:7431022                     |
| 143      | No match                                       |                                     |   |                         |                                  |  |                                       |   |                                |
| 144      | Aldose reductase-related protein (EC 1.1.1.21) | 201                                 |   | 31 / 6.9                | 14 / 31                          | 35.8 / 6.28                            | <i>A. fatua</i>                       | Q43320_AVEFA                                |                                |
| 145      | Translationally controlled tumor protein       | 229                                 |   | 23 / 4.65               | 10 / 33                          | 18.8 / 4.55                            | <i>T. aestivum</i>                    | Q8LRM8_WHEAT                                |                                |
| 146      | Aspartic proteinase                            | 269                                 |   | 27 / 4.8                | 14 / 21                          | 54.3 / 5.14                            | <i>T. aestivum</i>                    | Q401N7_WHEAT                                |                                |
| 147      | Aspartic proteinase                            | 360                                 |   | 27 / 4.9                | 12 / 16                          | 54.3 / 5.14                            | <i>T. aestivum</i>                    | Q401N7_WHEAT                                |                                |
| 148      | Heat shock protein HSP26                       | 274                                 |   | 23 / 5.2                | 19 / 53                          | 26.6 / 7.88                            | <i>T. aestivum</i>                    | Q9SBB6_WHEAT                                |                                |
| 149      | Small heat shock protein Hsp23.6 precursor     | 19                                  |   | 22 / 5.25               | 6 / 15                           | 23.6 / 5.25                            | <i>T. aestivum</i>                    | Q9ZP24_WHEAT                                |                                |
| 150      | Heat shock protein HSP26                       | 56                                  |   | 24 / 5.35               | 4 / 15                           | 26.5 / 9.36                            | <i>T. aestivum</i>                    | Q9ZSR6_WHEAT                                |                                |

**Table A-2.5** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)               | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 151      | Triosephosphate-isomerase                            | 284                                    |   | 25 / 5.6                   | 15 / 44                             | 26.8 / 5.38                            | <i>T. aestivum</i>                    | Q9FS79_WHEAT                                |                                |
| 152      | Wheat EST match                                      | 68                                     | Cupin family protein, expressed                       | 25 / 5.6                   | 6 / 30                              | 17.95 / 5.6                            | <i>O. sativa</i>                      | Ta132809-2                                  | GI:108706671                   |
| 153      | Wheat EST match                                      | 143                                    | 7S Globulin storage protein                           | 26.5 / 5.65                | 12 / 14                             | 60.7 / 8.24                            | <i>T. aestivum</i>                    | Ta2606253405-2                              | GI:170696                      |
| 154      | Wheat EST match                                      | 68                                     | Cupin family protein, expressed                       | 25 / 5.8                   | 8 / 55                              | 17.95 / 5.6                            | <i>O. sativa</i>                      | Ta132809-2                                  | GI:108706671                   |
| 155      | Wheat EST match                                      | 192                                    | 7S Globulin storage protein                           | 27 / 5.8                   | 10 / 11                             | 60.7 / 8.24                            | <i>T. aestivum</i>                    | Ta2606253405-2                              | GI:170696                      |
| 156      | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 157      | Wheat EST match                                      | 101                                    | Cupin family protein, expressed                       | 25 / 5.75                  | 11 / 64                             | 17.95 / 5.6                            | <i>O. sativa</i>                      | Ta132809-2                                  | GI:108706671                   |
| 158      | Putative proteasome subunit alpha type 3             | 179                                    |   | 26 / 5.8                   | 12 / 28                             | 27.2 / 5.76                            | <i>O. sativa</i>                      | Q6F321_ORYSA                                |                                |
| 159      | Wheat EST match                                      | 45                                     | 7S Globulin storage protein                           | 25 / 6.1                   | 10 / 15                             | 66.9 / 7.64                            | <i>T. aestivum</i>                    | Ta2606253404-2                              | GI:170696                      |
| 160      | Glutathione transferase (EC 2.5.1.18)                | 403                                    |   | 23 / 6.35                  | 20 / 42                             | 24.98 / 6.35                           | <i>T. aestivum</i>                    | Q8RW04_WHEAT                                |                                |
| 161      | Glutathione transferase (EC 2.5.1.18)                | 42                                     |   | 23 / 6.45                  | 16 / 39                             | 25.0 / 6.35                            | <i>T. aestivum</i>                    | Q8RW04_WHEAT                                |                                |
| 162      | Wheat EST match                                      | 99                                     | 7S Globulin storage protein                           | 25 / 6.55                  | 11 / 16                             | 60.7 / 8.24                            | <i>T. aestivum</i>                    | Ta2606253405-2                              | GI:170696                      |
| 163      | Wheat EST match                                      | 348                                    | 7S Globulin storage protein                           | 24 / 6.55                  | 14 / 18                             | 60.7 / 8.24                            | <i>T. aestivum</i>                    | Ta2606253405-2                              | GI:170696                      |
| 164      | Putative alpha 1 subunit of 20S proteasome           | 230                                    |   | 25 / 6.65                  | 14 / 31                             | 27.6 / 6.19                            | <i>O. sativa</i>                      | Q7G665_ORYSA                                |                                |
| 165      | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 166      | Small heat shock protein Hsp23.5 precursor           | 73                                     |   | 17 / 5.2                   | 7 / 27                              | 23.4 / 6.22                            | <i>T. aestivum</i>                    | Q9ZP25_WHEAT                                |                                |
| 167      | Heat shock protein HSP26                             | 55                                     |   | 18 / 5.4                   | 17 / 58                             | 26.6 / 7.88                            | <i>T. aestivum</i>                    | Q9ZSR5_WHEAT                                |                                |
| 168      | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 169      | Wheat EST match                                      | 207                                    | Unnamed protein product                               | 23 / 5.65                  | 37 / 54                             | 36.9 / 9.06                            | <i>T. aestivum</i>                    | Ta13211-3                                   | GI:21813                       |
| 170      | Heat shock protein HSP26                             | 33                                     |   | 20 / 5.75                  | 14 / 41                             | 26.5 / 9.36                            | <i>T. aestivum</i>                    | Q9ZSR6_WHEAT                                |                                |
| 171      | Wheat EST match                                      | 122                                    | Cupin family protein, expressed                       | 23.5 / 5.8                 | 6 / 31                              | 17.95 / 5.6                            | <i>O. sativa</i>                      | Ta132809-2                                  | GI:108706671                   |
| 172      | Wheat EST match                                      | 158                                    | Unnamed protein product                               | 23 / 5.95                  | 16 / 32                             | 27.9 / 7.27                            | <i>T. aestivum</i>                    | Ta13210-3                                   | GI:21813                       |
| 173      | Wheat EST match                                      | 83                                     | 7S Globulin storage protein                           | 17 / 6.7                   | 24 / 21                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 174      | Wheat EST match                                      | 238                                    | Unnamed protein product                               | 19 / 6.75                  | 26 / 44                             | 36.9 / 9.06                            | <i>T. aestivum</i>                    | Ta13211-3                                   | GI:21813                       |
| 175      | Endogenous alpha-amylase/subtilisin inhibitor (WASI) | 327                                    |   | 19 / 6.85                  | 17 / 60                             | 19.6 / 6.77                            | <i>T. aestivum</i>                    | IAAS_WHEAT                                  |                                |
| 176      | Wheat EST match                                      | 363                                    | 7S Globulin storage protein                           | 15.5 / 4.95                | 16 / 16                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |

**Table A-2.5** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)  | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|---|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 177      | Wheat EST match   | 387                                    | 7S Globulin storage protein                           | 15 / 5                     | 17 / 17                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 178      | Wheat EST match   | 347                                    | 7S Globulin storage protein                           | 5.2 / 5.1                  | 14 / 11                             | 78.9 / 8.75                            | <i>T. aestivum</i>                    | Ta2154253404-2                              | GI:170696                      |
| 179      | Wheat EST match   | 498                                    | 7S Globulin storage protein                           | 5.1 / 5.25                 | 18 / 17                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 180      | Wheat EST match   | 148                                    | 7S Globulin storage protein                           | 16 / 5.25                  | 11 / 32                             | 36.9 / 9.58                            | <i>T. aestivum</i>                    | Ta3079253404-3                              | GI:170696                      |
| 181      | Wheat EST match   | 638                                    | 7S Globulin storage protein                           | 15 / 5.35                  | 21 / 22                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 182      | Wheat EST match   | 571                                    | 7S Globulin storage protein                           | 15 / 5.4                   | 14 / 16                             | 61.7 / 8.08                            | <i>T. aestivum</i>                    | Ta3078253410-1                              | GI:170696                      |
| 183      | Wheat EST match   | 544                                    | 7S Globulin storage protein                           | 15 / 5.45                  | 23 / 25                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 184      | Wheat EST match   | 600                                    | 7S Globulin storage protein                           | 15 / 5.55                  | 14 / 16                             | 61.7 / 8.08                            | <i>T. aestivum</i>                    | Ta3078253410-1                              | GI:170696                      |
| 185      | Wheat EST match   | 227                                    | 7S Globulin storage protein                           | 15.5 / 5.6                 | 15 / 17                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 186      | Wheat EST match   | 248                                    | Heat shock protein 16.9B                              | 15.5 / 5.75                | 13 / 27                             | 31.2 / 7.76                            | <i>T. aestivum</i>                    | Ta238116-2                                  | GI:21805                       |
| 187      | Wheat EST match   | 318                                    | 7S Globulin storage protein                           | 16 / 5.7                   | 11 / 31                             | 23.1 / 5.3                             | <i>T. aestivum</i>                    | Ta9905-2                                    | GI:170696                      |
| 188      | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 189      | Wheat EST match   | 502                                    | 7S Globulin storage protein                           | 15 / 5.8                   | 20 / 27                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 190      | Wheat EST match   | 359                                    | 7S Globulin storage protein                           | 15 / 5.9                   | 13 / 14                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 191      | Wheat EST match   | 85                                     | OSJNBa0027P08.9                                       | 16 / 5.9                   | 15 / 22                             | 36.9 / 9.06                            | <i>O. sativa</i>                      | Ta13211-3                                   | GI:50924568                    |
| 192      | 16.9 kDa class I heat shock protein<br>(Low molecular weight heat shock<br>protein) | 76                                     |   | 15 / 6.15                  | 6 / 37                              | 16.9 / 5.83                            | <i>T. aestivum</i>                    | HSP11_WHEAT                                 |                                |
| 193      | 7S Globulin storage protein   | 79                                     |   | 17 / 6.2                   | 8 / 8                               | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 194      | Wheat EST match   | 419                                    | 7S Globulin storage protein                           | 15 / 6.25                  | 15 / 20                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 195      | Heat shock protein 17.9. Pennisetum<br>americanum                                   | 38                                     |   | 16 / 6.45                  | 4 / 13                              | 17.9 / 5.82                            | <i>P. glaucum</i>                     | Q40867_PENAM                                |                                |
| 196      | Wheat EST match   | 131                                    | 7S Globulin storage protein                           | 16 / 6.6                   | 13 / 35                             | 20.5 / 8.42                            | <i>T. aestivum</i>                    | Ta194625-1                                  | GI:170696                      |
| 197      | OJ991113_30.23 protein  | 28                                     |   | 15 / 6.9                   | 3 / 60                              | 4.8 / 4.53                             | <i>O. sativa</i>                      | Q7XSA9_ORYSA                                |                                |
| 198      | Wheat EST match   | 30                                     | Putative ribophorin I homologue                       | 13.5 / 4.6                 | 8 / 15                              | 21.7 / 9.4                             | <i>H. vulgare</i>                     | Ta127531-2                                  | GI:2894378                     |
| 199      | Wheat EST match   | 125                                    | 7S Globulin storage protein                           | 14.5 / 4.8                 | 10 / 28                             | 36.9 / 9.58                            | <i>T. aestivum</i>                    | Ta3079253404-3                              | GI:170696                      |
| 200      | Wheat EST match   | 317                                    | 7S Globulin storage protein                           | 14 / 4.85                  | 13 / 35                             | 36.9 / 9.58                            | <i>T. aestivum</i>                    | Ta3079253404-3                              | GI:170696                      |
| 201      | Wheat EST match   | 259                                    | 7S Globulin storage protein                           | 13.5 / 4.95                | 14 / 15                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 202      | Wheat EST match   | 447                                    | 7S Globulin storage protein                           | 14 / 5.0                   | 16 / 15                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 203      | Wheat EST match   | 436                                    | 7S Globulin storage protein                           | 15.5 / 5.05                | 17 / 21                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 204      | Wheat EST match   | 355                                    | 7S Globulin storage protein                           | 15 / 5.15                  | 15 / 17                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 205      | Wheat EST match   | 369                                    | 7S Globulin storage protein                           | 15 / 5.2                   | 16 / 16                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |

**Table A-2.5** (continued)

| Spot No. | Protein name<br>(Mascot cereal search) | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup>   | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 206      | Wheat EST match                        | 478                                    | 7S Globulin storage protein                             | 15 / 5.35                  | 16 / 17                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 207      | Wheat EST match                        | 561                                    | 7S Globulin storage protein                             | 15 / 5.3                   | 23 / 25                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 208      | Wheat EST match                        | 357                                    | 7S Globulin storage protein                             | 15 / 5.15                  | 13 / 14                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 209      | 16.9 kDa class I heat shock protein    | 181                                    |   | 15 / 5.7                   | 11 / 47                             | 16.9 / 5.83                            | <i>T. aestivum</i>                    | HSP11_WHEAT                                 |                                |
| 210      | No match                               |  |   |                            |                                     |  |                                       |   |                                |
| 211      | Wheat EST match                        | 555                                    | 7S Globulin storage protein                             | 14 / 6.1                   | 17 / 17                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 212      | Wheat EST match                        | 488                                    | 7S Globulin storage protein                             | 14 / 6.2                   | 13 / 13                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 213      | 7S Globulin storage protein            | 41                                     |   | 14 / 6.5                   | 28 / 29                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 214      | Wheat EST match                        | 556                                    | 7S Globulin storage protein                             | 14 / 6.7                   | 17 / 17                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 215      | Wheat EST match                        | 443                                    | 7S Globulin storage protein                             | 15 / 6.9                   | 15 / 17                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 216      | Wheat EST match                        | 197                                    | 7S Globulin storage protein                             | 12.5 / 4.9                 | 10 / 13                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 217      | Wheat EST match                        | 176                                    | 7S Globulin storage protein                             | 13 / 4.9                   | 12 / 19                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 218      | Wheat EST match                        | 405                                    | 7S Globulin storage protein                             | 14 / 14.95                 | 15 / 18                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 219      | Wheat EST match                        | 212                                    | 7S Globulin storage protein                             | 12 / 4.95                  | 10 / 13                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 220      | Wheat EST match                        | 91                                     | 7S Globulin storage protein                             | 13 / 5.2                   | 13 / 14                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 221      | Wheat EST match                        | 421                                    | 7S Globulin storage protein                             | 14 / 5.2                   | 18 / 19                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 222      | Wheat EST match                        | 489                                    | 7S Globulin storage protein                             | 14.95 / 5.2                | 15 / 30                             | 36.9 / 9.58                            | <i>T. aestivum</i>                    | Ta3079253404-3                              | GI:170696                      |
| 223      | Wheat EST match                        | 376                                    | 7S Globulin storage protein                             | 14 / 5.3                   | 16 / 17                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 224      | Wheat EST match                        | 338                                    | 7S Globulin storage protein                             | 14 / 5.35                  | 19 / 21                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 225      | Wheat EST match                        | 438                                    | 7S Globulin storage protein                             | 14 / 5.3                   | 18 / 21                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 226      | Wheat EST match                        | 332                                    | 7S Globulin storage protein                             | 14 / 5.5                   | 16 / 17                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 227      | Wheat EST match                        | 280                                    | Glyoxalase family protein, expressed                    | 12 / 5.55                  | 13 / 26                             | 31.3 / 9.25                            | <i>O. sativa</i>                      | Ta886253410-2                               | GI:108707474                   |
| 228      | Wheat EST match                        | 52                                     | 7S Globulin storage protein                             | 11 / 5.2                   | 6 / 11                              | 52.8 / 9.69                            | <i>T. aestivum</i>                    | Ta627253413-2                               | GI:170696                      |
| 229      | Wheat EST match                        | 518                                    | 7S Globulin storage protein                             | 13 / 5.75                  | 18 / 17                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 230      | ABA inducible protein                  | 19                                     |   | 12 / 5.9                   | 6 / 27                              | 17.5 / 5.95                            | <i>T. aestivum</i>                    | Q7XAP5_WHEAT                                |                                |
| 231      | Wheat EST match                        | 120                                    | 7S Globulin storage protein                             | 11 / 5.9                   | 9 / 11                              | 52.8 / 9.69                            | <i>T. aestivum</i>                    | Ta627253413-2                               | GI:170696                      |
| 232      | Wheat EST match                        | 138                                    | Glyoxalase family protein, expressed                    | 12 / 6.35                  | 9 / 18                              | 31.3 / 9.25                            | <i>O. sativa</i>                      | Ta886253410-2                               | GI:108707474                   |
| 233      | Wheat EST match                        | 213                                    | Nucleoside diphosphate kinase 1,<br>putative, expressed | 13 / 6.45                  | 10 / 25                             | 29.3 / 9.51                            | <i>O. sativa</i>                      | Ta14950-3                                   | GI:31433540                    |

