IMPACT OF DORMANCY GENOTYPES ON DIFFERENTIAL PROTEIN EXPRESSION PROFILES AND REDOX-SENSITIVE PROTEOME IN SEEDS OF HYBRID SPRING WHEAT LINES

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by

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ABSTRACT

Seed survival in the soil and cycling through states of dormancy is a key component determining entry and persistence in ecosystems, and seed dormancy is a major trait altered during domestication of wild species. The potential for dormancy is overcome through the time- and environment-sensitive process of after-ripening that occurs in the dry seed. The dormant condition is not a quiescent state; it is a dynamic state in which cell metabolism is active, although growth is repressed. Dormancy is thought to be under the control of two distinct processes: the accumulation of damaging Reactive Oxygen Species (ROS), a critical level of which leads to dormancy alleviation, and a hormonal balance that regulates dormancy directly and likely interacts with ROS and/or antioxidative pathways. The precise mechanisms by which ROS affect seed dormancy status and germination potential remain to be elucidated. Thiol-disulfide proteins are particularly important for redox-dependent regulation of metabolic and developmental activities in cells as functional 'hotspots' in the proteome. Differential proteomic analysis of six hybrid lines of spring wheat (*Triticum aestivum* L.) doubled haploid population, derived from the cross 8021-V2 (high dormancy) × AC Karma (low dormancy) segregating transgressively for dormancy phenotype, and two parent genotypes, was addressed to gain further insight into biochemical mechanisms underlying dormancy controlling events. The thiol redox-sensitive and the total proteome were quantitatively monitored by 2D-gel electrophoresis combined with solubility-based protein fractionation, fluorescent thiol-specific labelling, and mass spectrometry analysis in conjunction with wheat EST sequence libraries.

Quantitative differences between genotypes were found for 106 spots containing 64 unique proteins. Forty seven unique proteins displayed distinctive abundance pattern, and of

these 31 proteins contained 78 unique redox active cysteines. Seventeen unique proteins with 19 reactive modified cysteines were found to have differential post-translational thiol redox modification. The results give an insight into the dormancy-related alteration of thiol-redox profiles in seed proteins that function in a number of major processes in seed physiology. In dormant seeds, there is a shift in the accumulation of proteins from those active in biosynthesis and metabolism to those with roles in storage and protection against biotic and abiotic stresses. The proteomic data provide evidence for an increased capacity of potent antioxidant machinery in seeds of high non-deep physiological dormancy wheat genotypes, which could be coupled with their ability to regenerate antioxidant systems rapidly upon rehydration for dormancy maintenance.

Keywords: Dormancy; Germination; Reactive oxygen species; Thiol-redox regulation; *Triticum aestivum* L.; Plant proteomics; Two-dimensional polyacrylamide gel electrophoresis; Mass spectrometry

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LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

2-D IEF PAGE	two-dimensional isoelectric focusing polyacrylamide gel electrophoresis
2-DE	two-dimensional electrophoresis
ABA	abscisic acid
ACN	acetonitrile
AER	2-alkenal reductase
APS	ammonium persulfate
APX	ascorbate peroxidise
ASC	ascorbate
BSA	bovine serum abumin
CAT	catalases
CBB	Coomassie brilliant blue R-250
DDI	deionized
DH	alcohol dehydrogenase
DHA	dehydroascorbate
DHAR	dehydroascorbate reductase
DTT	dithiothreitol
ESP	embryo-specific protein
EST	expressed sequence tag
FW	fresh weight
GA	gibberellic acid
HMW-GSs	high molecular weight glutenins
IAM	iodoacetamide
IEF	isoelectric focusing
IPG	immobilized pH gradient
LC-MS/MS	liquid chromatography tandem mass spectrometry
LMW-GSs	low molecular weight glutenins
MALDI	matrix-assisted laser desorption/ionization
mBBr	monobromobimane
MDHA	monodehydroascorbate
MS	mass spectrometry
MS/MS	tandem mass spectrometry
m/z	mass to charge ratio
PCD	programmed cell death
PHS	pre-harvest sprouting
p*I*	isoelectric point
Prx	peroxiredoxin
PTM	post-translational modification
QTL	quantitative trait loci
ROS	reactive oxygen species
SDS	sodium dodecyl sulfate
SOD	superoxide dismutase
TCA	trichloroacetic acid

TEMED	tetramethylethylenediamine
Tris	tris (hydroxymethyl) aminomethane
Trx	thioredoxin

CO-AUTHORSHIP STATEMENT

I was in charge of all aspects of the project including literature review, data collection, data analysis, and finding alternative methods to better answer the research question, and preparation of the manuscript for publication.

Chapter one of my Thesis INTRODUCTION LITERATURE REVIEW was written by me and it was partially used for the paper published in *Phytochemistry* journal in year 2010.

The chapters two to five contributed into the paper written and supervised by Dr. Natalia Bykova, and co-authored with Brenda Hoehn (lab technician, AAFC), Dr. Christof Rampitsch (Scientist at AAFC, Winnipeg, MB), Jo-Ann Stebbing (lab technician, AAFC) and Dr. Ron Knox (Scientist at AAFC, Swift Current, SK). My contribution to this manuscript included setting the research question, running two-dimensional protein gels, mapping and quantifying differential protein expression and redox thiol modification, analyzing the bioinformatic data on protein sequencing and identification, functional assignment of proteins and their redox cysteine modification sites, and preparing data in tables and figures for the publication. I also assisted in the preparation and revision of the manuscript. Each biological replicate experiment contained 48 gels (48 × 2 biological replicates = 96 gels; 96 gels × 2 types of images UV and CBB = 192 images analyzed).

All co-authors in the paper contributed to the identification and design of the research project. The complete project published in *Phytochemistry* journal is a result of collaborative contribution by the authors in the following manner: Dr. Bykova is the corresponding author who designed research project, led the experimental work, data analysis and writing of the manuscript; Brenda Hoehn is a lab technician who worked initially on the development of the

methods for protein extraction/fractionation and differential labelling, optimal two-dimensional gel separation, run the replicate experiment to confirm the protein expression maps; Dr. Christof Rampitsch was in charge of the LTQ mass spectrometer contributing with raw protein sequencing and MASCOT search data (this type of instrument/analysis is not available at Memorial University of Newfoundland); Jo-Ann Stebbing contributed with growing wheat plants in growth chambers and greenhouse for the increase of the hybrid seed material, and Dr. Ron Knox is a wheat breeder who provided the hybrid wheat lines for our analysis.

1. INTRODUCTION AND LITERATURE REVIEW

1.1 Socio-economic impact of wheat

1.1.1 Economic and nutritional values of wheat

Wheat (*Triticum*) constitutes about one-third of the global production of cereals, and plays a dominant role in the grain trade attributable to its nutritional value, ease of cultivation and storage (Dubcovsky and Dvorak, 2007). The most financially and nutritionally significant species is the common or bread wheat (*Triticum aestivum* L.), which is modified by selective breeding for desired traits (Feuillet *et al.*, 2007). It provides the greatest number of high-yielding varieties of starchy grains, contributing to one-fifth of the calories consumed by humans.

1.1.2 Wheat classes and cultivation conditions

The species of wheat differ in their basic number of chromosomes. Each related genome (A, B, or D) contains seven chromosomes. For example, *T. monococcum* is diploid (2n=2×=14), *T. durum* tetraploid (2n=4×=28) and *T. aestivum* hexaploid (2n=6×=42) (Sears, 1966). Growing seasons and temperature-dependent flowering conditions separate wheat into the winter wheat and spring wheat. Moreover, according to the seed texture and the seed coat color, five marked classes of wheat are distinguished: Hard and Soft Red Winter (HRW and SRW), Hard Red Spring (HRS), durum (Durum), and White, which is classified in the following subclasses: Hard and Soft Winter (HWW and SWW), and Hard and Soft Spring (HWS and SWS) (Shuey, 1960). The grain

development of winter-types requires a lower temperature (3°C to 8 °C) for seedling emergence than that of spring-types (Feuillet *et al.,* 2007). After harvest, low moisture content and low temperature are essential for successful storage of grain for prolonged periods of time.

1.1.3 The primary uses of wheat

Wheat is an efficient source of protein and carbohydrate. Various classes of wheat are used for different purposes. Durum wheat is the main material for macaroni, spaghetti, and similar products. Soft wheat, with a lesser content of protein than hard wheat, is generally used in the manufacture of cakes, biscuits, and pastry flours. The major classes used for bread are hard-red spring and hard-red winter wheat. Hard white wheat is of higher quality than red wheat, but is prone to pre-harvest sprouting (PHS) (Bassoi and Flintham, 2005). PHS is a harmful phenomenon causing wheat seeds to germinate on the mother plant before harvest, and reducing grain quality and yield (Flintham and Gale, 1988). The susceptibility of wheat varieties to such sprouting is associated with low levels of seed dormancy, especially in white wheat.

1.2 General structure of wheat seeds

1.2.1 Wheat seed structural and functional features

The wheat grain has a complex structure with many individual components. The protective peripheral layers (14% of the grain dry weight) are composed of aleurone layer, testa (seed coat) and pericarp layer (Setter and Carlton, 2000) (Figure 1.1).

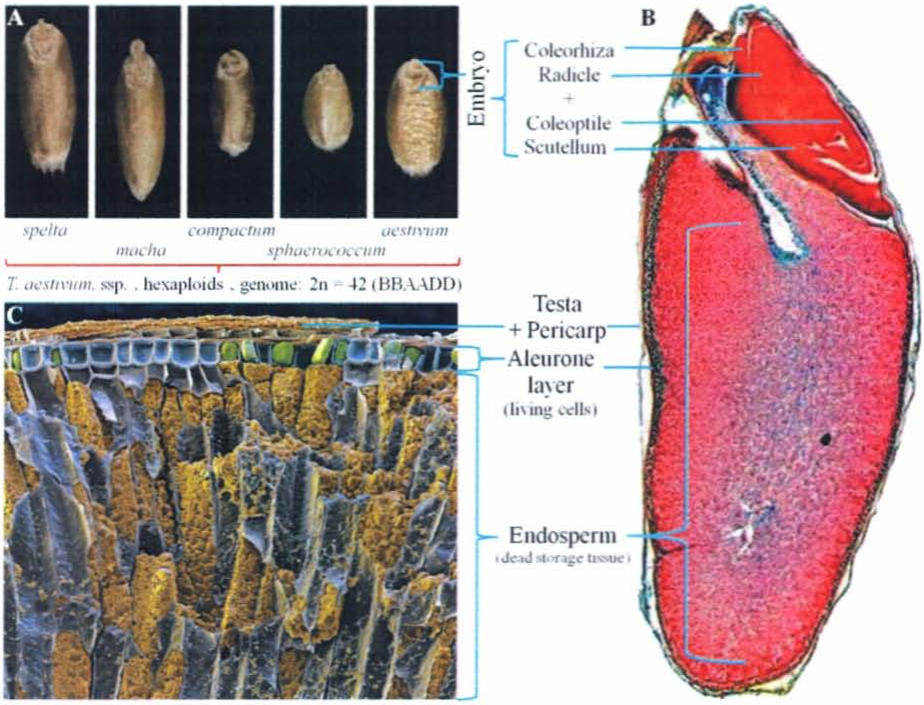


Figure 1.1 The structure of wheat grain and functions of different components in a grain.

A: Phenotypic diversity in grain morphology (e.g. size and shape) among *Triticum aestivium* ssp. *Triticum aestivium* L. is hexaploid, with six copies of seven different chromosomes, 2n = 42 (BBAADD) chromosomes in total. (Adapted from Gegas V C *et al*., 2010);

B: Light micrograph of a vertical section through a grain of common wheat, *Triticum aestivium* L. The main structure of wheat grain contains: ① an embryo (deep red, top), including a scutellum and radicle enclosed within the coleoptile and coleorhiza respectively. The scutellum and radicle are the structures by which the seed will germinate and develop a root and leaf system, developing into a new plant; ②an endosperm (red/blue, large area), in which the reserves of carbon and nitrogen accumulated in protein glutens and carbohydrate starch serve as nutrient sources to support the growth of embyro during embryogenesis and upon germination; ③ peripheral layers (white/grey, thin outer layer), including testa (seed coat) fused with pericarp (fruit coat) that act as protective covering against damages and aleurone layer (grey/black, thin layer adjacent to endosperm) that maintains high enzyme activity for wheat seed germination. Magnification: × 14 when printed 10 centimetres wide (Adapted from *SciencePhoto Library*);

C: Coloured scanning electron micrograph (SEM) of a section through a grain of common (bread) wheat, *Triticum aestivium* L. The majority of the seed comprises a store of starch grains (yellow) surrounded by cell walls (grey). Above this is aleurone layer, a single layer of cells containing protein crystals, such as acitve enzymes (green). The entire seed is covered in a seed coat (brown). Magnification: × 600 when printed 10cm wide (Adapted from *SciencePhoto Library*).

Molecules of nutritional interest (e.g. vitamins, minerals, dietary fibres, and anti-oxidants), and toxic pollutants (e.g. pesticide residues and heavy metals) are concentrated in peripheral layers. The storage endosperm, characterized by high content of starch and protein, contributes to 83% of the grain dry weight. At maturity, the endosperm (dead storage tissue) conteins inactive reserves, which provide energy for the developing embryo. Aleurone (a single layer of living cells) maintains high enzyme activity for wheat seed germination. Developed embryo (3% of the grain dry weight) is the embryonic wheat plant, and it functions in absorbing the nutrients from the endosperm and delivering them to the growing seedling (Shewry, 2009). Mobilization of the storage reserves in the endosperm requires an embryo signal (e.g. gibberellin), which induces the production and secretion of hydrolytic enzymes from the aleurone layer.

1.2.2 Wheat seed protein composition and criteria of wheat end-use quality

The structural composition of the mature endosperm (i.e. protein content, grain hardness and starch quality) determines the end-use quality of wheat products (Rhazi *et al.*, 2003; Veraverbeke and Delcour, 2002). Proteins and carbohydrates accumulated in the developing endosperm not only support germination and early seedling growth as sources of carbon, nitrogen and sulphur through storage proteolysis, but are also critical to humans and animals as food sources.

The endosperm proteome contain approximately 11% of defense- and stress-related proteins out of the total protein content. They protect the starch reserves by increasing resistance to biotic stress factors. For example, α-amylase and α-amylase/trypsin inhibitors protect the seed from attack by pathogens secreting proteases

(Kurek *et al.*, 2002). In addition, proteins related to abiotic environmental stresses were found in the endosperm, such as Heat Shock Protein (HSP) chaperones, which are related to drought stress and/or elevated temperature (Basha *et al.*, 2004; Flemetakis *et al.*, 2002; Johansen *et al.*, 2000). Moreover, a number of antioxidant proteins are present in the endosperm to prevent oxidative stress, notably by Reactive Oxygen Species (ROS) that are generated through different metabolic processes during early and late stages of endosperm development (Mandal and Mandal, 2000). In wheat, the majority of storage proteins are comprised of glutens. Their polymeric structures are formed through inter- and intra-chain disulphide bonds via cysteine residues, tyrosine-based cross-links and other covalent bindings during wheat endosperm formation (Shewry and Halford, 2002; Tilley *et al.*, 2001). They have been shown to undergo a change in thiol redox state as the grain matures, that is, conversion from a redox active (sulfhydryl) state to an oxidised, stable (disulfide) state (Kobrehel *et al.*, 1992). During germination, proteins in the endosperm show a reversal of this redox change, that is, reduction and conversion back to an activated state, thereby facilitating the mobilization of nitrogen and carbon for the developing seedling. A growing number of redox regulated processes demonstrated in previous studies (De Gara *et al.*, 2003; Wong *et al.*, 2003) was found to be controlled by way of a thioredoxin system.

Proteins identified in the embryo were found to be in functional categories associated with activating growth and development, such as transcription, translation, energy and general metabolism, protein assembly, transport, cell division, signalling processes, and components of the cytoskeleton construction (Agrawal and Rakwal, 2008; Pawłowski, 2009). Protein composition in the embryo provides the basis for a better

understanding of the dynamic mechanisms involved in grain dormancy and germination at the metabolic and molecular levels (Williams, 1999; Williams and Hochstrasser, 1997). Although wheat grain protein composition depends primarily on genotype, it is significantly affected by environment factors and their interactions (Ma *et al.*, 2009; Wieser, 2007). Therefore, an enhancement of wheat grain quality for yield development, biotic and abiotic stress resistance, is a major objective for common wheat breeding.

Comparison of protein profiles in the endosperm and embryo demonstrated that the endosperm contains more protein disulphide isomerase isoforms (Skylas *et al.*, 2000), the function of which is to catalyze the rearrangement of both inter-chain and intra-chain disulphide bonds during the folding and maturation of proteins containing disulphide bonds (Shimoni *et al.*, 1995). A larger number of metabolic enzymes were found in the embryo, whereas only oxidoreductases and isomerases were expressed in the endosperm during mid-development. The composition of superoxide dismutase (SOD) isoforms (e.g. Mn-SOD, Cu/Zn-SOD and Fe-SOD) was demonstrated to be different between the endosperm and embryo. The functional role of SOD is to reduce the superoxide radicals that are normally produced in actively respiring cells and can be highly toxic (Wu *et al.*, 1999). A larger number of expressed SOD isoforms present in the endosperm compared to the embryo suggests that the endosperm is likely to be exposed to more oxidative stress than the embryo.

1.3 Regulation of seed development, dormancy and germination

1.3.1 Wheat seed development and maturation

Wheat seed embryo and endosperm development and seed maturation phase comprise an orchestrated physiological process. The formation of embryo structure is followed by further cell differentiation and tissue establishment, to the maximum seed quality and potential longevity attained at physiological maturity (Gutierrez *et al.*, 2007). During embryogenesis, the basic architecture of the seed embryo is built, starting with the formation of a single-cell zygote, followed by cell division and the establishment of embryo structures. Subsequent events include embryo growth and expansion during seed filling, further cell differentiation of vegetative tissue and organ systems, until finally the embryo arrests during maturation to prepare for seed dormancy (Zhu and Khan, 2001). Endosperm development progresses to reach endosperm cell differentiation into tissue types such as starchy endosperm and aleurone (Young and Gallie, 2000). As in all monocotyledons, the endosperm of wheat seeds represents the main part of the mature seed (James *et al.*, 2003), which is an important organ for reserving storage compounds (carbohydrates, proteins and lipids) that are redistributed as nitrogen and carbon sources during germination.

During seed maturation, the developing seed is geared towards the concomitant increase in volume and mass due to significant cell expansion of the storage tissues. Seed maturation is completed when storage compounds have accumulated, water content has decreased, abscisic acid (ABA) levels have increased, and desiccation tolerance and primary dormancy are established (Bewley, 1997). At maturity, so-called orthodox seeds acquire desiccation tolerance during development and remain viable but dormant in a

highly dehydrated state when stored under dry and cold conditions (Arc *et al.*, 2011; Black *et al.*, 1999). This process allows seeds to delay germination until there are suitable environmental conditions.

1.3.2 Seed dormancy and germination

1.3.2.1 The definition and significance of seed dormancy

Developing seeds enter a physiological state where an intrinsic block to germination is built during the completion of seed maturation on the mother plant (Wilkinson *et al.*, 2002). This developmental state, in which a viable seed fails to germinate under favourable environmental conditions, has been termed as primary dormancy or seed dormancy (Finch-Savage and Leubner-Metzger, 2006). In general, fresh-harvested dormant seeds temporarily maintain dormancy in conditions adequate for germination, whereas non-dormant seeds will germinate easily. Numerous transcripts and proteins correlated with dormant versus non-dormant seeds have been identified through "-omics" approaches (Bykova *et al.*, 2011; Gerjets *et al.*, 2010; Somyong *et al.*, 2011). These global studies of functional aspects of entire genomes, transcriptomes, and proteomes complement genetic studies for a comprehensive understanding of the dormancy controlling systems.

Dormancy is not a single phenomenon but a condition with many contributing causes, which categorize dormancy into different types. Owing to embryonic immaturity or physiological constraints, dormancy has been classified as embryo dormancy if the controlling structure or substances are embryonic, or seed coat–imposed dormancy if the tissues surrounding the seed inhibit germination (Finch-Savage and Leubner-Metzger,

2006). Whereas primary dormancy is acquired during seed maturation, imbibed after-ripened seeds exposed to unfavourable temperature conditions or lacking adequate light or nitrate may enter a state of secondary dormancy (Finkelstein *et al.*, 2008). Moreover, based on a comprehensive classification system reflecting that dormancy is determined by both morphological and physiological properties of the seed, physiological dormancy (PD) (Baskin and Baskin, 2004) is the most prevalent type of dormancy in temperate seed banks and is the most abundant dormancy class in the crop field. Finch-Savage and Leubner-Metzger (2006) categorized PD into three levels as deep, intermediate and non-deep according to the germination requirements and conditions to break dormancy. The great majority of seeds have non-deep PD, including the spring wheat seeds discussed in this thesis. Baskin and Baskin (2004) demonstrated that non-deep PD can be broken by GA treatment, scarification, after-ripening in dry storage, and cold (0-10 °C) or warm (>15 °C) stratification. Embryos excised from these seeds produce normal seedlings.

Seed dormancy, a delay between seed shedding and germination, ensures the ability of a species to survive natural catastrophes, decreases competition between individual organisms of the same species, or prevents germination out of season, allowing seedling establishment according to seasonal changes and persistence of the population (Bahin *et al.*, 2011). Lack of seed dormancy is not desirable because it causes pre-harvest sprouting, which reduces seed longevity and damages seed quality.

1.3.2.2 The role of after-ripening in interaction between dormancy and germination

In the process of seed maturation, seed dormancy level increases and reaches a maximum in harvest-ripe seeds (Karssen *et al.*, 1983). Environmental conditions during

seed development affect the dormancy status of different genotypes when the dry seeds are freshly shed from the mother plant (Assmann, 2003). Following desiccation, metabolic processes, such as transcription and translation, are arrested and the embryo enters a period of dormancy during which germination will not occur, even under favourable environmental conditions (Chow and McCourt, 2004). A quiescent period during after-ripening allows the seed to fully release the maternal control, which prevents germination, and finalize the separation from the mother plant to become autonomous. The rudimentary embryo must develop into a full embryonic axis before germination can occur (Yamaguchi-Shinozaki and Shinozaki, 2007). Generally, primary dormancy may be broken during subsequent dry storage of the seeds (after-ripening) or/and stratification (Bewley and Black, 1994), which consist, respectively, of a warm temperature treatment to dry seeds and a low temperature treatment to imbibed seeds.

During after-ripening, the dormancy status reduces until seeds are able to complete germination when imbibed under favourable conditions. Upon imbibition, the quiescent seed is able to reboot its system by internal regulatory control under adequate external conditions. Germination commences with emergence of the radicle from the seed coat by taking up water, and terminates with the elongation of the embryonic axis (Bewley and Black, 1994). This process is driven metabolically by the hydrolysis of proteins and lipids stored during maturation, and subsequent reactivation of a cascade of metabolic activities including transcription, translation, DNA synthesis and cell division leading to the growth of the embryo (von Well and Fossey, 1998). The speed of after-ripening and dormancy status varies, influenced by external environmental cues during seed maturation, seed storage and germination conditions (Holdsworth *et al.*, 2008). A

seed population that previously exhibited a high level of dormancy on imbibition, will subsequently show a high level of germination under the same conditions (Donohue, 2002; Kucera *et al.*, 2005). Both temperature and moisture content influence the speed of after-ripening of unimbibed seeds (Bair *et al.*, 2006; Steadman *et al.*, 2003). The molecular mechanisms that decrease the dormancy status during after-ripening include the hormone-balance theory, which explains dormancy by the opposing action of hormones inhibiting (ABA) and stimulating (mainly gibberellins, GAs) germination (Karssen and Laçka, 1986), and the metabolic theory through enzymic and non-enzymic reactions, which postulates a specific perturbation of respiration in dormant seeds (Bewley and Black, 1994). In addition to gene expression differences between dormant and germinating seeds, non-enzymatic reactions play a role in dormancy release by a causal link between proteome modification via ROS and after-ripening (Oracz *et al.*, 2007). Wouters *et al.* (2010, 2011) demonstrated that the oxidation of proteins results in a modification of enzymatic or binding mechanisms, which leads to changes in the structure and/or function of the specifically carbonylated proteins involved in the dormancy release in dry seeds.

1.3.2.3 The controlling factors of seed dormancy and germination

Common wheat seeds are often dormant when they are shed, and then gradually lose dormancy through dry after-ripening (Steadman *et al.*, 2003). The controlling components have been identified as substances, of which accumulation in seeds correlates with the depth of dormancy (Baskin and Baskin, 2004). Seed dormancy has been investigated with regard to associated physiological, biochemical and molecular changes, as such it is highly regulated by both internal and external cues that determine the

dormancy status and the potential for germination (defined as the final percentage of germination) (Bewley, 1997). Environmental cues, such as temperature, light, oxygen and moisture that widen the requirements for germination, are by definition regarded as dormancy release factors (Finch-Savage and Leubner-Metzger, 2006). In addition to substantial environmental influences, genetic variations in structure and/or pigmentation of the seed coat (testa) affect the dormancy status of seed (Fofana *et al*, 2008). It has been demonstrated that dormancy genes are tightly linked to seed coat colour as determined by dominant R alleles (Flintham, 2000). In wheat, the strongest dormancy is associated with a red seed coat colour, whereas the lines with white seed coats are non-dormant or weakly dormant and therefore are susceptible to pre-harvest sprouting damage.

Dormancy and germination are physiological developmental processes mediated by a complex network of phytohormones, including ABA, GA, ethylene and auxin (Finkelstein *et al*., 2008; Kwak *et al*., 2006). Hormone controlling events depend on the combination of the hormone content (the net result of rates of synthesis and metabolism), and the sensitivity of the cells to the hormone (Bradford and Trewavas, 1994). Previous studies demonstrated that the onset of dormancy during embryo maturation is regulated by ABA biosynthesis (Brady and McCourt, 2003), ABA signal transduction (Kuhn and Schroeder, 2003) and maturation processes (To *et al*., 2006). During seed maturation, the ABA content increases and the changes in sensitivity to ABA are related to the maintenance of dormancy, tolerance to desiccation stress and inhibition of germination (Holdsworth *et al*., 2008). ABA is produced in maternal tissues and in the embryo, but only embryonic ABA is necessary to impose a lasting dormancy (Nambara and Marion-Poll, 2003), whereas maternal ABA, or ABA application during seed development, fails

to induce lasting seed dormancy (Rajjou and Debeaujon, 2008). However, *de novo* ABA synthesis in the embryo during imbibition allows maintenance of dormancy (Kucera *et al.*, 2005). The embryonic ABA content decreased quickly after imbibition in non-dormant grains (where germination occurred), but remained high in dormant imbibed grains (where germination was prevented) (Benech-Arnold *et al.* 2006). Deficiency of ABA during seed development is associated with the absence of primary dormancy in the mature seed, whereas the over-expression of ABA biosynthesis genes can increase seed ABA content and enhance seed dormancy or delay germination (Finkelstein *et al.*, 2002; Kushiro *et al.*, 2004). Therefore, ABA levels and the resulting dormancy are controlled by the combined action of differentially expressed enzymes involved in several steps of both ABA synthesis and catabolism.

On the contrary to the role of ABA controlling the establishment and maintenance of dormancy, the presence of GAs stimulates germination by inducing hydrolytic enzymes that weaken the barrier tissues such as the endosperm or seed coat, inducing mobilization of seed storage reserves, and stimulating expansion of the embryo (Grappin *et al.*, 2000). There are many instances where GA alone is ineffective for the induction of germination in dormant seeds, and GA is thought to be necessary but not sufficient for dormancy release (Gonai *et al.*, 2004). There is growing evidence that GA mediates metabolism of ABA and vice versa (Gubler *et al.*, 2008). In the aleurone layer of seed, the role of GAs is crucial after dormancy breakage, where it is required for reserve mobilization and germination (Gubler *et al.*, 2002). The balance of ABA/GA levels and sensitivity plays a critical role in regulating seed germination and dormancy status, and these hormones have reciprocal effects on their respective biosynthesis and inactivation

pathways (Cadman *et al.*, 2006; Oh *et al.*, 2007). Moreover, ethylene promotes germination, and auxins support plant growth and development throughout the plant life cycle (Allen *et al.*, 2007). It is necessary to characterize and quantify the germination and dormancy behaviour of seeds in response to a diverse range of physiological states and environmental conditions.

In addition to the role during seed dormancy and germination, phytohormones are known to control the mobilization of storage reserves in cereals (Lovegrove and Hooley, 2000). ABA and GA regulate the expression of genes encoding the enzymes required for storage protein and carbohydrate mobilization in wheat (Pulido *et al.*, 2009a). Studies on seed dormancy controlling events (Kim *et al.*, 2009; Kranner *et al.*, 2010) have revealed a highly complex interaction between environmental conditions, seed growth regulators, and the sensitivity of seeds to these parameters.

1.4 Environmental factors related to seed dormancy and germination

1.4.1 Interactions between phytohormones and the environment

Seed dormancy or germination outcomes are determined by a balance between pathways associated with GA and ABA, external environmental signals, and internal developmental signals (Brady and McCourt, 2003). The signalling pathways of these hormones are interconnected at several levels and interact with other hormones, such as ethylene and brassinosteroids, which both influence the ABA/GA balance by counteracting ABA effects and promoting germination (Weiss and Ori, 2007). It is likely that the crosstalk between different hormone signalling pathways contributes to the flexibility of seeds in their responses to developmental and environmental factors

(Alboresi *et al.*, 2005). Low temperatures and exposure to light are the major environmental factors that release seed dormancy and enable the completion of germination (Chiwocha *et al.*, 2005). Furthermore, ROS, nitrate, and nitric oxide have been suggested to affect GA and ABA pathways (Bethke *et al.*, 2007; Pulido *et al.*, 2009b) and was shown to accelerate the decrease in ABA levels that occurs during seed imbibition (Ali-Rachedi *et al.*, 2004; Koornneef *et al.*, 2002). However, the precise mechanisms by which ROS affect seed dormancy status and germination potential remain to be elucidated.

1.4.2 Pre-harvest sprouting

Dormancy at harvest is a desired trait because it prevents the precocious germination, the premature sprouting of grains in the head following exposure to cool moist conditions, known as pre-harvest sprouting (PHS) or vivipary (Bassoi and Flintham, 2005). PHS is the major cause of increased alpha-amylase hydrolytic enzyme activity during the hydrolysis of starch in the endosperm, which results in a decrease of grain weight conditions (King, 1993), and leads to a reduction in grain quality and viability of seed, therefore significant economic losses on end-product quality for the grain industry, especially in parts of the world where cool damp conditions prior to harvest are a possibility (Gubler *et al.*, 2005). Common wheat is susceptible to PHS due to a lack of sufficient degree of seed dormancy (McCaig and Depauw, 1992). Therefore, it is necessary to breed for increased resistance by enhancing seed dormancy to improve the tolerance to PHS.

Different between genotypes, PHS resistance is a complex trait that varies depending on the stage of maturity, which is affected by environmental conditions during grain ripening, and depends on spike and crop morphology, biotic and abiotic stress (Mares *et al.*, 2005). Significant efforts have been made to identify quantitative trait loci (QTLs) controlling seed dormancy and pre-harvest sprouting tolerance in wheat (Mori *et al.*, 2005; Torada *et al.*, 2008). PHS can be combated in part through manipulation of grain colour via the red grain (R) locus, which provides some resistance to sprouting (Kato *et al.*, 2001). Chen *et al.* (2007) demonstrated that QTL on chromosome 4A can influence dormancy level at harvest, as well as ABA sensitivity and PHS susceptibility.

A number of studies have analysed the relationships between dormancy, ABA sensitivity and susceptibility to PHS in wheat. Germination potential of wheat seeds increases with time of dry storage (Mares, 2005), and the kinetics of after-ripening are related to variety and environment (Mori *et al.*, 2005). PHS-resistant varieties exhibited enhanced dormancy characteristics of isolated embryos and enhanced responsiveness to applied ABA (Flintham, 2000). Conclusions from previous studies (Fofana *et al.*, 2008) show 1) dormancy at harvest and PHS susceptibility have been assumed to be linked phenomena; 2) understanding the phytohormone signalling mechanisms that control wheat seed dormancy will contribute to the targeted breeding of wheat varieties with storage proteins better protected against uncontrolled proteolysis caused by sprouting .

1.4.3 Abiotic and biotic stresses to wheat seed growth

Abiotic stresses, such as extreme temperatures, drought, salinity, chemical toxicity and oxidative stress, suppress the activities of cellular molecules and result in the

deterioration of the cellular environment, reduced growth and extensive losses in agricultural production by more than 50% (Benech-Arnold, 2006). Wheat seeds from temperate climates often exhibit primary dormancy at harvest that is most evident at warm temperatures (>15°C) (Leymarie *et al.*, 2008). Insufficient dormancy can result in pre-harvest sprouting in humid climates, while excessive dormancy can interfere with utilization of the grain for planting.

Common wheat has low drought resistance and is vulnerable to water deficit (McCaig and Depauw, 1992). Drought conditions (caused by weather conditions and/or soil type) have the potential to alter the dormancy status in seeds. Withholding water during seed development caused a decrease in the degree of seed dormancy (Rajjou and Debeaujon, 2008). During early seed development, drought causes the abortion of developing grains, resulting in the shrinking of grains, leading to yield losses (Blum, 1998). Lower seed mass affects the development and biomass of the seedlings (Aparicio *et al.*, 2002), and thus the carbohydrate reserves and yield of the next generation (Barnabás *et al.*, 2008). Late in seed development, upon imbibition, the quiescent seed embryo faces a hostile environment. The conditions of high heat and humidity accelerate seed ageing (Hajheidari *et al.*, 2007). It has demonstrated that imbibing embryos are capable of expressing an enhanced level of heat shock response, which is related to the seed's ability to germinate and establish under extremes of temperature and moisture (Wang *et al.*, 2004). The heat shock response during very early germination plays an important role in the survival and eventual germination of seeds under stressful conditions. Dormant seeds are deficient in heat shock response proteins and show a marked depression in their ability to survive and germinate following exposure to long periods of

high temperature and humidity, while non-dormant seeds demonstrate a high frequency of germination under the same stress (McElwain and Spiker, 1992). It was established in previous studies (Basha *et al.*, 2004) that a germinating wheat embryo is able to synthesize a complete set of heat shock responsive proteins to survive heat stress, when heat shock was initiated simultaneously with imbibition.

Soil salinity adversely affects physiological and metabolic processes, and finally diminishes crop growth and yield (Ashraf and Harris, 2004). The effect of dormancy-related growth regulators (e.g. GA, ABA, and ethylene) on germination has been studied in relation to their response to salinity (Athar *et al.*, 2008; Sairam *et al.*, 2005; Wahid *et al.*, 2007). Changes in growth regulators balance that are induced by salt stress may be related to the mechanisms inducing dormancy in seeds.

Seed growth reduction due to salinity is attributed to ion toxicity and nutrient imbalance, which lead to an oxidative stress manifested by accelerated production of ROS (Lee *et al.*, 2001). The balance ROS-formation and ROS-scavenging appears to represent a key stress tolerance trait (Kim *et al.*, 2009). Expression of antioxidant defense genes would, in turn, be triggered to defend the cells against oxidative damage (Blokhina *et al.*, 2003). Elimination of ROS is mainly achieved by antioxidant compounds such as ascorbic acid, glutathione, thioredoxine and caroteniods, and by ROS scavenging enzymes, such as superoxide dismutase, glutathione peroxidase and catalase (Colville and Kranner, 2010). This shows that oxidative stress tolerance is genetically controlled and it provides a wide scope for crop improvements enabling the breeding of more drought tolerant, or generally more stress tolerant crops.

1.5 Oxidative stress and thiol-based antioxidants in wheat seeds

1.5.1 Oxidative stress by reactive oxygen species

Aerobic metabolism produces oxygen derivatives including singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) or hydroxyl radicals (·OH), termed ROS (Apel and Hirt, 2004). Although ROS are produced as a consequence of aerobic metabolism, their levels are maintained relatively low under standard growth conditions (Asada, 2006; Gapper and Dolan, 2006). However, environmental stress, such as salinity, drought, high light intensity, low or high temperature, or pathogen attack disturb the cellular homeostasis and increase ROS production, thus causing oxidative stress (Gill and Tuteja, 2010). ROS accumulation has a toxic effect due to the high reactivity of these species that cause damage to the lipids, proteins and nucleic acids. Because proteins have numerous biological functions, their oxidation may result in modification of their enzymatic and binding properties and lead to diverse functional changes. Previous studies (Arc *et al.*, 2011; Davies, 2005) have shown that oxidation of proteins can occur through a number of different mechanisms, such as the formation of disulfide cross-links and glycoxidation adducts, nitration of tyrosine residues, and carbonylation of specific amino acid residues.

1.5.2 Oxidative stress during wheat seed development and germination

ROS are continuously produced during seed development, from embryogenesis to germination, but also during seed storage (Bailly, 2004). Bailly and El-Maarouf-Bouteau (2008) demonstrated that photosynthetic production of ROS is elevated at early stages of seed development; respiration is a significant source of ROS production during the early

stages of embryogenesis when moisture content is high enough to allow metabolic activities, but subsequently decreases during seed maturation and desiccation, a phase with a low metabolic activity.

At maturity, desiccated seeds enter into a dormant period when water is not available for biochemical reactions, and ABA induces desiccation tolerance, permitting their survival for long periods in a dry state. In this phase, the seed suffers oxidative stress due to the production of ROS that occurs as a consequence of massive loss of water (Leprince *et al.*, 1993). Desiccation tolerant is correlated with the generation of ROS and the occurrence of oxidative damage during dehydration (Pukacka and Ratajczak, 2007a), leading to the suggestion that desiccation tolerance depends in part on the ability to scavenge ROS compounds by antioxidant defense systems during after-ripening (storage in dry conditions) or during stratification (imbibition at low temperature), metabolic reactivation that implies resumption of respiration, and other non-enzymatic reactions are likely to occur such as lipid peroxidation (Tammela *et al.*, 2005; Pukacka and Ratajczak, 2007b), or the Amadori and Maillard reactions associated with free radical production and oxidation processes in the embryo (Murthy and Sun, 2000; Murthy *et al.*, 2003). During germinating and post-germinating, wheat seeds tissues are featured with a high rate of cell division and metabolic activity (Penfield and King, 2009). All of these tissues suffer oxidative stress and undergo programmed cell death (PCD) during development and germination, which can be accelerated by oxidative damage.

The degree of damage depends on the balance between formation of ROS and its removal by both enzymatic and non-enzymatic antioxidative scavenging systems and it appears to represent an important stress-tolerance trait. Among the non-enzymatic

systems, the presence of molecules with antioxidant activity in seed tissues including ascorbate, tocopherols and polyphenols has been shown (Howitt and Pogson, 2006; Sattler *et al.*, 2004). The non-enzymatic antioxidant systems have a relevant role in ageing seeds (Ahmad *et al.,* 2010). Among the enzymatic antioxidant systems, seeds express superoxide dismutases responsible for the conversion of superoxide anion to hydrogen peroxide, which is reduced to water by different peroxidases, such as ascorbate peroxidase, catalases, and peroxiredoxins (Bailly *et al.*, 2008). For example, maturing seeds have increased catalase activity (Berjak, 2006) and accumulate glutathione, peroxiredoxins (Haslekås *et al.*, 2003), or even storage proteins serving as substrates for oxidative carbonylation (Job *et al.*, 2005). Studies (Pulido *et al.*, 2009a, 2009b; Rajjou and Debeaujon, 2008) have shown that, in wheat seedlings, elimination of ROS is mainly achieved by antioxidant compounds (e.g. ascorbic acid, glutathione, thioredoxine and caroteniods), and by ROS scavenging enzymes (e.g. superoxide dismutase, glutathione peroxidase and catalase) mainly increased during development of orthodox seeds when acquisition of desiccation tolerance is associated with the synthesis of specific proteins, accumulation of oligosaccharides, and activation of antioxidant defense systems.

1.6 Signalling roles of ROS and redox regulation of proteins

1.6.1 Cellular signalling roles of ROS in seed dormancy and germination

Protein oxidation due to ROS is not necessarily a deleterious phenomenon in seed physiology (Job *et al.*, 2005). ROS have been invoked to play a role in cellular signalling, notably acting as regulators of growth and development, programmed cell death, hormone signalling and responses to biotic and abiotic stresses (Mittler *et al.*, 2004). The signalling

role of ROS is relevant for seeds during the transition from a developmental to a germinative mode and the alleviation of dormancy, raising the hypothesis that these compounds can facilitate the shift from a dormant to a non-dormant status in seeds. Plant hormones, such as ABA and GA, are considered as being the major signalling actors in these processes (Bethke *et al.*, 2007). Several studies (El-Maarouf-Bouteau and Bailly, 2008; Kwak *et al.*, 2006) have demonstrated that ROS can also interplay with the hormonal signalling pathways.

Increased generation of ROS occurs during the activation of a regulatory system controlled by intrinsic (dormancy) and extrinsic (environmental conditions) factors. The external factors control the balance between ROS scavenging and production (Schopfer *et al.*, 2001). The success of germination tightly depends on external factors such as temperature, light, oxygen and water availability (Bailly *et al.*, 2008). ROS act as messengers or transmitters of environmental cues to the internal sensors in seeds to complete the germination. Hydrogen peroxide, hydroxyl radicals and superoxide radicals have been shown to accumulate in the germination process and contribute to cell wall loosening during endosperm weakening, programmed cell death of aleurone layer of cereal grains or protection of the emerging seedling against pathogens (Wojtyla *et al.*, 2006). At the cellular level, ROS regulate the cellular redox status to cause the oxidation of proteins and to trigger specific gene expression through the changes in ROS homeostasis (Bailly, 2004). With regards to ROS metabolism during dormancy imposition and release, it has been proposed that ROS could be a ubiquitous signal involved in dormancy alleviation during storage of seeds in dry conditions (after-ripening), and that they could facilitate the shift from a dormant to a non-dormant status

in seeds. The accumulation of ROS by non-enzymatic metabolism and peroxidation products in the dry state lead to irreversible protein oxidation (carbonylation) in cells of embryonic axes, allowing a shift from a metabolism characteristic of dormant seeds to a metabolism characteristic of non-dormant (after-ripened) seeds (Ahmad *et al.*, 2010). Additional evidence on the putative role of ROS in dormancy control comes from studies on the interaction of these compounds with plant hormones as ABA, GA, which being well known for playing a major role in seed dormancy and germination (Bailly *et al.*, 2008). For example, H_2O_2 was shown to represent major reactive oxygen leading to cell death in aleurone cells (Bethke and Jones, 2001). GA initiates cell death of aleurone cells, whereas ABA inhibits cell death (Wong *et al.*, 2003; 2004). The activities of ROS-scavenging enzymes, including catalase, ascorbate peroxidase, and superoxide dismutase, are significantly down-regulated in GA_3-treated aleurone cells, thus rendering these cells sensitive to oxidative damage and cell death, whereas ABA caused increases in catalase activity (Fath *et al.*, 2002). ROS-scavenging mechanisms will be important for mediating and controlling these responses. A strong oxidative burst will cause cellular damage and death (Apel and Hirt, 2004). Furthermore, constitutive ROS elevations, even if not very high, could cause malfunction or desensitization of ROS-dependent signaling responses (Vandenabeele *et al.*, 2003). Several studies suggest that ROS-scavenger proteins play central roles in ABA signalling (Dietz, 2008). In addition, D'Autreaux and Toledano (2007) have shown that several ROS scavenging mRNAs are regulated in response to GA, ABA, and oxidative stress.

1.6.2 Dynamic thiol-disulfide redox regulation and modification of proteins

Cysteine (Cys) plays an important role in protein biochemistry. The unique chemical property and high reactivity of the free thiol group makes reduced cysteine a versatile component of catalytic centres and metal binding sites in proteins (Ghezzi, 2005). Cys is susceptible to a variety of modifications by ROS, which increases in abundance under unfavourable conditions like drought, salt or temperature stress. Under severe oxidative stress, the cell activates the programmed cell death, and sequential oxidation of Cys thiols yields sulfenic (–SOH), sulfinic ($–SO_2H$), or sulfonic ($–SO_3H$) acid derivatives, which are considered irreversible modifications by excess ROS (Møller *et al.*, 2007). Less severe oxidative stress triggers signalling cascades to readjust the redox environment through reversible oxidation of Cys thiols by limited amount of ROS, which result in a disulfide bridge forming between two thiols groups either within a protein chain or between protein chains (Hogg, 2003; Ghezzi *et al.*, 2005). Covalent postranslational modification by disulfide bond formation stabilizes the protein by maintaining its tertiary structure, which prevents denaturation and decreases susceptibility to proteolytic degradation prominent in secretory and storage proteins (Holdsworth *et al.*, 2008). In addition to stabilizing structure, cysteine-dependent regulation of protein activities associated with the conformational changes between reversibly oxidized (S−S) and reduced (−SH HS−) often alter functions of proteins or result in either a catalytic or regulatory change (Yano and Kuroda, 2006; Ströher and Dietz, 2008). Catalytic disulfides are often formed between two cysteines separated by one or two amino acids, which can physically affect the catalytic properties of the enzyme, such as NADP-malate dehydrogenase (Dalle-Donne *et al.*, 2007), of which the redox active site functions either

as an electron (hydrogen) donor or acceptor (Dixon *et al.*, 2005; Yano *et al.*, 2002). The interconversion of thiols to a disulfide equally provides a mechanism for the regulation of catalytic activity, and can be prevented with a mixed disulfide formed by cysteinyl residues that undergo reversible modification such as glutathionylation,

During wheat seed development and germination, seed tissues suffer oxidative stress. Among the different antioxidant mechanisms available in this processes, thioredoxin *h* (Trx *h*) system (Cazalis *et al.*, 2006; Montrichard *et al.*, 2009), NADPH-dependent thioredoxin reductase (NTR) system (Shahpiri *et al.*, 2008; Li *et al.*, 2009; Meyer *et al.*, 2009), and a thiol-based antioxidant system formed by 1-Cys peroxiredoxin (1-Cys Prx) (Dietz *et al.*, 2006) support the classical function proposed for the NTR/Trx redox system in the activation of storage mobilization, thus facilitating seed germination.

1.6.3 The NADP/Thioredoxin system of seeds

Thioredoxins (Trxs) are small proteins (12-14 kDa) with a conserved active site formed by the consensus sequence -Cys-Gly-Pro-Cys- (-CGPC-), in which the two Cys residues act as efficient disulfide reductants (Sokolov *et al.*, 2006). The redox conversion of disulfide/dithiol functions in a process of the NADPH-dependent Trx reductase (NTR)/Trx system, which accelerate seed germination by facilitating the mobilization of storage compounds in the starchy endosperm (Serrato *et al.*, 2002). Most of the proteins in the starchy endosperm are in the oxidized state, and reduction is required to facilitate the action of proteases involved in their degradation (Besse *et al.*, 1996). Trx *h* isoform is involved in the inactivation of α-amylase and trypsin inhibitors, thus facilitating starch and protein degradation (Shahpiri *et al.*, 2008). It was shown to promote the activation of

α-amylase, pullulanase, and proteases with the concomitant reduction of disulfide (S–S) groups in diverse seed proteins (e.g. storage proteins, enzymes, and enzyme inhibitors), resulting in the mobilization of carbohydrate and protein reserves that sustain seed germination (Lozano *et al.*, 1996; Yano *et al.*, 2001). A proteomics-based approach allowed identification of Trx targets in wheat starchy endosperm that confirmed the important role of this redox system in seed germination (Wong *et al.*, 2003). Besides the role of the NTR/Trx *h* system in facilitating starch and protein degradation in germinating seeds, it was reported that overexpressing Trx *h* showed enhanced gibberellin synthesis in the embryo, which affects the expression of α-amylase in aleurone cells, thus suggesting a functional role of this enzyme in communication between the embryo and aleurone tissues (Wong *et al.*, 2004). Thus, the localization of Trx *h* and NTR in seed tissues could be connected to Trx function as a redox signalling molecule in germinating seeds linked to the coordination of redox control of different tissues within seeds.

1.6.4 1-Cys peroxiredoxin expressed in seeds

Peroxiredoxins (Prxs) are thiol-based peroxidases that show a typical thioredoxin fold. Prxs represent an important family of sulfhydryl-linked antioxidant proteins, ubiquitously present in all known organisms (Kranner *et al.*, 2010). Peroxidase activity of Prxs is based on the action of one or two Cys residues that form the active site, in contrast with other peroxidises (Colville and Kranner, 2010). Regarding the seed peroxide scavenging systems, 1-Cys Prx is exclusively expressed in developing wheat seeds, where it accumulates in the endosperm, scutellum, and aleurone cells, with expression being at

the highest level during the desiccation stage (Dietz *et al.*, 2006). In the starchy endosperm of germinating seeds, the 1-Cys Prx was detected as a dimer, which could be a reflection of the highly oxidative environment in this tissue. In both aleurone and scutellum cells of germinating seeds, 1-Cys Prx was detected in a monomeric form, and decreased upon germination completion (Monteiro *et al.*, 2007). Furthermore, the nuclear localization, antioxidant activity, and characteristic expression pattern of a 1-Cys Prx in seed cells suffering oxidative stress suggested a possible role of this enzyme in the control of peroxide levels in the nucleus. This antioxidant system may be relevant not only as a mechanism to protect nuclear DNA from oxidative damage, but for the redox regulation of nuclear processes, such as transcription, splicing and protein trafficking (Guo *et al.*, 2007). In addition, 1-Cys Prx antioxidant system has an important function of ROS scavenging involved in a tolerance mechanism to oxidative stress and Programmed Cell Death (PCD) (Wahid *et al.*, 2007), which requires a source of reducing power in order to maintain activity, a function carried out in most organisms by thioredoxins (Trxs).

1.7 Proteomics analysis in wheat seeds

1.7.1 From genomics to proteomics

Newly emerging technologies encompassing 'genomics' (DNA), 'transcriptomics' (mRNA) and 'proteomics' (proteins) provide complementary tools and interactive sequence databases to elucidate interactions between DNA, RNA and proteins in living biological systems, and thereby bridge the gap between DNA and proteins, and between the genome and the proteome (Anderson and Seilhamer, 1997). Proteomics is the study of the full complement of polypeptides expressed by the genes of an organism in a specific

tissue, at a particular stage of development and under specified growth conditions (Skylasa *et al.*, 2005). Proteomics emphasise the functional aspects of genomic studies, involving the elucidation of downstream effects of the genome and taking into account the interplay of environment with the genome, to determine the characteristics of the organism.

1.7.2 Proteomics approaches

Proteomics studies the total complement of cell proteins or of defined sub-proteomes (Wang *et al.*, 2007). High-throughput separative and analytical techniques are used to detect changes in level/status of specific proteins. Most of the proteomic studies have used two-dimensional sodium dodecyl sulphate–polyacrylamide gel electrophoresis (2D SDS–PAGE) as a protein separation and quantification tool. The 'first dimension', known as isoelectric focusing (IEF), separates the proteins in an immobilised pH gradient (IPG). Proteins migrate and resolve to the point in which they have zero net charge (isoelectric point, p*I*). The 'second dimension' separates proteins according to their molecular mass alone, using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A uniform charge-to-mass ratio allows proteins to be separated from the mixture (Görg *et al.*, 2000). The separation power of 2D gels considerably simplifies the subsequent analysis by MS. The digestion of each spot on a 2D gives a few dozen peptides, producing a level of complexity that is managed by peptide fragment fingerprinting approaches based on matrix-assisted laser desorption/ionization (MALDI)-MS/MS, or nano-electrospray liquid chromatography (LC)-MS/MS approaches

(Shevchenko *et al.*, 2000). Mass spectrometric sequencing is used to allow protein databases or expressed sequence tag (EST) databases to be searched.

1.7.3 Proteomic analysis of wheat seeds

Proteomics has the potential to contribute to genetic and physiological studies in wheat science and to grain quality by elucidating the ways in which the genes are expressed during grain filling under given environmental conditions (Skylas *et al.*, 2002). For example, a major objective of proteome studies of the wheat grain is to elucidate the range of polypeptides and disulfide bonds in proteins that account for the unique dough-forming properties of wheat flour (Southan and MacRitchie, 1999). The proteome of the wheat amyloplast has been investigated with the objective of learning more about starch quality (Andon *et al.*, 2002). Moreover, the application of wheat proteomics is well-suited to studying the proteins associated with tolerance to heat-stress conditions during growth (Skylas *et al.*, 2002). The application of examining the effects of variations in growth conditions is based on the fact that relatively minor changes in the composition of a large number of proteins can be observed using image-analysis equipment (Majoul *et al.*, 2003). Therefore, it may be possible to identify marker proteins to select for tolerance to the dough-weakening effects of heat-stress, thereby eliminating the need to grow wheat genotypes under controlled conditions and test for dough quality.

1.7.4 Disulfide and redox proteomics of wheat grain proteins

The proteins synthesized in a cell undergo several post-translational modifications that are essential in their functional regulation. The change of the redox state of cysteine

residues represents a very dynamic and regulated balance. These reversible thiol modifications have found a use as regulatory nano-switches in an increasing number of redox sensitive proteins (Wormuth *et al.*, 2007). These redox-regulated proteins are able to adjust their activity quickly in response to changes in their redox environment (Wouters *et al.*, 2010, 2011). A number of techniques have been developed to quantitatively describe the extent of thiol modification *in vivo*, giving insight into the global thiol–disulfide state of proteins in the cell. An increasing amount of studies have been addressed to detect protein oxidation in plants through redox proteomics (Leonard *et al.*, 2011; Finnie and Svensson, 2009). They have been successfully used to find substrates of thiol–disulfide oxidoreductases and to discover novel redox-regulated proteins (Alvarez *et al.*, 2011). For example, a model study of thioredoxin-linked reactions in seed germination has been conducted by redox proteomics based on analysing disulfide proteome (Rinalducci *et al.,* 2008; Yano and Kuroda, 2006). Drought tolerance in wheat has been widely studies for redox regulation by proteomics approach (Hajheidari *et al.*, 2007). Moreover, the thiol-redox proteomics approach (Bykova *et al.*, 2011) was used recently to study dynamic changes in the redox-sensitive proteome upon seed dormancy release in wheat.

Proteomic analysis is a powerful tool to depict the posttranslational modifications of the proteome (Spickett *et al.*, 2006). Most of the proteomic studies of the oxidative stress response have used 2D SDS–PAGE to separate proteins based on charges and masses, coupled with MS/MS approaches as a protein characterization tool. Many post-translational modifications can be detected, such as some stages of cysteine oxidation (cysteine sulphinic and sulphonic acids), which are expected to induce p*I* changes.