**Table A-2.5** (continued)

| Spot No. | Protein name<br>(Mascot cereal search) | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(KDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 234      | Wheat EST match                        | 50                                     | 7S Globulin storage protein                           | 13 / 6.6                   | 13 / 16                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 235      | PR-4 (Fragment)                        | 47                                     |   | 12 / 6.8                   | 2 / 13                              | 13.1 / 6.28                            | <i>T. aestivum</i>                    | Q9SQG4_WHEAT                                |                                |
| 236      | Transposase (Fragment)                 | 24                                     |   | 12.5 / 6.9                 | 8 / 29                              | 14.6 / 9.48                            | <i>T. aestivum</i>                    | Q8W1P3_WHEAT                                |                                |
| 237      | Wheat EST match                        | 80                                     | 7S Globulin storage protein                           | 11 / 6.9                   | 6 / 6                               | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |

**Table A-2.6** Inner bran fraction (aleurone cells) proteins identified from 2D gel pH 6-11

| Spot No. | Protein name (Mascot cereal search) | Mascot or GPM <sup>1</sup> score | Protein name (Wheat EST BLAST search) <sup>2</sup> | Observed MW (KDa)/pI | Matching peptides / %Coverage | Theoretical <sup>3</sup> MW (Da)/pI | Species with homologous protein | Swiss-Prot accession number <sup>4</sup> | GenBank accession number |
|----------|-------------------------------------|----------------------------------|--|----------------------|-------------------------------|-------------------------------------|---------------------------------|--|--------------------------|
| 1        | Wheat EST match                     | 32                               | 7S Globulin storage protein                        | 78 / 6.7             | 28 / 39                       | 66.9 / 6.85                         | <i>T. aestivum</i>              | Ta3078253407-1                           | GI:170696                |
| 2        | 7S Globulin storage protein         | 62                               |  | 110 / 6.7            | 19 / 23                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 3        | 7S Globulin storage protein         | 48                               |  | 75 / 6.7             | 16 / 16                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 4        | Wheat EST match                     | 98                               | 7S Globulin storage protein                        | 78 / 6.8             | 31 / 42                       | 66.9 / 6.85                         | <i>T. aestivum</i>              | Ta3078253407-1                           | GI:170696                |
| 5        | 7S Globulin storage protein         | 69                               |  | 75 / 6.9             | 25 / 28                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 6        | Wheat EST match                     | 140                              | 7S Globulin storage protein                        | 78 / 7.1             | 28 / 36                       | 66.9 / 6.85                         | <i>T. aestivum</i>              | Ta3078253407-1                           | GI:170696                |
| 7        | Wheat EST match                     | 49                               | 7S Globulin storage protein                        | 78 / 7.2             | 33 / 45                       | 66.9 / 6.85                         | <i>T. aestivum</i>              | Ta3078253407-1                           | GI:170696                |
| 8        | 7S Globulin storage protein         | 43                               |  | 73 / 6.9             | 26 / 36                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 9        | 7S Globulin storage protein         | 37                               |  | 75 / 6.9             | 18 / 19                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 10       | 7S Globulin storage protein         | 113                              |  | 74 / 6.9             | 26 / 25                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 11       | 7S Globulin storage protein         | 80                               |  | 75 / 7.1             | 23 / 27                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 12       | Wheat EST match                     | 104                              | 7S Globulin storage protein                        | 74 / 7.4             | 35 / 41                       | 78.9 / 8.75                         | <i>T. aestivum</i>              | Ta2154253404-2                           | GI:170696                |
| 13       | 7S Globulin storage protein         | 50                               |  | 72 / 6.9             | 30 / 36                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 14       | Wheat EST match                     | 187                              | 7S Globulin storage protein                        | 72 / 6.9             | 36 / 49                       | 66.9 / 6.85                         | <i>T. aestivum</i>              | Ta3078253407-1                           | GI:170696                |
| 15       | Wheat EST match                     | 162                              | 7S Globulin storage protein                        | 74 / 6.95            | 33 / 44                       | 66.9 / 6.85                         | <i>T. aestivum</i>              | Ta3078253407-1                           | GI:170696                |
| 16       | 7S Globulin storage protein         | 56                               |  | 73 / 6.95            | 23 / 33                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 17       | 7S Globulin storage protein         | 63                               |  | 73 / 6.9             | 31 / 29                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 18       | 7S Globulin storage protein         | 67                               |  | 74 / 6.95            | 28 / 29                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 19       | 7S Globulin storage protein         | 120                              |  | 74 / 7.0             | 41 / 38                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 20       | 7S Globulin storage protein         | 98                               |  | 74 / 7.15            | 28 / 34                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 21       | 7S Globulin storage protein         | 95                               |  | 74 / 7.2             | 23 / 25                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 22       | 7S Globulin storage protein         | 117                              |  | 75 / 7.2             | 30 / 39                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 23       | 7S Globulin storage protein         | 114                              |  | 74 / 7.25            | 33 / 30                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 24       | 7S Globulin storage protein         | 102                              |  | 75 / 7.55            | 21 / 23                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 25       | 7S Globulin storage protein         | 49                               |  | 75 / 7.4             | 15 / 18                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 26       | 7S Globulin storage protein         | 88                               |  | 73 / 7.55            | 25 / 26                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 27       | 7S Globulin storage protein         | 89                               |  | 73 / 7.65            | 20 / 23                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 28       | 7S Globulin storage protein         | 77                               |  | 73 / 7.8             | 20 / 22                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 29       | Wheat EST match                     | 135                              | 7S Globulin storage protein                        | 73 / 7.85            | 38 / 40                       | 76.3 / 9.22                         | <i>T. aestivum</i>              | Ta3078253413-3                           | GI:170696                |



**Table A-2.6** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)     | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 30       | Cytosolic NADP malic enzyme                | 74                                     |   | 69 / 6.1                   | 27 / 32                             | 64.2 / 6.5                             | <i>T. aestivum</i>                    | Q5JKW5_ORYSA                                |                                |
| 31       | Wheat EST match                            | 50                                     | Cytosolic NADP malic enzyme                           | 71 / 6.15                  | 18 / 54                             | 25.8 / 8.9                             | <i>O. sativa</i>                      | Ta965253413-3                               | GI:38261493                    |
| 32       | Wheat EST match                            | 169                                    | 7S Globulin storage protein                           | 72 / 6.45                  | 30 / 40                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 33       | 7S Globulin storage protein                | 64                                     |   | 72 / 6.6                   | 26 / 31                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 34       | 7S Globulin storage protein                | 84                                     |   | 72 / 6.7                   | 28 / 28                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 35       | Wheat EST match                            | 209                                    | 7S Globulin storage protein                           | 72 / 6.75                  | 36 / 47                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 36       | 7S Globulin storage protein                | 86                                     |   | 73 / 6.95                  | 32 / 36                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 37       | 7S Globulin storage protein                | 64                                     |   | 72 / 6.95                  | 27 / 29                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 38       | 7S Globulin storage protein                | 34                                     |   | 72 / 7.0                   | 26 / 36                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 39       | 7S Globulin storage protein                | 122                                    |   | 73 / 7.05                  | 24 / 26                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 40       | 7S Globulin storage protein                | 135                                    |   | 73 / 7.15                  | 32 / 36                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 41       | 7S Globulin storage protein                | 72                                     |   | 72 / 7.2                   | 31 / 29                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 42       | 7S Globulin storage protein                | 108                                    |   | 74 / 7.35                  | 27 / 22                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 43       | 7S Globulin storage protein                | 102                                    |   | 73 / 7.4                   | 27 / 29                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 44       | 7S Globulin storage protein                | 109                                    |   | 74 / 7.5                   | 30 / 40                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 45       | 7S Globulin storage protein                | 89                                     |   | 73 / 7.6                   | 25 / 28                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 46       | 7S Globulin storage protein                | 80                                     |   | 73 / 7.7                   | 20 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 47       | Hypothetical protein P0431E05.13           | 38                                     |   | 73 / 7.9                   | 10 / 53                             | 16.5 / 11.34                           | <i>O. sativa</i>                      | Q653N9_ORYSA                                |                                |
| 48       | 7S Globulin storage protein                | 119                                    |   | 72 / 8.1                   | 11 / 7                              | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 49       | 7S Globulin storage protein                | 157                                    |   | 72 / 8.2                   | 17 / 15                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 50       | 7S Globulin storage protein                | 114                                    |   | 72 / 7.9                   | 14 / 15                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 51       | 7S Globulin storage protein                | 76                                     |   | 72 / 8.3                   | 12 / 10                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 52       | 7S Globulin storage protein                | 57                                     |   | 72 / 8.4                   | 12 / 11                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 53       | 7S Globulin storage protein                | 69                                     |   | 72 / 8.4                   | 12 / 10                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 54       | 7S Globulin storage protein                | 34                                     |   | 72 / 8.8                   | 14 / 12                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 55       | 7S Globulin storage protein                | 172                                    |   | 75 / 9.5                   | 14 / 12                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 56       | 7S Globulin storage protein                | 47                                     |   | 58 / 6.2                   | 25 / 25                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 57       | Putative dihydrolipoamide<br>dehydrogenase | 73                                     |   | 58 / 6.3                   | 25 / 44                             | 52.6 / 7.21                            | <i>O. sativa</i>                      | Q9ASP4_ORYSA                                |                                |

**Table A-2.6 (continued)**

| Spot No. | Protein name<br>(Mascot cereal search) | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 58       | 7S Globulin storage protein            | 44                                     |   | 71 / 6.7                   | 22 / 28                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 59       | Wheat EST match                        | 38                                     | Unknown protein                                       | 70 / 6.8                   | 14 / 39                             | 23.6 / 9.18                            | <i>O. sativa</i>                      | Ta1159253408-3                              | GI:55769693                    |
| 60       | 7S Globulin storage protein            | 84                                     |   | 58 / 6.9                   | 27 / 28                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 61       | 7S Globulin storage protein            | 47                                     |   | 69 / 6.9                   | 30 / 33                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 62       | 7S Globulin storage protein            | 73                                     |   | 61 / 7.1                   | 28 / 31                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 63       | 7S Globulin storage protein            | 79                                     |   | 71 / 6.95                  | 31 / 32                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 64       | Wheat EST match                        | 126                                    | 7S Globulin storage protein                           | 70 / 7.0                   | 34 / 45                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 65       | Wheat EST match                        | 90                                     | 7S Globulin storage protein                           | 71 / 7.05                  | 36 / 40                             | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 66       | 7S Globulin storage protein            | 89                                     |   | 71 / 7.0                   | 32 / 33                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 67       | 7S Globulin storage protein            | 78                                     |   | 60 / 7.0                   | 29 / 38                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 68       | 7S Globulin storage protein            | 91                                     |   | 69 / 7.0                   | 29 / 31                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 69       | Wheat EST match                        | 69                                     | 7S Globulin storage protein                           | 67 / 7.3                   | 20 / 30                             | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 70       | 7S Globulin storage protein            | 106                                    |   | 68 / 7.15                  | 32 / 32                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 71       | 7S Globulin storage protein            | 104                                    |   | 67 / 7.3                   | 24 / 29                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 72       | 7S Globulin storage protein            | 115                                    |   | 67 / 7.6                   | 25 / 29                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 73       | Putative globulin                      | 58                                     |   | 68 / 7.65                  | 9 / 13                              | 52.1 / 6.78                            | <i>T. aestivum</i>                    | Q852L2_ORYSA                                |                                |
| 74       | Wheat EST match                        | 29                                     | 7S Globulin storage protein                           | 56 / 6.65                  | 29 / 18                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 75       | Wheat EST match                        | 57                                     | 7S Globulin storage protein                           | 56 / 6.85                  | 32 / 22                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 76       | Wheat EST match                        | 31                                     | 7S Globulin storage protein                           | 57 / 6.9                   | 29 / 40                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 77       | Wheat EST match                        | 45                                     | 7S Globulin storage protein                           | 60 / 7.1                   | 25 / 29                             | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 78       | 7S Globulin storage protein            | 87                                     |   | 58 / 7.15                  | 26 / 36                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 79       | Wheat EST match                        | 62                                     | 7S Globulin storage protein                           | 60 / 7.2                   | 30 / 32                             | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 80       | 7S Globulin storage protein            | 51                                     |   | 67 / 7.25                  | 23 / 26                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 81       | 7S Globulin storage protein            | 113                                    |   | 59 / 7.25                  | 24 / 26                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 82       | 7S Globulin storage protein            | 128                                    |   | 50 / 7.35                  | 34 / 36                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 83       | 7S Globulin storage protein            | 46                                     |   | 59 / 7.35                  | 23 / 32                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 84       | 7S Globulin storage protein            | 97                                     |   | 59 / 7.5                   | 29 / 34                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 85       | 7S Globulin storage protein            | 95                                     |   | 67 / 7.4                   | 22 / 25                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 86       | 7S Globulin storage protein            | 36                                     |   | 70 / 7.5                   | 14 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |

**Table A-2.6 (continued)**

| Spot No. | Protein name<br>(Mascot cereal search) | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 87       | 7S Globulin storage protein            | 105                                    |   | 67 / 7.4                   | 29 / 33                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 88       | 7S Globulin storage protein            | 28                                     |   | 55 / 7.6                   | 15 / 24                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 89       | 7S Globulin storage protein            | 83                                     |   | 66 / 7.8                   | 22 / 22                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 90       | Putative cell death associated protein | 35                                     |   | 59 / 7.8                   | 11 / 34                             | 36 / 5.26                              | <i>O. sativa</i>                      | Q8GSJ3_ORYSA                                |                                |
| 91       | 7S Globulin storage protein            | 108                                    |   | 67 / 7.8                   | 18 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 92       | 7S Globulin storage protein            | 104                                    |   | 62 / 7.9                   | 23 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 93       | Globulin-like protein                  | 36                                     |   | 55 / 8.0                   | 5 / 10                              | 52 / 6.78                              | <i>O. sativa</i>                      | Q8L8I0_ORYSA                                |                                |
| 94       | 7S Globulin storage protein            | 39                                     |   | 72 / 8.0                   | 11 / 12                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 95       | 7S Globulin storage protein            | 72                                     |   | 70 / 8.2                   | 13 / 11                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 96       | Wheat EST match                        | 41                                     | Alanine aminotransferase                              | 50 / 6.2                   | 18 / 28                             | 47.1 / 9.35                            | <i>H. vulgare</i>                     | Ta209289-1                                  | GI:469148                      |
| 97       | Wheat EST match                        | 99                                     | 7S Globulin storage protein                           | 52 / 6.4                   | 28 / 36                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 98       | 7S Globulin storage protein            | 52                                     |   | 53 / 6.6                   | 28 / 32                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 99       | 7S Globulin storage protein            | 70                                     |   | 53 / 6.5                   | 21 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 100      | Wheat EST match                        | 30                                     | 7S Globulin storage protein                           | 53 / 6.9                   | 22 / 56                             | 41.3 / 9.42                            | <i>T. aestivum</i>                    | Ta3078253409-1                              | GI:170696                      |
| 101      | 7S Globulin storage protein            | 71                                     |   | 53 / 7.0                   | 21 / 24                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 102      | Wheat EST match                        | 41                                     | 7S Globulin storage protein                           | 56 / 7.0                   | 21 / 26                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 103      | 7S Globulin storage protein            | 64                                     |   | 52 / 7.1                   | 23 / 25                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 104      | 7S Globulin storage protein            | 47                                     |   | 50 / 6.9                   | 22 / 31                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 105      | Globulin-like protein                  | 37                                     |   | 57 / 7.1                   | 13 / 21                             | 52.0 / 6.78                            | <i>O. sativa</i>                      | Q8L8I0_ORYSA                                |                                |
| 106      | 7S Globulin storage protein            | 62                                     |   | 50 / 7.2                   | 18 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 107      | Wheat EST match                        | 141                                    | 7S Globulin storage protein                           | 50 / 7.35                  | 21 / 33                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 108      | 7S Globulin storage protein            | 116                                    |   | 50 / 7.2                   | 21 / 23                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 109      | Globulin-like protein                  | 51                                     |   | 48 / 7.2                   | 5 / 8                               | 52 / 6.78                              | <i>O. sativa</i>                      | Q8L8I0_ORYSA                                |                                |
| 110      | Wheat EST match                        | 45                                     | 7S Globulin storage protein                           | 56 / 7.15                  | 35 / 34                             | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 111      | Wheat EST match                        | 32                                     | 7S Globulin storage protein                           | 51 / 7.25                  | 33 / 40                             | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 112      | Wheat EST match                        | 35                                     | 7S Globulin storage protein                           | 49 / 7.25                  | 22 / 47                             | 41.3 / 9.42                            | <i>T. aestivum</i>                    | Ta3078253409-1                              | GI:170696                      |
| 113      | Wheat EST match                        | 29                                     | 7S Globulin storage protein                           | 49 / 7.4                   | 20 / 47                             | 41.3 / 9.42                            | <i>T. aestivum</i>                    | Ta3078253409-1                              | GI:170696                      |
| 114      | 7S Globulin storage protein            | 141                                    |   | 50 / 7.5                   | 17 / 17                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |

**Table A-2.6 (continued)**

| Spot No. | Protein name<br>(Mascot cereal search)                         | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 115      | 7S Globulin storage protein                                    | 32                                     |   | 72 / 7.5                   | 13 / 15                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 116      | 7S Globulin storage protein                                    | 122                                    |   | 55 / 7.7                   | 21 / 23                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 117      | 7S Globulin storage protein                                    | 112                                    |   | 50 / 7.8                   | 12 / 8                              | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 118      | 7S Globulin storage protein                                    | 97                                     |   | 52 / 7.9                   | 24 / 28                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 119      | 7S Globulin storage protein                                    | 166                                    |   | 50 / 7.9                   | 18 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 120      | 7S Globulin storage protein                                    | 151                                    |   | 49 / 8.0                   | 24 / 25                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 121      | 7S Globulin storage protein                                    | 131                                    |   | 50 / 8.2                   | 19 / 18                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 122      | 7S Globulin storage protein                                    | 68                                     |   | 50 / 8.3                   | 14 / 12                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 123      | 7S Globulin storage protein                                    | 145                                    |   | 50 / 8.1                   | 18 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 124      | 7S Globulin storage protein                                    | 135                                    |   | 50 / 8.2                   | 17 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 125      | 7S Globulin storage protein                                    | 80                                     |   | 50 / 8.3                   | 10 / 7                              | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 126      | 7S Globulin storage protein                                    | 164                                    |   | 50 / 8.4                   | 20 / 20                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 127      | 7S Globulin storage protein                                    | 170                                    |   | 50 / 8.5                   | 20 / 17                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 128      | 7S Globulin storage protein                                    | 157                                    |   | 51 / 8.6                   | 16 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 129      | 7S Globulin storage protein                                    | 190                                    |   | 50 / 8.6                   | 22 / 23                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 130      | 7S Globulin storage protein                                    | 147                                    |   | 49 / 8.7                   | 18 / 15                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 131      | 7S Globulin storage protein                                    | 155                                    |   | 50 / 8.8                   | 19 / 24                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 132      | 7S Globulin storage protein                                    | 138                                    |   | 50 / 8.9                   | 17 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 133      | 7S Globulin storage protein                                    | 162                                    |   | 50 / 9.0                   | 17 / 18                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 134      | 7S Globulin storage protein                                    | 160                                    |   | 50 / 9.1                   | 17 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 135      | 7S Globulin storage protein                                    | 156                                    |   | 50 / 9.2                   | 13 / 12                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 136      | 7S Globulin storage protein                                    | 165                                    |   | 50 / 9.1                   | 16 / 20                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 137      | Elongation factor 1-alpha<br>(EF-1-alpha)                      | (EF-1-alpha) 41                        |   | 50 / 9.3                   | 9 / 14                              | 49.1 / 9.2                             | <i>T. aestivum</i>                    | EF1A_WHEAT                                  |                                |
| 138      | Elongation factor 1-alpha<br>(EF-1-alpha)                      | (EF-1-alpha) 74                        |   | 50 / 9.4                   | 10 / 18                             | 49.1 / 9.2                             | <i>T. aestivum</i>                    | EF1A_WHEAT                                  |                                |
| 139      | Elongation factor 1-alpha<br>(EF-1-alpha)                      | (EF-1-alpha) 60                        |   | 50 / 9.5                   | 11 / 23                             | 49.1 / 9.2                             | <i>T. aestivum</i>                    | EF1A_WHEAT                                  |                                |
| 140      | Formate dehydrogenase, mitochondrial<br>precursor (EC 1.2.1.2) | 93                                     |   | 45 / 6.4                   | 15 / 30                             | 41.5 / 6.9                             | <i>H. vulgare</i>                     | FDH_HORVU                                   |                                |

**Table A-2.6 (continued)**

| Spot No. | Protein name<br>(Mascot cereal search)                                  | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|---|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 141      | Formate dehydrogenase (Fragment)  | 165                                    |   | 45 / 6.5                   | 14 / 48                             | 28.99 / 8.61                           | <i>T. aestivum</i>                    | Q7X9L3_WHEAT                                |                                |
| 142      | Citrate synthase, eukaryotic  | 43                                     |   | 47 / 6.6                   | 13 / 27                             | 52.3 / 7.71                            | <i>O. sativa</i>                      | Q2R339_ORYSA                                |                                |
| 143      | Wheat EST match   | 53                                     | 7S Globulin storage protein                           | 45 / 6.6                   | 35 / 43                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 144      | Wheat EST match   | 48                                     | 7S Globulin storage protein                           | 42 / 6.9                   | 21 / 26                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 145      | Glyceraldehyde-3-phosphate<br>dehydrogenase, cytosolic<br>(EC 1.2.1.12) | 51                                     |   | 47 / 7.1                   | 19 / 39                             | 36.5 / 6.67                            | <i>H. vulgare</i>                     | G3PX_HORVU                                  |                                |
| 146      | 7S Globulin storage protein   | 103                                    |   | 50 / 7.1                   | 28 / 35                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 147      | Wheat EST match   | 150                                    | 7S Globulin storage protein                           | 48 / 7.1                   | 17 / 29                             | 61.7 / 8.08                            | <i>T. aestivum</i>                    | Ta3078253410-1                              | GI:170696                      |
| 148      | 7S Globulin storage protein   | 72                                     |   | 50 / 7.25                  | 24 / 30                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 149      | 7S Globulin storage protein   | 62                                     |   | 51 / 7.4                   | 12 / 14                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 150      | 7S Globulin storage protein   | 94                                     |   | 50 / 7.3                   | 23 / 28                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 151      | 7S Globulin storage protein   | 84                                     |   | 50 / 7.4                   | 25 / 26                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 152      | 7S Globulin storage protein   | 136                                    |   | 47 / 7.5                   | 18 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 153      | 7S Globulin storage protein   | 115                                    |   | 50 / 7.6                   | 21 / 24                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 154      | 7S Globulin storage protein   | 79                                     |   | 50 / 7.7                   | 21 / 25                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 155      | 7S Globulin storage protein   | 106                                    |   | 45 / 7.6                   | 14 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 156      | 7S Globulin storage protein   | 141                                    |   | 50 / 7.8                   | 18 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 157      | 7S Globulin storage protein   | 102                                    |   | 47 / 7.9                   | 12 / 7                              | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 158      | 7S Globulin storage protein   | 112                                    |   | 49 / 7.8                   | 14 / 13                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 159      | 7S Globulin storage protein   | 89                                     |   | 47 / 7.9                   | 14 / 13                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 160      | 7S Globulin storage protein   | 125                                    |   | 50 / 7.7                   | 15 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 161      | Transposase   | 23                                     |   | 50 / 8                     | 5 / 75                              | 5.58 / 12.19                           | <i>X. oryzae</i>                      | Q5GYA8_XANOR                                |                                |
| 162      | Wheat EST match   | 112                                    | 7S Globulin storage protein                           | 45 / 8.0                   | 17 / 27                             | 61.7 / 8.08                            | <i>T. aestivum</i>                    | Ta3078253410-1                              | GI:170696                      |
| 163      | 7S Globulin storage protein   | 148                                    |   | 50 / 8.1                   | 18 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 164      | 7S Globulin storage protein   | 157                                    |   | 47 / 8.2                   | 17 / 14                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 165      | 7S Globulin storage protein   | 153                                    |   | 47 / 8.3                   | 15 / 13                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 166      | 7S Globulin storage protein   | 120                                    |   | 47 / 8.5                   | 13 / 11                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 167      | 7S Globulin storage protein   | 178                                    |   | 47 / 8.5                   | 18 / 15                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |

**Table A-2.6 (continued)**

| Spot No. | Protein name<br>(Mascot cereal search)                                  | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(KDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|---|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 168      | 7S Globulin storage protein   | 167                                    |   | 47 / 8.5                   | 16 / 12                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 169      | 7S Globulin storage protein   | 173                                    |   | 47 / 8.6                   | 16 / 15                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 170      | 7S Globulin storage protein   | 128                                    |   | 47 / 8.8                   | 15 / 12                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 171      | 7S Globulin storage protein   | 128                                    |   | 47 / 9.2                   | 18 / 17                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 172      | 7S Globulin storage protein   | 87                                     |   | 47 / 9.1                   | 21 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 173      | 7S Globulin storage protein   | 122                                    |   | 47 / 9.3                   | 18 / 18                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 174      | 7S Globulin storage protein   | 143                                    |   | 47 / 9.4                   | 16 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 175      | 7S Globulin storage protein   | 101                                    |   | 46 / 9.6                   | 12 / 11                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 176      | 7S Globulin storage protein   | 113                                    |   | 47 / 9.5                   | 11 / 8                              | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 177      | 7S Globulin storage protein   | 138                                    |   | 46 / 9.6                   | 20 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 178      | 7S Globulin storage protein   | 95                                     |   | 44 / 9.6                   | 12 / 14                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 179      | Cytoplasmic aldolase  | 31                                     |   | 43 / 6.1                   | 20 / 36                             | 38.7 / 6.56                            | <i>O. sativa</i>                      | Q40676_ORYSA                                |                                |
| 180      | Enolase (EC 4.2.1.11)   | 86                                     |   | 44 / 6.2                   | 26 / 48                             | 47.9 / 5.41                            | <i>O. sativa</i>                      | ENO_ORYSA                                   |                                |
| 181      | Aspartate aminotransferase precursor<br>(EC 2.6.1.1)                    | 29                                     |   | 43 / 6.2                   | 11 / 16                             | 47.6 / 6.71                            | <i>P. miliaceum</i>                   | Q43305_PANMI                                |                                |
| 182      | Glyceraldehyde-3-phosphate<br>dehydrogenase, cytosolic (EC<br>1.2.1.12) | 47                                     |   | 42 / 6.3                   | 15 / 36                             | 36.5 / 6.67                            | <i>H. vulgare</i>                     | G3PX_HORVU                                  |                                |
| 183      | Glyceraldehyde-3-phosphate<br>dehydrogenase, cytosolic (EC<br>1.2.1.12) | 482                                    |   | 42 / 6.5                   | 24 / 43                             | 36.5 / 6.67                            | <i>H. vulgare</i>                     | G3PX_HORVU                                  |                                |
| 184      | Putative fructose 1-,6-biphosphate<br>aldolase (Fragment)               | 44                                     |   | 38 / 6.5                   | 5 / 19                              | 29.1 / 8.71                            | <i>T. aestivum</i>                    | Q8VWM9_WHEAT                                |                                |
| 185      | Glyceraldehyde-3-phosphate<br>dehydrogenase, cytosolic (EC<br>1.2.1.12) | 157                                    |   | 42 / 6.8                   | 22 / 39                             | 36.5 / 6.67                            | <i>H. vulgare</i>                     | G3PX_HORVU                                  |                                |
| 186      | Wheat EST match   | 88                                     | 7S Globulin storage protein                           | 42 / 6.8                   | 9 / 9                               | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 187      | Wheat EST match   | 50                                     | 7S Globulin storage protein                           | 42 / 7.1                   | 14 / 56                             | 23.6 / 9.99                            | <i>T. aestivum</i>                    | Ta136340-2                                  | GI:170696                      |
| 188      | Wheat EST match   | 203                                    | 7S Globulin storage protein                           | 43 / 7.2                   | 19 / 25                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 189      | Cytosolic glyceraldehyde-3-phosphate<br>dehydrogenase GAPDH (Fragment)  | 191                                    |   | 42 / 7.2                   | 8 / 29                              | 25.3 / 7.83                            | <i>T. aestivum</i>                    | Q9M4V4_WHEAT                                |                                |

**Table A-2.6** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)                                | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|---|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 190      | Wheat EST match   | 87                                     | 7S Globulin storage protein                           | 42 / 7.25                  | 15 / 14                             | 78.9 / 8.75                            | <i>T. aestivum</i>                    | Ta2154253404-2                              | GI:170696                      |
| 191      | Wheat EST match   | 53                                     | 7S Globulin storage protein                           | 42 / 7.3                   | 14 / 68                             | 23.6 / 9.99                            | <i>T. aestivum</i>                    | Ta136340-2                                  | GI:170696                      |
| 192      | Wheat EST match   | 158                                    | 7S Globulin storage protein                           | 48 / 7.5                   | 19 / 19                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 193      | Wheat EST match   | 25                                     | RNA recognition motif family<br>protein, expressed    | 42 / 7.45                  | 10 / 40                             | 26.5 / 10.010                          | <i>O. sativa</i>                      | Ta212753-3                                  | GI:108707669                   |
| 194      | Wheat EST match   | 166                                    | 7S Globulin storage protein                           | 43 / 7.5                   | 22 / 23                             | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 195      | Wheat EST match   | 101                                    | 7S Globulin storage protein                           | 43 / 7.7                   | 13 / 14                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 196      | Wheat EST match   | 50                                     | 7S Globulin storage protein                           | 42 / 7.9                   | 9 / 30                              | 20.5 / 8.42                            | <i>T. aestivum</i>                    | Ta194625-1                                  | GI:170696                      |
| 197      | Wheat EST match   | 65                                     | 7S Globulin storage protein                           | 42 / 7.9                   | 8 / 18                              | 41.3 / 9.42                            | <i>T. aestivum</i>                    | Ta3078253409-1                              | GI:170696                      |
| 198      | Xylanase inhibitor (Fragment)   | 84                                     |   | 42 / 8.1                   | 8 / 19                              | 40.2 / 8.41                            | <i>T. aestivum</i>                    | Q53IQ4_WHEAT                                |                                |
| 199      | 7S Globulin storage protein   | 92                                     |   | 42 / 8.3                   | 15 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 200      | Wheat EST match   | 121                                    | 7S Globulin storage protein                           | 42 / 8.6                   | 30 / 33                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |
| 201      | Xylanase inhibitor precursor (Xylanase<br>inhibitor TAXI-I)           | 48                                     |   | 42 / 8.6                   | 4 / 6                               | 40.9 / 8.18                            | <i>T. aestivum</i>                    | Q8H0K8_WHEAT                                |                                |
| 202      | Wheat EST match   | 91                                     | 7S Globulin storage protein                           | 43 / 9.1                   | 11 / 9                              | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 203      | 7S Globulin storage protein   | 103                                    |   | 43 / 9.2                   | 11 / 9                              | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 204      | Malate dehydrogenase  | 69                                     |   | 36 / 6.1                   | 18 / 29                             | 35.4 / 8.74                            | <i>O. sativa</i>                      | Q7FSL4_ORYSA                                |                                |
| 205      | Wheat EST match   | 288                                    | O-methyltransferase                                   | 37 / 6.15                  | 27 / 37                             | 51.9 / 7.64                            | <i>T. aestivum</i>                    | Ta94253412-2                                | GI:4098238                     |
| 206      | Putative fructose 1-,6-biphosphate<br>aldolase (Fragment)             | 76                                     |   | 37 / 6.4                   | 8 / 33                              | 29.1 / 8.71                            | <i>T. aestivum</i>                    | Q8VWM9_WHEAT                                |                                |
| 207      | Cytosolic glyceraldehyde-3-phosphate<br>dehydrogenase (Fragment)      | 105                                    |   | 37 / 6.4                   | 13 / 58                             | 18.2 / 6.34                            | <i>T. aestivum</i>                    | Q7XJJ1_WHEAT                                |                                |
| 208      | Wheat EST match   | 175                                    | Putative malate dehydrogenase                         | 36 / 6.45                  | 9 / 21                              | 52.4 / 9.39                            | <i>O. sativa</i>                      | Ta2794-3                                    | GI:50932771                    |
| 209      | Hypothetical protein P0686H11.1<br>(Hypothetical protein P0605H02.41) | 34                                     |   | 37 / 6.5                   | 7 / 74                              | 8.92 / 5.66                            | <i>O. sativa</i>                      | Q6Z8U5_ORYSA                                |                                |
| 210      | Wheat EST match   | 160                                    | 7S Globulin storage protein                           | 38 / 6.9                   | 15 / 56                             | 23.6 / 9.99                            | <i>T. aestivum</i>                    | Ta136340-2                                  | GI:170696                      |
| 211      | Putative glyceraldehyde-3-phosphate<br>dehydrogenase                  | 27                                     |   | 37 / 6.5                   | 14 / 29                             | 36.5 / 7.68                            | <i>O. sativa</i>                      | Q6K5G8_ORYSA                                |                                |