Oxidative stress can cause changes in levels of specific proteins detectable by protein staining and image analysis. Similarly, redox-based processes altering the p*I* or M_r of proteins (i.e., charge isomerization, protein backbone cleavage, and crosslinking) are detected as altered 2D SDS–PAGE spots. Moreover, single fluorescence-based labelling has been widely used to detect oxidized or reduced Cys residue by 2D gels. For example, the IAM-derivatives 5-iodoacetamidofluorescein (Chiappetta *et al.*, 2010) and monobromobimane (Yano, 2003), a Cys-specific reagent that fluoresces upon UV irradiation, have been used to reveal the extent of Cys residue oxidation by 2D gels. Reduced Cys residues were blocked by alkylation with IAM, and oxidized residues were labeled with the fluorescent Cys-reagent. Labeled proteins were visualized on 2D gels using a fluorescence imaging system, and the indexes of protein-thiol oxidation were determined by spots intensity (Hochgrafe *et al.*, 2005). The MS/MS approaches allow both the description and the localization of the modification in the modified peptide. However, Chouchani *et al.* (2011) demonstrated some modifications that cause the MS signal to decrease considerably. For example, sulphation and cysteine oxidation in sulphinic or sulphonic acid alter the charge of the peptide, often making it negative. Carbonylation favours peptide-peptide interactions, which in turn decrease the peptide extraction yields and thus the signal.

1.7.5 Functional diversity of targeted proteins subject to cysteine oxidation

ROS driven oxidative modification of proteins comprise an important class of post-translational modifications. Produced throughout the variety of stages during seed development and maturation, ROS can directly affect the thiol side chains of protein

cysteines to regulate the activity of proteins. The free thiol functional groups (-SH) are characterized by high reactivity, making cysteine very sensitive to redox transformations. As cysteine often participates in electron transfer catalytic reactions, in metal binding (i.e., Zn^{2+} and Fe^{2+}), and plays structural roles in proteins, oxidoreductive modifications can adopt a variety of oxidation states and profoundly influence protein function (Wouters *et al.*, 2010). Depending on the cysteine oxidation state that is formed, such modifications can be reversible or irreversible. The thiol sulfhydryl/disulfide exchange and sulfenation (SOH) are best known forms of reversible oxidoreductive post-translational modification to cysteine residues in proteins affected by restricted levels of ROS. While there are some beneficial roles of ROS, when they are over produced, they can cause damage. Excessive levels of ROS oxidize thiol groups in cysteine residues to generate irreversible oxygen derivatives in proteins, such as sulfenic (RSOH), sulfinic (RSO_2H), and sulfonic (RSO_3H) acid, resulting in oxidative stress to cells (Jeong *et al.*, 2011). In addition, the thiol side chain of cysteine participates as a nucleophile in numerous post-translational modifications including S-acylation, S-nitrosation and protein splicing. In cereal seeds, the regulatory sulfhydryl/disulfide bond system alters the structure and activity of proteins controlling an array of events in seed germination such as mobilization of storage proteins, activation of proteases, transcription, cell division, radical scavenging and detoxification (Montrichard *et al.*, 2009; Jacob and Ba, 2011). Together, these proteins and enzymes form an extensive and significant network to sense changes in the intracellular redox environment and to trigger a measured, appropriate and rapidly reversible response, in the form of gene expression, antioxidant defence or by inducing apoptosis.

1.8 Thesis hypothesis and objectives

1.8.1 Hypothesis

Seed dormancy is a discrete developmental pathway associated with specific metabolic networks. While the dormancy condition is often thought of as a quiescent state; it is in fact a dynamic state in which cell metabolism is active, but growth is repressed. During the dormancy stage, there is a biochemical shift from active biosynthesis and metabolism to storage and protection against biotic and abiotic stresses. Moreover, a higher antioxidant capacity related to sensing of a threshold redox potential and balancing the existing redox pools is needed for wheat seeds to maintain high dormancy. The capacity to maintain, or to rapidly re-establish a number of antioxidant protein systems upon rehydration is needed for wheat seeds to prevent ROS damage during a dormancy stage.

1.8.2 Objectives

The objectives of this research project are: 1). to identify and characterize differential proteome expressed in fresh harvest-ripe seeds of closely related hybrid genotypes of spring wheat lines varying in dormancy properties in order to identify the functional protein network and metabolic regulation associated with germination potential and dormancy as a developmental state; 2). to examine the role of thiol redox control under varying dormancy conditions. The reactive landscape of the proteome, redox active proteins with specific modification sites that undergo reversible cysteine oxidation under physiological conditions and responding differentially in dormant and non-dormant seed protein extracts, is probed by a thiol-redox proteomics approach.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

Spring wheat (*Triticum aestivum* L.) double haploid hybrid lines obtained by the cross AC Karma and 94C15/9014 = 8021-V2 segregating transgressively were part of a breeding program at Semiarid Prairie Agricultural Research Centre in Agriculture and Agri-Food Canada (Clarke *et al.*, 2005). One parent line, AC Karma (Knox *et al.*, 1995), has white seed coat with low PHS resistance. The other parent line, 8021-V2 (DePauw *et al.*, 1992), has white seed coat and high PHS resistance. Two parent and 12 spring wheat double haploid hybrid lines used as sources of white-grained dormancy in this study are part of a larger population of 380 doubled haploid lines from the cross AC Karma/SC8021V2 previously characterized for expression of dormancy (Clarke *et al.*, 2005).

2.1.2 Plant growth and development conditions

The spring wheat hybrid lines were seeded in early spring to reach physiological maturity under long days. Plants were grown in a climate-controlled greenhouse at 15 °C with 16 h day-light at 50% relative humidity. The temperature was raised to 18 °C at 8 weeks after seeding and to 21 °C at 10 weeks after seeding. Natural light was supplemented with 100 W high-pressure sodium lamps. During watering, fertilizer (Plant-Prod 20–20–20, 500 ml of 15 g/L per pot per day, 1 plant per pot) was applied. To

maximise the genetic expression of dormancy, a number of precautions were taken to avoid environmental stress and reduce environmental differences between experiments. Pots were watered from the top every second day to prevent salt accumulation on the surface of the potting mix. Water recirculation system was supplied to provide a reliable water supply for the fast growing wheat plants (Hickey *et al.* 2010). Three replications were randomly arranged within each block to minimize the environmental sources of variability in the glasshouse.

2.1.3 Collecting harvest-ripe grains

Harvest-ripe grain was collected using a standardized procedure. Individual heads on the primary and secondary tiller were harvested at physiological maturity, the stage of critical importance for imposing maximum dormancy (Nyachiro *et al.*, 2002). For determination of physiological maturity, three criterion were met: 1) all green colour from the seed disappeared and the seed was taking on a pale yellow colour; 2) the seed was slightly soft upon touch with forceps; 3) upon squishing the embryo end of the seed, the seed bursted under firm pressure with no free liquid present. When all three conditions were met, the moisture content at physiological maturity was approximately 35-42% (Clarke *et al.*, 2005). Heads were immediately dried down at 35 °C for 5 days in an air-forced dehydrator to reduce grain moisture content below 12%. The grain was then removed from the heads by gentle hand threshing, with care taken to minimize any damage to the seed coat and embryo, then stored at -20 °C to maintain dormancy (Mares, 1993; Nyachiro *et al.*, 2002) until all samples were collected for further use (Figure 2.1).

2.2 Methods

2.2.1 Seed germination assay

The germination test was used to assess the dormancy level retained following after-ripening treatment. Germination was scored as seed coat rupture over the embryo. Prior to experiments seeds were surface sterilized for 20 min on a horizontal shaker with 2.5 ml of 1% 'No Damp' solution per 20 seeds (2.5% oxine benzoate fungicide stock solution, Plant Products Co. Ltd.) followed by three rinses in deionized water. Twenty of the surface-sterilized seeds were placed crease facing down into a Petri dish with filter paper hydrated with water, and incubated at 15 °C and 40% relative humidity in the dark for 21 days. Plates were examined daily, and seeds with radicals and signs of visible pericarp rupture were counted as germinated and removed. On day 21, the ungerminated seeds were treated for 1 h with 0.5 mM GA_3 in water, placed at 4°C in the dark, and daily counts were continued for another 5 days to test seed viability.

A weighted germination index, GR (days), was calculated using the equation as described previously (Gordon, 1971), which provides time to 50% germination of germinated seeds:

$$GR = \frac{\frac{d_1}{2}(n_1)+[\frac{d_2+d_1}{2}(n_2)]+\ldots+[\frac{d_i+d_{i-}}{2}(n_i)]}{N} \text{ days}$$

where d_1, d_2, ..., d_i are the first, second to i th day of germination counts, n_1, n_2, n_3, ... n_d are the number of seeds germinated on first, second, third to d th day, and N is the total number of seeds germinated.

2.2.2 Preparation of labelled protein fractions

2.2.2.1 Fluorescent labelling of reduced proteins with monobromobimane

Proteins were extracted with simultaneous fractionation based on their solubility from intact dry seeds and seeds subject to 24 h of imbibition. The thiolyte monobromobimane (mBBr, dissolved in acetonitrile, Calbiochem, San Diego, CA) labelling of redox reactive available -SH groups in wheat seed proteins was performed simultaneously with protein extraction (Figure 2.2).

2.2.2.2 Total SDS-soluble protein extraction

For each parent and hybrid line, 20 whole seeds (600–800 mg FW) of the same size were selected and ground to a fine powder in liquid nitrogen with 7 mL 63 mM Tris–HCl, pH 6.8 extraction buffer containing 2% (w/v) SDS, 0.25 mM mBBr (100 mM stock solution in ACN) and protease inhibitor cocktail (Complete, ROCHE) added directly to the pre-chilled mortar (Rhazi *et al.*, 2003). Extraction and labelling of samples was carried out by continuously vortexing for 30 min at room temperature followed by incubation at 60 °C for 2 h with occasional vortexing every 15 minutes. Samples were cooled down to room temperature, transferred to 10 ml screw-capped centrifuge tubes (Oakridge centrifuge tubes, max 10 000 ×g / 9300 rpm on SS 34), and centrifuged at 16,000 ×g for 30 min at 22 °C. The supernatants were collected, aliquoted, stored at -80 °C and used further as the total protein extracts. Protein concentration was determined using a Bradford dye-binding assay (Bio-Rad Laboratories) with Bovine serum albumin (BSA) as a standard.

2.2.2.3 Aqueous protein extraction

For each parent and hybrid line, 20 intact seeds (600-800 mg Fresh Weight, FW) of the same size were selected and ground in liquid nitrogen to a fine powder with 5 mM Tris-HCl, pH 7.5 buffer containing 1 mM $CaCl_2$, 0.25 mM mBBr, and protease inhibitor cocktail (Complete, ROCHE), using a ratio 1 g sample / 7 ml buffer. The mixture was transferred to a 10 ml screw-capped centrifuge tube (Oakridge centrifuge tubes, max 10 000 ×g) and vortexed for 30 min at 4 °C followed by centrifugation at 16,000 ×g for 25 min at 4 °C. The collected supernatant was used as a fraction containing water soluble proteins and was kept separately in a polypropylene falcon tube, store at 4 °C as collection of aqueous protein extraction. Protein concentrations were determined by Bradford dye-binding assay (Bio-Rad Laboratories) with BSA as a standard, and fractions were aliquoted and stored at −80 °C.

2.2.2.4 Remaining SDS-soluble protein fraction

The pellet remaining after the aqueous protein extraction was further resuspended with 5 mM Tris–HCl, pH 7.5 containing 50% (v/v) propan-1-ol, 0.25 mM mBBr (100 mM stock solution in acetonitrile) and protease inhibitor cocktail (Complete, ROCHE), vortexed for 1 h at room temperature and centrifuged again at 16,000 ×g for 25 min at 20 °C. The supernatant was collected and the pellet was washed in the same buffer but containing 70% (v/v) propan-1-ol without label. After centrifugation the supernatant was combined with the 50% propan-1-ol supernatant. The propan-1-ol soluble fraction was discarded due to the lack of detectable differences in gliadin and glutenin storage proteins.

The pellet was resuspended with 63 mM Tris–HCl, pH 6.8 containing 2% SDS and protease inhibitor cocktail, without label, and vortexed for 30 min at room temperature. The protein extraction was continued at 60 °C for 2 h with occasional vortexing followed by centrifugation at 16,000 ×g for 30 min at 20 °C. Supernatant was collected and used as SDS-soluble protein fraction. Protein concentrations were determined using a Bradford dye-binding assay (Bio-Rad Laboratories) with BSA as a standard, and fractions were aliquoted and stored at −80 °C.

2.2.3 Determination of protein concentrations by Bradford dye-binding assay

Bio-Rad Protein Assay method (Microassay for protein levels from 2.5 to 25 μg/ml) was used for determination of protein concentrations in the extracted protein fractions. The lyophilized BSA standard (Bio-Rad Laboratories) was reconstituted with 20 ml of deionized water to obtain a stock solution of 1.43 mg/ml, then aliquoted and frozen at -20 °C. The further diluted standard stock solution of BSA at protein concentration of 20 μg/ml was prepared. One blank solution with 800 μl of water and six dilutions of BSA standard with different protein concentrations in the range between 1 to 20 μg/ml in a final volume of 800 μl were prepared and used for the standard curve. Protein Assay Dye Reagent Concentrate (Bio-Rad, solution containing dye, phosphoric acid, and methanol) in 200 μl volume was added to each standard and protein solution. The solutions were further incubated at room temperature (23 °C) for at least 5 min, and the absorbance at 595 nm was measured. The absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 dye shifts from 465 nm to 595 nm when binding to

protein occurs, then the value of $O.D._{595}$ as *y* axis and diluted protein concentration (μg/ml) as *x* axis was plotted to determine protein concentrations in extracted protein fractions.

2.2.4 Removal of contaminants and SDS by acetone protein precipitation

To remove salts and other compounds that interfere with protein isoelectric focusing (IEF), 800 μg of each aqueous protein fraction was transferred into a 15 ml borosilicate glass centrifuge tube (Kimble® High Strength borosilicate, up to 13,100 ×g with an accessory rubber adapter sleeve in a 50 ml rotor cavity), and vortexed well at 1050 rmp for 10 min with 8x sample volume of ice-cold acetone at a final concentration of 80% (v/v) acetone and 0.07% (w/v) dithiothreitol (DTT). DTT was used to reduce the disulfide bonds of proteins and to prevent intramolecular and intermolecular disulfide bonds from forming between cysteine residues in proteins during protein precipitation. The washing was followed by overnight precipitation at -20 °C and centrifugation at 10,000 rpm for 30 min at 4 °C (Mandel Scientific, Sorvall RC-6 Plus Refrigerated Centrifuge; Fisher Scientific, Sorvall SS-34 Rotor F21-8*50y, 21000 rpm, 52,600*G). The supernatant liquid was gently discarded and the pellet of protein extract was broken up with a glass rod to make a homogenous mixture with another portion of ice-cold acetone at a final concentration of 80% (v/v) acetone and 0.07% (w/v) DTT, followed by washing and precipitation as described above. After five repetitions of protein precipitation and washing, the final protein pellet was carefully dried under nitrogen gas and stored at -80 °C.

For the fractions containing 2% SDS, protein samples (1.0 mg for total SDS-soluble protein extract, 800 μg for SDS fraction protein) were first diluted with 2% (w/v)

CHAPS solution to achieve a dilution of SDS from 2% to 0.25% (w/v) with a ratio of 8:1 or greater of CHAPS to SDS, and vortexed occasionally for 1 h before being precipitated and washed as described above for the aqueous protein fraction, and then dried and stored at -80 °C.

2.2.5 2D IEF/ SDS-PAGE

2.2.5.1 Rehydration of IPG strips

In the case of aqueous protein fractions, 800 μg of protein in dried acetone powder was dissolved for 1.5 h in 500 μl of IEF rehydration solution containing 7 M urea, 2 M thiourea, 50 mM dithiothreitol (DTT), 4% (w/v) CHAPS, 1% (w/v) ampholyte (40% Biolyte 3–10, Bio-Rad Laboratory, Mississauga, ON, Canada), and 0.002% (w/v) bromophenol blue. For the fractions containing SDS, 1.0 mg of the total SDS-soluble protein extract or 800 μg of the SDS fraction protein were solubilized for 1.5 h in 500 μl of IEF rehydration solution containing 5 M urea, 2 M thiourea, 50 mM DTT, 2% (w/v) CHAPS, 2% (w/v) N-decyl-N,N-dimethyl-3-ammonio-1-propane sulfonate (SB 3–10), 1% (w/v) ampholyte, and 0.002% (w/v) bromophenol blue. Each protein fraction was transferred to a 1.5 ml siliconized centrifuge tube and subject to flash freezing in liquid nitrogen, followed after thawing by additional 1 h solubilisation with 20mM DTT (5 ul of 2 M stock to each 500 ul sample). To achieve maximum solubility of extracted proteins, samples were sonicated on ice with six 10 s cycles, each consisting of 5 s sonication with consistant amplitude at 5 followed by a 5 s break (to prevent overheating of the samples). Special care was taken to avoid sample foaming. After the sonication samples were transferred to ultra-centrifuge tubes and centrifuged at 100 000 ×g (53000 rpm) for 30

min. at 23 °C (Beckman Coulter TLA-100.2 Rotor). Rehydration of IPG strips with extracted protein samples was carried out overnight at room temperature in a custom made teflon rehydration/equilibration tray.

2.2.5.2 The first dimension IEF separation of proteins

IEF was conducted with the Ettan IPGphor II system and Manifold tray (GE Healthcare, Piscataway, NJ, USA) using 24-cm ReadyStrip IPG strips according to the manufacturer's instructions (Berkelman and Stenstedt, 1998). Analysis of different isoelectric point (p*I*) ranges for 2D gels demonstrated that the optimal resolution and coverage of proteins from the SDS-soluble extracts were obtained from IPG strips with non-linear pH gradient 3–10 and for the aqueous fraction with linear pH gradient range 5–8. IEF was performed in the following manner with the maximum current of 50 microamp per strip at 20 °C: in the first step the voltage increased gradually from zero to 250 V for 2 h; in the second step the voltage was kept at 250 V for the next 1 h; in the third step the voltage gradually increased from 250 V to 1000 V for 2 h; the fourth step continued by gradient voltage increase from 1000 V to 8000 V for the following 6 h; and in the final fifth step the voltage was set at 8000 V for 9 h. The proteins in IPG strips were focused for a total of 100 kVhr during 20 h of run time.

2.2.5.3 The second dimension SDS-PAGE separation of proteins

The second dimension Tris–glycine SDS-PAGE was carried out with linear gradient 10-20% acrylamide separating gels, containing 0.375 M Tris-HCl at pH 8.8, 10% to 20% acrylamide, 0% to 21.4% glycerol, 0.1% SDS, 0.03% to 0.021%

Tetramethylethylenediamine (TEMED), 0.03% to 0.021% ammonium persulfate (APS). The stacking gels contained 0.125 M Tris-HCl buffer at pH 6.8, 4% acrylamide, 0.1% SDS, 0.08% TEMED, 0.08% APS, as described for the Laemmli SDS-PAGE system (Laemmli, 1970). The Ettan DALT six gradient maker and gel caster were used for casting 1.0 mm thick large format gels of 25.5 × 20.5 cm with a volume of approximately 52 ml in casting cassettes of 27 × 22 cm (GE Healthcare, Piscataway, NJ, USA). IPG gel strips were incubated with an equilibration buffer 1 containing 50 mM Tris-HCl at pH 8.8, 4% SDS, 6 M Urea, 30% Glycerol, 0.002% bromophenol blue, 1% DTT, followed by the equilibration buffer 2 containing 50 mM Tris-HCl at pH 8.8, 4% SDS, 6 M Urea, 30% Glycerol, 0.002% bromophenol blue, 2.5% iodoacetamide, for 10 min in each buffer, and subsequently rinsed with the Tris–glycine electrophoresis buffer containing 25 mM Tris, 192 mM glycine, 0.2% SDS. IPG gel strips were placed tightly against the upper edge of the stacking gel to avoid air bubbles. Electrophoresis was performed at 26 °C, first with 100 V, 50 mA, 10 W for 45 min and then with 500 V, 240 mA, 100 W for 5 h, using Ettan DALT six system (GE Healthcare, Piscataway, NJ, USA) apparatus.

2.2.6 Visualization of protein thiol modifications and image analysis

2.2.6.1 Detection of fluorescent protein signal

After electrophoresis, gels were fixed in 12.5% (w/v) Trichloroacetic acid (TCA) for 2 h in the dark, then transferred into a solution of 40% (v/v) methanol and 10% (v/v) acetic acid and incubated in the dark for 4 h or overnight to reduce the background. Protein spots on the 2-DE gel were first visualized under a UV light source (365 nm) with

an exposure time of 2 to 4 s, to detect mBBr labelling of reduced thiol (-SH) groups in the fluorescence images (Transilluminator Kodac).

2.2.6.2 Staining and visualization for the total protein content

Gels were stained overnight with 0.15% (w/v) Coomassie brilliant blue R-250 in 50% ethanol, 7% (v/v) acetic acid/, followed by destaining in 25% (v/v) ethanol, 7% (v/v) acetic acid for 1 hour to reveal the total protein pattern. The protein extractions and fractionations were replicated two times (biological replicates), and 2–3 gel run replicates per extracted protein sample under each condition were performed. The gels were transferred to 7% acetic acid, vacuum sealed, and stored at 4 °C. The gels stained for the total protein content were scanned and analyzed using densitometry by video imaging (ImageScanner III, GE Healthcare).

2.2.6.3 UV fluorescent image analysis

The intensity of fluorescent spots is proportional to the number of available -SH groups in the protein, since the reaction between mBBr and –SH groups is stoichiometric, rapid and complete (O'Keefe, 1994). The extent of protein reduction, and the total protein content detected by staining with Coomassie Blue R250 as described above were quantified using densitometry by video imaging with Quantity One software (Bio-Rad). Normalized conditions were used throughout with respect to protein extraction, labelling and loading on the gels, fluorescent signal detection and Coomassie staining visualization. The normalized protein spot intensities were analysed in both the fluorescence images and the images of the total protein pattern. The ratio of fluorescence intensity to protein

level was determined by dividing the intensity of a protein spot on the fluorescence image by the intensity of the corresponding Coomassie Blue-stained protein spot. The fluorescence to protein ratio is a reflection of the number of labelled/reduced Cys residues in the protein sequence, and of protein expression level. For the same protein spots of replicate gel images, a mean ratio of the protein abundance or the ratio of fluorescence signal intensity or fluorescence/protein ratio was calculated and included if the standard deviation was less than 30%. In order to evaluate the redox state of thiols in proteins from dormant dry seeds and after imbibition, the thiol modification ratio of a protein spot was calculated by dividing the mean fluorescence/protein ratio of the dormant samples by the mean fluorescence/protein ratio of the non-dormant samples as described previously (Wolf *et al.*, 2008).

2.2.7 Statistical analysis

Results were analysed with Origin data analysis and graphing software v8.0.63.988 (OriginLab Corporation, Northampton, MA, USA). Only protein spots that showed consistent differences in two biological replicates were considered for quantitative and statistical analysis, thus preventing the assignment of normalized volume values to missing spots. Another issue related to proteomics data is the correlation between spot normalized volume and spot variance, described in some proteomic studies (Valledor *et al.*, 2008), which means that the higher the mean intensity of a spot, the higher the variance, this being explained by a scale phenomenon related to data acquisition (Gustafsson *et al.*, 2004). To reduce this variance-mean dependence between different spot intensities and sample sets, spots were considered to be variable if they

showed statistically significant quantitative differences according to the one-away ANOVA test. P-values of 0.05 or below were calculated for the majority of protein abundance ratios, fluorescence signal intensity and thiol modification ratios of 1.5 or higher. Therefore, redox thiol modification for a specific protein spot was considered to be significantly increased, when the thiol modification ratio was 1.5, or significantly decreased, when the thiol modification ratio was 0.67. Only protein spots with P-value of 0.05 or below are discussed in the manuscript. In the analysis of variance, it is assumed that different samples have equal variances, which is commonly called homogeneity of variance. The Levene test and Brown–Forsythe test as part of the one-way ANOVA algorithms were used to verify the assumption. The ratios with low variance (high statistically significant homogeneity) are highlighted in bold in results (Tables 3.1, 3.2, and 3.3; Supplemental Tables S1, S2, and S3; Appendices I, II, and III).

2.2.8 LC-MS/MS identification of mBBr labelled proteins

Protein spots were excised from 2-D gels, washed with 100 mM NH_4HCO_3, reduced with 10 mM DTT for 45 min at 56 °C, alkylated with 55 mM iodoacetamide for 30 min in the dark at room temperature, washed again with 100 mM NH_4HCO_3, and digested overnight at 37 °C with modified trypsin (Promega, sequencing-grade). Tryptic peptides were extracted from the gel as previously described (Rampitsch *et al.*, 2006). Automated nano-flow LC-MS/MS analysis of peptide digests was performed using a linear ion trap Finnigan LTQ (Thermo Finnigan, San Jose, CA, USA) mass spectrometer connected on-line with nano-HPLC (Dionex UltiMate™ 3000) essentially as previously described (Bykova *et al.*, 2011). Briefly, chromatographic separation was accomplished

with a 17 cm reversed-phase nano-column (75 μm ID, 360 μm OD; packed in-house with Vydac C18, 5 μm bead and 300 Å pore size resin) with flow rate delivered at 250 nl/min and peptide elution using a linear gradient of 4–40% (v/v) ACN for 40 min, followed by a short gradient 40–80% (v/v) ACN for 3 min, and 80% (v/v) ACN for 2 min in 1% (v/v) formic acid, 0.5% (v/v) acetic acid. The analytical column was directly connected to a distally coated, fused silica emitter (New Objective, Cambridge, MA, USA) (360 μm OD/20 μm ID/10 μm tip ID) biased to 1.8 kV. The mass spectrometer was operated in the positive ion mode with source temperature 200 °C, and was tuned in nano-spray mode using 10 μM (Glu)-Fibrinopeptide B (GluFib) singly charged ion at m/z 1552.67. Data-dependent analysis was employed with one MS m/z range 450–2000 and MS/MS of five most abundant ions in each cycle, 20 s dynamic exclusion.

2.2.9 Database searching, protein sequence analysis and bimane-Cys assignment

The LC-MS/MS data were interpreted using MASCOT v. 2.1.01 search engine (Matrix Science, UK) first with the NCBInr protein database (Viridiplantae taxonomy, 6573034 sequences) followed by querying 1361178 wheat EST sequences (in-house database). The Finnigan Xcalibur (LTQ) raw data were converted into the DTA format and used for protein identification and modification screening. The bimane-Cys was incorporated into MASCOT and used as variable modification for automated analysis. The following parameters were used for database search: (1) trypsin as digestion enzyme with maximum one missed cleavage; (2) monoisotopic peptide masses were used; (3) the peptide mass tolerance at 1 Da; and the fragment ions mass tolerance at 0.5 Da for LTQ nano-flow LC-MS/MS; (4) variable modifications bimane (C), carbamidomethyl (C),

deamidation (NQ), oxidation (M); (5) peptide charge states +1, +2 and +3. The bimane modification was added to the MASCOT list of variable modifications with elemental composition C(10) H(10) N(2) O(2) and mass average/monoisotopic 190.2016/190.0742 for covalent bimane adduct to Cys residue. Only Triticum aestivum protein and EST complementary sequence matches were used for identification of proteins and their post translational modifications. A single peptide probability of identification MOWSE score greater than 52–55 indicated identity using the EST database. Peptide matches indicating identity or extensive homology were considered for confident protein identification. Providing that the protein had at least 1–3 confidently identified peptides, peptide matches with bimane-Cys modification were manually verified using the GPMAW 7.0 (Lighthouse Data, Odense, Denmark) software.