**Table A-2.6 (continued)**

| Spot No. | Protein name (Mascot cereal search)                           | Mascot or GPM <sup>1</sup> score | Protein name (Wheat EST BLAST search) <sup>2</sup> | Observed MW (kDa)/pI | Matching peptides / %Coverage | Theoretical <sup>3</sup> MW (Da)/pI | Species with homologous protein | Swiss-Prot accession number <sup>4</sup> | GenBank accession number |
|----------|---|----------------------------------|--|----------------------|-------------------------------|-------------------------------------|---------------------------------|--|--------------------------|
| 212      | Cytosolic glyceraldehyde-3-phosphate dehydrogenase (Fragment) | 28                               |  | 37 / 7.1             | 7 / 39                        | 18.2 / 6.34                         | <i>T. aestivum</i>              | Q7XJJ1_WHEAT                             |                          |
| 213      | Wheat EST match   | 124                              | 7S Globulin storage protein                        | 42 / 7.2             | 15 / 21                       | 67.9 / 8.86                         | <i>T. aestivum</i>              | Ta628253404-2                            | GI:170696                |
| 214      | Wheat EST match   | 197                              | 7S Globulin storage protein                        | 41 / 7.25            | 13 / 50                       | 30.0 / 7.22                         | <i>T. aestivum</i>              | Ta628253407-1                            | GI:170696                |
| 215      | Globulin-like protein   | 57                               |  | 41 / 7.4             | 5 / 9                         | 52 / 6.78                           | <i>O. sativa</i>                | Q8L8I0_ORYSA                             |                          |
| 216      | Cytosolic glyceraldehyde-3-phosphate dehydrogenase GAPC4      | 159                              |  | 39 / 7.5             | 15 / 34                       | 36.4 / 6.61                         | <i>Z. mays</i>                  | Q43359_MAIZE                             |                          |
| 217      | Wheat EST match   | 289                              | 7S Globulin storage protein                        | 41 / 7.7             | 16 / 42                       | 30.0 / 7.22                         | <i>T. aestivum</i>              | Ta628253407-1                            | GI:170696                |
| 218      | Wheat EST match   | 48                               | Putative S-formylglutathione hydrolase             | 35 / 6.2             | 14 / 26                       | 41.4 / 8.35                         | <i>O. sativa</i>                | Ta56703-2                                | GI:57900400              |
| 219      | Wheat EST match   | 271                              | Glucose and ribitol dehydrogenase homolog          | 36 / 6.45            | 31 / 43                       | 48.3 / 8.17                         | <i>H. vulgare</i>               | Ta631253406-3                            | GI:7431022               |
| 220      | Wheat EST match   | 273                              | Glucose and ribitol dehydrogenase homolog          | 36 / 6.3             | 24 / 46                       | 48.3 / 8.17                         | <i>H. vulgare</i>               | Ta631253406-3                            | GI:7431022               |
| 221      | Stress responsive protein                                     | 28                               |  | 36 / 6.5             | 7 / 32                        | 22.2 / 7.64                         | <i>T. aestivum</i>              | Q4U0C9_WHEAT                             |                          |
| 222      | Putative aldose reductase                                     | 68                               |  | 36 / 6.7             | 12 / 26                       | 35.6 / 6.32                         | <i>O. sativa</i>                | Q65WW3_ORYSA                             |                          |
| 223      | Caleosin 1  | 34                               |  | 35 / 6.5             | 14 / 39                       | 34.2 / 6.11                         | <i>H. vulgare</i>               | Q6UFY8_HORVU                             |                          |
| 224      | Wheat EST match   | 145                              | 7S Globulin storage protein                        | 37 / 6.7             | 18 / 23                       | 67.9 / 8.86                         | <i>T. aestivum</i>              | Ta628253404-2                            | GI:170696                |
| 225      | Wheat EST match   | 116                              | 7S Globulin storage protein                        | 36 / 6.95            | 15 / 56                       | 23.6 / 9.99                         | <i>T. aestivum</i>              | Ta136340-2                               | GI:170696                |
| 226      | Aldose reductase-related protein (EC 1.1.1.21)                | 177                              |  | 36 / 6.9             | 12 / 27                       | 35.8 / 6.28                         | <i>A. fatua</i>                 | Q43320_AVEFA                             |                          |
| 227      | Putative aldose reductase                                     | 168                              |  | 36 / 7               | 11 / 21                       | 35.6 / 6.32                         | <i>O. sativa</i>                | Q65WW3_ORYSA                             |                          |
| 228      | Wheat EST match   | 91                               | 7S Globulin storage protein                        | 37 / 7.1             | 10 / 25                       | 41.3 / 9.42                         | <i>T. aestivum</i>              | Ta3078253409-1                           | GI:170696                |
| 229      | Wheat EST match   | 41                               | 7S Globulin storage protein                        | 38 / 7.15            | 12 / 61                       | 23.6 / 9.99                         | <i>T. aestivum</i>              | Ta136340-2                               | GI:170696                |
| 230      | 7S Globulin storage protein                                   | 40                               |  | 37 / 7.3             | 25 / 30                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 231      | Wheat EST match   | 29                               | 7S Globulin storage protein                        | 37 / 7.3             | 13 / 51                       | 23.6 / 9.99                         | <i>T. aestivum</i>              | Ta136340-2                               | GI:170696                |
| 232      | 7S Globulin storage protein                                   | 74                               |  | 37 / 7.45            | 25 / 23                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 233      | Wheat EST match   | 401                              | 7S Globulin storage protein                        | 37 / 7.6             | 30 / 34                       | 69.9 / 8.42                         | <i>T. aestivum</i>              | Ta628253405-1                            | GI:170696                |
| 234      | 7S Globulin storage protein                                   | 81                               |  | 37 / 7.7             | 20 / 20                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |
| 235      | 7S Globulin storage protein                                   | 66                               |  | 36 / 7.75            | 23 / 21                       | 72.2 / 6.8                          | <i>T. aestivum</i>              | Q7DMU0_WHEAT                             |                          |



**Table A-2.6** (continued)

| Spot No. | Protein name<br>(Mascot cereal search) | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 236      | Wheat EST match                        | 82                                     | 7S Globulin storage protein                           | 36 / 7.75                  | 10 / 16                             | 39.3 / 11.17                           | <i>T. aestivum</i>                    | Ta5443-3                                    | GI:170696                      |
| 237      | 7S Globulin storage protein            | 45                                     |   | 36 / 8.0                   | 18 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 238      | 7S Globulin storage protein            | 95                                     |   | 36 / 8.2                   | 21 / 23                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 239      | 7S Globulin storage protein            | 101                                    |   | 37 / 8.2                   | 20 / 22                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 240      | 7S Globulin storage protein            | 63                                     |   | 36.5 / 8.4                 | 24 / 27                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 241      | 7S Globulin storage protein            | 94                                     |   | 37 / 8.5                   | 22 / 23                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 242      | 7S Globulin storage protein            | 112                                    |   | 36.5 / 8.7                 | 20 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 243      | 7S Globulin storage protein            | 68                                     |   | 37 / 8.8                   | 18 / 18                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 244      | 7S Globulin storage protein            | 142                                    |   | 37 / 8.8                   | 20 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 245      | 7S Globulin storage protein            | 121                                    |   | 37 / 9.1                   | 19 / 20                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 246      | 7S Globulin storage protein            | 149                                    |   | 36.5 / 9.2                 | 24 / 22                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 247      | 7S Globulin storage protein            | 156                                    |   | 37 / 9.2                   | 20 / 20                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 248      | 7S Globulin storage protein            | 144                                    |   | 37 / 9.3                   | 22 / 22                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 249      | 7S Globulin storage protein            | 148                                    |   | 38 / 9.4                   | 17 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 250      | 7S Globulin storage protein            | 81                                     |   | 38 / 9.4                   | 13 / 13                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 251      | 7S Globulin storage protein            | 153                                    |   | 38 / 9.5                   | 18 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 252      | 7S Globulin storage protein            | 107                                    |   | 36 / 9.5                   | 11 / 8                              | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 253      | 7S Globulin storage protein            | 134                                    |   | 36 / 9.8                   | 16 / 22                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 254      | Wheat EST match                        | 97                                     | 7S Globulin storage protein                           | 34 / 6.2                   | 14 / 16                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 255      | 7S Globulin storage protein            | 58                                     |   | 32 / 6.2                   | 7 / 8                               | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 256      | Wheat EST match                        | 316                                    | Glucose and ribitol dehydrogenase homolog             | 35 / 6.65                  | 18 / 53                             | 29.6 / 9.51                            | <i>H. vulgare</i>                     | Ta631253408-2                               | GI:7431022                     |
| 256      | Wheat EST match                        | 53                                     | Cupin family protein, expressed                       | 31 / 6.2                   | 10 / 17                             | 60.7 / 8.24                            | <i>O. sativa</i>                      | Ta2606253405-2                              | GI:108708022                   |
| 257      | Wheat EST match                        | 85                                     | Glucose and ribitol dehydrogenase homolog             | 35 / 6.3                   | 28 / 38                             | 48.3 / 8.17                            | <i>H. vulgare</i>                     | Ta631253406-3                               | GI:7431022                     |
| 258      | 7S Globulin storage protein            | 53                                     |   | 34 / 6.4                   | 12 / 16                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 260      | Wheat EST match                        | 191                                    | Glucose and ribitol dehydrogenase homolog             | 36 / 6.65                  | 11 / 24                             | 26.8 / 11.79                           | <i>H. vulgare</i>                     | Ta631253407-3                               | GI:7431022                     |
| 261      | Putative aldose reductase              | 77                                     |   | 35 / 6.9                   | 7 / 16                              | 35.6 / 6.32                            | <i>O. sativa</i>                      | Q65WW3_ORYSA                                |                                |
| 262      | Putative aldose reductase              | 117                                    |   | 35 / 7.1                   | 8 / 17                              | 35.6 / 6.32                            | <i>O. sativa</i>                      | Q65WW3_ORYSA                                |                                |

**Table A-2.6** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)                                  | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|---|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 263      | Wheat EST match   | 34                                     | Glucose and ribitol dehydrogenase homolog             | 33 / 7.1                   | 14 / 31                             | 29.6 / 9.51                            | <i>H. vulgare</i>                     | Ta631253408-2                               | GI:7431022                     |
| 264      | Wheat EST match   | 34                                     | Putative serine/threonine protein kinase              | 30 / 7.15                  | 13 / 36                             | 41.6 / 9.98                            | <i>O. sativa</i>                      | Ta57592-2                                   | GI:50916410                    |
| 265      | Xylanase inhibitor XIP-III  | 57                                     |   | 30 / 7.6                   | 15 / 43                             | 33.3 / 7.14                            | <i>T. aestivum</i>                    | Q4W6G2_WHEAT                                |                                |
| 266      | 7S Globulin storage protein   | 47                                     |   | 30 / 7.9                   | 17 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 267      | Lipoprotein-like  | 105                                    |   | 30 / 8.0                   | 9 / 17                              | 28 / 7.79                              | <i>O. sativa</i>                      | Q94J20_ORYSA                                |                                |
| 268      | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) | 416                                    |   | 30 / 8.4                   | 22 / 43                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 269      | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) | 415                                    |   | 28 / 8.4                   | 23 / 56                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 270      | 7S Globulin storage protein   | 85                                     |   | 36 / 8.5                   | 21 / 24                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 271      | 7S Globulin storage protein   | 96                                     |   | 36 / 8.9                   | 19 / 20                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 272      | 30S ribosomal protein S2  | 62                                     |   | 29 / 8.8                   | 10 / 27                             | 29.5 / 8.73                            | <i>X. oryzae</i>                      | Q2P4A5_XANOR                                |                                |
| 273      | Class II chitinase (EC 3.2.1.14)  | 31                                     |   | 35 / 8.9                   | 4 / 10                              | 28.2 / 8.66                            | <i>T. aestivum</i>                    | Q4Z8L7_WHEAT                                |                                |
| 274      | 7S Globulin storage protein   | 100                                    |   | 36 / 9.1                   | 18 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 275      | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 276      | 7S Globulin storage protein   | 141                                    |   | 36.5 / 9.2                 | 24 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 277      | 7S Globulin storage protein   | 134                                    |   | 36 / 9.2                   | 18 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 278      | Voltage dependent anion channel<br>(VDAC) (Fragment)                    | 61                                     |   | 35 / 9.3                   | 16 / 59                             | 29.3 / 9.33                            | <i>T. aestivum</i>                    | Q41590_WHEAT                                |                                |
| 279      | 7S Globulin storage protein   | 147                                    |   | 36.5 / 9.3                 | 20 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 280      | 7S Globulin storage protein   | 126                                    |   | 37 / 9.2                   | 21 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 281      | 7S Globulin storage protein   | 160                                    |   | 37 / 9.3                   | 21 / 20                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 282      | 7S Globulin storage protein   | 149                                    |   | 37 / 9.4                   | 21 / 17                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 283      | 7S Globulin storage protein   | 150                                    |   | 37 / 9.5                   | 21 / 20                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 284      | Caleosin 1  | 52                                     |   | 26 / 6.2                   | 16 / 37                             | 34.2 / 6.11                            | <i>H. vulgare</i>                     | Q6UFY8_HORVU                                |                                |

**Table A-2.6 (continued)**

| Spot No. | Protein name<br>(Mascot cereal search)                                  | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|---|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 285      | Hypothetical protein P0686H11.1<br>(Hypothetical protein P0605H02.41)   | 27                                     |   | 30 / 6.3                   | 5 / 60                              | 8.92 / 5.66                            | <i>O. sativa</i>                      | Q6Z8U5_ORYSA                                |                                |
| 286      | Wheat EST match   | 41                                     | Cupin family protein, expressed                       | 26 / 6.4                   | 6 / 30                              | 18.9 / 10.14                           | <i>O. sativa</i>                      | Ta189317-3                                  | GI:108706671                   |
| 287      | Putative alpha 1 subunit of 20S<br>proteasome                           | 50                                     |   | 26 / 6.5                   | 10 / 34                             | 27.6 / 6.19                            | <i>O. sativa</i>                      | Q7G665_ORYSA                                |                                |
| 288      | Putative aldose reductase   | 42                                     |   | 24 / 6.5                   | 12 / 27                             | 35.6 / 6.32                            | <i>O. sativa</i>                      | Q65WW3_ORYSA                                |                                |
| 289      | Wheat EST match   | 80                                     | 7S Globulin storage protein                           | 25 / 6.5                   | 17 / 60                             | 23.6 / 9.99                            | <i>T. aestivum</i>                    | Ta136340-2                                  | GI:170696                      |
| 290      | Putative aldose reductase   | 140                                    |   | 24 / 6.7                   | 6 / 16                              | 35.6 / 6.32                            | <i>O. sativa</i>                      | Q65WW3_ORYSA                                |                                |
| 291      | Putative aldose reductase   | 100                                    |   | 24 / 6.9                   | 6 / 16                              | 35.6 / 6.32                            | <i>O. sativa</i>                      | Q65WW3_ORYSA                                |                                |
| 292      | Wheat EST match   | 28                                     | Squamosa promoter binding protein 2-<br>like          | 24 / 7.05                  | 9 / 39                              | 26.2 / 9.91                            | <i>O. sativa</i>                      | Ta3299253405-1                              | GI:53791916                    |
| 293      | Wheat EST match   | 31                                     | Cupin family protein, expressed                       | 23 / 7.1                   | 16 / 22                             | 66.9 / 7.64                            | <i>O. sativa</i>                      | Ta2606253404-2                              | GI:108708022                   |
| 294      | Wheat EST match   | 31                                     | Annexin   | 24 / 7.5                   | 8 / 28                              | 28.7 / 9.4                             | <i>T. aestivum</i>                    | Ta164763-3                                  | GI:38606205                    |
| 295      | Wheat EST match   | 226                                    | Cupin family protein, expressed                       | 23 / 7.5                   | 23 / 23                             | 66.9 / 7.64                            | <i>O. sativa</i>                      | Ta2606253404-2                              | GI:108708022                   |
| 296      | Class II chitinase (EC 3.2.1.14)  | 107                                    |   | 24 / 7.9                   | 8 / 30                              | 28.2 / 8.66                            | <i>T. aestivum</i>                    | Q4Z8L7_WHEAT                                |                                |
| 297      | Xylanase inhibitor protein 1 precursor<br>(Class III chitinase homolog) | 273                                    |   | 30 / 8.1                   | 23 / 50                             | 33.3 / 8.66                            | <i>T. aestivum</i>                    | XIP1_WHEAT;<br>Q8L5C6                       |                                |
| 298      | No match  |  |   |                            |                                     |  |                                       |   |                                |
| 299      | Class II chitinase (EC 3.2.1.14)  | 94                                     |   | 25 / 8.6                   | 9 / 37                              | 28.2 / 8.66                            | <i>T. aestivum</i>                    | Q4Z8L7_WHEAT                                |                                |
| 300      | Class II chitinase (EC 3.2.1.14)  | 99                                     |   | 25 / 8.8                   | 5 / 21                              | 28.2 / 8.66                            | <i>T. aestivum</i>                    | Q4Z8L7_WHEAT                                |                                |
| 301      | 26 kDa endochitinase 1 precursor (EC<br>3.2.1.14)                       | 123                                    |   | 27 / 9.1                   | 8 / 17                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | CHII_HORVU                                  |                                |
| 302      | Actin and related proteins  | 212                                    |   | 30 / 9.2                   | 13 / 31                             | 28.5 / 5.31                            | <i>A. oryzae</i>                      | Q2U9E1_ASPOR                                |                                |
| 303      | Actin and related proteins  | 137                                    |   | 27 / 9.2                   | 12 / 31                             | 28.5 / 5.31                            | <i>A. oryzae</i>                      | Q2U9E1_ASPOR                                |                                |
| 304      | Actin and related proteins  | 106                                    |   | 30 / 9.3                   | 6 / 23                              | 28.5 / 5.31                            | <i>A. oryzae</i>                      | Q2U9E1_ASPOR                                |                                |
| 305      | Class II chitinase (EC 3.2.1.14)  | 74                                     |   | 25 / 9.2                   | 8 / 40                              | 28.2 / 8.66                            | <i>T. aestivum</i>                    | Q4Z8L7_WHEAT                                |                                |
| 306      | 26 kDa endochitinase 1 precursor (EC<br>3.2.1.14)                       | 119                                    |   | 25 / 9.4                   | 8 / 28                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | CHII_HORVU                                  |                                |
| 307      | Wheat EST match   | 74                                     | 7S Globulin storage protein                           | 29 / 9.5                   | 19 / 37                             | 39.3 / 11.17                           | <i>T. aestivum</i>                    | Ta5443-3                                    | GI:170696                      |

**Table A-2.6 (continued)**

| Spot No. | Protein name<br>(Mascot cereal search)                               | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 308      | Putative 40S ribosomal protein S3                                    | 76                                     |   | 25 / 9.5                   | 9 / 22                              | 25.4 / 9.63                            | <i>T. aestivum</i>                    | Q8L804_WHEAT                                |                                |
| 309      | 1-Cys-peroxiredoxine   | 26                                     |   | 24 / 6.1                   | 5 / 19                              | 23.95 / 6.08                           | <i>T. aestivum</i>                    | Q6W8Q2_WHEAT                                |                                |
| 310      | Wheat EST match  | 90                                     | 7S Globulin storage protein                           | 26 / 6.2                   | 21 / 72                             | 23.6 / 9.99                            | <i>T. aestivum</i>                    | Ta136340-2                                  | GI:170696                      |
| 311      | 1-Cys-peroxiredoxine   | 50                                     |   | 24 / 6.4                   | 17 / 52                             | 23.95 / 6.08                           | <i>T. aestivum</i>                    | Q6W8Q2_WHEAT                                |                                |
| 312      | Wheat EST match  | 28                                     | 1-Cys-peroxiredoxine                                  | 25 / 6.45                  | 12 / 29                             | 34.6 / 8.33                            | <i>T. aestivum</i>                    | Ta1386253404-1                              | GI:34539782                    |
| 313      | Prohibitin protein Wph   | 50                                     |   | 23 / 6.6                   | 5 / 15                              | 30.1 / 6.0                             | <i>T. aestivum</i>                    | Q84VJ0_WHEAT                                |                                |
| 314      | Wheat EST match  | 218                                    | Cupin family protein, expressed                       | 21 / 7.1                   | 10 / 14                             | 66.9 / 7.64                            | <i>O. sativa</i>                      | Ta2606253404-2                              | GI:108708022                   |
| 315      | Endogenous alpha-amylase/subtilisin inhibitor (WASI)                 | 59                                     |   | 22 / 7.2                   | 10 / 61                             | 19.6 / 6.77                            | <i>T. aestivum</i>                    | IAAS_WHEAT                                  |                                |
| 316      | Wheat EST match  | 138                                    | Cupin family protein, expressed                       | 19 / 7.5                   | 14 / 20                             | 66.9 / 7.64                            | <i>O. sativa</i>                      | Ta2606253404-2                              | GI:108708022                   |
| 317      | 7S Globulin storage protein  | 52                                     |   | 21 / 8.4                   | 21 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 318      | 7S Globulin storage protein  | 79                                     |   | 21 / 8.5                   | 26 / 29                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 319      | 7S Globulin storage protein  | 60                                     |   | 21 / 8.7                   | 12 / 15                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 320      | Wheat EST match  | 73                                     | P0481E12.28   | 24 / 8.7                   | 14 / 36                             | 37.7 / 7.75                            | <i>O. sativa</i>                      | Ta369-2                                     | GI:34909516                    |
| 321      | Wheat EST match  | 68                                     | 7S Globulin storage protein                           | 25 / 9.05                  | 11 / 20                             | 33.7 / 11.52                           | <i>T. aestivum</i>                    | Ta5446-1                                    | GI:170696                      |
| 322      | Class II chitinase (EC 3.2.1.14)                                     | 102                                    |   | 25 / 9.2                   | 6 / 30                              | 28.2 / 8.66                            | <i>T. aestivum</i>                    | Q4Z8L7_WHEAT                                |                                |
| 323      | Basic endochitinase C precursor (EC 3.2.1.14) (Rye seed chitinase-c) | 43                                     |   | 26 / 9.2                   | 5 / 21                              | 28.3 / 8.82                            | <i>S. cereale</i>                     | CHIC_SECCE                                  |                                |
| 324      | Wheat EST match  | 76                                     | 7S Globulin storage protein                           | 23 / 9.3                   | 10 / 21                             | 39.3 / 11.17                           | <i>T. aestivum</i>                    | Ta5443-3                                    | GI:170696                      |
| 325      | Wheat EST match  | 131                                    | 7S Globulin storage protein                           | 24 / 9.6                   | 14 / 31                             | 39.3 / 11.17                           | <i>T. aestivum</i>                    | Ta5443-3                                    | GI:170696                      |
| 326      | Wheat EST match  | 108                                    | 7S Globulin storage protein                           | 23 / 9.55                  | 14 / 27                             | 38.0 / 9.56                            | <i>T. aestivum</i>                    | Ta5441-3                                    | GI:170696                      |
| 327      | Glutathione transferase (EC 2.5.1.18)                                | 71                                     |   | 23 / 6.3                   | 17 / 52                             | 24.98 / 6.35                           | <i>T. aestivum</i>                    | Q8RW04_WHEAT                                |                                |
| 328      | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 329      | Manganese superoxide dismutase (EC 1.15.1.1)                         | 159                                    |   | 23 / 6.3                   | 13 / 39                             | 25.3 / 7.9                             | <i>T. aestivum</i>                    | P93606_WHEAT                                |                                |
| 330      | Manganese superoxide dismutase (EC 1.15.1.1)                         | 217                                    |   | 23 / 6.4                   | 15 / 45                             | 25.3 / 7.9                             | <i>T. aestivum</i>                    | Q96185_WHEAT                                |                                |

**Table A-2.6** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)               | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 331      | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 332      | Wheat EST match                                      | 42                                     | Unnamed protein product                               | 18 / 6.2                   | 22 / 50                             | 27.9 / 7.27                            | <i>T. aestivum</i>                    | Ta13210-3                                   | GI:21813                       |
| 333      | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 334      | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 335      | Endogenous alpha-amylase/subtilisin inhibitor (WASI) | 231                                    |   | 17 / 6.9                   | 23 / 82                             | 19.6 / 6.77                            | <i>T. aestivum</i>                    | IAAS_WHEAT                                  |                                |
| 336      | Endogenous alpha-amylase/subtilisin inhibitor (WASI) | 94                                     |   | 15 / 7.3                   | 19 / 76                             | 19.6 / 6.77                            | <i>T. aestivum</i>                    | IAAS_WHEAT                                  |                                |
| 337      | Wheat EST match                                      | 159                                    | Cupin family protein, expressed                       | 18 / 8.4                   | 8 / 10                              | 66.9 / 7.64                            | <i>T. aestivum</i>                    | Ta2606253404-2                              | GI:108708022                   |
| 338      | 7S Globulin storage protein                          | 50                                     |   | 21 / 9                     | 12 / 14                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 339      | Actin and related proteins                           | 168                                    |   | 20 / 9                     | 14 / 37                             | 28.5 / 5.31                            | <i>A. oryzae</i>                      | Q2U9E1_ASPOR                                |                                |
| 340      | Wheat EST match                                      | 50                                     | 7S Globulin storage protein                           | 19 / 9.0                   | 10 / 20                             | 39.3 / 11.17                           | <i>T. aestivum</i>                    | Ta5443-3                                    | GI:170696                      |
| 341      | Wheat EST match                                      | 49                                     | 7S Globulin storage protein                           | 20 / 9.05                  | 14 / 15                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 342      | Secretory protein                                    | 73                                     |   | 21 / 9.3                   | 6 / 25                              | 24.2 / 9.32                            | <i>T. aestivum</i>                    | Q9SWZ5_WHEAT                                |                                |
| 343      | 7S Globulin storage protein                          | 64                                     |   | 20 / 9.3                   | 14 / 15                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 344      | 7S Globulin storage protein                          | 55                                     |   | 20 / 9.3                   | 11 / 12                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 345      | Secretory protein                                    | 49                                     |   | 21 / 9.5                   | 7 / 24                              | 24.2 / 9.32                            | <i>T. aestivum</i>                    | Q9SWZ5_WHEAT                                |                                |
| 346      | 7S Globulin storage protein                          | 63                                     |   | 20 / 9.4                   | 15 / 18                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 347      | Wheat EST match                                      | 81                                     | 7S Globulin storage protein                           | 21 / 9.5                   | 10 / 18                             | 40.0 / 11.75                           | <i>T. aestivum</i>                    | Ta9894-2                                    | GI:170696                      |
| 348      | 7S Globulin storage protein                          | 47                                     |   | 19 / 9.4                   | 11 / 10                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 349      | 7S Globulin storage protein                          | 45                                     |   | 21 / 9.5                   | 18 / 17                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 350      | 7S Globulin storage protein                          | 59                                     |   | 19 / 9.5                   | 18 / 18                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 351      | 7S Globulin storage protein                          | 56                                     |   | 20 / 9.6                   | 15 / 17                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 352      | Wheat EST match                                      | 75                                     | 7S Globulin storage protein                           | 18 / 9.6                   | 13 / 29                             | 38.0 / 9.56                            | <i>T. aestivum</i>                    | Ta5441-3                                    | GI:170696                      |
| 353      | 7S Globulin storage protein                          | 49                                     |   | 20 / 9.8                   | 8 / 4                               | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 354      | Wheat EST match                                      | 49                                     | 7S Globulin storage protein                           | 18 / 9.75                  | 12 / 26                             | 38.0 / 9.56                            | <i>T. aestivum</i>                    | Ta5441-3                                    | GI:170696                      |
| 355      | Wheat EST match                                      | 76                                     | 7S Globulin storage protein                           | 22 / 10.2                  | 9 / 8                               | 78.9 / 8.75                            | <i>T. aestivum</i>                    | Ta2154253404-2                              | GI:170696                      |
| 356      | Wheat EST match                                      | 151                                    | 7S Globulin storage protein                           | 21 / 10.75                 | 16 / 12                             | 78.9 / 8.75                            | <i>T. aestivum</i>                    | Ta2154253404-2                              | GI:170696                      |

**Table A-2.6 (continued)**

| Spot No. | Protein name<br>(Mascot cereal search)               | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 357      | Nucleoside diphosphate kinase<br>2.7.4.6) (Fragment) | 62                                     |   | 13 / 6.3                   | 2 / 50                              | 2.68 / 4.87                            | <i>A. sativa</i>                      | Q9S8M2_AVESA                                |                                |
| 358      | Wheat EST match                                      | 125                                    | 7S Globulin storage protein                           | 12 / 6.2                   | 19 / 38                             | 38.9 / 9.17                            | <i>T. aestivum</i>                    | Ta3078253411-3                              | GI:170696                      |
| 359      | Wheat EST match                                      | 49                                     | Heat shock protein 16.9B                              | 15 / 6.4                   | 12 / 27                             | 25.8 / 6.43                            | <i>T. aestivum</i>                    | Ta238185-3                                  | GI:21805                       |
| 360      | Wheat EST match                                      | 63                                     | 7S Globulin storage protein                           | 14 / 6.4                   | 31 / 39                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 361      | Wheat EST match                                      | 54                                     | 7S Globulin storage protein                           | 15 / 6.45                  | 16 / 26                             | 38.0 / 9.56                            | <i>T. aestivum</i>                    | Ta5441-3                                    | GI:170696                      |
| 362      | Wheat EST match                                      | 33                                     | 7S Globulin storage protein                           | 15 / 6.6                   | 11 / 19                             | 41.3 / 9.42                            | <i>T. aestivum</i>                    | Ta3078253409-1                              | GI:170696                      |
| 363      | 7S Globulin storage protein                          | 60                                     |   | 14 / 6.75                  | 16 / 23                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 364      | Wheat EST match                                      | 66                                     | Stress-inducible membrane pore<br>protein             | 15 / 6.9                   | 8 / 12                              | 39.3 / 10.09                           | <i>B. inermis</i>                     | Ta58534-1                                   | GI:16555405                    |
| 365      | Wheat EST match                                      | 458                                    | 7S Globulin storage protein                           | 12 / 6.9                   | 18 / 20                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 366      | Wheat EST match                                      | 394                                    | 7S Globulin storage protein                           | 14 / 7.05                  | 20 / 23                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 367      | Hypothetical protein<br>OSJNBb0094P23.22             | 39                                     |   | 15 / 7.3                   | 4 / 92                              | 5.6 / 11.88                            | <i>O. sativa</i>                      | Q6Z1K0_ORYSA                                |                                |
| 368      | No match   |  |   |                            |                                     |  |                                       |   |                                |
| 369      | Prohibitin protein Wph                               | 107                                    |   | 16 / 9                     | 6 / 15                              | 30.1 / 6.0                             | <i>T. aestivum</i>                    | Q84VJ0_WHEAT                                |                                |
| 370      | OSJNBa0093F12.16 protein                             | 89                                     |   | 16 / 9.2                   | 10 / 53                             | 17.7 / 9.23                            | <i>O. sativa</i>                      | Q7XNU2_ORYSA                                |                                |
| 371      | Wheat EST match                                      | 110                                    | 7S Globulin storage protein                           | 18 / 9.3                   | 17 / 26                             | 33.7 / 11.52                           | <i>T. aestivum</i>                    | Ta5446-1                                    | GI:170696                      |
| 372      | Wheat EST match                                      | 46                                     | 7S Globulin storage protein                           | 17 / 9.45                  | 11 / 27                             | 39.3 / 11.17                           | <i>T. aestivum</i>                    | Ta5443-3                                    | GI:170696                      |
| 373      | Wheat EST match                                      | 107                                    | 7S Globulin storage protein                           | 17 / 9.55                  | 12 / 17                             | 38.0 / 9.56                            | <i>T. aestivum</i>                    | Ta5441-3                                    | GI:170696                      |
| 374      | 7S Globulin storage protein                          | 75                                     |   | 17 / 9.6                   | 11 / 11                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 375      | 7S Globulin storage protein                          | 67                                     |   | 17 / 9.6                   | 17 / 17                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 376      | Wheat EST match                                      | 85                                     | 7S Globulin storage protein                           | 17 / 10.75                 | 8 / 5                               | 78.9 / 8.75                            | <i>T. aestivum</i>                    | Ta2154253404-2                              | GI:170696                      |
| 377      | Putative nucleoside diphosphate kinase               | 82                                     |   | 10 / 6.2                   | 8 / 30                              | 25.9 / 8.88                            | <i>O. sativa</i>                      | Q5TKF4_ORYSA                                |                                |
| 378      | Wheat EST match                                      | 188                                    | 7S Globulin storage protein                           | 13 / 6.5                   | 13 / 16                             | 76.3 / 9.22                            | <i>T. aestivum</i>                    | Ta3078253413-3                              | GI:170696                      |

**Table A-2.6 (continued)**

| Spot No. | Protein name<br>(Mascot cereal search) | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 379      | 7S Globulin storage protein            | 45                                     |   | 9 / 6.4                    | 17 / 19                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 380      | 16.9 kDa class I heat shock protein    | 153                                    |   | 9 / 6.6                    | 7 / 37                              | 16.9 / 5.83                            | <i>T. aestivum</i>                    | HSP11_WHEAT                                 |                                |
| 381      | R2R3MYB-domain protein (Fragment)      | 35                                     |   | 9 / 7                      | 6 / 81                              | 4.85 / 8.2                             | <i>Z. mays</i>                        | Q9SQC0_MAIZE                                |                                |
| 382      | Wheat EST match                        | 53                                     | 7S Globulin storage protein                           | 7 / 7.3                    | 27 / 28                             | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 383      | Putative nucleoside diphosphate kinase | 50                                     |   | 9 / 7.4                    | 9 / 24                              | 25.9 / 8.88                            | <i>O. sativa</i>                      | Q5TKF4_ORYSA                                |                                |
| 384      | Wheat EST match                        | 56                                     | OSJNBa0027P08.9                                       | 9 / 7.5                    | 25 / 37                             | 36.9 / 9.06                            | <i>O. sativa</i>                      | Ta13211-3                                   | GI:50924568                    |
| 385      | No match                               |  |   |                            |                                     |  |                                       |   |                                |
| 386      | Putative 60S ribosomal protein L12     | 205                                    |   | 16 / 9.35                  | 10 / 41                             | 17.7 / 9.23                            | <i>O. sativa</i>                      | Q6Z8E0_ORYSA                                |                                |
| 387      | Wheat EST match                        | 65                                     | 40S subunit ribosomal protein                         | 14 / 9.5                   | 6 / 16                              | 19.5 / 9.84                            | <i>O. sativa</i>                      | Ta33749-2                                   | GI:55775376                    |
| 388      | 7S Globulin storage protein            | 64                                     |   | 16 / 9.5                   | 10 / 11                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 389      | Oleosin (Fragment)                     | 88                                     |   | 14 / 9.5                   | 4 / 35                              | 5.79 / 8.59                            | <i>H. vulgare</i>                     | Q43474_HORVU                                |                                |
| 390      | 7S Globulin storage protein            | 80                                     |   | 16 / 9.7                   | 11 / 12                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 391      | 7S Globulin storage protein            | 66                                     |   | 16 / 9.8                   | 9 / 8                               | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 392      | 7S Globulin storage protein            | 71                                     |   | 16 / 11                    | 13 / 10                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 393      | Wheat EST match                        | 123                                    | 7S Globulin storage protein                           | 16 / 10.75                 | 17 / 33                             | 33.7 / 11.52                           | <i>T. aestivum</i>                    | Ta5446-1                                    | GI:170696                      |
| 394      | Wheat EST match                        | 60                                     | 7S Globulin storage protein                           | 13 / 10.7                  | 10 / 19                             | 39.3 / 11.17                           | <i>T. aestivum</i>                    | Ta5443-3                                    | GI:170696                      |
| 395      | Wheat EST match                        | 71                                     | 7S Globulin storage protein                           | 9 / 6.35                   | 10 / 15                             | 66.9 / 6.85                            | <i>T. aestivum</i>                    | Ta3078253407-1                              | GI:170696                      |
| 396      | Putative nucleoside diphosphate kinase | 61                                     |   | 8 / 6.3                    | 7 / 23                              | 25.9 / 8.88                            | <i>O. sativa</i>                      | Q5TKF4_ORYSA                                |                                |
| 397      | 7S Globulin storage protein            | 63                                     |   | 7 / 6.25                   | 14 / 18                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 398      | Putative nucleoside diphosphate kinase | 59                                     |   | 9 / 6.4                    | 6 / 22                              | 25.9 / 8.88                            | <i>O. sativa</i>                      | Q5TKF4_ORYSA                                |                                |
| 399      | 7S Globulin storage protein            | 52                                     |   | 9 / 6.5                    | 6 / 5                               | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 400      | Wheat EST match                        | 82                                     | 7S Globulin storage protein                           | 8 / 6.5                    | 6 / 13                              | 29.7 / 9.12                            | <i>T. aestivum</i>                    | Ta3078253412-1                              | GI:170696                      |
| 401      | 7S Globulin storage protein            | 82                                     |   | 6 / 6.7                    | 16 / 22                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 402      | 7S Globulin storage protein            | 53                                     |   | 7 / 6.75                   | 17 / 21                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |