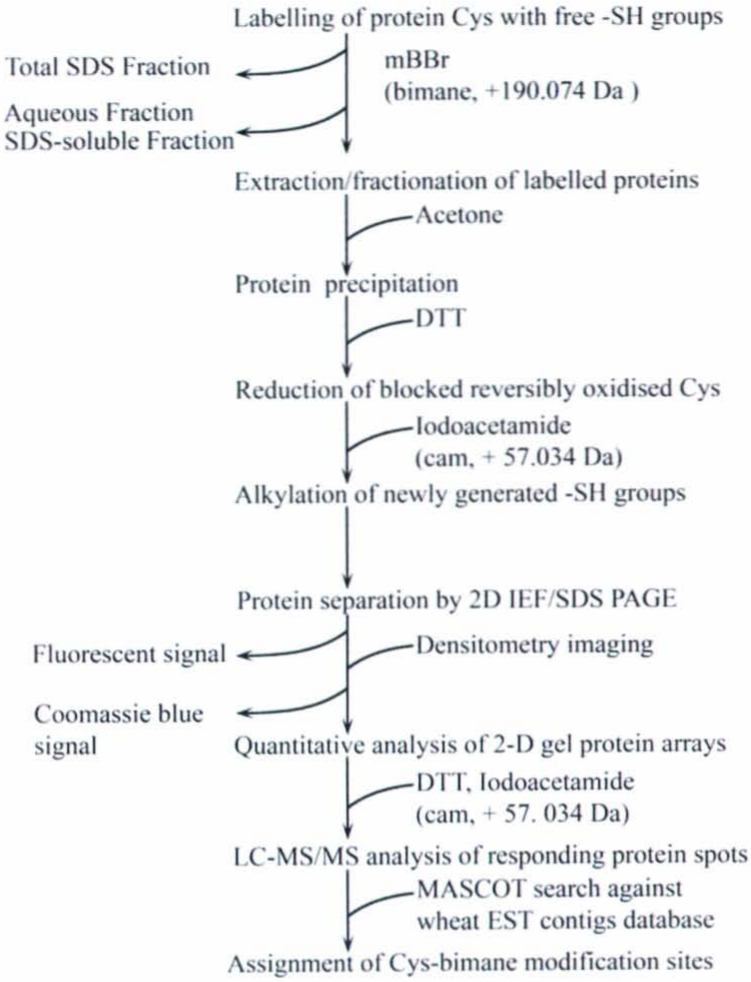


Figure 2.1 Experimental design for the detection of thiol redox-sensitive proteins. Thiol redox-sensitive proteome in dormant and non-dormant genotypes of wheat were followed by fluorescent mBBr in situ labelling of protein cysteines with free SH groups in native seed protein extracts. After labelling, proteins were either solubilized into a total protein extract or fractionated into aqueous and SDS-soluble extracts. In addition to the bimane derivatized protein cysteines, unlabelled cysteines remained oxidised. Proteins with remaining reversibly oxidised cysteines, disulfide bonds and mixed disulfides, were completely reduced with DTT and alkylated with iodoacetamide (carbamidomethylation, CAM) prior to separation by 2D IEF/SDS PAGE as well as prior to in-gel digestion of individual protein spots.

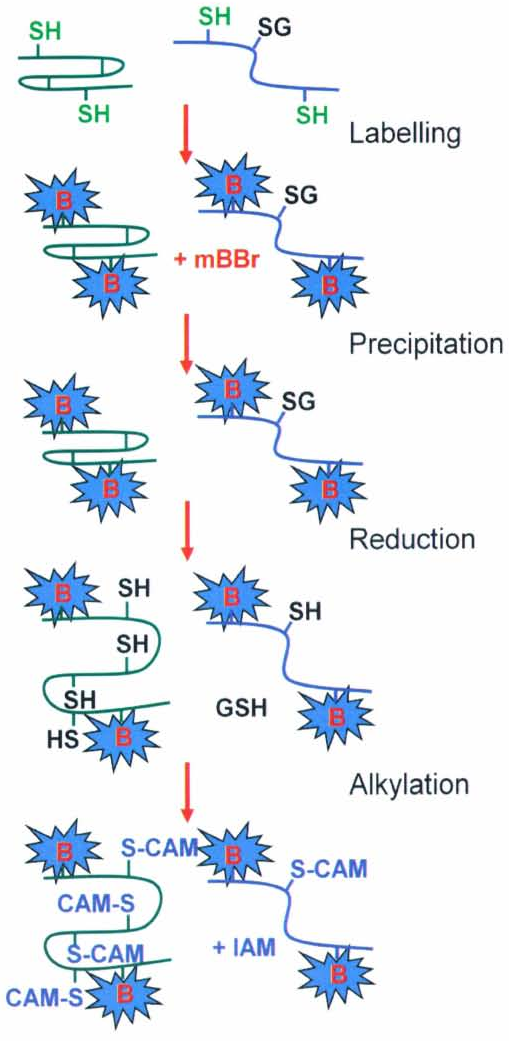


Figure 2.2 Labelling scheme of proteins with different redox state. Differential changes in the protein redox state were followed by fluorescent mBBr in situ labelling of protein cysteines with free SH groups in native wheat seed protein extracts. In addition to the bimane-derivatized protein cysteines, unlabelled cysteines remained oxidized. Inter- or intra-molecular disulphide bonds (S-S) and mixed disulphides are shown as examples of blocked reversibly oxidized cysteines. After labelling, all proteins were completely reduced and alkylated with iodoacetamide (cam) prior to separation by 2-D IEF/SDS-PAGE as well as prior to in-gel digestion of individual protein spots. A combined fluorescent and mass spectrometric detection and analysis procedure for quantifying and identifying redox status of cysteine residues in proteins under native conditions were performed based on this labelling scheme. Adapted from Bykova *et al.*, 2011.

3. RESULTS

3.1 Monitoring phenotypic trait for germination resistance

The germination index assay was used to test different levels of PHS resistance between twelve doubled haploid hybrid and two parent lines of spring wheat (*Triticum aestivum* L.) (Figure 3.1). It has been demonstrated that, under controlled environmental growing conditions, dormant and non-dormant fixed lines can be clearly differentiated and achieve similar levels of grain dormancy to field-grown plants (Nyachiro *et al.*, 2002; Clarke *et al.*, 2005; Hickey *et al.*, 2010). Based on the germination resistance test, four hybrid lines and the parent standard line 8021-V2 showed consistently high levels of seed dormancy. Six hybrid lines displayed germination characteristics that reflected a level of dormancy even slightly lower than non-dormant donor parent AC Karma. The spring wheat hybrid lines used in this study are part of a larger population of 380 doubled haploid lines derived from the cross AC Karma/SC8021V2 previously characterized for the expression of dormancy. There was significant bidirectional transgressive segregation in both the glasshouse and field environments (Clarke *et al.*, 2005). Three hybrid lines (BB, AF, BH) with one parent line (8021-V2) showing highest PHS resistance, and three susceptible hybrid lines (AL, AN, BQ) with one parent line (AC Karma), a total of 8 lines (Figure 3.1), were chosen as phenotypic extremes for proteomic analysis.

3.2 The differential expression profiling of total proteome in dormant and non-dormant hybrid wheat lines

3.2.1 Proteome maps and identification of wheat proteins

Qualitative and quantitative changes in proteins were analysed by comparing the protein abundance of individual spots on 2D SDS-PAGE proteome maps from dormant and non-dormant seeds in two states, dry and imbibed for 24 h. A number of protein spots displaying significant up- or down-regulation between dormant and non-dormant genotypes were detected in the Coomassie stained 2D SDS-PAGE images but were absent in the fluorescence images, indicating the absence of labelled Cys residues in corresponding protein sequences. These protein spots were regarded as candidates that maintain dormancy-related differential expression between dormant and non-dormant lines based on the quantification and statistical analysis, and were then subject to MS analysis for protein identification. A number of spots identified as the same protein could correspond either to post-translational modification (PTM) of the same protein or to different protein isoforms.

The strategy for protein identification was based on considerable bioinformatic resources and an available extensive database, hence the proteomics approach was combined with transcriptomic resources to study differential wheat proteomes. This was essential for achieving a high rate of successful protein identification by LC-MS/MS (Tables 3.1, 3.2, and 3.3; Supplemental Tables S1, S2, S3, and S4; Appendices I, II, III, and IV). As rich sources of comparative sequence information, publicly available EST databases have grown exponentially and represent the largest collection of genetic sequences. However, due to the large size and redundancy of EST databases, their

application in high throughput functional proteomic analyses makes both automatic MASCOT searches and results interpretation very inefficient. In our approach, we used a custom database where contig sequences were constructed from wheat EST entries to reduce the size of the database (Supplemental Table S4; Appendices IV). In addition to 51% of spots matched to *Triticum aestivum* protein sequences (54 spots), 40% of spots could be uniquely identified from the wheat EST database (42 spots), 9% could be identified from both approaches (10 spots), and in most cases sets of peptides unique to protein or EST sequences were used.

3.2.2 Protein abundance differences in dormant and non-dormant lines

A set of proteins displayed quantitative differences in abundance between dry and/or imbibed for 24 h seeds of dormant and non-dormant genotypes. Among the spots that discriminated the genotypes, sixteen unlabelled proteins in 25 spots were found in the protein expression maps (Table 3.1; Supplemental Table S1; Appendix I; Figure 3.2 A–D). Among these were one enzyme of glycolysis/gluconeogenesis (spot 1) and one TCA-cycle enzyme (in the same spot 1), four enzymes of starch metabolism (spots 2–12, Figure 3.2 B–D), one enzyme of amino acid metabolism (spot 13, Figure 3.2 D), two proteins involved in protein folding (spot 14 in Figure 3.2 B and spot 15 in Figure 3.2 D), one component of intracellular protein transport and signal transduction system (spot 16, Figure 3.2 C), one enzyme involved in antioxidative defence response (spots 17, 18 in Figure 3.2 B and spot 19 in Figure 3.2 C), and five storage proteins (spots 20–25, Figure 3.2 C).

Most of these proteins (14 proteins in 22 spots) were found to be more abundant in dry seeds of dormant lines. Three proteins phosphoglycerate kinase, beta-chain of succinyl-CoA ligase, and one of the isoforms of beta amylase showed higher expression level in dry seeds of non-dormant versus dormant lines. Three identified proteins from dormant lines, mitochondrial manganese superoxide dismutase (SOD), small Ran-related GTP-binding protein, and one of the beta amylase isoforms displayed significantly increased protein abundance ratio in response to 24 h of imbibition (Table 3.1; Supplemental Table S1; Appendix I). Interestingly, two spots from aqueous fraction containing SOD protein were differentially expressed, with spot 17 higher and spot 18 slightly lower in abundance in dormant lines (Figure 3.2 B), indicating possible post-translational modification variants.

3.2.2.1 Differential expression of dormancy-related proteins in dry seeds of dormant and non-dormant wheat lines

Twelve proteins found to be more abundant in dry seeds of dormant lines (Figure 3.2) include 0.19 dimeric alpha-amylase inhibitor (spots 2, 3, 4), dimeric alpha-amylase inhibitor (spot 6), one of the isoforms of beta amylase (spot 11), betaine aldehyde dehydrogenase (spot 13), heat-shock protein-14.5kDa (spot 14), protein disulfide isomerise 3 (spot 15), manganese SOD (spot 17), glutenin HMW subunit 1Ax1 (spot 20), glutenin HMW subunit 12 (spot 22), glutenin HMW subunit 1Dx5' (spot 23), triticin (spot 24), and glutenin HMW subunit B3-2 (spot 25).

Three proteins, phosphoglycerate kinase (spot 1), beta-chain of succinyl-CoA ligase (spot 1), and one of the isoforms of beta amylase (spots 9, 10), showed higher expression level in dry seeds of non-dormant versus dormant lines.

3.2.2.2 Differential expression of dormancy-related proteins in imbibed seeds of dormant and non-dormant wheat lines

Seventeen proteins were found to have higher expression level in the seeds of dormant lines after 24 h of imbibition (Figure 3.2), including phosphoglycerate kinase (spot 1), beta-chain of succinyl-CoA ligase (spot 1), 0.19 dimeric alpha-amylase inhibitor (spots 2, 3, 4), dimeric alpha-amylase inhibitor (spots 5, 6), alpha-amylase/subtilisin inhibitor (spot 8), one of the isoforms of beta amylase (spot 12), betaine aldehyde dehydrogenase (spot 13), protein disulfide isomerise 3 (spot 15), small Ran-related GTP-binding protein (spot 16), manganese SOD (spot 17), glutenin HMW subunit 1Ax1 (spot 20), glutenin HMW subunit 1Dx5' (spot 23), triticin (spot 24), and glutenin HMW subunit B3-2 (spot 25).

Only one of the isoforms of beta amylase (spots 9, 10) showed higher expression level in the seeds of non-dormant lines responding to 24 h of imbibitions.

3.2.2.3 Higher level of germination-related protein expression in dry and imbibed seeds from non-dormant lines

Phosphoglycerate kinase (spot 1) and beta-chain of succinyl-CoA ligase (spot 1) from dry seeds displayed significant up-regulation in protein expression in non-dormant hybrid lines. However, the expression level of the two proteins changed dramatically after

24 h of imbibition and was found to be approximately 2 fold lower in the imbibed seeds from dormant lines. It is important to mention that there were two unique proteins present in one spot (spot 1), and therefore the protein abundance ratio results from the sum of two protein amounts, making the quantitative analysis tentative only. One of the beta amylase isoforms (spots 9, 10) from non-dormant lines displayed increased protein abundance ratio in response to 24 h of imbibition.

3.2.3.4 Higher level of dormancy-related protein expression in dry and imbibed seeds from dormant lines

Five identified proteins 0.19 dimeric alpha-amylase inhibitor (spot 4), alpha-amylase/subtilisin inhibiter (spot 7), one of the isoforms of beta amylase (spot 11), heat-shock protein-14.5 kDa (spot 14), and glutenin HMW subunit 12 (spot 22) from dormant lines showed statistically significant differential expression level in dry seeds, while these differences either decreased (spot 4) or were completely eliminated after 24 h of imbibition.

Five identified proteins from dormant lines, alpha-amylase/subtilisin inhibiter (spot 8), one of the isoforms of beta amylase (spot 12), betaine aldehyde dehydrogenase (spot 13), mitochondrial manganese SOD (spot 17), and small Ran-related GTP-binding protein (spot 16) with no apparent changes in the expression level in dry seeds, displayed significantly increased protein abundance ratio in response to 24 h of imbibition.

Two spots from aqueous fraction containing SOD protein were differentially expressed, with spot 17 higher and spot 18 slightly lower in abundance in dormant lines (Figure 3.2 B), indicating possible post-translational modification variants.

3.3 Differential expression of the dormancy-related thiol redox-sensitive proteome

3.3.1 Monitoring of differential expression profiles of the dormancy-related thiol redox-sensitive proteome

Dormancy-related differential changes in the protein sulphydryl status were monitored using fluorescent monobromobimane (mBBr) in situ labelling of active-free –SH groups of protein thiols in native wheat seed protein extracts, followed by the two-dimensional isoelectric focusing polyacrylamide gel electrophoresis (2-D IEF/SDS PAGE) separation. The bimane-derivatized proteins were first visualized under a UV light source for detection of fluorescently labelled Cys residues in proteins and subsequently Commassie Brilliant Blue (CBB) stained for the total protein content in the same 2-D gel. The 2-D IEF/SDS PAGE comparison of the fluorescence intensity with the total protein expression level revealed two groups of proteins with different fluorescence and protein staining characteristics. Some protein spots were observed in the fluorescence image as well as in the stained gel. A few protein spots produced a strong fluorescent signal but were either absent or faint in the total protein image, indicating low-abundance highly labelled Cys-containing proteins in these positions.

The fluorescence to protein ratio is a reflection of the number of labelled/reduced Cys residues in the protein sequence, and of protein expression level. Three types of differences were observed: 1) the first type of response was due to differences in protein expression for protein spots with about the same level of Cys reduction/oxidation (no change in the fluorescence to protein ratio, Table 3.2, Supplemental Table S2, Appendix II); 2) the second type of response was caused by changes in both reduction/oxidation of Cys and protein expression level, where the fluorescent and protein signals showed

differences yet the fluorescence to protein ratio was not necessarily different between the genotypes (overlapping proteins in Tables 3.2 and 3.3; Supplemental Tables S2 and S3; Appendices II and III); 3) the third type of response resulted from the reduction/oxidation of Cys residues without prominent changes in the protein expression level (increase/decrease in fluorescence to protein ratio) (Table 3.3; Supplemental Table S3; Appendix III). The thiol modification ratio was used to reflect changes in Cys redox state.

3.3.2 Identification of bimane-labelled proteins and assignment of modified cysteine thiols

Specific labelling of reduced free Cys with mBBr helped to identify and distinguish redox responding proteins even from a mixture of proteins overlapping in one spot. This often happens with proteins on 2D gels either because they have very close pI values or their pI can be shifted due to post-translational modifications. Several spots (spots 27, 28, 32, 33, 34, 52, 56 in Table 3.2; Supplemental Table S2; Appendix II. Spot 94 in Table 3.3; Supplemental Table S3; Appendix III) contained more than one protein with identified bimane-Cys residues and for these proteins the given quantitative fluorescence to protein ratio is tentative only. Many proteins were represented by more than one isoform, 11 proteins had two, and one protein, dimeric alpha-amylase inhibitor (spots 43, 44, 45 in Figure 3.3 A), had three isoforms.

To characterize Cys functionality in native proteomes based on reactivity profiling, a strategy for differential alkylation was applied with either mBBr during protein extraction or with iodoacetamide prior to in-gel digestion. This allowed us to discriminate redox active from inactive cysteines and detect Cys residues with mixed redox

modifications. During mass spectrometry (MS) analysis, all Cys residues differentially reduced at the time of protein extraction, had a covalent bimane modification (bimane, +190.074 Da monoisotopic mass), whereas non-labelled (oxidised) Cys were distinguished by carbamidomethylation (cam, +57.034 Da monoisotopic mass) modification, performed prior to digestion. In MS analysis, metastable decomposition product ions containing free cysteine due to partial photolytic fragmentation of bimane-modified peptides was also observed. In collision induced dissociation (CID) tandem MS (MS/MS) spectra fragment y- and b-type ions provided an easily interpretable peptide sequence information with covalently bound bimane-Cys remaining intact in peptide fragmentation product ions. Two specific diagnostic signatures for the site of modification were present in mass spectra: 1) in MS spectra – a mass increment of 190.074 Da between bimane-derivatized peptide ions and metastable decomposition product ions containing free cysteine; 2) in CID MS/MS spectra – fragment y- and b-type ions with a mass difference corresponding to bimane-Cys and Cys-SH for a modified and unmodified peptide ion signal, respectively. The presence of all three modified forms bimane-Cys, cam-Cys and Cys-SH increased confidence in the peptide sequencing and in the assignment of Cys modification sites.

A total of 97 redox modified Cys were detected in 93 peptides from 64 unique proteins responding differentially in dormant and non-dormant closely related wheat genotypes. The identified cysteines with known functional roles perform important catalytic and/or regulatory functions for their parent proteins, or correspond to sites for glutathionylation, nitrosylation and disulfide formation, and therefore offer points of protein control by oxidative stress pathways.

3.3.3 Differences between dormant and non-dormant genotypes in expression of proteins with reduced cysteines

A number of labelled thiol redox active proteins displayed differential expression in dormant compared to non-dormant genotypes (Table 3.2; Supplemental Table S2; Appendix II; Figure 3.3). Redox responding proteins from the functional groups of carbohydrate metabolism, starch and sucrose metabolism, biosynthesis of secondary metabolites, energy and amino acid metabolism, genetic information processing and cell cycle, antioxidative defence and storage proteins were found in all three solubility fractions (total SDS, aqueous and SDS-soluble) of the whole seed protein extracts (Table 3.2; Supplemental Table S2; Appendix II; Figure 3.3 A–F). Storage globulins (spots 79, 80, 81, 82, 83) as intact proteins and fragments were found in the total protein extract and in the SDS-soluble fraction (Figure 3.3 A, B, E and F).

3.3.3.1 Labelled thiol redox active proteins with higher level of expression in dormant compared to non-dormant dry seeds

A set of 16 proteins from dry seeds identified in 25 spots displayed increased relative protein abundance ratio in dormant lines, and upon imbibition their abundance ratio either decreased or remained at the same level (Figure 3.3). These represented the enzymes of glycolysis/gluconeogenesis metabolism, including triosephosphate isomerase (spot 30) and phosphoglucomutase (spot 31), alcohol dehydrogenase (DH) (spot 33), cytosolic phosphoglycerate kinase (spot 38), monomeric alpha-amylase inhibitor (spot 42), 0.19 dimeric alpha-amylase inhibitor (spot 43), dimeric alpha-amylase inhibitor (spot 45), alpha-amylase/trypsin inhibitor CM3 (spot 46), glucose and ribitol DH (spots 47–51),

decarboxylase (spot 52), cytosolic malate DH (spots 58, 59), two enzymes of amino acid metabolism alanine aminotransferase (spots 32, 61) and aspartate aminotransferase (spots 52, 56), three serine protease inhibitors serpin-Z1A (spots 62, 63), serpin-Z2B (spot 65), and serpin-Z1C (spot 66), cell division control protein 48 (spot 68), two enzymes of antioxidative defence response 27K thioredoxin family protein (spots 69) and aldehyde DH (spot 75).

3.3.3.4 Labelled thiol redox active proteins with higher level of expression in non-dormant compared to dormant imbibed seeds

Upon imbibition of seeds from non-dormant lines, a significant increase in expression level could be detected for NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (DH) (spots 35, 36), NADP-specific isocitrate DH (spot 57), serpin-Z1B (spot 64), and serpin Z1C (spot 66). A moderate increase was observed for triosephosphate isomerase (spot 29), phosphofructokinase (spot 32), alanine aminotransferase (spot 32), alcohol dehydrogenase ADH1A (spot 34), mitochondrial formate dehydrogenase (spot 34), serpin-Z1A (spots 63), and 27K thioredoxin family protein (spot 69).

In addition, some identified proteins showed differential expression in aqueous and SDS-based protein fractions indicating possible variation in their solubility. Among these were alcohol DH (spots 33, 34), mitochondrial formate DH (spots 55, 33, 56, 34), alanine aminotransferase (spots 32, 60, 61), and 27 K thioredoxin family protein (spots 71, 72). Other proteins were identified in several spots that displayed variation in protein abundance indicating possible differences in post-translational protein modification

between dormant and non-dormant protein extracts. These were triosephosphate isomerase (spots 29, 30), alanine aminotransferase (spots 32, 60), and 27 K thioredoxin family protein (spots 69, 70).

3.4 Monitoring of Cys oxidoreduction without prominent changes in the protein expression level

A comparative analysis of fluorescently cysteine labelled and protein stained two-dimensional electrophoresis (2-DE) maps identified a set of 36 proteins with quantitative thiol modification differences between dormant and non-dormant genotypes (Table 3.3; Supplemental Table S3; Appendix III; Figure 3.3). Seventeen unique proteins with 19 reactive modified cysteines were found to have differential post-translational thiol redox modification without differences in protein expression level. Among these, a significantly higher thiol modification ratio in dry seeds from dormant lines was found in enolase (spot 26), glucose and ribitol dehydrogenase (DH) (spots 48, 49), O-methyltransferase ZRP4 (spot 90), NADP-specific malic enzyme (spot 53), alanine aminotransferase (spot 32), serpin-Z2A (spot 98), 1-Cys peroxiredoxin PER1 (spot 73), embryo-specific protein (spot 76), globulin 3 full length (spot 102) and fragments (in spots 103, 104), and lipoprotein-like protein (spot 106). Higher thiol modification ratio in protein extracts of imbibed dormant seeds from dormant lines included enolase (spot 26), pyruvate orthophosphate dikinase 1 (spot 37), monomeric alpha-amylase inhibitor (spot 42), mitochondrial formate dehydrogenase (spot 55), serpin-Z1A (spot 63), serpin-Z2A (spot 98), serpin-Z1C (spot 66), globulin 3 full length (spot 102) and fragments (in spots 103, 104), and lipoprotein-like protein (spot 106).

The most abundant in aqueous extracts of imbibed dormant seeds with prominent response were proteinaceous inhibitors of α-amylases, including 0.19 dimeric alpha-amylase inhibitor (spot 87), alpha-amylase/trypsin inhibitor CM3 (spot 88). Four unique Cys residues in seven peptides (Tables 3.2 and 3.3; Supplemental Tables S2 and S3; Appendices II and III) were found to be reduced in three isoforms of dimeric alpha-amylase inhibitor (spots 43–45 in Figure 3.3 A, 87 in Figure 3.3 B).

Besides cytosolic phosphoglycerate kinase (spot 38), beta amylase (spot 86) that displayed differences in protein expression level, a set of thiol redox-modified proteins without differences in protein expression level was more reduced in non-dormant dry seeds. These represented phosphoglucomutase (spot 31), fructose 1-,6- biphosphate aldolase (spot 39), granule-bound starch synthase (spot 89), mitochondrial formate dehydrogenase (spot 92), cytosolic malate dehydrogenase (spot 94), alanine aminotransferase (spots 61, 96), heat shock 70 kDa protein (HSP70) (spot 99), putative 60S acidic ribosomal protein P0 (spot 94), r40g2 protein (spot 105). In non-dormant seeds imbibed for 24h, several proteins showed changes in only reduction/oxidation of Cys without differences in protein expression level, including enolase (spot 84), triosephosphate isomerise (spot 29), NAD-dependent glyceraldehydes-3-phosphate dehydrogenase (spot 85), glucose and ribitol dehydrogenase (spot 50), thiamine biosynthetic enzyme (spot 91), cytosolic malate dehydrogenase (spots 93, 58, 94), mitochondrial serine hydroxymethyltransferase (spot 95), alanine aminotransferase (spot 60), serpin-Z2A (spot 97), serpin-Z1B (spot 64), cell division control protein 48 (spot 68), putative 60S acidic ribosomal protein P0 (spot 94), 2-alkenal reductase (spot 100), aldehyde dehydrogenase (spots 101, 75), embryo-specific protein (spot 77).