**Table A-2.6 (continued)**

| Spot No. | Protein name<br>(Mascot cereal search) | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 403      | Wheat EST match                        | 96                                     | 7S Globulin storage protein                           | 6 / 6.9                    | 5 / 5                               | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 404      | 7S Globulin storage protein            | 73                                     |   | 7 / 7                      | 7 / 8                               | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 405      | Hypothetical protein OJ1124_E11.8.     | 43                                     |   | 9 / 7.2                    | 9 / 32                              | 10.9 / 9.52                            | <i>O. sativa</i>                      | Q6K875_ORYSA                                |                                |
| 406      | 7S Globulin storage protein            | 77                                     |   | 6 / 7.25                   | 18 / 24                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 407      | Wheat EST match                        | 62                                     | 7S Globulin storage protein                           | 5 / 7.25                   | 9 / 8                               | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 408      | 7S Globulin storage protein            | 49                                     |   | 7 / 7.5                    | 19 / 23                             | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 409      | No match                               |  |   |                            |                                     |  |                                       |   |                                |
| 410      | Embryo globulin                        | 67                                     |   | 9 / 9.1                    | 10 / 10                             | 72.2 / 6.8                             | <i>H. vulgare</i>                     | Q03678_HORVU                                |                                |
| 411      | Wheat EST match                        | 43                                     | 7S Globulin storage protein                           | 9 / 9.2                    | 9 / 8                               | 61.7 / 8.08                            | <i>T. aestivum</i>                    | Ta3078253410-1                              | GI:170696                      |
| 412      | Wheat EST match                        | 90                                     | Unknown protein                                       | 9 / 9.4                    | 7 / 19                              | 27.1 / /10.16                          | <i>O. sativa</i>                      | Ta1007253406-2                              | GI:52353767                    |
| 413      | Wheat EST match                        | 89                                     | 7S Globulin storage protein                           | 10 / 9.65                  | 12 / 15                             | 52.8 / 9.69                            | <i>T. aestivum</i>                    | Ta627253413-2                               | GI:170696                      |
| 414      | Wheat EST match                        | 126                                    | Unknown protein                                       | 9 / 9.65                   | 6 / 13                              | 21.99 / 9.69                           | <i>O. sativa</i>                      | Ta131241-1                                  | GI:52353767                    |
| 415      | No match                               |  |   |                            |                                     |  |                                       |   |                                |
| 416      | Disease-resistant-related protein      | 45                                     |   | 9 / 11                     | 8 / 23                              | 15.4 / 11.16                           | <i>O. sativa</i>                      | Q8SA80_ORYSA                                |                                |
| 417      | Wheat EST match                        | 51                                     | 7S Globulin storage protein                           | 10 / 10.7                  | 11 / 19                             | 39.3 / 11.17                           | <i>T. aestivum</i>                    | Ta5443-3                                    | GI:170696                      |
| 418      | 50S ribosomal protein L31              | 31                                     |   | 5 / 6.9                    | 5 / 60                              | 9.36 / 9.22                            | <i>X. oryzae</i>                      | Q2P6M2_XANOR                                |                                |
| 419      | 7S Globulin storage protein            | 67                                     |   | 6 / 7                      | 8 / 10                              | 72.2 / 6.8                             | <i>T. aestivum</i>                    | Q7DMU0_WHEAT                                |                                |
| 420      | No match                               |  |   |                            |                                     |  |                                       |   |                                |
| 421      | Wheat EST match                        | 104                                    | 7S Globulin storage protein                           | 5 / 7.0                    | 21 / 24                             | 52.8 / 9.69                            | <i>T. aestivum</i>                    | Ta627253413-2                               | GI:170696                      |
| 422      | Wheat EST match                        | 103                                    | 7S Globulin storage protein                           | 6 / 7.1                    | 7 / 8                               | 52.8 / 9.69                            | <i>T. aestivum</i>                    | Ta627253413-2                               | GI:170696                      |
| 423      | Wheat EST match                        | 109                                    | 7S Globulin storage protein                           | 5 / 7.4                    | 13 / 15                             | 69.9 / 8.42                            | <i>T. aestivum</i>                    | Ta628253405-1                               | GI:170696                      |
| 424      | Wheat EST match                        | 119                                    | 7S Globulin storage protein                           | 5 / 7.9                    | 10 / 11                             | 52.8 / 9.69                            | <i>T. aestivum</i>                    | Ta627253413-2                               | GI:170696                      |
| 425      | Wheat EST match                        | 97                                     | 7S Globulin storage protein                           | 5 / 8.05                   | 5 / 5                               | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 426      | No match                               |  |   |                            |                                     |  |                                       |   |                                |
| 427      | Wheat EST match                        | 77                                     | 7S Globulin storage protein                           | 5 / 8.1                    | 20 / 25                             | 67.9 / 8.86                            | <i>T. aestivum</i>                    | Ta628253404-2                               | GI:170696                      |
| 428      | Wheat EST match                        | 115                                    | 7S Globulin storage protein                           | 5.5 / 9.2                  | 9 / 13                              | 52.8 / 9.69                            | <i>T. aestivum</i>                    | Ta627253413-2                               | GI:170696                      |
| 429      | Wheat EST match                        | 107                                    | 7S Globulin storage protein                           | 6 / 9.25                   | 7 / 10                              | 52.8 / 9.69                            | <i>T. aestivum</i>                    | Ta627253413-2                               | GI:170696                      |



**Table A-2.6** (continued)

| Spot No. | Protein name<br>(Mascot cereal search)            | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|---|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 430      | Wheat EST match                                   | 125                                    | 7S Globulin storage protein                           | 5 / 9.4                    | 7 / 8                               | 52.8 / 9.69                            | <i>T. aestivum</i>                    | Ta627253413-2                               | GI:170696                      |
| 431      | Elongation factor 1-alpha<br>(EF-1-alpha)         | 61                                     |   | 9 / 9.4                    | 14 / 23                             | 49.1 / 9.2                             | <i>T. aestivum</i>                    | EF1A_WHEAT                                  |                                |
| 432      | Wheat EST match                                   | 109                                    | Elongation factor 1-alpha, putative,<br>expressed     | 9 / 9.5                    | 5 / 17                              | 29.2 / 9.84                            | <i>O. sativa</i>                      | Ta3484253413-3                              | GI:108706480                   |
| 433      | Transposase IS1478                                | 49                                     |   | 9.5 / 9.5                  | 10 / 35                             | 21.5 / 11.55                           | <i>X. oryzae</i>                      | Q5H473_XANOR                                |                                |
| 434      | Wheat EST match                                   | 51                                     | Histone H3  | 4 / 10.75                  | 8 / 26                              | 15.7 / 10.98                           | <i>O. sativa</i>                      | Ta3497253410-1                              | GI:34898304                    |
| 435      | 26 kDa endochitinase 1 precursor (EC<br>3.2.1.14) | 121                                    |   | 23 / 9.2                   | 6 / 13                              | 33.4 / 8.54                            | <i>H. vulgare</i>                     | CHI1_HORVU                                  |                                |

**Table A-2.7** Identification of proteins in supernatant from imbibed grain

| Spot No. | Protein name<br>(Mascot cereal search) | Mascot<br>or GPM <sup>1</sup><br>score | Protein name<br>(Wheat EST BLAST search) <sup>2</sup> | Observed<br>MW<br>(kDa)/pI | Matching<br>peptides /<br>%Coverage | Theoretical <sup>3</sup><br>MW (Da)/pI | Species with<br>homologous<br>protein | Swiss-Prot accession<br>number <sup>4</sup> | GenBank<br>accession<br>number |
|----------|--|--|---|----------------------------|-------------------------------------|--|---------------------------------------|---|--------------------------------|
| 1        | Oxalate oxidase precursor              | 90                                     |   | 67 / NA                    | 5 / 18                              | 23.5 / 6.9                             | <i>T. aestivum</i>                    | Q70PK0_WHEAT                                |                                |
| 2        | Polyphenol oxidase (Fragment)          | 391                                    |   | 65 / NA                    | 27 / 50                             | 46.6 / 5.23                            | <i>T. aestivum</i>                    | Q6PLQ9_WHEAT                                |                                |
| 3        | Peroxidase (EC 1.11.1.7) (Fragment)    | 52                                     |   | 33 / NA                    | 4 / 37                              | 8.36 / 4.34                            | <i>T. aestivum</i>                    | PERX_WHEAT                                  |                                |
| 4        | Endochitinase precursor                | 304                                    |   | 30 / NA                    | 15 / 48                             | 33.6 / 7.38                            | <i>T. aestivum</i>                    | Q41539_WHEAT                                |                                |
| 5        | PSBGer3 protein                        | 73                                     |   | 27 / NA                    | 4 / 16                              | 23.4 / 6.18                            | <i>T. aestivum</i>                    | P93600                                      |                                |
| 6        | Histone H4 variant TH011               | 33                                     |   | 14 / NA                    | 5 / 56                              | 11.3 / 11.48                           | <i>T. aestivum</i>                    | H41_WHEAT                                   |                                |

<sup>1</sup> GPM scores -log(e) values

<sup>2</sup> Wheat EST NCBI BLAST search matching to proteins with e-values less than 0.0001

<sup>3</sup> MW and pI are of fragment and wheat EST match sequence and not of actual complete protein

<sup>4</sup> Accession numbers beginning with Ta are for the Wheat EST database

***A-3 Construction of Wheat EST Database for  
searching using XTandem and Mascot***

Python Program. Requires Biopython installation see [Biopython.org](http://Biopython.org):

```
#!C:\Program files\Python24\python.exe
```

```
import os, sys, string
```

```
from Bio import Fasta, Translate, Alphabet, Seq
```

```
from Bio.Alphabet import IUPAC
```

```
from Bio.Seq import Seq
```

```
my_alpha = IUPAC.unambiguous_dna
```

```
standard_translator = Translate.unambiguous_dna_by_id[1]
```

```
parser = Fasta.RecordParser()
```

```
translated = ""
```

```
# The file sequences.txt must be in the folder in which the program is running
```

```
# Sequences.txt is the FASTA Wehaet EST-contig file
```

```
file_to_parse = open("sequences.txt", 'r')
```

```
iterator = Fasta.Iterator(file_to_parse, parser)
```

```
while 1:
```

```
    cur_record = iterator.next()
```

```
    translate_all = []
```

```
    new_seq = []
```

```

if cur_record is None:

    break

    # create reverse complement

    complement_table = {"A": "T", "T": "A", "C": "G", "G": "C", "N": "N", "R": "Y",
                        "Y": "R", "K": "M", "M": "K", "S": "W", "W": "S", "B": "D", "D": "B", "H": "V", "V":
                        "H"}

    for letter in cur_record.sequence:

        complement_letter = complement_table[letter]

        new_seq.append(complement_letter)

    new_seq.reverse()

    rev_seq = "".join(new_seq)

    # append the title to the translated list

    title_atoms = string.split(cur_record.title)

    old_accession = string.split(title_atoms(PCT/DK98/00460), '|')

    int_accession = string.split(old_accession[0:1], ",")

    new_accession = "Ta" + int_accession(PCT/DK98/00460)

    title_atoms2 = ''.join(title_atoms[0:])

    title_1 = "> " + new_accession + "-1" + " " + title_atoms2 + "\n"

    title_2 = "> " + new_accession + "-2" + " " + title_atoms2 + "\n"

    title_3 = "> " + new_accession + "-3" + " " + title_atoms2 + "\n"

    title_4 = "> " + new_accession + "-4" + " " + title_atoms2 + "\n"

    title_5 = "> " + new_accession + "-5" + " " + title_atoms2 + "\n"

    title_6 = "> " + new_accession + "-6" + " " + title_atoms2 + "\n"

```

```

translate_all.append(title_1)

for_seq = Seq(cur_record.sequence, my_alpha)

prot_for = standard_translator.translate(for_seq)

for_prot = ".join(prot_for) + "\n"

translate_all.append(for_prot)

translate_all.append(title_2)

prot_for = standard_translator.translate(for_seq[1:])

for_prot = ".join(prot_for) + "\n"

translate_all.append(for_prot)

translate_all.append(title_3)

prot_for = standard_translator.translate(for_seq[2:])

for_prot = ".join(prot_for) + "\n"

translate_all.append(for_prot)

translate_all.append(title_4)

reverse_seq = Seq(rev_seq, my_alpha)

prot_rev = standard_translator.translate(reverse_seq)

rev_prot = ".join(prot_rev) + "\n"

translate_all.append(rev_prot)

translate_all.append(title_5)

prot_rev = standard_translator.translate(reverse_seq[1:])

rev_prot = ".join(prot_rev) + "\n"

translate_all.append(rev_prot)

translate_all.append(title_6)

```

```
prot_rev = standard_translator.translate(reverse_seq[2:])  
rev_prot = ".join(prot_rev) + "\n"  
translate_all.append(rev_prot)  
translated = ".join(translate_all)  
result_file_name = os.path.join(os.getcwd(), 'TranslatedESTs.fasta')  
result_file = open(result_file_name, 'a')  
result_file.write(translated)  
result_file.close()
```

***A-4 Wheat EST alignments for cupin domain  
containing proteins***



**Table A-4.1** Wheat EST alignments with cupin containing proteins

Alignment: C:\Documents and Settings\tjerkovi\My Documents\seaview\ALL\_mod2.aln  
Seaview [blocks=10 fontsize=10 A4] on Fri Sep 15 16:52:22 2006

|                |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|
|                | 1          |            |            |            |            |            |
| Ta2154253404-2 | GTVRNTGRPR | VGDRAVITP  | LLFLLGTSLL | FAAAVSASHD | DEEDRRGGHS | LOQCVRCCQ  |
| Ta9905-2       | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta3078253410-1 | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta3078253407-1 | ---EGARQAE | QTMAIRATIP | LLFLLGTSLL | FAAAVSASRD | DEEDRRGGHS | LOQCVRCCQ  |
| Ta3078253412-1 | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta3078253409-1 | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta3078253413-3 | ---QLASST  | MATRGRATIP | LLFLLGTSLL | FAAAVSASHD | DEEDRRGGHS | LOQCVRCCQ  |
| Ta5446-1       | -----      | -----      | -----      | -----      | -----      | -----      |
| AAA34269       | -----      | MATRAKATIP | LLFLLGTSLL | FAAAVSASHD | DEEDRRGGHS | LOQCVRCCQ  |
| Q03678 HORVU   | -----      | MATRAKATIP | LLFLLGTSLL | FAAAVSASHD | DEEDRRGGHS | LOQCVRCCQ  |
| Ta194625-1     | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta5441-3       | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta9894-2       | -----      | -----      | -----      | -----      | -----      | -----      |
| Q852L2 ORYSA   | -----      | -----      | -----      | -----      | -----      | -----      |
| Q8L8I0 ORYSA   | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta628253404-2  | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta628253407-1  | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta136340-2     | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta627253413-2  | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta628253405-1  | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta132809-2     | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta189317-3     | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta2606253404-2 | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta2606253405-2 | -----      | -----      | -----      | -----      | -----      | -----      |
| Q70PK0 WHEAT   | -----      | -----      | -----      | -----      | -----      | -----      |
| ATEG_07804     | -----      | -----      | -----      | -----      | -----      | -----      |
|                | 61         |            |            |            |            |            |
| Ta2154253404-2 | DRFRYSHARC | VQDCRGDQQQ | HGRHEEE-Q  | GRGRGRHGEG | EREEDGGGR  | GRHGGEREE  |
| Ta9905-2       | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta3078253410-1 | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta3078253407-1 | DRFRYSHARC | VQDCREDQQQ | HGRHEEE-Q  | GRGRGRHGEG | EREEDGGGR  | GRHGGEREE  |
| Ta3078253412-1 | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta3078253409-1 | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta3078253413-3 | DRFRYSHARC | VQDCRDDQQQ | HGRHEEE-Q  | GRGRGRHGEG | EREEDGGGR  | GRHGGEREE  |
| Ta5446-1       | -----      | -----      | -----      | -----      | -----      | -----      |
| AAA34269       | ERFRYSHARC | VQDCRDDQQQ | HGRHEEE-Q  | GRGRGRHGEG | EREEDGGGR  | GRHGGEREE  |
| Q03678 HORVU   | ERFRYSHARC | VQDCRDDQQQ | HGRHEEE-Q  | GRGRGRHGEG | EREEDGGGR  | GRHGGEREE  |
| Ta194625-1     | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta5441-3       | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta9894-2       | -----      | -----      | -----      | -----      | -----      | -----      |
| Q852L2 ORYSA   | MA         | KKKTSSSMAR | S--QLAALLI | SLCF-LSLAS | NAVWGR     | RGEREEDER  |
| Q8L8I0 ORYSA   | MA         | KKKTSSSMAR | S--QLAALLI | SLCF-LSLAS | NAVWGR     | RGEREEDER  |
| Ta628253404-2  | SPT        | SIIMKSTVVR | SPWLALALVL | SLCLSLSFAS | WDAEDVGRGS | RRWEGGDDE  |
| Ta628253407-1  | SPT        | SIIMKSTVVR | SPWLALALVL | SLCLSLSFAS | WDAEDVGRGS | RRWEGGDDE  |
| Ta136340-2     | VSSSRT     | TITMKS-AVR | SPWLVLALVL | SLCLSLSFAS | WDAEDVGRGS | RRWEGGDDE  |
| Ta627253413-2  | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta628253405-1  | -----      | -----      | D          | HHWLVLALVL | SLCLSLSFAS | WDAEDVGRGS |
| Ta132809-2     | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta189317-3     | -----      | -----      | -----      | -----      | -----      | -----      |
| Ta2606253404-2 | -----      | -----      | -----      | EEGEGE     | WRPEEEAKGG | GGGGGTGKGL |
| Ta2606253405-2 | -----      | -----      | -----      | AVR        | WRPEEEAKGG | GGGGGTGKGL |
| Q70PK0 WHEAT   | -----      | -----      | -----      | -----      | -----      | -----      |
| ATEG_07804     | -----      | -----      | -----      | -----      | -----      | -----      |