Four antioxidative defence-responsive proteins with modified thiols displayed higher protein abundance level in dormant versus non-dormant seed protein extracts. Three proteins, 1-Cys peroxiredoxin (Prx), DHAR, and aldehyde DH, showed elevated expression upon 24 h of imbibition. The thioredoxin family 27 K protein was found in four protein spots 69–72 containing two identified isoforms (Table 3.2; Supplemental Table S2; Appendix II; Figure 3.3) with spots 70 and 71 more abundant and spots 69 and 72 less abundant in dry dormant seeds, indicating possible post-translational modification variants. A moderately decreased thiol modification ratio in imbibed dormant seeds was observed for 2-alkenal reductase and aldehyde DH (Table 3.2; Figure 3.3). It was also demonstrated that 1-Cys Prx expression level was higher in dormant dry and imbibed seeds, whereas the redox state was moderately decreased in imbibed dormant compared to imbibed non-dormant wheat seeds (Tables 3.2 and 3.3; Supplemental Tables S2 and S3; Appendices II and III). One protein involved in protein degradation 20S proteasome subunit alpha 7A had higher expression level in dormant than in non-dormant seeds, and also contained redox active thiol (Table 3.2; Supplemental Table S2; Figure 3.3). It was shown in this study that serpin-Z1B was more abundant in dormant seeds with significantly increased expression level upon imbibition, whereas serpin-Z1A, serpin-Z2B and serpin-Z1C were more abundant in non-dormant seeds (Table 3.2; Supplemental Table S2; Appendix II; Figure 3.3). Two isoforms serpin-Z1A and serpin-Z1C showed elevated thiol reduction level in dormant seeds upon imbibition, and one isoform serpin-Z2A was found to be significantly more reduced in dry and imbibed dormant seeds without systematic differences in protein expression between dormant and non-dormant genotypes (Table 3.3; Supplemental Table S3; Figure 3.3). Other two isoforms serpin-

Z2A and serpin-Z1B displayed higher thiol reduction level in non-dormant seeds. This indicates differential serpin isoform variants.

Table 3.1 Identification of proteins that displayed systematic expression differences in dormant and non-dormant hybrid genotypes

No.[a]	Putative Identity[b]	Accession N, Homolog, EST matches – % identity[c]	MASCOT[d]			ANOVA[e]				Fraction[f]
			Score	MP	SC	Dry		24 HI		
						p-Value	D/ND ratio	p-Value	D/ND ratio	
1. Metabolism										
1.1 Carbohydrate Metabolism										
1.1.1 Glycolysis / Gluconeogenesis										
1	Phosphoglycerate kinase, cytosolic	gi|129916 *Triticum aestivum*	1231	18	63	6.77918E-9	0.27	0.00216	1.97	Aqueous
1.1.2 Tricarboxylic Acid Cycle (TCA cycle)										
1	Succinyl-CoA ligase, beta-chain	gi|115447367 *Oryza sativa* EST CL1Contig602 – 93	1245	23	39	6.77918E-9	0.27	0.00216	1.97	Aqueous
1.1.3 Starch and Sucrose Metabolism										
2,	0.19 dimeric alpha-amylase inhibitor	gi|54778507 *Triticum aestivum*	737	10	95	7.47232E-7	1.92	6.18063E-4	**1.53**	Aqueous
3,			590	9	95	1.77609E-7	**2.66**	4.09172E-9	**3.66**	Aqueous
4			547	10	95	2.79675E-8	3.15	7.23665E-5	**1.61**	Aqueous
5,	Dimeric alpha-amylase inhibitor	gi|65993829 *Triticum aestivum*	105	3	31	ns	-	1.33782E-6	1.50	SDS fraction
6		gi|65993731 *Triticum aestivum*	236	3	20	9.16234E-5	2.77	0.00428	2.07	Total SDS
7,	Alpha-amylase/	gi|123975	786	15	78	0.00307	**1.48**	ns	-	SDS fraction
8	subtilisin inhibitor	*Triticum aestivum*	695	15	80	ns	-	3.77435E-5	1.79	SDS fraction
9,	Beta amylase	gi|75107132 *Hordeum vulgare*	1129	24	45	4.72071E-5	0.24	0.00311	**0.42**	Aqueous
10		EST CL1Contig11939 – 86	1277	27	49	7.2508E-8	0.12	0.00275	**0.48**	Aqueous

11, 12	Beta amylase	gi|32400764 *Triticum aestivum*	917 1126	17 19	74 71	0.03859	2.48	ns	-	Aqueous
		gi|75107132 *Hordeum vulgare* EST CL1Contig11939 – 86	2058	32	58	ns	-	1.28624E-5	2.68	Aqueous
1.2 Amino Acid Metabolism										
13	Betaine aldehyde dehydrogenase	gi|21747870 *Triticum aestivum*	535	11	35	0.00642	**1.60**	1.39082E-7	3.30	Total SDS
2. Genetic Information Processing										
2.1 Folding, Sorting and Degradation										
14	Heat-shock protein, 14.5 kDa	gi|186886566 *Triticum aestivum*	282	6	44	0.01627	3.56	ns	-	Aqueous
15	Protein disulfide isomerase 3	gi|13925728 *Triticum aestivum*	1575	32	66	7.49238E-4	2.91	2.54793E-4	2.82	Total SDS
3. Environmental Information Processing										
3.1 Transport										
16	Small Ran-related GTP-binding protein	gi|19919694 *Triticum aestivum*	250	7	44	ns	-	0.00203	**1.81**	SDS fraction
3.2 Antioxidative Defence Response										
17,	Superoxide dismutase, manganese	gi|1621627 *Triticum aestivum*	822	14	80	1.36747E-5	**1.67**	2.34196E-4	2.58	Aqueous
18,			922	15	82	6.00238E-4	**0.66**	7.76118E-5	0.58	Aqueous
19			818	14	70	ns	-	5.55614E-4	1.46	Total SDS
4. Storage Proteins										
20	Glutenin HMW, subunit 1Ax1	gi|21743 *Triticum aestivum*	609	10	18	3.02401E-4	**1.80**	0.00355	2.14	SDS fraction
21,	Glutenin HMW,	gi|121452	542	10	21	0.02487	1.47	ns	-	SDS fraction
22	subunit 12	*Triticum aestivum*	506	11	21	8.48934E-4	**1.80**	ns	-	SDS fraction

23	Glutenin HMW, subunit 1Dx5'	gi|296280726 *Triticum aestivum*	304	6	32	0.00919	**1.70**	4.44089E-16	2.03	SDS fraction
24	Triticin	gi|171027826 *Triticum aestivum* EST CL1Contig3986 – 99	619	14	25	8.19942E-5	1.66	9.10612E-4	1.61	SDS fraction
25	Glutenin LMW, subunit B3-2	gi|269854581 *Triticum aestivum* EST CL1Contig3975 – 98	106	5	17	5.74153E-8	**1.71**	3.66969E-4	1.95	SDS fraction

[a]Spot numbers correspond to 2D gels in Figure 3.2.
[b]Proteins were identified by LC-MS/MS analysis and 'Mascot' search of MS/MS spectra against protein NCBInr and EST wheat databases followed by homology identification of EST sequences with BLAST. All identifications met statistical confidence criteria according to 'Mascot' and BLAST scoring schemes. See supplementary data for information on peptide matches (Supplemental Table S1).
[c]When the only match was to EST *Triticum aestivum*, the accession number and taxonomy of protein to which the EST clone has the most similarity is given, followed by the EST GenBank accession number or a contig name, and percentage of sequence identity. See supplementary data for accession numbers corresponding to contigs (Supplemental Table S4).
[d]Probability based MOWSE score, number of matched peptides (MP), and percentage of sequence coverage (SC) are shown.
[e] *p*-value given for the genotype effect from the one-way ANOVA analysis performed for dry seeds (Dry) and after 24 hour of imbibition (24 HI). D/ND ratio represents the average ratio of the protein abundance (the fold change in Coomassie stained signal of individual protein spots from 2D gel maps) between three high dormancy (D) and three low dormancy (ND) hybrid lines of spring wheat (*Triticum aestivum* L.) double haploid population, derived from the cross 94C15/9014 = 8021-V2 (high dormancy, white seed coat) and AC Karma (low dormancy, white seed coat) segregating transgressively, and parent lines. Numbers in bold indicate values with low intra-line variation according to the homogeneity of variance Levene's test; ns stands for not significant differences in protein abundance.
[f]Proteins were found in different solubility fractions as indicated: the aqueous buffer fraction of whole seed protein extracts (Aqueous) followed by the SDS-soluble fraction (SDS fraction), or total SDS-soluble whole seed protein extracts (Total SDS).

Table 3.2 Identification of proteins with reduced Cys that displayed systematic expression differences in dormant and non-dormant genotypes

No.[a]	Putative Identity[b]	Accession number, Homolog organism, EST matches – % identity[c]	Score/ MP/ SC[d]	ANOVA[e] Dry p-Value	Dry D/ND ratio	24 HI p-Value	24 HI D/ND ratio	Peptides with bimane-Cys sites[f]	Structure and Function[g]
1. Metabolism									
1.1 Carbohydrate Metabolism									
1.1.1 Glycolysis / Gluconeogenesis									
26/ A,	Enolase	gi|110288667 *Oryza sativa* EST	2151/ 41/67	2.06659 E-4	**0.68**	1.9984 E-15	**0.61**	^{18}GNPTVEVDV**[bC]**CSDGTFAR^{35}	Unspecified
27/ SF,		CL1Contig14818 – 94	1720/ 24/50	6.40957 E-4	0.55	ns	-	^{347}S**[bC]**NALLLK^{354}	Near active site K^{354}
28/ TS			2085/ 35/64	9.33252 E-6	0.48	ns	-	^{18}GNPTVEVDV**[bC]**CSDGTFAR^{35} ^{103}NEWGW**[bC]**K^{109} ^{112}LGANAILAVSLAV**[bC]**K^{126} ^{347}S**[bC]**NALLLK^{354}	Identical Unspecified Unspecified Identical
29/ A,	Triosephosphate isomerase	gi|11124572 *Triticum aestivum*	713/ 13/52	ns	-	0.00111	**0.63**	^{56}LRPEIQVAAQN**[bC]**WVK^{70} ^{124}VIA**[bC]**VGETLEQR^{135} ^{207}IIYGGSVTGAS**[bC]**K^{219}	Unspecified Unspecified S-thiolation
30/ A			506/ 10/51	0.00227	**1.93**	6.90855 E-5	**1.60**	^{124}VIA**[bC]**VGETLEQR^{135} ^{207}IIYGGSVTGAS**[bC]**K^{219}	Identical Identical
31/ A	Phosphogluco-mutase	gi|18076790 *Triticum aestivum*	1325/ 30/69	0.04047	4.59	0.03337	**1.50**	^{222}FSF**[bC]**FDGLHGVAGAYAK^{238} ^{240}MFVDELGASESSLLN**[bC]**VPK^{258} ^{370}FFGNLMDAGM**[bC]**SV**[bC]**GEE SFGTGSDHIR^{396}	Unspecified Unspecified Unspecified
32/ A	**Phosphofructo-kinase, putative**	gi|116310015 *Oryza sativa* EST	337/ 5/34	1.95838 E-5	**0.3**	3.8225 E-13	**0.65**	^{111}AALVT**[bC]**GGL**[bC]**PGLNTVL R^{128}	Near active site G^{118}

		gi|24993241 – 80							
33/ A,	Alcohol dehydrogenase ADH1A	gi|119388723 *Triticum aestivum*	1230/ 22/69	2.96566 E-4	1.89	ns	-	^{107}SAESNM[**bC**]DLLR^{117} ^{172}V[**bC**]VLSCGISTGLGASINVAK PPK^{194} ^{238}FG[**bC**]TEFVNPK^{247} ^{41}ILFTSL[**bC**]HTDVYFWEAK^{57}	Zinc 2 binding site Cys^{177} Zinc 1 catalytic site Unspecified Zinc 1 catalytic site
34/ TS			893/ 17/60	0.00166	0.64	0.00122	0.66	^{107}SAESNM[**bC**]DLLR^{117} ^{238}FG[**bC**]TEFVNPK^{247} ^{41}ILFTSL[**bC**]HTDVYFWEAK^{57}	Identical Identical Identical
35/ A,	Glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent	gi|148508784 *Triticum aestivum*	1396/ 24/78	ns	-	1.90597 E-6	0.55	^{144}SDIDIVSNAS[**bC**]TTN[**bC**]LAPL AK^{164}	Cys^{154} Active site
36/ A			1497/ 26/77	ns	-	2.34979 E-12	0.34	^{75}EVAVFG[**bC**]R^{82} ^{129}DAPMFV[**bC**]GVNEK^{140} ^{144}SDIDIVSNAS[**bC**]TTN[**bC**]LAPL AK^{164}	Unspecified Unspecified Identical
37/ SF	Pyruvate, orthophosphate dikinase 1	gi|75254569 *Oryza sativa* EST CL1Contig6941 – 90	1734/ 30/44	2.15369 E-4	0.32	8.28404 E-18	**0.25**	^{844}VGI[**bC**]GEHGGEPSSVAFFAK^{862} ^{863}AGLDYVS[**bC**]SPFR^{874} ^{713}EL[**bC**]AETGAAEDDVLAR^{728}	Substrate binding sites V^{844} and G^{845} Mg binding site G^{845} Unspecified Unspecified
38/ TS	Phosphoglycerate kinase, cytosolic	gi|129916 *Triticum aestivum*	968/ 20/61	0.0405	1.95	ns	-	^{87}LSELLGLEVVMAPD[**bC**]IGEEVE K^{95}	Unspecified
39/ TS	Fructose 1-,6-biphosphate aldolase	gi|18496065 *Triticum aestivum*	624/ 13/66	1.8729 E-7	0.58	ns	-	^{78}YAII[**bC**]QENGLVPIVEPEILVDGP HDIDR^{105}	Unspecified
1.1.2 Starch and Sucrose Metabolism									
40/ A,	Beta amylase	gi|32400764 *Triticum aestivum*	980/ 16/69	ns	-	5.52539 E-4	**1.96**	^{31}ASLNFT[**bC**]AEMR^{41}	Unspecified
41/ TS			328/ 7/37	ns	-	4.93288 E-5	1.74	^{187}YPSYPQSHGWSFPGIGEFI[**bC**]Y DK^{209}	Unspecified

42/ A	Monomeric alpha-amylase inhibitor	gi|229615618 *Triticum turgidum* EST gi|49520392– 91	97/4/ 22	2.11458 E-7	1.92	ns	-	^{57}LQ**[bC]**VGSQVPEAVLR^{70}	Disulfide bond
27/ SF,	ADP-glucose pyrophosphorylase small subunit	gi|7340287 *Triticum aestivum*	1182/ 20/61	6.40957 E-4	0.55	ns	-	^{345}VLDADVTDSVIGEG**[bC]**VIK^{362} ^{76}LIDIPVSN**[bC]**LNSNISK^{91}	Unspecified Unspecified
28/ TS			1160/ 23/68	9.33252 E-6	0.48	ns	-	^{345}VLDADVTDSVIGEG**[bC]**VIK^{362}	Identical
43/ TS	0.19 dimeric alpha-amylase inhibitor	gi|54778511 *Triticum aestivum*	692/ 11/95	1.39066 E-5	2.43	5.4878 E-6	**2.10**	^{40}EC**[bC]**QQLADISEWCR^{53} ^{101}LPIVIDASGDGAYV**[bC]**K^{116}	Alpha-amylase binding site, 49…51 Disulfide bond
44/ TS,	Dimeric alpha-amylase inhibitor	gi|65993731 *Triticum aestivum*	768/ 11/41	0.03223	**1.76**	0.01005	1.98	^{43}LQ**[bC]**NGSQVPEAVLR^{56} ^{118}LPIVVDASGDGAYV**[bC]**K^{133}	Disulfide bond Disulfide bond
45/ TS		gi| 65993925 *Triticum aestivum* EST gi|93726383 – 99	802/ 10/41	3.79172 E-5	1.97	3.67026 E-4	**1.54**	^{43}LQ**[bC]**NGSQVPEAVLR^{56} ^{57}DCCQQLAHISEW**[bC]**R^{70} ^{118}LPIVVDASGDGAYV**[bC]**K^{133}	Identical Disulfide bond Identical
46/ TS	Alpha-amylase/ trypsin inhibitor CM3	gi|123957 *Triticum aestivum*	612/ 9/73	2.75307 E-6	**1.91**	5.94047 E-5	**1.62**	^{116}SGNVGESGLIDLPG**[bC]**PR^{132}	Unspecified
1.1.3 Other Carbohydrate Metabolism									
47/ A,	Glucose and ribitol dehydrogenase	gi|7431022 *Hordeum vulgare* EST CL1Contig4351 – 97	400/ 8/23	0.00559	1.51	ns	-	^{127}VDILVNNAAEQYVRP**[bC]**ITEIS EQDLER^{153}	Unspecified
48/ A			1310/ 24/47	1.47162 E-4	1.69	ns	-	^{6}FPPQQQD**[bC]**QPGK^{17} ^{101}ALAGDLGYEEN**[bC]**R^{113} ^{127}VDILVNNAAEQYVRP**[bC]**ITEIS EQDLER^{153}	Unspecified Unspecified Identical
49/ A,	Glucose and ribitol dehydrogenase	gi|7431022 *Hordeum vulgare* EST CL1Contig3679 – 97	1086/ 20/47	6.62527 E-4	1.67	ns	-	^{6}FPPQQQD**[bC]**QPGK^{17} ^{127}VDILVNNAAEQYVRP**[bC]**ITEI SEQDLER^{153}	Identical Identical

50/ A,			939/ 20/48	6.63511 E-6	2.88	1.59315 E-5	**2.76**	^{101}ALAGDLGYEEN[**bC**]R^{113} ^{127}VDILVNNAAEQYVRP[**bC**]ITEIS EQDLER^{153}	Identical Identical
51/ SF			216/7 /18	2.76034 E-4	1.59	3.7469 E-4	**1.50**	^{127}VDILVNNAAEQYVRP[**bC**]ITEIS EQDLER^{153}	Identical
1.2 Biosynthesis of Secondary Metabolites									
52/ A	**UDP-D-glucuronate decarboxylase**	gi|50659026 *Hordeum vulgare* EST CL1Contig9364 – 99	536/ 11/27	0.00298	0.53	ns	-	^{134}HDVTEPLLVEVDQIYHLA [**bC**]PASPIFYK^{160}	Unspecified
1.3 Energy Metabolism									
53/ A,	Malic enzyme, NADP-specific	gi| 38261493 *Oryza sativa* EST CL1Contig7328 – 89	1090/ 18/53	0.00404	**1.49**	0.0014	1.94	^{446}SE[**bC**]TAEQAYSWSQGR^{460}	Unspecified
		EST CL1Contig7283 – 90	650/ 14/45					^{30}ATEDQPVTPWAV[**bC**]IASGHSL LR^{51}	Unspecified
54/ A		CL1Contig7328 – 89	627/ 15/52	ns	-	0.01529	**1.57**		
		CL1Contig7383 – 90	431/ 12/49					^{30}ATEDQPVTPWAV[**bC**]IASGHSL LR^{51}	Identical
55/ A,	Formate dehydrogenase,	gi|21263612 *Hordeum vulgare*	1204/ 23/58	5.01937 E-7	1.52	9.45733 E-8	1.89	^{45}NPNFVG[**bC**]VEGALGIR^{59}	Unspecified
33/ A,	mitochondrial	EST CL1Contig17645 – 95	706/ 20/50	2.96566 E-4	1.89	ns	-	^{45}NPNFVG[**bC**]VEGALGIR^{59}	Identical
56/ SF,			858/ 14/43	0.03651	0.58	0.00316	**0.58**	^{45}NPNFVG[**bC**]VEGALGIR^{59}	Identical
34/ TS			844/ 18/47	0.00166	0.64	0.00122	0.66	^{210}LKPFN[**bC**]NLLYHDR^{222} ^{248}[**bC**]DVIVINTPLTEK^{260}	Unspecified Unspecified

57/ A	Isocitrate dehydrogenase, NADP-specific	gi|5007084 *Oryza sativa* EST CL1Contig210 – 94	1561/ 39/48	ns	-	1.30978 E-9	**0.59**	[261]SEGGYVWA[**bC**]K[270]	Unspecified
58/ SF,	Malate dehydrogenase, cytosolic	gi|49343245 *Triticum aestivum*	1417/ 19/71	3.24728 E-4	**0.62**	ns	-	[69]GVVATTDVVEA[**bC**]TGVNVAV MVGGFPR[94] [113]AQASALEAHAAPN[**bC**]K[127] [153]NIS[**bC**]LTR[159] [242]LSSALSAASSA[**bC**]DHIR[257]	NAD binding site, 89..92 Unspecified NAD binding site L[157] Dimer interface, 244..247, 250
59/ TS			800/1 6/61	4.03814 E-8	0.54	ns	-	[113]AQASALEAHAAPN[**bC**]K[127] [242]LSSALSAASSA[**bC**]DHIR[257] [326]ALAYS[**bC**]LA[333]	Identical Identical C-terminal protein
1.4 Amino Acid Metabolism									
32/ A,	Alanine aminotransferase	gi|1703227 *Hordeum vulgare* EST CL1Contig13096 – 96	2011/ 36/55	1.95838 E-5	**0.30**	3.8225 E-13	**0.65**	[69]EVLAL[**bC**]DHPDLLK[81] [285]LEGIT[**bC**]NK[392] [393]AEGAMYLFPQI[**bC**]LPQK[408]	Unspecified Unspecified Unspecified
60/ A,			1474/ 28/52	2.00087 E-7	**1.61**	ns	-	[69]EVLAL[**bC**]DHPDLLK[81] [328]IASVNL[**bC**]SNITGQILASLVMN PPK[351] [393]AEGAMYLFPQI[**bC**]LPQK[408] [454][**bC**]TILPQEEK[462]	Identical Unspecified Identical Unspecified
61/ SF			2137/ 34/56	3.44666 E-4	**0.55**	1.36606 E-10	**0.49**	[69]EVLAL[**bC**]DHPDLLK[81] [285]LEGIT[**bC**]NK[392] [393]AEGAMYLFPQI[**bC**]LPQK[408] [453][**bC**]TILPQEEK[461]	Identical Identical Identical Identical
52/ A,	Aspartate aminotransferase	gi|164471780 *Triticum aestivum*	1127/ 21/65	0.00298	0.53	ns	-	[80]VATVQ[**bC**]LSGTGSLR[93]	Unspecified
56/ SF			684/ 12/42	0.03651	0.58	0.00316	**0.58**	[80]VATVQ[**bC**]LSGTGSLR[93]	Identical
2. Genetic Information Processing									
2.1 Folding, Sorting and Degradation									
62/	Serpin-Z1A	gi|75282265	1506/	7.30061	0.64	5.7683	0.57	[302][**bC**]LGLQLPF**G**DEADFSEMVD	Unspecified

A,		*Triticum aestivum*	26/78	E-4		E-7		SLM**PQ**GLR[328]	
		gi|75282265 *Triticum aestivum* EST CL1Contig18337 – 96	1326/ 25/63					[109]VAFANGVFVDASLLLKPSFQE LAV**[bC]**K[124]	Unspecified
63/ SF		gi|75282265 *Triticum aestivum*	1497/ 21/74	2.16318 E-5	0.40	5.45747 E-4	0.56	[302]**[bC]**LGLQLPF**G**DEADFSEMVD SLM**PQ**GLR[328]	Identical
64/ TS	Serpin-Z1B	gi|75279910 *Triticum aestivum*	1208/ 18/67	8.95975 E-4	1.68	8.54751 E-8	4.08	[99]VAFANGVFVDASLLLKPSFQEL AV**[bC]**K[124]	Identical
								[302]**[bC]**LGLQLPF**S**DEADFSEMVDS PM**PQ**GLR[328]	Similar
65/ TS	Serpin-Z2B	gi|75279909 *Triticum aestivum*	1172/ 17/55	0.04839	**0.65**	0.0021	0.64	[99]VAFANGVFVDASLLLKPSFQEL AV**[bC]**K[124]	Identical
66/ A	Serpin-Z1C	gi|75313848 *Triticum aestivum*	1239/ 20/64	ns	-	2.94004 E-4	0.55	[302]**[bC]**LGLQLPF**S**NEADFSEMVDS PM**AH**GLR[328]	Similar
67/ SF	**20S Proteasome subunit alpha type-7-A**	gi| 75131289 *Oryza sativa* EST CL1Contig10503 – 98	516/ 10/28	-	new	2.38477 E-6	1.63	[63]IASLDTHIALA**[bC]**AGLK[78]	Unspecified
2.2 Cell Cycle									
68/ SF	Cell division control protein 48	gi| 108706222 *Oryza sativa* EST CL1Contig9579 – 96	2060/ 34/37	2.15369 E-4	0.32	2.69628 E-10	**0.28**	[103]LGDVVSVHQ**[bC]**PDVK[116]	Unspecified
								[536]AIANE**[bC]**QANFISIK[549]	Unspecified
								[684]YTQGFSGADITEI**[bC]**QR[699]	Unspecified
3. Environmental Information Processing									
3.1 Antioxidative Defence Response									
69/ A,	**27K thioredoxin family protein**	gi|30793446 *Triticum aestivum*	265/ 7/53	8.3866 E-7	0.14	7.86515 E-5	0.33	[12]VHVAIYYESL**[bC]**PYS**V**R[27]	Unspecified
70/ A			373/ 8/59	8.19937 E-5	**1.72**	ns	-	[12]VHVAIYYESL**[bC]**PYS**V**R[27]	Identical

71/ A, 72/ TS	**27K thioredoxin family protein**	gi|290350670 *Triticum aestivum* EST CL1Contig17267 – 88	499/ 11/48 543/ 12/49	1.09893 E-6 2.45486 E-4	1.89 0.61	ns ns	- -	^{24}VHVAIYYESL**[bC]**PYSAR^{39} ^{24}VHVAIYYESL**[bC]**PYSAR^{39}	Similar Identical
73/ A	1-Cys peroxiredoxin PER1	gi|75324900 *Triticum aestivum*	813/ 16/69	0.0031 2	1.44	1.02001 E-5	1.85	^{67}LLGIS**[bC]**DDVQSHK^{79} ^{140}LSFLYPS**[bC]**TGR^{150} ^{170}VATPANWNPGE**[bC]**VVIAPGV SDDEAKK^{195}	Redox active, Trx Redox active, Trx K^{195} Bipartite nuclear localization signal
74/ A	Dehydroascorbate reductase	gi|28192421 *Triticum aestivum*	782/ 15/85	ns	-	2.47556 E-7	1.54	^{8}AAVGHPDTLGD**[bC]**PFSQR^{24}	Cys^{19} redox active site
75/ A	Aldehyde dehydrogenase	gi|11995457 *Oryza sativa* EST CL1Contig4569 – 90 EST CL1Contig5198 – 92	1016/ 19/37 985/ 16/52	1.79399 E-4	**0.50**	0.00749	1.75	^{110}ILPEGIGEVQEIIDM[**bC**]DYAVG LSR^{133} ^{264}**[bC]**LLELSGNNAIIVMDDADIP LAVR^{287} ^{440}WIGPHGSD[**bC**]GIVNVNIPTNG AEIGGAFGGEK^{470}	Unspecified Unspecified Unspecified
4. Storage Proteins									
76/ A,	Embryo-specific protein	gi|4105692 *Oryza sativa* EST	620/ 12/40	9.10383 E-15	1.89	ns	-	^{55}QVEAHHF**[bC]**AHLNEDVR^{70}	Unspecified
77/ TS		CL183Contig1 – 86	646/ 14/38	ns	-	7.81499 E-4	2.28	^{55}QVEAHHF**[bC]**AHLNEDVR^{70} ^{71}Q**[bC]**LVFDGPDAGAR^{83} ^{174}QDLAD**[bC]**VEK^{182}	Identical Unspecified Unspecified
78/ SF	Globulin 2 (11S globulin, legumin)	gi|228310 *Zea mays* EST CL1Contig3675 – 58	480/ 14/18	7.40514 E-8	**1.77**	2.30183 E-4	1.83	^{258}FHQITGDQ**[bC]**HHLR^{270}	Unspecified
79/ SF, 80/ SF, 81/ TS,	Globulin 3 (storage protein 7S, vicilin)	gi|215398470 *Triticum aestivum*	389/ 8/16 1260/ 29/54 453/ 9/19	ns ns 6.11125 E-4	- - 1.72	5.89628 E-4 2.69987 E-4 3.62546 E-7	2.07 1.55 2.13	^{489}GSSNLQVV**[bC]**FEINAER^{504} ^{397}LAVVLEGEGEVEIV**[bC]**PHLG R^{416} ^{489}GSSNLQVV**[bC]**FEINAER^{504}	Unspecified Unspecified Identical

82/		348/	2.44249		5.24495		^{489}GSSNLQVV**[bC]**FEINAER^{504}	
TS,		6/11	E-15	**3.28**	E-7	2.12		Identical
83/		400/	0.02124		0.00293		^{489}GSSNLQVV**[bC]**FEINAER^{504}	
TS	EST gi|39568029 - 92	7/23		**1.76**		1.95		Identical

[a]Spot numbers correspond to 2D gels in Figure 3.3. Proteins were found in different solubility fractions as indicated: the aqueous buffer fraction of whole seed protein extracts (A) followed by the SDS-soluble Fraction (SF), or total SDS-soluble whole seed protein extracts (TS).

[b]Putative names of the identified proteins are listed. Protein digests were analyzed by LC-MS/MS analysis and 'Mascot' search of MS/MS spectra against protein NCBInr and EST wheat databases followed by homology identification of EST sequences with BLAST. All identifications met statistical confidence criteria according to 'Mascot' and BLAST scoring schemes. See supplementary data for details (Supplemental Table S2). Proteins in bold were not previously shown as potential Trx targets.

[c]When the only match was to EST *Triticum aestivum*, the accession number and taxonomy of protein to which the EST clone has the most similarity is given, followed by the EST GenBank accession number or a contig name, and percentage of sequence identity. See supplementary data for accession numbers corresponding to contigs (Supplemental Table S4).

[d]Probability based MOWSE scores are given; MP means number of matched peptides, and SC represents sequence coverage according to Mascot Search Results.

[e] *p*-value given for the genotype effect from the one-way ANOVA analysis performed for dry seeds (Dry) and after 24 hour of imbibition (24 HI). D/ND ratio represents the average ratio of the protein abundance (the fold change in protein staining or fluorescence signal of individual protein spots from 2D gel maps of labelled protein extracts) between three high dormancy (D) and three low dormancy (ND) hybrid lines of spring wheat (*Triticum aestivum* L.) double haploid population, derived from the cross 94C15/9014 = 8021-V2 (high dormancy, white seed coat) and AC Karma (low dormancy, white seed coat) segregating transgressively, and parent lines. Numbers in bold indicate values with low intra-line variation according to the homogeneity of variance Levene's test; ns stands for not significant differences in protein abundance.

[f]Peptide sequences with Cys-bimane [bC] sites of modification. Single amino acid substitutions in protein isoforms are shown in bolds. The amino acid residue numbering corresponds to the sequence numbering of annotated protein to which the EST clone has the most similarity (shown in column 3).

[g]Putative function of labelled Cys site predicted by similarity from annotated UniProtKB, specific references are discussed in the text.

Table 3.3 Identification of redox-sensitive proteins with reduced cysteine residue differences in dormant and non-dormant hybrid genotypes

No.[a]	Putative Identity[b]	Accession number, Homolog organism, EST matches – % identity[c]	Score/ MP/ SC[d]	ANOVA[e] Dry p-Value	Dry TM ratio	24 HI p-Value	24 HI TM ratio	Peptides with bimane-Cys sites[f]	Structure and Function[g]
1. Metabolism									
1.1 Carbohydrate Metabolism									
1.1.1 Glycolysis / Gluconeogenesis									
26/ A,	Enolase	gi|110288667 *Oryza sativa* EST CL1Contig14818 – 94	2151/ 41/67	4.60102 E-8	3.18	0.01179	2.46	^{18}GNPTVEVDV**[bC]**CSDGTFAR^{35}	Unspecified
84/ A			2430/ 36/65	ns	-	0.03037	0.61	^{18}GNPTVEVDV**[bC]**CSDGTFAR^{35}	Identical
29/ A	Triosephosphate isomerase	gi|11124572 *Triticum aestivum*	713/ 13/52	ns	-	0.03536	0.18	^{56}LRPEIQVAAQN**[bC]**WVK^{70} ^{124}VIA**[bC]**VGETLEQR^{135} ^{207}IIYGGSVTGAS**[bC]**K^{219}	Unspecified Unspecified S-thiolation
31/ A	Phosphogluco-mutase	gi|18076790 *Triticum aestivum*	1325/ 30/69	0.02078	0.64	0.02079	0.14	^{222}FSF**[bC]**FDGLHGVAGAYA K^{238} ^{240}MFVDELGASESSLLN**[bC]**VPK^{258} ^{370}FFGNLMDAGM**[bC]**SV**[bC]**GEE SFGTGSDHIR^{396}	Unspecified Unspecified Unspecified
85/ A	Glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent	gi|253783729 *Triticum aestivum*	1175/ 21/52	ns	-	2.40974 E-9	0.25	^{142}YTSDVNIVSNAS**[bC]**TTN**[bC]**L APLAK^{164}	Cys^{154} Active site Cys^{158} unspecified
37/ SF	Pyruvate, orthophosphate dikinase 1	gi|75254569 *Oryza sativa* EST CL1Contig6941 – 90	1734/ 30/44	ns	-	2.92879 E-5	2.51	^{863}AGLDYVS**[bC]**SPFR^{874} ^{713}EL**[bC]**AETGAAEDDVLAR^{728} ^{844}VGI**[bC]**GEHGGEPSSVAFFAK^{862}	Unspecified Unspecified Substrate binding sites V^{844} and G^{845} Mg binding site G^{845}

38/ TS	Phosphoglycerate kinase, cytosolic	gi|129916 *Triticum aestivum*	968/ 20/61	3.81125 E-4	0.49	ns	-	^{87}LSELLGLEVVMAPD**[bC]**IGEEVE K^{95}	Unspecified
39/ TS	Fructose 1-,6- biphosphate aldolase	gi|18496065 *Triticum aestivum*	624/ 13/66	2.85879 E-7	0.42	ns	-	^{78}YAII**[bC]**QENGLVPIVEPEILVDGP HDIDR^{105}	Unspecified
1.1.2 Starch and Sucrose Metabolism									
86/ TS	Beta amylase	gi|75107132 *Hordeum vulgare* EST CL1Contig11939 – 86	1216/ 23/44	4.44761 E-5	**0.53**	0.00143	0.31	^{187}YPSYPQSHGWSFPGIGEFI**[bC]**Y DK^{209}	Unspecified
								^{29}HHASLNFT**[bC]**AEMR^{41}	Unspecified
42/ A	Monomeric alpha-amylase inhibitor	gi|229615618 *Triticum turgidum* EST gi|49520392– 91	97/ 4/22	ns	-	5.67878 E-4	**1.82**	^{57}LQ**[bC]**VGSQVPEAVLR^{70}	Disulfide bond
87/ A	0.19 dimeric alpha-amylase inhibitor	gi|54778507 *Triticum aestivum*	777/ 15/96	0.00845	**1.47**	9.85526 E-4	1.73	^{40}EC**[bC]**QQLADISEWCR^{53}	Disulfide bond
								^{101}LPIVIDASGDGAYV**[bC]**K^{116}	Disulfide bond
88/ A	Alpha-amylase/trypsin inhibitor CM3	gi|123957 *Triticum aestivum*	598/ 9/69	ns	-	7.75392 E-4	1.70	^{81}LYC**[bC]**QELAEISQQCR^{95}	Unspecified
89/ TS	**Granule-bound starch synthase**	gi|4588609 *Triticum aestivum*	1373/ 29/63	6.35031 E-8	**0.52**	ns	-	^{192}FSLL**[bC]**QAALEVPR^{204}	Unspecified
								^{264}VAF**[bC]**IHNISYQGR^{276}	Unspecified
								^{490}FEP**[bC]**GLIQLQGMR^{502}	Unspecified
								^{503}YGTP**[bC]**A**[bC]**ASTGGLVDTIV EGK^{523}	Unspecified
								^{531}LSVD**[bC]**NVVEPADVK^{544}	Unspecified
1.1.3 Other Carbohydrate Metabolism									
48/ A,	Glucose and ribitol dehydrogenase	gi|7431022 *Hordeum vulgare* EST CL1Contig4351 – 97	1310/ 24/47	0.00157	3.27	ns	-	^{6}FPPQQQD[**bC**]QPGK^{17}	Unspecified
								^{101}ALAGDLGYEEN[**bC**]R^{113}	Unspecified
								^{127}VDILVNNAAEQYVRP[**bC**]ITEIS EQDLER^{153}	Unspecified
49/ A,			516/ 12/36	3.46116 E-4	**2.57**	ns	-	^{6}FPPQQQD[**bC**]QPGK^{17}	Identical
								^{127}VDILVNNAAEQYVRP[**bC**]ITEIS	Identical

								EQDLER[153]	
50/ A		EST CL1Contig3679 – 97	939/20/48	ns	-	0.00273	0.53	[101]ALAGDLGYEEN[**bC**]R[113] [127]VDILVNNAAEQYVRP[**bC**]ITEIS EQDLER[153]	Identical Identical
1.2 Biosynthesis of Secondary Metabolites									
90/ A	**O-Methyltransferas ZRP4**	gi|16924034 *Oryza sativa* EST CL1339Contig2 – 56	692/ 14/33	0.01147	1.97	ns	-	TLFEVAHG**[bC]**TR WVM**[bC]**LWQDEDAVK	Unspecified Unspecified
91/ SF	Thiamine biosynthetic enzyme	gi|32352138 *Oryza sativa* EST CL1Contig18963 – 73	807/ 13/36	ns	-	5.52713 E-5	0.54	[228]IVVSS[**bC**]GHEGLFSANGK[244] [298]M[**bC**]PTFGATIISGQK[311]	Unspecified Unspecified
1.3 Energy Metabolism									
53/ A	Malic enzyme, NADP-specific	gi| 38261493 *Oryza sativa* EST CL1Contig7328 – 89	1090/ 18/53	0.03521	1.70	4.96198 E-9	0.43	[446]SE**[bC]**TAEQAYSWSQGR[460]	Unspecified
		EST CL1Contig7283 – 90	650/ 14/45					[30]ATEDQPVTPWAV**[bC]**IASGHSL LR[51]	Unspecified
55/ A,	Formate dehydrogenase, mitochondrial	gi|21263612 *Hordeum vulgare* EST CL1Contig17645 – 95	1204/ 23/58	ns	-	0.01269	1.56	[45]NPNFVG[**bC**]VEGALGIR[59]	Unspecified
92/ TS			981/ 19/49	0.00812	0.60	ns	-	[45]NPNFVG**[bC]**VEGALGIR[59] [210]LKPFN**[bC]**NLLYHDR[222] [248]**[bC]**DVIVINTPLTEK[260]	Identical Unspecified Unspecified
93/ A,	Malate dehydrogenase, cytosolic	gi|49343245 *Triticum aestivum*	1369/ 21/71	ns	-	0.00457	0.66	[242]LSSALSAASSA[bC]DHIR[257] [326]ALAYS[bC]LA[333]	Unspecified Unspecified
58/ SF,			1417/ 19/71	ns	-	1.05754 E-6	0.49	[39]GVVATTDVVEA[bC]TGVNVAV MVGGFPR[64]	Unspecified

								^{83}AQASALEAHAAPN[bC]K^{97}	Unspecified
								^{153}NIS[bC]LTR^{159}	Unspecified
								^{212}LSSALSAASSA[bC]DHIR^{227}	Identical
94/ SF			639/ 12/55	7.72734 E-4	0.54	0.00977	0.57	^{83}AQASALEAHAAPN[bC]K^{97}	Identical
								^{212}LSSALSAASSA[bC]DHIR^{227}	Identical
1.4 Amino Acid Metabolism									
95/ A	Serine hydroxy-methyltransferase, mitochondrial	gi|108862549 *Oryza sativa* EST CL1Contig11606 – 84	1016/ 18/38	ns	-	0.00201	0.22	^{128}YYGGNEVIDEVEEL**[bC]**R^{143}	Unspecified
32/ A,	Alanine aminotransferase	gi|1703227 *Hordeum vulgare* EST CL1Contig13096 – 96	1091/ 22/45	0.0067	**1.76**	ns	-	^{393}AEGAMYLFPQI**[bC]**LPQK^{408}	Unspecified
60/ A,			1474/ 28/52	ns	-	0.04547	**0.58**	^{69}EVLAL**[bC]**DHPDLLK^{81}	Unspecified
								^{300}GYYGE**[bC]**GK^{307}	Unspecified
								^{328}IASVNL**[bC]S**NI**T**GQILASLVM NPPK^{351}	Unspecified
								^{393}AEGAMYLFPQI**[bC]**LPQK^{408}	Identical
								^{454}**[bC]**TILPQEEK^{462}	Unspecified
61/ SF,			2137/ 34/56	3.62009 E-5	**0.67**	0.01996	**0.66**	^{69}EVLAL**[bC]**DHPDLLK^{81}	Identical
								^{393}AEGAMYLFPQI**[bC]**LPQK^{408}	Identical
96/ TS			1776/ 33/52	0.01674	**0.24**	0.04539	0.19	^{17}**[bC]**EYAVR^{22}	Unspecified
								^{69}EVLAL**[bC]**DHPDLLK^{81}	Identical
								^{285}LEGIT**[bC]**NK^{392}	Unspecified
								^{393}AEGAMYLFPQI**[bC]**LPQK^{408}	Identical
								^{453}**[bC]**TILPQEEK^{461}	Identical
2. Genetic Information Processing									
2.1 Folding, Sorting and Degradation									
63/ SF	Serpin-Z1A	gi|75282265 *Triticum aestivum*	1497/ 21/74	ns	-	1.71833 E-5	2.35	^{302}**[bC]**LGLQLPF**G**DEADFSEMVD **SL**MPQGLR^{328}	Unspecified

97/ A	Serpin-Z2A Serpin 3	gi|75313847 *Triticum aestivum* gi|224589270 *Triticum aestivum*	1237/ 18/67 1134/ 17/64	ns	-	6.08366 E-5	0.54	^{99}V**T**FANGVFVDASL**P**LKPSFQEL AV**[bC]**K^{124} ^{99}V**A**FANGVFVDASL**L**LKPSFQEL AV**[bC]**K^{124} ^{302}**[bC]**LGLQLPF**S**DEADFSEMVD S**P**MPQGLR^{328}	Unspecified Similar Similar
98/ TS	Serpin-Z2A	gi|75313847 *Triticum aestivum*	869/ 14/49	0.00107	5.22	2.28817 E-13	**3.24**	^{99}V**T**FANGVFVDASL**P**LKPSFQEL AV**[bC]**K^{124}	Identical
64/ SF	Serpin-Z1B	gi|75279910 *Triticum aestivum*	1208/ 18/67	ns	-	3.26971 E-6	0.45	^{109}V**A**FANGVFVDASL**L**LKPSFQEL AV**[bC]**K^{124}	Identical
66/ A	Serpin-Z1C	gi|75313848 *Triticum aestivum*	1239/ 20/64	ns	-	0.02787	3.19	^{302}**[bC]**LGLQLPF**S**NEADFSEMVDS **P**MAHGLR^{328}	Identical
99/ TS	Heat Shock 70 kDa Protein, HSP70	gi|2827002 *Triticum aestivum*	1907/ 33/61	0.00361	0.55	8.98409 E-4	0.38	^{4}GEGPAIGIDLGTTYS**[bC]**VGVWQ HDR^{27} ^{603}ELEGI**[bC]**NPIIAK^{614}	Unspecified Unspecified
	2.2 Cell Cycle								
68/ SF	Cell division control protein 48	gi| 108706222 *Oryza sativa* EST CL1Contig9579 – 96	2060/ 34/37	ns	-	1.90832 E-6	0.34	^{103}LGDVVSVHQ**[bC]**PDVK^{116} ^{536}AIANE[bC]QANFISIK^{549} ^{684}YTQGFSGADITEI**[bC]**QR^{699}	Unspecified Unspecified Unspecified
94/ SF	60S acidic ribosomal protein P0, putative	gi| 77552928 *Oryza sativa* EST CL1Contig4238– 93	1069/ 5/38	7.72734 E-4	0.54	0.00977	0.57	^{18}L**[bC]**QLLEEYTK^{27}	Unspecified
	3. Environmental Information Processing								
	3.1 Antioxidative Defence Response								
73/ A	1-Cys peroxiredoxin PER1	gi|75324900 *Triticum aestivum*	813/ 16/69	0.00801	1.53	0.00801	0.65	^{67}LLGIS**[bC]**DDVQSHK^{79} ^{140}LSFLYPS**[bC]**TGR^{150} ^{170}VATPANWNPGE**[bC]**VVIAPGV SDDEAKK^{195}	Redox active, Trx Redox active, Trx K^{195} Bipartite nuclear localization signal
100/ A	**2-Alkenal reductase**	gi|62765876 *Hordeum vulgare* EST CL1Contig3486– 92	583/ 12/35	ns	-	0.00362	0.64	^{180}ISG**[bC]**YVVGSAGSDEK^{194} ^{253}VSV**[bC]**GLISQYNLEQSEG VR^{271} ^{272}NLF**[bC]**IITK^{279}	Nucleotide binding Nucleotide binding Nucleotide binding

101/ A,	Aldehyde dehydrogenase	gi|11995457 *Oryza sativa* EST	873/ 13/49	ns	-	2.63025 E-5	0.39	^{440}WIGPHGSD**[bC]**GIVNVNIPTNG AEIGGAFGGEK^{470}	Unspecified
75/ A		CL1Contig5198 – 92	985/1 6/52	ns	-	4.95049 E-6	0.32	^{440}WIGPHGSD**[bC]**GIVNVNIPTNG AEIGGAFGGEK^{470}	Identical
		CL1Contig4569 – 90 EST	1016/ 19/37					^{110}ILPEGIGEVQEIIDM[**bC**]DYAVG LSR^{133}	Unspecified
								^{264}**[bC]**LLELSGNNAIIVMDDADIP LAVR^{287}	Unspecified
4. Storage Proteins									
76/ A,	Embryo-specific protein	gi|4105692 *Oryza sativa* EST	620/ 12/40	2.57239 E-12	2.53	ns	-	^{55}QVEAHHF**[bC]**AHLNEDVR^{70}	Unspecified
77/ TS		CL183Contig1 – 86 EST	646/ 14/38	ns	-	0.00341	0.47	^{55}QVEAHHF**[bC]**AHLNEDVR^{70}	Identical
		CL183Contig3 – 87						^{71}Q**[bC]**LVFDGPDAGAR^{83}	Unspecified
								^{174}QDLAD**[bC]**VEK^{182}	Unspecified
102/ TS,	Globulin 3 (storage protein 7S, vicilin)	gi|215398470 *Triticum aestivum* EST	1057/ 22/40	7.44229 E-4	2.13	7.83529 E-4	1.95	^{489}GSSNLQVV**[bC]**FEINAER^{504}	Unspecified
103/ TS,		CL1Contig9410 – 93 EST	386/ 8/28	0.01044	2.32	0.0022	**1.57**	^{489}GSSNLQVV**[bC]**FEINAER^{504}	Identical
104/ TS		gi|39568029 – 92	318/ 7/21	0.00429	2.53	2.78196 E-4	3.94	^{489}GSSNLQVV**[bC]**FEINAER^{504}	Identical
5. Unknown Function									
105/ SF	**r40g2 protein**	gi|34394517 *Oryza sativa* EST	1039/ 18/61	6.57531 E-4	0.50	ns	-	^{201}ASEDY**[bC]**LAVR^{210}	Unknown
		CL1Contig9406 – 84						^{211}NGTV**[bC]**LAPTNPR^{222}	Unknown
106/ TS	**Lipoprotein-like protein**	gi|14209547 *Oryza sativa* EST	284/ 6/23	1.37735 E-4	**1.85**	2.10286 E-8	**2.41**	^{80}LNQDVLQ[**bC**]PVYDSDDK^{95}	Unknown

[a]Spot numbers correspond to 2D gels in Figure 3.3. Proteins were found in different solubility fractions as indicated: the aqueous buffer fraction of whole seed protein extracts (A) followed by the SDS-soluble Fraction (SF), or total SDS-soluble whole seed protein extracts (TS).

[b]Putative names of the identified proteins are listed. Protein digests were analyzed by LC-MS/MS analysis and 'Mascot' search of MS/MS spectra against protein NCBInr and EST wheat databases followed by homology identification of EST sequences with BLAST. All identifications met statistical confidence criteria according to 'Mascot' and BLAST scoring schemes. See supplementary data for details (Supplemental Table S3). Proteins in bold were not previously shown as potential Trx targets.

[c]When the only match was to EST *Triticum aestivum*, the accession number and taxonomy of protein to which the EST clone has the most similarity is given, followed by the EST GenBank accession number or a contig name, and percentage of sequence identity. See supplementary data for accession numbers corresponding to contigs (Supplemental Table S4).

[d]Probability based MOWSE scores are given; MP means number of matched peptides, and SC represents sequence coverage according to Mascot Search Results.

[e] *p*-value given for the genotype effect from the one-way ANOVA analysis performed for dry seeds (Dry) and after 24 hour of imbibition (24 HI). TM ratio represents the average ratio of the protein thiol modification calculated as follows: the fluorescence/protein ratio of a specific protein spot on 2D gels from fluorescently labelled seed protein extracts of three hybrid and 8021-V2 parent high dormancy lines was divided by the fluorescence/protein ratio of corresponding protein spot on 2D gels from fluorescently labelled seed protein extracts of three hybrid and AC Karma parent low dormancy lines. Numbers in bold indicate values with low intra-line variation according to the homogeneity of variance Levene's test; ns stands for not significant differences in protein abundance.

[f]Peptide sequences with Cys-bimane [bC] sites of modification. Single amino acid substitutions in protein isoforms are shown in bolds. The amino acid residue numbering corresponds to the sequence numbering of annotated protein to which the EST clone has the most similarity (shown in column 3).

[g]Putative function of labelled Cys site predicted by similarity from annotated UniProtKB, specific references are discussed in the text.

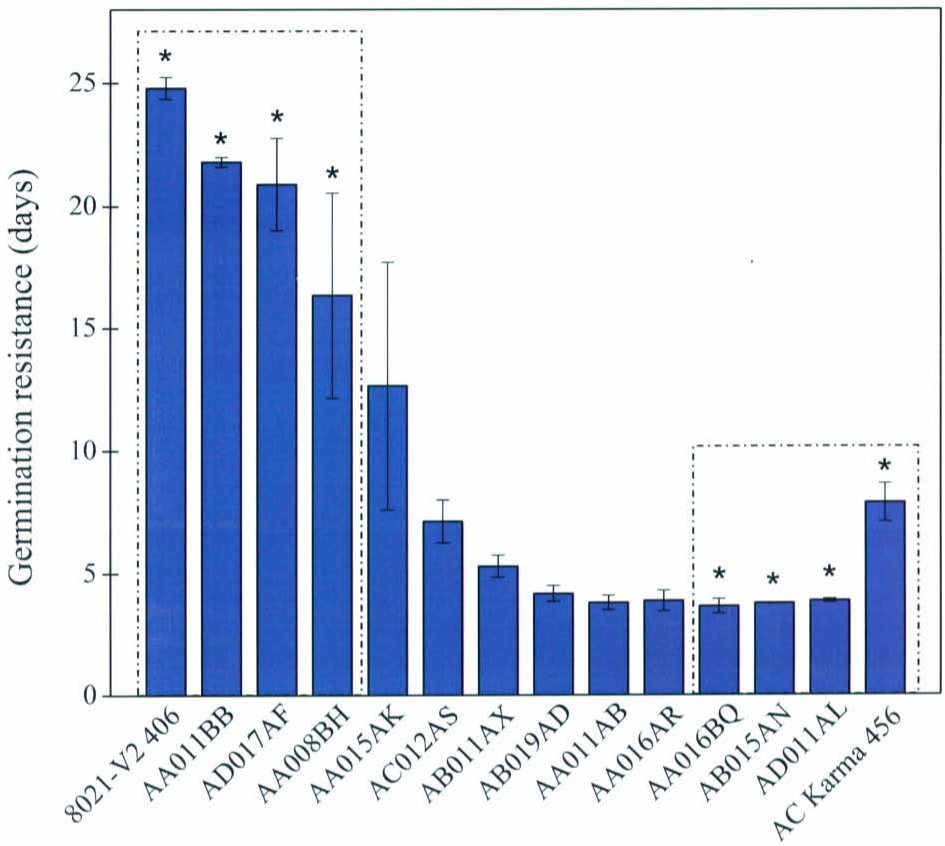


Figure 3.1 Germination resistance test for 12 hybrid lines of spring wheat (*Triticum aestivum* L.) Double haploid population, derived from the cross 94C15/9014 = 8021-V2 (high PHS resistance, white seed coat) and AC Karma (low PHS resistance) segregating transgressively for superior levels of grain dormancy. Dormant and non-dormant parents were used as standards.Mean germination resistance values ± standard errors are shown. The lines with values included in dotted square were chosen for further redox proteomic analysis.

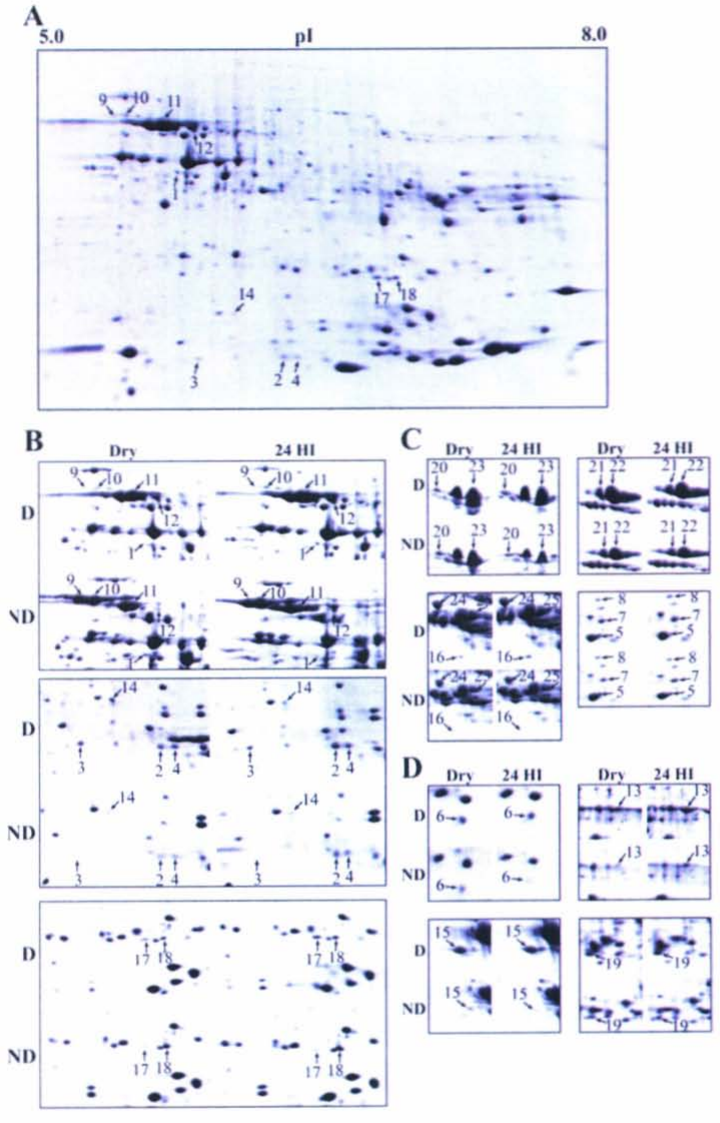


Figure 3.2 Differential protein expression in mature dry and imbibed seeds of dormant and non-dormant closely related genotypes. Reference 2-DE map of aqueous protein fraction (A) extracted from dry seed material of dormant (AD017AF) hybrid line. Close-up of protein expression differences in aqueous (B), total SDS-soluble (C) protein extracts, and SDS-soluble protein fraction (D) between dry and imbibed for 24 h, 24 HI, seeds of representative dormant, D, (AD017AF) and non-dormant, ND, (AD011AL) hybrid lines. Identified protein spots with systematic abundance differences between dormant and non-dormant genotypes are marked with arrows and numbers.