**Table A-4.1** Wheat EST alignments with cupin containing proteins (continued)

|                |            |            |            |            |             |             |            |
|----------------|------------|------------|------------|------------|-------------|-------------|------------|
|                | 121        |            |            |            |             |             |            |
| Ta2154253404-2 | EEGRGRGRHG | DGER       | ----       | ----       | ----        | EEHGRH      | DGRGRRGEG  |
| Ta9905-2       | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta3078253410-1 | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta3078253407-1 | EEGRGRGRHG | DGER       | ----       | ----       | ----        | EEHGRH      | DGRGRRGEG  |
| Ta3078253412-1 | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta3078253409-1 | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta3078253413-3 | EQGRGRGRHG | DGER       | ----       | ----       | ----        | DEE         | ----       |
| Ta5446-1       | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| AAA34269       | EHGRGRGRHG | DGEREEERGR | GHGRHGEGER | EEERGRGRGR | HGEGEREEEEE | GRGRGRRGEG  | GRGRGRRGEG |
| Q03678 HORVU   | EHGRGRGRHG | DGEREEERGR | GHGRHGEGER | EEERGRGRGR | HGEGEREEEEE | GRGRGRRGEG  | GRGRGRRGEG |
| Ta194625-1     | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta5441-3       | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta9894-2       | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Q852L2 ORYSA   | RRHGGE     | ----       | ----       | ----       | ----        | ----        | ----       |
| Q8L8I0 ORYSA   | RRHGGE     | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta628253404-2  | GRSGG      | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta628253407-1  | RRSGE      | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta136340-2     | GRSGG      | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta627253413-2  | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta628253405-1  | GRSGG      | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta132809-2     | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta189317-3     | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta2606253404-2 | ESEGG      | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta2606253405-2 | ESEGG      | ----       | ----       | ----       | ----        | ----        | ----       |
| Q70PK0 WHEAT   | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| ATEG_07804     | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
|                | 181        |            |            |            |             |             |            |
| Ta2154253404-2 | ERDEEGGSR  | RPYVFGPRNF | RSIIRSDHGF | VKALRPFDEV | SRLLEGIRNY  | RVAIMEVNPR  | ----       |
| Ta9905-2       | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta3078253410-1 | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta3078253407-1 | ERDEEHGDSR | RPYVFGPRNF | RSIIRSDHGF | VKALRPFDEV | SRLLEGIRNY  | RVAIMEVNPR  | IMEVNPR    |
| Ta3078253412-1 | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta3078253409-1 | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta3078253413-3 | HGDGR      | RPYVFGPRSF | RSIIRSDHGF | VKALRPFDEV | SRLLEGIRNY  | RVAIMEVNPR  | ----       |
| Ta5446-1       | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| AAA34269       | ERDEEGGDSR | RPYVFGPRSF | RSIIRSDHGF | VKALRPFDQV | SRLLEGIRDY  | RVAIMEVNPR  | ----       |
| Q03678 HORVU   | ERDEEGGDSR | RPYVFGPRSF | RSIIRSDHGF | VKALRPFDQV | SRLLEGIRDY  | RVAIMEVNPR  | ----       |
| Ta194625-1     | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta5441-3       | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta9894-2       | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Q852L2 ORYSA   | GG         | RPYHLGEESF | RHWTRTRHGR | FSVLERFDE  | QVVGAAVGGY  | RVAVLEAAPR  | ----       |
| Q8L8I0 ORYSA   | GG         | RPYHLGEESF | RHWTRTRHGR | FSVLERFDE  | QVVGAAVGGY  | RVAVLEAAPR  | ----       |
| Ta628253404-2  | SG         | RPYHFGESF  | REWAKSRHGH | FSVLERF-DH | ELLRGSIGDY  | RVACLDAAAPR | ----       |
| Ta628253407-1  | SG         | RPYHFGESF  | REWAKSRHGH | FSVLERF-DH | ELLRGSIGDY  | RVACLDAAAPR | ----       |
| Ta136340-2     | SG         | RPYHFGESY  | REWAKSRHGH | FSVLERF-DH | ELLRGSIGDY  | RVAYLDAAAPR | ----       |
| Ta627253413-2  | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta628253405-1  | SG         | RPYHFGESY  | REWAKSRHGH | FSVLERF-DH | ELLRGSIGDY  | RVAYLDAAAPR | ----       |
| Ta132809-2     | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta189317-3     | ----       | ----       | ----       | ----       | ----        | ----        | ----       |
| Ta2606253404-2 | SV         | HVVRGLPASG | VPEAPWQHGG | WSAG--CGAC | REGLMHIG    | ----        | FITMEPK    |
| Ta2606253405-2 | SV         | HVVRGLPASG | VPEAPWQHGG | WSAGAGCGAC | REGLMHIG    | ----        | FITMEPK    |
| Q70PK0 WHEAT   | MGYS       | KILVAGLFAT | LLLAPVVLAT | DEDF       | ----        | ----        | LDQFCVADLD |
| ATEG_07804     | MKISDLA    | LTLALLAGT  | GAARPTARDG | AGNPFLRGSE | DLLGYSASNK  | LSFHSTEDIK  | ----       |

**Table A-4.1** Wheat EST alignments with cupin containing proteins (continued)

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|                |             |            |            |             |            |            |
|----------------|-------------|------------|------------|-------------|------------|------------|
| Ta2154253404-2 | AFVVPGLTDA  | DGVGYVAGGE | GVLTVIENG  | KRSYTVRQGD  | VIV--APAGS | IMHLANTDGR |
| Ta9905-2       |             |            |            |             |            |            |
| Ta3078253410-1 | AFVVPGLTDA  | DGVGYVAGGE | GVLTVIENG  | KRSYTVRQGD  | VIV--APAGS | IMHLANTDGR |
| Ta3078253407-1 | SPVVPGLTDA  | DGVGYVAGGE | GVLTVIENG  | RRSYTVRQGD  | VIV--APAGS | IMHLANTDGR |
| Ta3078253412-1 |             |            |            | VRSYTVRQGR  | MLIRCAPAGS | IMHLANTDCR |
| Ta3078253409-1 |             |            |            | SAR-G       |            | TDGR       |
| Ta3078253413-3 | AFVVPGLTDA  | DGVGYVAGGE | GVLTVIENG  | KRSYTVRQGD  | VIV--APAGS | IMHLANTDGR |
| Ta5446-1       |             | YVAGGE     | GVLTVIENG  | KRSYTVRQGD  | VIV--APAGS | IMHLANTDGR |
| AAA34269       | AFVVPGLTDA  | DGVGYVAGGE | GVLTVIENG  | KRSYTVRQGD  | VIV--APAGS | IMHLANTDGR |
| Q03678_HORVU   | AFVVPGLTDA  | DGVGYVAGGE | GVLTVIENG  | KRSYTVRQGD  | VIV--APAGS | IMHLANTDGR |
| Ta194625-1     |             |            |            |             |            |            |
| Ta5441-3       |             |            | TTH        | ASANTECLASS | TMA        | TRAR       |
| Ta9894-2       |             |            |            |             |            |            |
| Q852L2_ORYSA   | AFLQPSHYDA  | DEVFVYKGE  | GVIVLLRGR  | RESFCVREGD  | AMV--IPAGA | IVYSANTHSS |
| Q8L8I0_ORYSA   | AFLQPSHYDA  | DEVFVYKGE  | GVIVLLRGR  | RESFCVREGD  | AMV--IPAGA | IVYSANTHSS |
| Ta628253404-2  | AFLQPSHYDA  | DEIAFVRGE  | GVIVLLRNGK | RESFCVREGD  | VFV--IPAGS | IVYSANTHRS |
| Ta628253407-1  | AFLQPSHYDA  | DEIAFVRGE  | GVIVLLRNGK | RESFCVREGD  | VFV--IPAGS | IVYSANTHRS |
| Ta136340-2     | AFLQPSHYDA  | DEIAFVRGE  | GVIVLLRNGK | RESFCVREGD  | VIV--IPAGS | IVYSANTHRS |
| Ta627253413-2  |             |            | G          | R--         | --LAS      | IVYSANTHRS |
| Ta628253405-1  | AFLQPSHYDA  | DEIAFVRGE  | GVIVLLRNGK | RESFCVREGD  | VIV--IPAGS | IVYSANTHRS |
| Ta132809-2     |             |            |            |             |            |            |
| Ta189317-3     |             |            |            |             |            |            |
| Ta2606253404-2 | TLFVPPQYIDS | NLILFVQGD  | VKVGWIHKG  | LVEKQLKMGD  | VLQ--IDAGS | TFYMWNTGKG |
| Ta2606253405-2 | TLFVPPQYIDS | NLILFVQGD  | VKVGWIHKG  | LVEKQLKMGD  | VLQ--MDAGS | TFYMWNTGKG |
| Q70PK0_WHEAT   | GKAVS       |            | VNG-HPCKP  | MSKAGD      | --DFLPSS   | KLAKAGNTST |
| ATEG_07804     | YTLASGSKDD  | ADLGVYLDPE | TVDNPPQIRG | ELGGTDGPR   | NYAYDRINDS | KLAPPGTDSG |

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|                |            |            |            |      |           |            |            |
|----------------|------------|------------|------------|------|-----------|------------|------------|
| Ta2154253404-2 | RKLVIKILH  | --TISVPGRF | QYFSAKP    | ---- | LLASL     | SKRVLRAALK | TS-DEQLGRL |
| Ta9905-2       |            |            |            |      |           |            |            |
| Ta3078253410-1 | RKLVIKILH  | --TISVPGMF | QYFSAKP    | ---- | LLASL     | SKRVLRAALK | TS-DEQLGRL |
| Ta3078253407-1 | RKLVIKILH  | --TISVPGKF | QYFSAKP    | ---- | LLASL     | SKRVLTAALK | TS-DEQLGRL |
| Ta3078253412-1 | RKLVIKILH  | --TISVPGPF | QYFSAKP    | ---- | LLASL     | SKRVLTAALK | TS-DEQLGRL |
| Ta3078253409-1 | RKLVIKILH  | --TISVPGKF | QYFSAKP    | ---- | LLASL     | SKRVLRAALK | TS-DEQLDRL |
| Ta3078253413-3 | RKLVIKILH  | --TISVPGKF | QYFSAKP    | ---- | LLASL     | SKRVLTAALK | TS-DEQLGSL |
| Ta5446-1       | RKLVIKILH  | --TISVPGKF | QYFSAKP    | ---- | LLASL     | SKRVLTAALK | TS-DEQLGSL |
| AAA34269       | RKLVIKILH  | --TISVPGKF | QYFSAKP    | ---- | LLASL     | SKRVLRAAFK | TS-DEQLGRL |
| Q03678_HORVU   | RKLVIKILH  | --TISVPGKF | QYFSAKP    | ---- | LLASL     | SKRVLRAAFK | TS-DEQLGRL |
| Ta194625-1     |            |            |            |      |           |            |            |
| Ta5441-3       | VTIPLLFLLG | --TSLLFAAA | VSASHDE    | ---- | EDRR      | GGRSLQCVG  | RC-HQDRPRY |
| Ta9894-2       |            |            | GDP        |      | VVPR      | GRHGEGERE  | HG-KHEQGR  |
| Q852L2_ORYSA   | KWFRVVMMLN | --FVSTPGHF | EEYFPVG    | -G   | DRPESFSAF | SDDVLQAAPN | TR-REELEKV |
| Q8L8I0_ORYSA   | KWFRVVMMLN | --FVSTPGHF | EEYFPVG    | -G   | DRPESFSAF | SDDVLQAAPN | TR-REELEKV |
| Ta628253404-2  | KWFRVVMMLN | --FVSTPGSF | QEPSPIGFGG |      | EQPQSFSVF | SDEVIAQAFN | TRQREDVDRV |
| Ta628253407-1  | KWFRVVMMLN | --FVSTPGSF | QEPSPIGFGG |      | EQPQSFSVF | SDEVIAQAFN | TRQREDVDRV |
| Ta136340-2     | KWLRVVMFIN | --FVSTPGRF | QELFLIG    |      |           |            | SRRR--APAV |
| Ta627253413-2  | KWLRVVMFIN | --FVSTPGSF | QEPSPIGFGG |      | EQPQSFSVF | SDEVIAQAFN | TRQREDVDRV |
| Ta628253405-1  | KWLRVVMFIN | --FVSTPGSF | QEP        |      |           |            |            |



**Table A-4.1** Wheat EST alignments with cupin containing proteins (continued)

|                |             |            |             |             |             |             |  |
|----------------|-------------|------------|-------------|-------------|-------------|-------------|--|
|                | 361         |            |             |             |             |             |  |
| Ta2154253404-2 | LGRRGQKEED  | SRSISIVRAS | EDQLRELSRQ  | ASEGGQGHWW  | PLPPFRGDSR  | DTFNLLDQRP  |  |
| Ta9905-2       |             |            |             |             |             |             |  |
| Ta3078253410-1 | LDPRQGQEKI  | GGSMSIVRAS | EDQLHELRSQ  | ASEGGQGHWW  | PLPPFRGDSR  | DTYNLLDQRP  |  |
| Ta3078253407-1 | LPGRRGQQEE  | ESSISIVRAS | EDQLRELRRQ  | ASEGGQGHWW  | PLPPFRGDSR  | DTFNLLDQRP  |  |
| Ta3078253412-1 | LPGRRGQQEE  | ESSISIVRAS | EDQLRELRRQ  | ASEGGQGHWW  | PLPPFRGDSR  | DTFNLLDQRP  |  |
| Ta3078253409-1 | LPGRRGQQEE  | ESSISIVRAS | EDQLRELRRQ  | ASEGGQGHWW  | PLPPFRGDSR  | DTYNLLDQRP  |  |
| Ta3078253413-3 | LGSRGQKEED  | EKSISIVRAS | EDQLRELRRQ  | ASEGGQGHWW  | PLPPFRGDSR  | DTFNLLDQRP  |  |
| Ta5446-1       | LGSRG--E--  |            |             |             |             |             |  |
| AAA34269       | FNQRQ--GQEK | TRSVSIVRAS | EDQLRELRRQ  | AAEGGQGHWW  | PLPPFRGDSR  | DTFNLLDQRP  |  |
| Q03678 HORVU   | FNQRQ--GQEK | TRSVSIVRAS | EDQLRELRRQ  | AAEGGQGHWW  | PLPPFRGDSR  | DTFNLLDQRP  |  |
| Ta194625-1     | --AR--      |            |             | --GRGHGR    | --HGEGR     | R--         |  |
| Ta5441-3       | SHARQ--     | --VQECR    | DEQQQHGRHE  | QEEQGRGHGR  | --HGEGR     | R--         |  |
| Ta9894-2       | --GRR--     |            |             |             | --GEGR      | R--         |  |
| Q852L2_ORYSA   | FERQREG--   | --GEITTAP  | EDQIRELSKS  | CSRGG--GG   | GSQSE--WEIK | PS--SLTGKSP |  |
| Q8L8I0_ORYSA   | FERQREG--   | --GEITTAP  | EDQIRELSKS  | CSRGG--GG   | GSQSE--WEIK | PP--SLTGKSP |  |
| Ta628253404-2  | FQKSRGE--   | --GQISEGS  | EDQIRELSRS  | CSRGGRGGGG  | GSQSEKEDIQ  | PR--SLTGKSP |  |
| Ta628253407-1  | FETKSRGQ--  | --GQISEGS  | EDQIRELSRS  | CSRGGRGGGG  | GSQ--       |             |  |
| Ta136340-2     | LLER--      |            | --LQR--     | --R--       |             |             |  |
| Ta627253413-2  | FQKSRGE--   | --GQISEGS  | EDQIRELSRS  | CSRGGRGGGG  | GSQSEKEDIQ  | PR--SLTGKSP |  |
| Ta628253405-1  | FESKSKGE--  | --GEIYDAS  | EDQIRELSRS  | CSRGGRGGGG  | --SGSEKEDIQ | PR--SLTGKSP |  |
| Ta132809-2     | FTSNAINC--  |            |             |             |             |             |  |
| Ta189317-3     | FTSNAINC--  |            |             |             |             |             |  |
| Ta2606253404-2 | LPVKPLGPFV  | SYTTESG--G | KEHGQGDKRD  | VGNGRESEF   | WRPVGRGDD   | --          |  |
| Ta2606253405-2 | LPVRTGGPFV  | SYTTESGSGG | KEHGQ--D    | VGNGRESEF   | WRPVGRGDD   | DD--        |  |
| Q70PK0 WHEAT   | LLVGILGS--  |            | LDGKGLYSR   | VVRAGETFLI  | FRGLMH--    |             |  |
| ATEG_07804     | CRIGAIN--   |            | --ENGETFIDD | VT--EGDVWFF | PPGVPHSIQA  | LD--        |  |
|                | 421         |            |             |             |             |             |  |
| Ta2154253404-2 | KIANRHGRLY  | EADARSFHAL | AQHDVVRVAV  | NITPGSMTAP  | YLNTQSFKLA  | VVLEG--EGEV |  |
| Ta9905-2       |             | -----HAS   | AQHDV--ASPG | PTSRRALDRA  | YLNTQSFKLA  | VVLEG--EGEV |  |
| Ta3078253410-1 | KIANRHGRLY  | EADARSFHAL | AQHDVVRVAV  | NITPGSMTAP  | YLNTQSFKLA  | VVLEG--EGEV |  |
| Ta3078253407-1 | KIANRHGRLY  | EADARSFHAL | AQHDVVRVAV  | NITPGSMTAP  | YLNTQSFKLA  | VVLEG--EGEV |  |
| Ta3078253412-1 | KIANRHGRLY  | EADARSFHAL | AQHDVVRVAV  | NITPGSMTAP  | YLNTQSFKLA  | VVLEG--EGEV |  |
| Ta3078253409-1 | KIANRHGRLY  | EADARSFHAL | AQHDVVRVAV  | NITPGSMTAP  | YLNTQSFKLA  | VVLEG--EGEV |  |
| Ta3078253413-3 | KIANRHGRLY  | EADARSFHAL | AQHDVVRVAV  | NITPGSMTAP  | YLNTQSFKLA  | VVLEG--EGEV |  |
| Ta5446-1       |             |            |             |             |             |             |  |
| AAA34269       | KIANRHGRLY  | EADARSFHAL | ANQDVRVAV   | NITPGSMTAP  | YLNTQSFKLA  | VVLEG--EGEV |  |
| Q03678 HORVU   | KIANRHGRLY  | EADARSFHAL | ANQDVRVAV   | NITPGSMTAP  | YLNTQSFKLA  | VVLEG--EGEV |  |
| Ta194625-1     | --EEEQGR--  | --GRGRHGQ  | GEREEEGRG   | HGRHGQGER   | --EEEQGRG   | RGRHG--QER  |  |
| Ta5441-3       | --EEEQGR--  | --GRGRHGQ  | GEREEEGRG   | RGRHGBGER   | --EEEQGRG   | RGRHG--QER  |  |
| Ta9894-2       | --DEEHGD--  | --SRFPYVF  | GPRNFRSII   | S--DHGFVKAL | RPFDEVSRL   | R--         |  |
| Q852L2_ORYSA   | YFSNNHGKLF  | ELTGDECRHL | KKLDLQIGLA  | NITRGSMTAP  | YNTNTRATKLA | VVLEG--SGYF |  |
| Q8L8I0_ORYSA   | YFSNNHGKLF  | ELTGDECRHL | KKLDLQIGLA  | NITRGSMTAP  | YNTNTRATKLA | VVLEG--SGYF |  |
| Ta628253404-2  | RYSNKHGRFH  | QITGDQCHHL | RKLDMDVTLV  | NITRGSMTAL  | RYTTRSTRY   | IVVEGRDGYF  |  |
| Ta628253407-1  |             |            |             |             |             |             |  |
| Ta136340-2     |             |            |             |             |             |             |  |
| Ta627253413-2  | RYSNKHGRFH  | QITGDQCHHL | RKLDMDVTLV  | NITRGSMTAL  | RYTTRSTRY   | IVVEGRDGYF  |  |
| Ta628253405-1  | RYSNKHGRFH  | QITGDQCHHL | RKLDMDVTLV  | NITRGSMTAL  | RYTTRSTRY   | IVVEGRDGYF  |  |
| Ta132809-2     |             | --DDPSHP-- | --KSEAYSSVS | NLLRG--     | -----FEVK   | ILRQGFVSA   |  |
| Ta189317-3     |             | --DDPSHP-- | --TSEAYSSVS | NLLRG--     | -----FDVK   | ILRQGFVSA   |  |
| Ta2606253404-2 | --ERGS--GQ  | STWTWSWRKL | MSRFIGGELN  | KKSDKTVRAP  | EPYNLFDHEP  | SFRNTYGSWI  |  |
| Ta2606253405-2 | --ERGS--GQ  | STWTWSWRKL | MSRFIGGELN  | KKSDKTVRAP  | EPYNLFDHEP  | SFRNTYGSWI  |  |
| Q70PK0 WHEAT   |             | LQFNVG--K  | TEASMVVSFN  | SONFGIVF--  | --VPLTLFS   | SNP--       |  |
| ATEG_07804     | --TGVEFL    | LVFDDGSFSE | DNTFLASEVF  | AHNPKSVLAK  | DLGLPISAFE  | NIPDELYIF   |  |