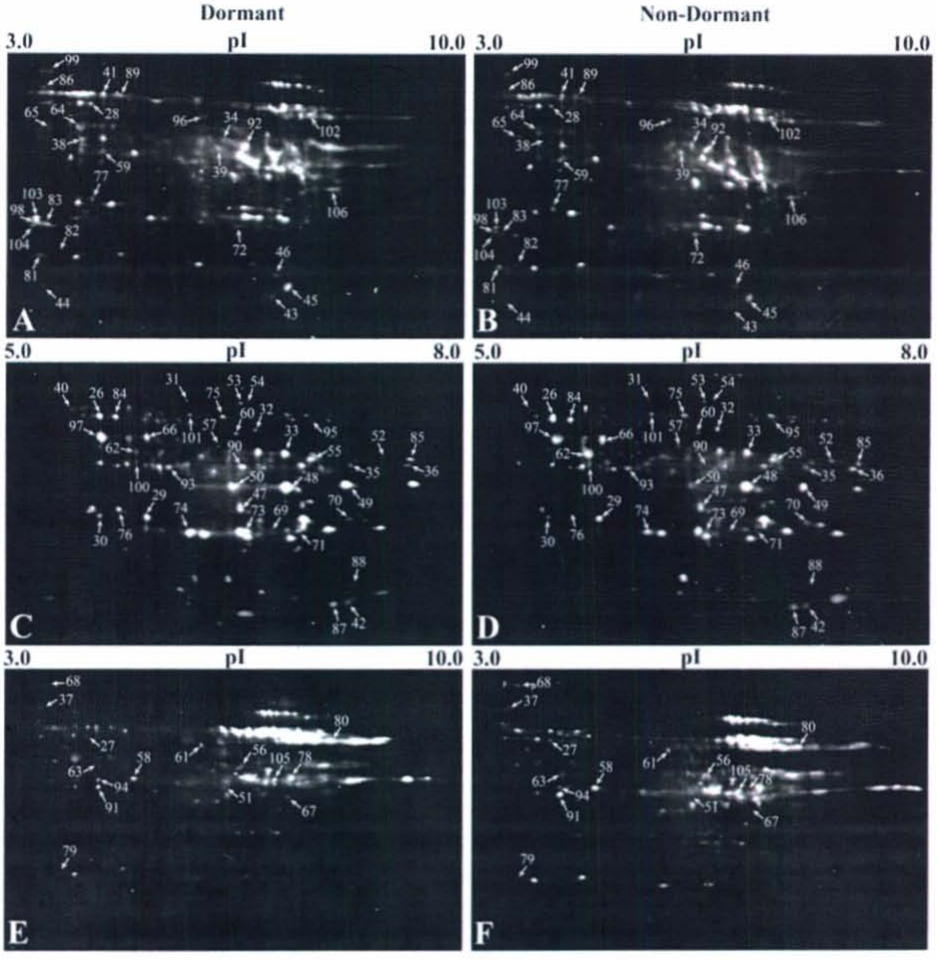


Figure 3.3 2-DE UV images of Cys-bimane fluorescently labelled proteins with differential thiol reduction. Differential protein thiol reduction in mature dry seeds of dormant (A, C, E) and non-dormant (B, D, F) closely related genotypes. Representative 2-DE UV images of Cys-bimane fluorescently labelled total SDS-soluble proteins in the pH range 3–10 NL (A and B), aqueous protein extracts in the pH range 5–8 (C and D), and SDS-soluble protein fraction in the pH range 3–10 NL (E and F) from dry seed material of dormant (AD017AF) and non-dormant (AD011AL) hybrid lines. Proteins indicated by arrows and numbers were analysed by LC-MS/MS (Tables 3.1, 3.2, and 3.3). More complete raw data sets for spot identifications are given in Supplemental Tables S1, S2, and S3 (Appendices I, II, and III).

4. DISCUSSION

4.1 Protein abundance differences in dormant and non-dormant lines

It is still not clear how the germination process is being induced in non-dormant seeds and suppressed in dormant seeds. This research aimed to address this question by studying wheat seed protein expression profiles associated with germination potential. In our study, a number of proteins showed higher expression level in dormant seeds versus non-dormant seeds. One of the most prominent identified proteins was Ran GTPase, which is involved in diverse biological functions such as nuclear transport, spindle formation during mitosis, DNA replication, and cell division. The functions of Ran signalling in nuclear transport and mitotic progression are well conserved in plants and animals and are essential for viability in every tested organism. This protein was recently demonstrated to be a direct target for NO_2-induced cellular molecular response (Heo, 2008; Heo and Campbell, 2006). The oxidative stress-induced perturbation of the Ran-mediated nuclear import suggests that the unique structural redox architecture of Ran could be a factor in the regulation of cell signal transduction pathways associated with this protein.

A strong, negative correlation was found between germination capacity and ROS, such as superoxide radical and hydrogen peroxide, as well as with lipid hydroxyperoxides (Bailly *et al.*, 2008). After-ripening of dormant sunflower (*Helianthus annuus* L.) seeds entailed a progressive accumulation of ROS, namely superoxide anions and hydrogen peroxide, in cells of embryonic axes. This accumulation occurred concomitantly with

lipid peroxidation and oxidation (carbonylation) of specific embryo proteins (Oracz *et al.*, 2007; Oracz *et al.*, 2009). Manganese SOD is involved in superoxide radical detoxification in the mitochondrial matrix. In mitochondria, ROS production is generally caused by an over-reduction of the electron transport chain. In the course of seed development and germination, energy-demanding processes relying on respiratory metabolism and seed mitochondria are exposed to water stress, desiccation, imbibitions, and hypoxic conditions. A number of proteins oxidatively damaged by carbonylation (including SOD) have been identified in the mitochondrial matrix, and the level of protein carbonylation was higher in the mitochondria than in other organelles such as chloroplasts and peroxisomes (Møller *et al.*, 2007). Noticeably, SOD qualifies for both ROS-detoxifying enzymes and enzymes that produce ROS as part of their normal catalytic cycle. Elevated expression level of SOD in highly dormant wheat lines firstly can play a protective role against oxidative stress damage during seed aging or desiccation, and secondly can be involved in regulation of a delicate balance between production and scavenging in ROS homeostasis for perception of environmental factors by seeds during dormancy maintenance.

4.2 Protein thiol redox modification in hybrid dormancy lines

The most abundant proteins in aqueous extracts of imbibed dormant seeds with prominent response were proteinaceous inhibitors of α-amylases. The cereal-type α-amylase inhibitor proteins contain five disulfide bonds and are known for their action on α-amylases from birds, bacilli, mammals and insects. All identified α-amylase inhibitor proteins were shown to be putative Trx *h*-reducible disulfide targets in wheat and barley

(Wong *et al.*, 2004; Maeda *et al.*, 2005). The disulfide bonds were proposed to be essential for the activity of some α-amylase inhibitor proteins *in vitro* (Kobrehel *et al.*, 1991). The findings suggest that the redox mobilization of defence proteins has a specific role in the high dormancy genotypes.

Globulin storage proteins legumins (11S globulin 2) and vicilins (7S globulin 3) were highly reduced in dry dormant seeds (Tables 3.2 and 3.3, Supplemental Tables S2 and S3, Appendices II and III, Figure 3.3). Both storage proteins are synthesized as precursors that undergo molecular maturation by limited proteolysis before deposition. Similar proteinases catalyse the proteolytic processing of these proteins and contribute to their complete breakdown during germination and seedling growth (Shutov *et al.*, 2003). Therefore, the pattern of well-defined partial proteolytic fragments may reflect a transient synthesis or activation of one or more proteases at this stage of development. Trx was shown to reduce the major storage proteins of wheat seeds *in vitro* (Montrichard *et al.*, 2009; Wong *et al.*, 2004). In a recent study on the effects of endogenous and recombinant Trx induction on rice bran proteins, it was demonstrated that the activation of a cysteine protease was accompanied by unfolding of its substrate, the embryo-specific protein (ESP) (Yano and Kuroda, 2006). Similar to ESP, globulins in wheat seeds are considered to have a folded, possibly protease-resistant structure before imbibition. Meanwhile, it has been suggested that Trx is inactive before imbibition, and seed proteins are degraded in specific stages of germination through the presence of a Trx-dependent mobilization mechanism. Thus it is likely that Trx fine-tunes the complete degradation of globulin 3 storage protein in non-dormant wheat seeds during germination. This finding suggests

that in dormant seeds globulins undergo a change (i.e. partial proteolysis) that renders them amenable to digestion following reduction by Trx.

4.3 Impact of dormancy genotypes on functional thiol-redox proteome

Four antioxidative defence-responsive proteins with modified thiols displayed higher protein abundance level in dormant versus non-dormant seed protein extracts. Three proteins, 1-Cys peroxiredoxin (Prx), dehydroascorbate reductase (DHAR), and aldehyde dehydrogenase (DH) showed elevated expression upon 24 h of imbibition. Prxs are ROS-scavenging enzymes that provide cells with highly efficient machinery for detoxifying H_2O_2, alkyl hydroperoxides, and $OH^{\cdot}$ and were shown to prevent radical attack of lipids, enzymes, and DNA *in vitro*. Moreover, Prxs were also reported to function as redox sensors, linking the redox signalling and ROS networks of cells (Dietz, 2008). Prxs are widely distributed and can be divided into 1-Cys and 2-Cys Prx groups based on the number of conserved cysteine residues in their catalytic cycle. 1-Cys Prx is almost exclusively expressed in seeds, the protein accumulates in the nucleus of aleurone and embryo cells (Stacy *et al.*, 1999) and exhibits antioxidant activity rather than dormancy-related function (Haslekås *et al.*, 2003). When coupled to a thiol-reducing system, 1-Cys Prxs display peroxidase activity. Reduction of 1-Cys Prxs by Trx *h* was previously observed in wheat (Montrichard *et al.*, 2009) and, in barley seeds, Trx *h* significantly reduced cysteine residues identical to Cys72 and Cys147 (Hägglund *et al.*, 2008). It was proposed that the antioxidant function of 1-Cys Prx resulted from the protection of nuclear DNA in seed cells suffering oxidative stress. Our findings demonstrate that the 1-Cys Prx expression level was higher in dormant dry and imbibed

seeds, whereas the redox state was moderately decreased in imbibed dormant compared to imbibed non-dormant wheat seeds (Tables 3.2 and 3.3, Supplemental Tables S2 and S3, Appendices II and III).

Antioxidant strategies are used by seeds to protect themselves against oxidative stress damage during desiccation and rehydration. One of the enzymes that maintains the redox status and regenerates antioxidants in their active form in the ascorbate-glutathione cycle is DHAR. Interaction between DHAR and Trx *h* was previously demonstrated in *Arabidopsis thaliana*, wheat and barley (Montrichard *et al.*, 2009). A recent quantitative proteomic study revealed that Cys19, the proposed catalytic residue of DHAR enzyme (Dixon *et al.*, 2002), was extensively reduced (over 60%) by Trx *h* (Hägglund *et al.*, 2008). Hence the reduction of DHAR by Trx *h* is likely to modulate its function. The conserved Cys19 forms mixed disulfides with glutathione GSSG, which preserve the enzyme from being inactivated. Such S-glutathionylation of enzymes protects essential cysteinyl residues from irreversible oxidation to the sulfinic acid and sulfonic acid derivatives during redox stress (Dixon *et al.*, 2002). It was found that the reduced DHAR form increased in abundance in imbibed seeds of dormant white-grained wheat genotypes versus imbibed seeds of non-dormant genotypes, and this could possibly indicate that the enzyme was present in a more active state.

A moderately decreased thiol modification ratio in imbibed dormant seeds was observed for 2-alkenal reductase (spot 100 in Table 3.3, Supplemental Table S3, Appendix III, Figure 3.3). Degradation of lipid peroxides leads to the formation of cytotoxic 2-alkenals and oxenes, collectively designated as reactive carbonyls. The NADPH-dependent oxidoreductase 2-alkenal reductase (AER) catalyzes the reduction of

the α, β-unsaturated bond of reactive carbonyls (Youn *et al.*, 2006). Specific and irreversible protein carbonylation during seed after-ripening has been recently associated with oxidative attack and dormancy alleviation (Oracz *et al.*, 2007). The putative AER proteins from Triticum shares 65% identity with At5g16970, one of 11 homologues in *Arabidopsis thaliana* (Youn *et al.*, 2006). The identified labelled conserved Cys183, Cys256 and Cys275 are located in the nucleotide binding domain of the folded At5g16970. The precise role of the Cys residues for AER function has to be further elucidated.

A major group of identified thiol redox active proteins is involved in carbohydrate, energy and amino acid metabolism. Enzymes of carbohydrate and energy metabolism were redox responding to genetic dormancy variation, including many DHs whose activity is dependent on NAD^+ or $NADP^+$ (Table 3.2, Supplemental Table S2, Appendix II). Maintaining a highly reducing internal environment in plant cells is made possible by interactions between the major soluble non-protein redox couples (NAD^+, $NADP^+$, glutathione and ascorbate). In addition, therefore, to key roles in primary and secondary metabolism, these compounds are at the center of the complex network of reactions surrounding ROS generation and control. Although their physiological functions are not limited to stress conditions, the status of each of them can influence stress responses. Local perturbation of this NAD(P)-dependent buffering system is likely an important process in the transmission of ROS signals (Noctor, 2006). Redox thiol change is one of the biochemical mechanisms through which the status of redox couples could be sensed. Many metabolic proteins identified in this study have been linked to Trx (Montrichard *et al.*, 2009; Wong *et al.*, 2004; Marchand *et al.*, 2006). One protein involved in protein

degradation, 20S proteasome subunit alpha 7A, had higher expression level in dormant than in non-dormant seeds, and also contained a redox active thiol (Table 3.2, Supplemental Table S2, Appendix II, Figure 3.3). In plants, protein-processing and degradation genes regulate many cellular events leading to development and division, and degrade unwanted or inhibitor proteins during cellular processes (Vierstra, 2003). A number of proteasome subunits were shown to be potential Trx targets. Trx was shown to enhance proteolysis in cereals by its ability to reductively activate proteases and increase the solubility and proteolytic susceptibility of storage proteins on the one hand, and inactivate protease inhibitors on the other (Montrichard *et al.*, 2009). High-level expression of this protein during imbibition of dormant seeds may affect a rapid degradation of dormancy-alleviating or germination-inducing proteins. Serine proteinase inhibitors of chymotrypsin-like enzymes displayed differential expression and thiol-reduction pattern in dormant and non-dormant genotypes. Six distinct serpins were previously identified in grains of hexaploid bread wheat but their physiological functions have not been completely elucidated (Østergaard *et al.*, 2000). In our study, serpin-Z1B was more abundant in dormant seeds with significantly increased expression level upon imbibition, whereas serpin-Z1A, serpin-Z2B and serpin-Z1C were more abundant in non-dormant seeds (Table 3.2, Supplemental Table S2, Appendix II, Figure 3.3). Two isoforms serpin-Z1A and serpin-Z1C showed elevated thiol reduction level in dormant seeds upon imbibition, and one isoform serpin-Z2A was found to be significantly more reduced in dry and imbibed dormant seeds without systematic differences in protein expression between dormant and non-dormant genotypes (Table 3.3, Supplemental Table S3, Appendix III, Figure 3.3). Other two isoforms serpin-Z2A and serpin-Z1B displayed

higher thiol reduction level in non-dormant seeds. This possibly indicates differential functional role of serpin isoform variants. Plant serpins are likely to use their irreversible mechanism in the inhibition of endogenous and exogenous proteinases capable of breaking down seed storage prolamins, such as digestive proteinases of insect pests or fungal pathogens (Østergaard *et al.*, 2000). The role of oxidative changes in serpins during dormancy control remains to be investigated.

5. CONCLUSIONS AND FUTURE STUDIES

5.1 Conclusions

The results demonstrated that harvest-ripe grains of closely related genotypes of wheat with either a dormant or a non-dormant phenotype, differentially express many proteins involved in metabolism, genetic and environmental information processing, antioxidative defence response and storage (Appendix VI). It was also demonstrated that in non-dormant seeds, thiol redox changes in proteins are associated with conversion to an active state, thereby facilitating the mobilization of nitrogen and carbon for germination and developing seedling. In dormant seeds, there was a biochemical shift in the accumulation of proteins from those active in biosynthesis and metabolism to those with roles in storage and protection against biotic and abiotic stresses. We observed in imbibed dormant seeds higher abundance of antioxidant proteins and enzymes important for redox control, ROS scavenging and detoxification. The proteomic data obtained provide evidence for an increased capacity of potent antioxidant machinery in seeds of high non-deep physiological dormancy genotypes, which could be coupled with their ability to rapidly regenerate antioxidant systems upon rehydration cycles for dormancy maintenance. Approximately 83% of the proteins identified in this study, 44 out of 53 redox active, have been shown *in vitro* to be potential or established Trx targets in land plants (Montrichard *et al.*, 2009). Nine redox sensitive proteins were not previously reported as potential Trx targets (shown in bold in Tables 3.2, 3.3). The results presented here support the hypothesis that antioxidative defence mechanisms could be involved in

imposing dormancy. This study is a further step toward a more comprehensive analysis of the genetic and biochemical endodormancy control in wheat. In order to identify low abundance proteins, alternative gel-free mass spectrometry-based quantitative approaches in conjunction with different liquid chromatography methods will need to be employed. Further research in this area will be informative for analysing the effect of the environmental conditions on dormancy controlling events to provide molecular fingerprints for dormancy and PHS resistance.

5.2 Future directions

Dormancy control through after-ripening is an essential physiological process for many agriculturally important crop species. The critical interaction between dormancy and after-ripening has received little attention, and in the long-term it should be addressed. The hypothesis is that after-ripening is a discrete developmental pathway associated with specific metabolic networks. I propose to characterize the molecular mechanisms of after-ripening. By varying dormancy and holding after-ripening constant, we can probe the modifications that after-ripening contributes to the expression of the dormancy-related functional proteomes. In these experiments, the proteome in seeds of closely related genotypes of *Triticum aestivum*, with either dormant or non-dormant phenotypes, will be investigated using hybrid lines of a spring wheat double haploid population derived from the cross 94C15/9014 = 8021-V2 (high pre-harvest sprouting resistance, white seed coat) and AC Karma (low pre-harvest sprouting resistance) segregating transgressively.

Furthermore, protein thiol redox patterns differ in dormant and non-dormant states and are additionally modified by after-ripening. The thiol redox-sensitive proteome in

dormant and non-dormant hybrid genotypes from fresh and after-ripened seeds will be detected from series of 2-DE gels and characterized by LC-MS/MS analysis. It will also be informative to follow changes in the redox ratios of oxidized and reduced forms for the key non-protein redox metabolite couples in the aqueous phase glutathione, NAD, NADP and ascorbate. The activities of the ascorbate-glutathione cycle enzymes ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase will be monitored.

Another area of research will focus on spatial tissue-specific distribution of metabolic pathways within the seed and whether subcomponents of the seed have different functions in the after-ripening regulation of dormancy. Dormancy is a function of the intact seed and in cereal grains it is partially associated with the embryo (embryo dormancy) and partially with the covering layers (Finch-Savage and Leubner-Metzger, 2006). To investigate after-ripening and dormancy-related functional tissue differentiation, the aleurone layer (plus seed coat), and the embryo (plus scutellum) will be dissected from seeds of spring-type wheat genotype with extreme dormancy. This approach in conjunction with IEF 2-DE will allow analysis of protein groups that otherwise would not be detectable in the intact seed proteome as described above. Blue-Native 2-DE will be used to analyse membrane-bound intact protein complexes (Bykova *et al.*, 2003; Eubel H *et al.*, 2005). This allows separation of hydrophobic protein complexes and provides information on their native interactions. In a preliminary study (unpublished results), several membrane protein complexes in the total membrane preparations were found to be more abundant and differentially modified in the aleurone/seed coat tissues following after-ripening treatment of a dormant line.

To provide a more complete documentation of protein expression profiles, gel-free multi-dimensional protein identification technologies will also be necessary to employ using protein sample prefractionation, Strong Cation Exchange fractionation of peptide digests and reverse-phase LC-MS/MS (Motoyama *et al.*, 2006). Finally, a strategy for differential label-free quantitative LC-MS/MS analysis of diagnostic biomarkers based on Mass and Time Tag Proteomic approach will be developed. In this approach, the combination of peptide mass accuracy (mass tags), MS/MS fragmentation for the unambiguous confirmation of biomarker, reproducible chromatographic separation (accurate LC elution time tag) and standardized conditions for sample preparation will give us a means for comparative nano-scale analysis of complex peptide samples (Griffin *et al.*, 2010; Wang *et al.*, 2006) permitting more rigorous quantification. Moreover, candidate biomarker proteins will be confirmed by Western and ELISA techniques or using PCR-based detection. The Western blotting detection and relative quantification experiments for some of the found candidate biomarker proteins (such as triosisphospate isomerise and alcohol dehydrogenase, for example) are in progress.

REFERENCES

Agrawal GK, Rakwal R. 2008. Rice Proteome at a Glance. In: GK Agrawal, R Rakwal (eds). *Plant proteomics: technologies, strategies, and applications.* John Wiley & Sons, Inc., Hoboken, NJ, USA, 165-289.

Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S. 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Cri. Rev. Biotech.* 30(3):161-175.

Alboresi A, Gestin C, Leydecker MT, Bedu M, Meyer C, Truong HN. 2005. Nitrate, a signal relieving seed dormancy in *Arabidopsis*. *Plant, Cell & Environment* 28:500-512.

Ali-Rachedi S, Bouinot D, Wagner MH, Bonnet M, Sotta B, Grappin P, Jullien M. 2004. Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of *Arabidopsis thaliana*. *Planta* 219:479-488.

Allen PS, Benech-Arnold RL, Batlla D, Bradford KJ. 2007. Modeling of seed dormancy. In: KJ Bradford, H Nonogaki (eds). *Seed Development, Dormancy and Germination.* Blackwell Publishing, Oxford, 72-112.

Alvarez S, Galant A, Jez JM, Hicks LM. 2011. Redox-regulatory mechanisms induced by oxidative stress in *Brassica juncea* roots monitored by 2-DE proteomics. *Proteomics* 11:1346-1350.

Anderson L, Seilhamer J. 1997. A comparison of selected mRNA and protein abundances in human liver. *Electrophoresis* 18:533-537.

Andon NL, Hollingworth S, Koller A, Geenland AJ, Yates JR, Haynes PA. 2002. Proteomic characterization of wheat amyloplasts using identification of proteins by tandem mass spectroscopy. *Proteomics* 2:1156-1168.

Aparicio N, Villegas D, Araus JL, Blanco R, Royo C. 2002. Seedling development and biomass as affected by seed size and morphology in durum wheat. *J. Agric. Sci.* 139:143-150.

Apel K, Hirt H. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Bio.* 55:373-399.

Arc E, Galland M, Cueff G, Godin B, Lounifi I, Job D, Rajjou L. 2011. Reboot the system thanks to protein post-translational modifications and proteome diversity: How

quiescent seeds restart their metabolism to prepare seedling establishment. *Proteomics* 11:1606-1618.

Asada K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol.* 141:391-96.

Ashraf M, Harris PJC. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* 166:3-6.

Assmann SM. 2003. OPEN STOMATA1 opens the door to ABA signalling in Arabidopsis guard cells. *Trends Plant Sci.* 8:151-153.

Athar HR, Khan A, Ashraf M. 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ. Exp. Bot.* 63:224-231.

Bahin E, Bailly C, Sotta B, Kranner I, Corbineau F, Leymarie J. 2011. Crosstalk between reactive oxygen species and hormonal signalling pathways regulates grain dormancy in barley. *Plant, Cell & Environment* 34:980-993.

Bailly C, El-Maarouf-Bouteau H, Corbineau F. 2008. From intracellular signalling networks to cell death: the dual role of reactive oxygen species in seed physiology. *C. R. Biologies* 331:806-814.

Bailly C. 2004. Active oxygen species and antioxidants in seed biology. *Seed Sci. Res.* 14:93-107.

Bair NB, Meyer SE, Allen PS. 2006. A hydrothermal after-ripening time model for seed dormancy loss in *Bromus tectorum* L. *Seed Sci. Res.* 16:17-28.

Barnabás B, Jäger K, Fehér A. 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ.* 31:11-38.

Basha E, Lee GJ, Demeler B, Vierling E. 2004. Chaperone activity of cytosolic small heat shock proteins from wheat. *Eur. J. Biochem.* 271:1426-1436.

Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. *Seed Sci. Res.* 14:1-16.

Bassoi MC, Flintham J. 2005. Relationship between grain colour and preharvest sprouting-resistance in wheat. *Pesq. Agropec. Bras.* 40:981-988.

Benech-Arnold RL, Gualano N, Leymarie J, Côme D, Corbineau F. 2006. Hypoxia interferes with ABA metabolism and increases ABA sensitivity in embryos of dormant barley grains. *J. Exp. Bot.* 57:1423-1430.

Berjak P. 2006. Unifying perspectives of some mechanisms basic to desiccation tolerance across life forms. *Seed Sci. Res.* 16:1-15.

Berkelman T, Stenstedt T. 1998. *2-D Electrophoresis using Immobilized pH Gradients: Principles and Methods*, Amersham Pharmacia Biotech, Piscataway, NJ, 17-46.

Besse I, Wong JH, Kobrehel K, Buchanan BB. 1996. Thiocalsin: a thioredoxin-linked, substrate-specific protease dependent on calcium. *Proc. Natl. Acad. Sci.* USA. 93(8):3169-3175.

Bethke PC, Jones RL. 2001. Cell death of barley aleurone protoplasts is mediated by reactive oxygen species. *Plant J.* 25:19-29.

Bethke PC, Libourel IG, Aoyama N, Chung YY, Still DW, Jones RL. 2007. The Arabidopsis aleurone layer responds to nitric oxide, gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. *Plant Physiol.* 143:1173-1188.

Bewley JD, Black M. 1994. *Seeds. Physiology of Development and Germination (2nd edition).* Plenum Press, New York.

Bewley JD. 1997. Seed germination and dormancy. *Plant Cell* 9:1055-1066.

Black M, Corbineau F, Gee H, Come D. 1999. Water content, rafinose, and dehydrins in the induction of desiccation tolerance in immature wheat embryos. *Plant Physiol.* 120(2):463-72.

Blokhina O, Virolainen E, Fagerstedt KV. 2003. Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: a Review. *Ann. Bot.* 91(2):179-194.

Blum A. 1998. Improving wheat grain filling under stress by stem reserve mobilisation. *Euphytica* 100:77-83.