**Table A-4.1** Wheat EST alignments with cupin containing proteins (continued)

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|                |             |            |            |            |           |              |
|----------------|-------------|------------|------------|------------|-----------|--------------|
| Ta2154253404-2 | EIVCFPHLGRD | SER--REHGK | -GRWSEEEED | DRRQQ---   | ---       | RRHGSGSESD   |
| Ta9905-2       | EIVCFPHLGRD | SER--REHGK | -GRWSEEEED | DRRQQ---   | ---       | RRHGSGSESD   |
| Ta3078253410-1 | QIVCFPHLGRD | SER--REHGK | -GRWSEEEED | DRRQQ---   | ---       | RRHGSGSESD   |
| Ta3078253407-1 | QIVCFPHLGRD | SERE-HEHGK | -GRRSEEEED | DRRQQ---   | ---       | RRRGSGSESD   |
| Ta3078253412-1 | QIVCFPHLGRD | SERE-HEHGK | -GRRSEEEED | DRRQQ---   | ---       | R---         |
| Ta3078253409-1 | QIVCFPHLARD | SER--HKQK  | -GRRSEEEED | DRRQQ---   | ---       | RRRGSGSESD   |
| Ta3078253413-3 | EIVCFPHLGRD | SERRDREHGK | GRWRSEEEED | DRRQQ---   | ---       | RRRGSGSESD   |
| Ta5446-1       | ---         | ---        | ---        | ---        | ---       | ---          |
| AAA34269       | QIVCFPHLGRD | SESE-REHGK | -GRRSEEEED | DRRQQ---   | ---       | RRRGSGSESD   |
| Q03678_HORVU   | QIVCFPHLGRD | SESE-REHGK | -GRRSEEEED | DRRQQ---   | ---       | RRRGSGSESD   |
| Ta194625-1     | E---EHGRRE  | QEE--GGGR  | -GRRGEGERD | EEHGD---   | ---       | SRR---       |
| Ta5441-3       | E---EHGKHE  | Q-----GR   | -GRRGEGERD | EEHGD---   | ---       | SRR---       |
| Ta9894-2       | ELFRRRHGSE  | P-----GL   | VRRAGTHGRG | RRRLR---   | ---       | RSRRGGAD---  |
| Q852L2_ORYSA   | EMACPHVSGG  | GSSE-RRE-- | --REREHG-  | RRREE---   | ---       | -EGEEE---    |
| Q8L8I0_ORYSA   | EMACPHVSGG  | GSSE-RRE-- | --REREHG-  | RRREE---   | ---       | -EGEEE---    |
| Ta628253404-2  | EMACPHVSSS  | GRSE-RREHE | QEREEREHG  | RRSEERG    | PH---     | -GRRSEEEEH   |
| Ta628253407-1  | ---         | ---        | ---        | ---        | ---       | ---          |
| Ta136340-2     | ---         | ---        | ---        | ---        | ---       | ---          |
| Ta627253413-2  | EMACPHVSSS  | GRSE-RREHE | QEREEREHG  | RRSEEREREH | GGRRSEERK | DEGGRQEEDG   |
| Ta628253405-1  | EMACPHISSS  | GRSE-RREHE | QEREEREHG  | RRSEEREREH | GGRRSEERE | DEGGRQEEDG   |
| Ta132809-2     | EVVEAIQSAK  | SPQS-----I | ITYNPDQKE  | ERARR---   | ---       | ---          |
| Ta189317-3     | EVVEAIRSAK  | SPQS-----I | ITYNPDQKE  | EKS---     | ---       | ---          |
| Ta2606253404-2 | SVDKHDYHPL  | DHSDIGVYLV | NLTAGSMMAF | HVNFR---   | ---       | ATEYGVVLAG   |
| Ta2606253405-2 | SVDKHDYEP   | DHSDIGVYLV | NLTAGSMMAF | HVNFR---   | ---       | ATEYGVVLAG   |
| Q70PK0_WHEAT   | ---PIPTPVL  | TKALRVENG  | VEL---     | ---        | ---       | ---          |
| ATBG_07804     | PGTAPAKDIE  | QONVTTAAGV | VPLKDSYSYH | FSEQPA---  | ---       | HQVAGGSVK--- |

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|                |            |            |            |             |             |            |
|----------------|------------|------------|------------|-------------|-------------|------------|
| Ta2154253404-2 | SE--EEQDQ  | RYETVRAVRS | RGSFVVPFG  | HPVVEIS--SS | QGSSNLQVVC  | FEINAERNER |
| Ta9905-2       | SE--EEQDQ  | RYETVRAVRS | RGSFVVPFG  | HPVVEIS--SS | QGSSNLQVVC  | FEINAERNER |
| Ta3078253410-1 | SESEEEQDQ  | RYQTVRAVRS | RGSFVVPFG  | HPVVEIA--SS | QGSSNLQVVC  | FEINAERNER |
| Ta3078253407-1 | ---EEQDQ   | RYETVRAVRS | RGSFVVPFG  | HPVVEIA--SS | QGSSNLQVVC  | FEINAERNER |
| Ta3078253412-1 | ---        | ---        | ---        | ---         | ---         | ---        |
| Ta3078253409-1 | SS--EEQDQ  | RYETVRAVRS | RGSFVVPFG  | HPVVEIA--SS | QGSSNLQVVC  | FEINAERNER |
| Ta3078253413-3 | EE---QDQ   | RYETVRAVRS | RGSFVVPFG  | HPVVEIA--SS | QGSSNLQVVC  | FEINAERNER |
| Ta5446-1       | ---        | ---        | ---        | ---         | ---         | ---        |
| AAA34269       | ---        | ---        | ---        | ---         | ---         | ---        |
| Q03678_HORVU   | ---        | ---        | ---        | ---         | ---         | ---        |
| Ta194625-1     | ---FYVF    | GPRSFRIIR  | SDHGFPVKAL | RPFDEVSRLL  | RGIRNYRVAI  | MEVMPRAFVV |
| Ta5441-3       | ---FYVF    | GERNFSRIIR | SDHGFPVKAL | RPFDEVSRLL  | RGIRNYRVAI  | MEVMPRAFVV |
| Ta9894-2       | ---GDRE    | RREAVLLRQA | RR-----    | ---         | RGAG-----GV | HHAPGQHRRP |
| Q852L2_ORYSA   | -HGERGEKAR | RYHKVRAQVR | EESVIVIPAS | HPATIVA--   | SEGESLAVVC  | FFVGANHDEK |
| Q8L8I0_ORYSA   | -HGERGEKAR | RYHKVRAQVR | EGSVIVIPAS | HPATIVA--   | SEGESLAVVC  | FFVGANHDEK |
| Ta628253404-2  | HGGERGEKSR | GYRQVRAQIK | GVSVIVLPAG | HPATFVA--   | GNEGNLALLS  | FGVGANNDEE |
| Ta628253407-1  | ---        | ---        | ---        | ---         | ---         | ---        |
| Ta136340-2     | ---        | ---        | ---        | ---         | ---         | ---        |
| Ta627253413-2  | GRGEEQEKSR | GYRQVRAQIK | VGSVIVLPAG | HPATFVA--   | GNEGNLALLS  | FGVGANNDEE |
| Ta628253405-1  | HGGERGEKSR | GYRQVRAQIK | VGSVIVLPAG | HPATFVA--   | GNEGNLALLS  | FGVGANNDEE |
| Ta132809-2     | ---EKE     | RRQR---    | ---        | ---         | ---         | ---        |
| Ta189317-3     | ---        | ---        | ---        | ---         | ---         | ---        |
| Ta2606253404-2 | EGVIQVVPFN | GSLAMSAAER | AGDVFVIFRH | FPFVQVA--   | SRGGPFVFFG  | FTTSARRNKP |
| Ta2606253405-2 | EGVIQVVPFN | GSLAMSAAER | AGDVFVIFRH | FPFVQVA--   | SRGGPFVFFG  | FTTSARRNKP |
| Q70PK0_WHEAT   | ---LKS     | KFAAGF---  | ---        | ---         | ---         | ---        |
| ATBG_07804     | VDEATEPIAS | KFAAAIVTVG | PGGMREIHW  | PSSDEWTFFI  | RGGRATLFE   | APSTATTFFD |

**Table A-4.1** Wheat EST alignments with cupin containing proteins (continued)

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|                |             |            |             |            |             |            |
|----------------|-------------|------------|-------------|------------|-------------|------------|
| Ta2154253404-2 | VWLAGRNNVI  | AKLDSPAQEL | TFGRPARREVQ | EVFRAKDQD  | EGFVAGPDD   | SHEQDQERG  |
| Ta9905-2       | VWLAGRNNVI  | AKLDSPAQEL | TFGRPARREVQ | EVFRAKDQD  | EGFVAGPDD   | SHEQDQERG  |
| Ta3078253410-1 | VWLAGRNNVI  | GKLDNPAQEL | TFGRPARREVQ | EVFRAKDQD  | EGFVAGPDD   | ----DQERG  |
| Ta3078253407-1 | VWLAGRNNVI  | GKLDNPAQEL | TFGRPARREVQ | EVFRAKDQD  | EGFVAGPDD   | DQERGDERR  |
| Ta3078253412-1 | VWLAGRNNVI  | GKLDNPAQEL | TFGRPARREVQ | EVFRAKDQG  | RR-ASSPDP   | SRTRSAAGTA |
| Ta3078253409-1 | VWLAGRNNVI  | AKLDDPAQEL | AFGRPARREVQ | EVFRAKDQD  | EGFVAGPDD   | ---QHERG   |
| Ta5446-1       |             |            |             |            |             |            |
| AAA34269       | VWLAGRNNVI  | GKLGSPAQEL | TFGRPARREVQ | EVFRAQDQ-D | EGFVAGPDD   | SREQDQDQER |
| Q03678 HORVU   | VWLAGRNNVI  | GKLGSPAQEL | TFGRPARREVQ | EVFRAQDQ-D | EGFVAGPDD   | SREQDQDQER |
| Ta194625-1     | PGLTDADG-V  | GYVAQGGVVL | TVIENG--EK  | RSYTVRE--  |             |            |
| Ta5441-3       | PGLTDADG-V  | GYVAQGGVVL | TVIENG--ER  | RSYTVRQGDV | IVAPAGSITMH | LAINTDGRRL |
| Ta9894-2       | EEAGHRQDP   | HHLRARQVFL | FLGQASSREF  | QTRAESGSG  | DLG-        |            |
| Q852L2_ORYSA   | VFLAGRNSPL  | RQLDDPAKKL | VFGGSAAREA  | DRVLAAPPEQ | ILLRGPHGRG  | SVSDM----  |
| Q8L8I0_ORYSA   | VFLAGRNSPL  | RQLDDPAKKL | VFGGSAAREA  | DRVLAAPPEQ | ILLRGPHGRG  | SVSDM----  |
| Ta628253404-2  | VFVTGGNSVL  | KQLDEAAKAL | AFPPQARELA  | DRVIRAQPE  | VFVAGPQQQR  | RVADM----  |
| Ta628253407-1  |             |            |             |            |             |            |
| Ta136340-2     |             |            |             |            |             |            |
| Ta627253413-2  | VFVTGGNSVL  | KQLDDAAKAL | AFPPQARELA  | DRVIRAQPE  | VFVAGPQQQR  | RVADM----  |
| Ta628253405-1  | VFVTGGNSVL  | KQLDEAAKAL | SFPQARELA   | DRVIRAQPE  | VFVAGPQQQR  | RVADM----  |
| Ta132809-2     | EEERERREEKE | RKQREBERA  | RREKERERE   | EEEAARREQE | EE-         |            |
| Ta189317-3     | ---NWTEEIF  | DALWGDESPL | NKKKKKKKKK  |            |             |            |
| Ta2606253404-2 | QFLTGFSTVL  | RMMLGPFLAA | GLGVPE--KE  | LKVMEAAQKV | AVIEPPLPEK  | EKGKEREFP  |
| Ta2606253405-2 | QFLTGFSTVF  | RMMLGPFLAA | GLGVPE--KE  | LKVMEAAQKV | AVIEPPLPEK  | EKGKEREFP  |
| Q70PK0 WHEAT   |             |            |             |            |             |            |
| ATEG_07804     | SAGDVGYFP   | SRSHYIENTG | DEELMFLEVL  | QADSFTDIAL | GQWIGSTFKQ  | IVADTLKLPE |

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|                |            |            |       |
|----------------|------------|------------|-------|
| Ta2154253404-2 | DRRRGDRGRG | DDAVGAFLRM | ATGAF |
| Ta9905-2       | DRRRGD---- |            |       |
| Ta3078253410-1 | DRRRGDRGRG | DEAVEAFLRM | ATAAL |
| Ta3078253407-1 | GDR-----   |            |       |
| Ta3078253412-1 |            |            |       |
| Ta3078253409-1 | AVVTAAAATK | FWRPS      |       |
| Ta3078253413-3 | DRRRGDRGRG | DEAVEAFLRM | ATAAL |
| Ta5446-1       |            |            |       |
| AAA34269       | HRRRGDRGRG | DEAVETFLRM | ATGAI |
| Q03678 HORVU   | HRRRGDRGRG | DEAVETFLRM | ATGAI |
| Ta194625-1     |            |            |       |
| Ta5441-3       | VIAKILHTSP | CPASSSILGQ | ALLRV |
| Ta9894-2       |            |            |       |
| Q852L2_ORYSA   |            |            |       |
| Q8L8I0_ORYSA   |            |            |       |
| Ta628253404-2  |            |            |       |
| Ta628253407-1  |            |            |       |
| Ta136340-2     |            |            |       |
| Ta627253413-2  |            |            |       |
| Ta628253405-1  |            |            |       |
| Ta132809-2     |            |            |       |
| Ta189317-3     |            |            |       |
| Ta2606253404-2 | VMKQVAME-- |            |       |
| Ta2606253405-2 | VMKQVARE-- |            |       |
| Q70PK0 WHEAT   |            |            |       |
| ATEG_07804     | SALSKLRTK  | QYVVGSGNET | SVAQR |

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Home-Grown Cereals Authority (HGCA) [www.wheatbp.net](http://www.wheatbp.net)

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The Australian Wheat Board (AWB) [www.awb.com.au](http://www.awb.com.au)

Mascot [www.matrixscience.com](http://www.matrixscience.com)