Bradford KJ, Trewavas AJ. 1994. Sensitivity thresholds and variable time scales in plant hormone action. *Plant Physiol.* 105:1029-1036.

Brady SM, McCourt P. 2003. Hormone cross-talk in seed dormancy. *J. Plant Growth Regulation* 22:25-31.

Bykova NV, Egsgaard H, Møller IM. 2003. Identification of 14 new phosphoproteins involved in important plant mitochondrial processes. *FEBS Lett.* 540(1-3):141-146.

Bykova NV, Hoehn B, Rampitsch C, Banks T, Stebbing J-A, Fan T, Konx R. 2011. Redox-sensitive proteome and antioxidant strategies in wheat seed dormancy control. *Proteomics* 11:865-882.

Cadman CSC, Toorop PE, Hilhorst HWM, Finch-Savage WE. 2006. Gene expression profiles of Arabidopsis *Cvi* seeds during dormancy cycling indicate a common underlying dormancy control mechanism. *Plant J.* 46:805-822.

Cazalis R, Pulido P, Aussenac T, Pérez-Rulz JM, Cejudo FJ. 2006. Cloning and characterization of three thioredoxin *h* isoforms from wheat showing differential expression in seeds. *J. Exp. Bot.* 57(10):2165-2172.

Chen C-X, Cai S-B, Bai G-H. 2007. A major QTL controlling seed dormancy and preharvest sprouting resistance on chromosome 4A in a Chinese wheat landrace. *Mol. Breed* 21:351-358.

Chiappetta G, Ndiaye S, Igbaria A, Kumar C, Vinh J, Toledano MB. 2010. Proteome Screens for Cys Residues Oxidation: The Redoxome. *Methods in Enzymology* 473:199-216.

Chiwocha SDS, Cutler AJ, Abrams SR, Ambrose SJ, Yang J, Ross ARS, Kermode AR. 2005. The *etr1-2* mutation in *Arabidopsis thaliana* affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy, moist-chilling and germination. *Plant J.* 42:35-48.

Chouchani ET, James AM, Fearnley IM, Lilley KS, Murphy MP. 2011. Proteomic approaches to the characterization of protein thiol modification. *Cur. Opi. Chem. Biol.*15:120-128.

Chow B, McCourt P. 2004. Hormone signalling from a developmental context. *J. Exp. Bot.* 55:247-251.

Clarke FR, Knox RE, DePauw RM. 2005. Expression of dormancy in a spring wheat cross grown in field and controlled environment conditions. *Euphytica* 143:297-300.

Colville L, Ilse Kranner I. 2010. Desiccation tolerant plants as model systems to study redox regulation of protein thiols. *Plant Growth Regul.* 62:241-255.

D'Autreaux B, Toledano MB. 2007. ROS as signalling molecules: Mechanisms that generate specificity in ROS homeostasis. *Nat. Rev. Mol. Cell Biol.* 8:813-824.

Dalle-Donne I, Rossi R, Giustarini D, Colombo R, Milzani A. 2007. S-glutathionylation in protein redox regulation. *Free Radic. Bio. Med.* 43:883-898.

Davies MJ. 2005. The oxidative environment and protein damage. *Biochim. Biophys. Acta.* 1703:93-109.

De Gara L, de Pinto MC, Moliterni VMC, D'Egidio MG. 2003. Redox reguraion and storage processes during maturation in kernels of Triticum durum. *J. Exp. Bot.* 54:249-258.

DePauw RM, McCaig TN, Clarke JM, McLeod JG, Knox RE, Fernandez MR. 1992. Registration of sprouting-resistant white-kerneled wheat germplasms SC8019R1 and SC8021V2. *Crop Sci.* 32:838.

Dietz KJ, Jacob S, Oelze ML, Laxa M, Tognetti V, de Miranda SM, Baier M, Finkemeier I. 2006. The function of peroxiredoxins in plant organelle redox metabolism. *J. Exp. Bot.* 57:1697-1709.

Dietz KJ. 2008. Redox signal integration: from stimulus to networks and genes. *Physiol. Plant.* 133:459-468.

Dixon DP, Skipsey M, Grundy NM, Edwards R. 2005. Stress-induced protein S-glutathionylation in Arabidopsis. *Plant Physiol.* 138:2233-2244.

Donohue K. 2002. Germination timing influences natural selection on life-history characters in Arabidopsis thaliana. *Ecology* 83:1006-1016.

Dubcovsky J, Dvorak J. 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science* 316:1862-1866.

El-Maarouf-Bouteau H, Bailly C. 2008. Oxidative signalling in seed germination and dormancy. *Plant Signal Behav.* 3:175-182.

Eubel H, Braun HP, Millar AH. 2005. Blue-native PAGE in plants: a tool in analysis of protein-protein interactions. *Plant Methods* 1(1):11.

Fath A, Bethke P, Beligni V, Jones RL. 2002. Active oxygen and cell death in cereal aleurone layers. *J. Exp. Bot.* 53:1273-1282.

Feuillet C, Langridge P, Waugh R. 2007. Cereal breeding takes a walk on the wild side. *Trends in Genetics* 24:24-32.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *New Phytol.* 171:501-523.

Finkelstein R, Gampala S, Rock C. 2002. Abscisic acid signaling in seeds and seedlings. *Plant Cell* 14:S15-45.

Finkelstein R., Reeves W, Ariizumi T, Steber C. 2008. Molecular aspects of seed dormancy. *Annu. Rev. Plant Biol.* 59:61387-415.

Finnie C, Svensson B. 2009. Barley seed proteomics from spots to structures. *Proteomics* 72:315-324.

Flemetakis E, Agalou A, Kavroulakis N, Dimou M, Martsikovskaya A, Slater A, Spaink HP, Roussis A, Katinakis P. 2002. *Lotus japonicus* gene *Ljsbp* is highly conserved among plants and animals and encodes a homologue to the mammalian selenium-binding proteins. *Mol. Plant Microbe. Interact.* 15:313-322.

Flintham JE, Gale MD. 1988. Genetics of pre-harvest sprouting and associated traits in wheat: review. *Plant Varieties Seeds* 1:87-9.

Flintham JE. 2000. Different genetic components control coat-imposed and embryo-imposed dormancy in wheat. *Seed. Sci. Res.*10:43-50.

Fofana B, Humphreys G, Rasul G, Cloutier S, Somers D. 2008. Assessment of molecular diversity at QTLs for preharvest sprouting resistance in wheat using microsatellite markers. *Genome* 51:375-386.

Gapper C, Dolan L. 2006. Control of plant development by reactive oxygen species. *Plant Physiol.* 141:341-345.

Gegas V C, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, Sayers L, Doonan J H, Snape J W.2010. A genetic framework for grain size and shape variation in wheat. *Plant Cell* 22:1046-1056.

Gerjets T, Scholefield D, Foulkes MJ, Lenton JR, Holdsworth MJ. 2010. An analysis of dormancy, ABA responsiveness, after-ripening and pre-harvest sprouting in hexaploid wheat (*Triticum aestivum* L.) caryopses. *J. Exp. Bot.* 61(2):597-607.

Ghezzi P, Bonetto V, Fratelli M. 2005. Thiol-disulfide balance: from the concept of oxidative stress to that of redox regulation. *Antioxid. Redox Signal* 7:964-972.

Ghezzi P. 2005. Oxidoreduction of protein thiols in redox regulation. *Biochemical Society Transactions* 33:1378-1381.

Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 48:909-930.

Gonai T, Kawahara S, Tougou M, Satoh S, Hashiba T, Hirai N, Kawaide H, Kamiya Y, Yoshioka T. 2004. Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. *J. Exp. Bot.* 55:111-118.

Gordon AG. 1971. The germination resistance test - a new test for measuring germination quality of cereals. *Can. J. Plant Sci.* 51:181-183.

Görg A, Obermaier C, Boguth G, Harder A, Scheibe B, Wildgruber R, Weiss W. 2000. The current state of two-dimensional electrophoresis with immobilised pH gradients. *Electrophoresis* 21:1037-1053.

Grappin P, Bouinot D, Sotta B, Miginiac E, Jullien M. 2000. Control of seed dormancy in Nicotiana plumbaginifolia: post-imbibition abscisic acid synthesis imposes dormancy maintenance. *Planta* 210:279-285.

Griffin NM, Yu J, Long F, Oh P, Shore S, Li Y, Koziol JA, Schnitzer JE. 2010. Label-free, normalized quantification of complex mass spectrometry data for proteomic analysis. *Nat. Biotechnol.* 28(1):83-89.

Gubler F, Chandler PM, White RG, Llewellyn DJ, Jacobsen JV. 2002. Gibberellin signaling in barley aleurone cells. Control of SLN1 and GAMYB expression. *Plant Physiol.* 129:191-200.

Gubler F, Hughes T, Waterhouse P, Jacobsen J. 2008. Regulation of dormancy in barley by blue light and after-ripening: effects on abscisic acid and gibberellin metabolism. *Plant Physiol.* 147:886-896.

Gubler F, Millar AA, Jacobsen JV. 2005. Dormancy release, ABA and pre-harvest sprouting. *Cur. Opin. Plant Biol.* 8:183-187.

Guo H, Yin J, Ren J, Wang Z, Chen H. 2007. Changes in proteins within germinating seeds of transgenic wheat with an antisense construct directed against the thioredoxin. *Mol. Biol.* 33:18-24.

Gustafsson JS, Robert C, Glasbey CA, Blomberg A, Rudemo M. 2004. Statistical exploration of variation in quantitative two-dimensional gel electrophoresis data, *Proteomics* 4:3791-3799.

Gutierrez L, Van Wuytswinkel O, Castelain M, Bellini C. 2007. Combined networks regulating seed maturation. *Trends Plant Sci.* 12:294-300.

Hägglund P, Bunkenborg J, Maeda K, Svensson B. 2008. Identification of thioredoxin disulfide targets using a quantitative proteomics approach based on isotope-coded affinity tags. *J. Proteome Res.* 7:5270-5276.

Hajheidari M, Eivazi A, Buchanan BB, Wong JH, Majidi I, Salekdeh GH. 2007. Proteomics uncovers a role for redox in drought tolerance in wheat. *J. Proteome Res.* 6:1451-1460.

Haslekås C, Viken MK, Grini PE, Nygaard V, Nordgard SH, Meza TJ, Aalen RB. 2003. Seed 1-cysteine peroxiredoxin antioxidants are not involved in dormancy, but contribute to inhibition of germination during stress. *Plant Physiol.* 133:1148-1157.

Heo J, Campbell SL. 2006. Ras regulation by reactive oxygen and nitrogen species. *Biochemistry* 45:2200-2210.

Heo J. 2008. Redox regulation of Ran GTPase. *Biochem. Biophys. Res. Commun.* 376:568-572.

Hickey LT, Dieters MJ, DeLacy IH, Christopher MJ, Kravchuk OY, Banks PM. 2010. Screening for grain dormancy in segregating generations of dormant × non-dormant crosses in white-grained wheat (*Triticum aestivum* L.). *Euphytica* 172:183-195.

Hochgrafe F, Mostertz J, Albrecht D, Hecker M. 2005. Fluorescence thiol modification assay: oxidatively modified proteins in Bacillus subtilis. *Mol. Microbiol.* 58:409-425.

Hogg PJ. 2003. Disulfide bonds as switches for protein function. *Trends Biochem. Sci.* 28:210-214.

Holdsworth MJ, Bentsink L, Soppe WJJ. 2008. Molecular networks regulating *Arabidopsis* seed maturation, afterripening, dormancy and germination. *New Phytol.*179: 33-54.

Howitt CA, Pogson BJ. 2006. Carotenoid accumulation and function in seeds and non-green tissues. *Plant Cell and Environment* 29:435-445.

Jacob C, Ba LA. 2011. Open Season for Hunting and Trapping Post-translational Cysteine Modifications in Proteins and Enzymes. *Chem. Bio. Chem.* 12(6):841-844.

James MG, Denyer K, Myers AM. 2003. Starch synthesis in the cereal endosperm. *Cur. Opin. Plant Biol.* 6: 215-222.

Jeong J, Jung Y, Na S, Jeong J, Lee E, Kim MS, Choi S, Shin DH, Paek E, Lee HY, Lee KJ. 2011. Novel Oxidative Modifications in Redox-Active Cysteine Residues. *Mol. Cell. Proteomics* 10, M110.000513.

Job C, Rajjou L, Lovigny Y, Belghazi M, Job D. 2005. Patterns of protein oxidation in *Arabidopsis* seeds and during germination. *Plant Physiol.* 138:790-802.

Johansen KS, Svendsen I, Rasmussen SK. 2000. Purification and cloning of the two domain glyoxalase I from wheat bran. *Plant Sci.* 155:11-20.

Karssen CM, Brinkhorst-van der Swan DLC, Breekland AE, Koornneef M. 1983. Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotype of *Arabidopsis thaliana* L. Heynh. *Planta* 157:158-65.

Karssen CM, Laçka E. 1986. A revision of the hormone balance theory of seed dormancy: studies on gibberellin and/or abscisic acid-deficient mutants of Arabidopsis thaliana. In: M Bopp (ed). *Plant growth substances*. Springer Berlin, Heidelberg, 315-323.

Kato K, Nakamura W, Tabiki T, Miura H, Sawada S. 2001. Detection of loci controlling seed dormancy on group 4 chromosomes of wheat and comparative mapping with rice and barley genomes. *Theor. Appl. Genet.* 102:980-985.

Kim HS, Kim YS, Hahn KW, Joung H, Jeon JH. 2009. Reactive Oxygen Species: Regulation of Plant Growth and Development. *Adv. Bot. Res.* 52:22-25.

King RW. 1993. Manipulation of grain dormancy in wheat. *J. Exp. Bot.* 44:1059-1066.

Knox RE, DePauw RM, McCaig TN, Clarke JM, McLeod JG, Fernandez MR. 1995. AC Karma white spring wheat. *Can. J. Plant Sci.* 75:899-901.

Kobrehel K, Wong JH, Balogh A, Kiss F, Yee BC, Buchanan BB. 1992. Specific reduction of wheat storage proteins by thioredoxin *h*. *Plant Physiol.* 99:919-924.

Kobrehel K, Yee BC, Buchanan BB. 1991. Role of the NADP/thioredoxin system in the reduction of alpha-amylase and trypsin inhibitor proteins. *J. Biol. Chem.* 266:16135-16140.

Koornneef M, Bentsink L, Hilhorst H. 2002. Seed dormancy and germination. *Cur. Opin. Plant Biol.* 5:33-36.

Kranner I, Farida V. Minibayeva FV, Beckett RP, Seal CE. 2010. What is stress? Concepts, definitions and applications in seed science. *New Phytologist* 188(3): 655-673.

Kucera B, Cohn MA, Leubner-Metzger G. 2005. Plant hormone interactions during seed dormancy release and germination. *Seed Sci. Res.* 15:281-307.

Kuhn J, Schroeder J. 2003. Impacts of altered RNA metabolism on abscisic acid signalling. *Curr. Opin. Plant Biol.* 6:463-469.

Kurek I, Pirkl F, Fischer E, Buchner J, Breiman A. 2002. Wheat FKBP73 functions in vitro as a molecular chaperone independently of its peptidyl prolyl cis-trans isomerase activity. *Planta* 215:119-126.

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S. 2004. The Arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. *EMBO J.* 23:1647-56.

Kwak JM, Nguyen V, Schroeder JI. 2006. The role of reactive oxygen species in hormonal responses. *Plant Physiol.* 141:323-329.

Lee DH, Kim YS, Lee CB. 2001. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *J. Plant Phyiol.* 158:737-45.

Leonard SE, Carroll KS. 2011. Chemical 'omics' approaches for understanding protein cysteine oxidation in biology. *Cur. Opi. Chem. Biol.* 15:88-102.

Leprince O, Hendry GAF, McKersie BD. 1993. The mechanisms of desiccation tolerance in developing seeds. *Seed Sci. Res.* 3:231-246.

Leymarie J, Robayo-Romero ME, Gendreau E, Benech-Arnold RL, Corbineau F. 2008. Involvement of aba in induction of secondary dormancy in barley (*Hordeum vulgare* L.) Seeds. *Plant Cell Physiol.* 49(12):1830-1838.

Li YC, Ren JP, Cho MJ, Zhou SM, Kim YB, Guo HX, Wong JH, Niu HB, Kim HK, Morigasaki S, Lemaux PG, Frick OL, Y J, Buchanan BB. 2009. The level of expression of thioredoxin is linked to fundamental properties and applications of wheat seeds. *Mol. Plant* 2(3):430-441.

Lovegrove A, Hooley R. 2000. Gibberellin and abscisic acid signalling in aleurone. *Trends Plant Sci.* 5:1360-1385.

Lozano RM, Wong JH, Yee BC, Peters A, Kobrehel K, Buchanan BB. 1996. New evidence for a role for thioredoxin *h* in germination and seedling development. *Planta* 200:100-106.

Ma L, Yang C, Zeng D, Cai J, Li X, Ji Z, Xia Y, Qian Q, Bao J. 2009. Mapping QTLs for heading synchrony in a doubled haploid population of rice in two environments. *J. Genet Genomics* 36:297-304.

Maeda K, Finnie C, Svensson B. 2005. Identification of thioredoxin *h*-reducible disulphides in proteomes by differential labelling of cysteines: insight into recognition and regulation of proteins in barley seeds by thioredoxin *h*. *Proteomics* 5:1634-1644.

Majoul T, Bancel E, Triboi E, Ben Hamida J, Branlard G. 2003. Proteomic analysis of the effect of heat stress on hexaploid wheat grain: characterization of heat-responsive proteins from total endosperm. *Proteomics* 3:175-183.

Mandal S, Mandal RK. 2000. Seed storage proteins and approaches for improvement of their nutritional quality by genetic engineering. *Curr. Sci.* 79:576-589.

Marchand C, Le Maréchal P, Meyer Y, Decottignies P. 2006. Comparative proteomic approaches for the isolation of proteins interacting with thioredoxin. *Proteomics* 6:6528-6537.

Mares DJ, Mrva K, Cheong J, Williams K, Watson B, Storlie E, Sutherland M, Zou Y. 2005. A QTL located on chromosome 4A associated with dormancy in white- and red-grained wheats of diverse origin. *Theor. Appl. Genet.* 111:1357-1364.

Mares DJ. 1993. Preharvest sprouting in wheat. 1. Influence of cultivar, rainfall and temperature during grain ripening. *Australian Journal of Agricultural Research* 44:1249-1272.

McCaig TN, Depauw RM. 1992. Breeding for pre-harvest sprouting tolerance in white seed colour spring wheat. *Crop Sci.* 32:19-23.

McElwain EF,Spiker S. 1992. Molecular and Physiological Analysis of a Heat-Shock Response in Wheat. *Plant Physiol.* 99(4):1455-1460.

Meyer Y, Buchanan BB, Reichheld JP. 2009. Thioredoxins and Glutaredoxins: Unifying Elements in Redox Biology. *Annu. Rev. Genet.* 43:335-67.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. 2004. Reactive oxygen gene network of plants. *Trends Plant Sci.* 9:490-98.

Møller IM, Jensen PE, Hansson A. 2007. Oxidative modifications to cellular components in plants. *Ann. Rev. Plant Biol.* 58:459-481.

Monteiro G, Horta BB, Pimenta DC, Augusto O, Netto LES. 2007. Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin C. *Proc. Natl Acad. Sci.* USA 104:4886-4891.

Montrichard F, Alkhalfioui F, Yano H, Vensel WH, Hurkman WJ, Buchanan BB. 2009. Thioredoxin targets in plants: the first 30 years. *J. Proteomics* 72:452-474.

Mori M, Uchino N, Chono M, Kato K, Miura H. 2005. Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect. *Theor. Appl. Genet.* 110:1315-1323.

Motoyama A, Venable JD, Ruse CI, Yates JR 3rd. 2006. Automated ultra-high-pressure multidimensional protein identification technology (UHP-MudPIT) for improved peptide identification of proteomic samples. *Anal Chem.* 78(14):5109-5118.

Murthy UMN, Sun WQ. 2000. Protein modification by Amadori and Maillard reactions during seed storage: roles of sugar hydrolysis and lipid peroxidation. *J. Exp. Bot.* 51:1221-1228.

Murthy UMN, Kumar PP, Sun WQ. 2003. Mechanisms of seed ageing under different storage conditions for *Vigna radiate* L. Wilczek: lipid peroxidation, sugar hydrolysis,

Maillard reactions and their relationship to glass state transition. *J. Exp. Bot.* 54:1057-1067.

Nambara E, Marion-Poll A. 2003. ABA action and interactions in seeds. *Trends Plant Sci.* 8:213-17.

Noctor G. 2006. Metabolic signalling in defence and stress: the central roles of soluble redox couples. *Plant Cell Environ.* 29:409-425.

Nyachiro JM, Clarke FR, DePauw RM, Knox RE, Armstrong KC. 2002. Temperature effects on seed germination and expression of seed dormancy in wheat. *Euphytica* 126:123-127.

O'Keefe DO. 1994. Quantitative electrophoretic analysis of proteins labeled with monobromobimane. *Anal. Biochem.* 222:86-94.

Oh E, Yamaguchi S, Hu J, Yusuke J, Jung B, Paik I, Lee HS, Sun TP, Kamiya Y, Choi G. 2007. PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds. *Plant Cell* 19:1192-1208.

Oracz K, El-Maarouf Bouteau H, Farrant JM, Cooper K, Belghazi M, Job C, Corbineau F, Bailly C. 2007. ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. *Plant J.* 50:452-465.

Oracz K, El-Maarouf-Bouteau H, Kranner I, Bogatek R, Corbineau F, Bailly C. 2009. The mechanisms involved in seed dormancy alleviation by hydrogen cyanide unravel the role of reactive oxygen species as key factors of cellular signalling during germination. *Plant Physiol.* 150:494-505.

Østergaard H, Rasmussen SK, Roberts TH, Hejgaard J. 2000. Inhibitory serpins from wheat grain with reactive centers resembling glutamine-rich repeats of prolamin storage proteins. Cloning and characterization of five major molecular forms. *J. Biol. Chem.* 275:33272-33279.

Pawłowski TA. 2009. Proteome analysis of Norway maple (Acer platanoides L.) seeds dormancy breaking and germination: influence of abscisic and gibberellic acids. *BMC Plant Biology* 9:48-60.

Penfield S, King J. 2009. Towards a systems biology approach to understanding seed dormancy and germination. *Biol. Sci.* 276: 3561-3569.

Pukacka S, Ratajczak E. 2007a. Ascorbate and glutathione metabolism during development and desiccation of orthodox and recalcitrant seeds of the genus Acer. *Funct. Plant Biol.* 34:601-613.

Pukacka S, Ratajczak E. 2007b. Age-related biochemical changes during storage of beech (*Fagus sylvatica* L.) seeds. *Seed Sci. Res.* 17: 45-53.

Pulido P, Cazalis R, Cejudo FJ. 2009a. An antioxidant redox system in the nucleus of wheat seed cells suffering oxidative stress. *Plant J.* 57:132-145.

Pulido P, Domînguez F, Cejudo FJ. 2009b. Oxidative stress and thiol-based antioxidants in cereal seeds. *Adv. Bot. Res.* 52:437-460.

Rajjou L, Debeaujon I. 2008. Seed longevity: survival and maintenance of high germination ability of dry seeds. *C. R. Biol.* 331:796-805.

Rhazi L, Cazalis R, Aussenac T. 2003. Sulfhydryl-disulfide changes in storage proteins of developing wheat grain: influence on the SDS-unextractable glutenin polymer formation. *J. Cereal. Sci.* 38:3-13.

Rinalducci S, Murgiano L, Zolla L. 2008. Redox proteomics: basic principles and future perspectives for the detection of protein oxidation in plants. *Journal of Experimental Botany* 59(14):3781-3801.

Sairam R, Srivastava G, Agarwal S, Meena R. 2005. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biol. Plant.* 9:85-91.

Sattler SE, Gilliland LU, Magallanes-Lundback M, Pollard M, DellaPenna D. 2004. Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. *Plant Cell* 16:1419-1432.

Sears ER. 1966. Nullisomic-tetrasomic combinations in wheat. In: R Riley, KR Lewis (eds). *Chromosome Manipulation and Plant Genetics.* Oliver and Boyd, Edinburgh, 29-45.

Serrato AJ, Pérez-Ruiz JM, Cejudo FJ. 2002. Cloning of thioredoxin *h* reductase and characterization of the thioredoxin reductase-thioredoxin *h* system from wheat. *Biochem. J.* 367:491-497.

Setter TL, Carlton G. 2000. The structure and development of the cereal plant. In: WK Anderson, JR Garlinge (eds). *The Wheat Book Principles and Practice.* Agriculture Western Australia, 23-36.

Shahpiri A, Svenson B, Finnie C. 2008. The NADPH-dependent thioredoxin reductase/thioredoxin system in germinating barley seeds: gene expression, protein profiles, and interactions between isoforms of thioredoxin *h* and thioredoxin reductase. *Plant Physiol.* 146:789-799.

Shevchenko A, Loboda A, Ens W, Standing KG. 2000. MALDI quadrupole time-of-flight mass spectrometry: a powerful tool for proteomic research. *Analytical Chemistry* 72:2132-2141.

Shewry PR, Halford NG. 2002. Cereal seed storage proteins: Structures, properties and role in grain utilization. *J. Exp. Bot.* 53:947-958.

Shewry PR. 2009. Wheat. *J. Exp. Bot.* 60(6):1537-1553.

Shimoni Y, Zhu Xz, Levanony H, Segal G, Galili G. 1995. Purification, characterization, and intracellular localization of glycosylated protein disulfide isomerase from wheat grains plant. *Physiology* 108(1):327-334.

Shuey WC. 1960. A wheat sizing technique for predicting flour milling yield. *Cereal Science Today* 5:71.

Shutov AD, Bäumlein H, Blattner FR, Müntz K. 2003. Storage and mobilization as antagonistic functional constraints on seed storage globulin evolution. *J. Exp. Bot.* 54:1645-1654.

Skylas DJ, Cordwell SJ, Hains PG, Larsen MR, Basseal DJ, Walsh BJ, Blumenthal C, Rathmell WG, Copeland L, Wrigley CW. 2002. Heat shock of wheat during grain filling: characterisation of proteins associated with heat-tolerance using a proteome approach. *J. Cereal Sci.* 35:175-188.

Skylas DJ, Dyke DV, Wrigley CW. 2005. Proteomics of wheat grain. *J. Cereal Sci.* 41(2):165-179.

Skylas DJ, Mackintosh JA, Cordwell SJ, Basseal DJ, Walsh BJ, Harry J, Blumenthal C, Copeland L, Wrigley CW, Rathmell W. 2000. Proteome approach to the characterisation of protein composition in the developing and mature wheat-grain endosperm. *J. Cereal Sci.* 32:169-188.

Sokolov L, Dominguez-Solis J, Allary A, Buchanan B, Luan S. 2006. A redox-regulated chloroplast protein phosphatase binds to starch diurnally and functions in its accumulation. *Proc. Natl. Acad. Sci.* USA 103 9732-9737.

Somyong S, Munkvold JD, Tanaka J, Benscher D, Sorrells ME. 2011. Comparative genetic analysis of a wheat seed dormancy QTL with rice and *Brachypodium* identifies candidate genes for ABA perception and calcium signalling. *Funct. Integr. Genomics* doi:10.1007/s10142-011-0219-2

Southan M, MacRitchie F.1999. Molecular weight distribution of wheat proteins. *Cereal Chemi.* 76:827-836.

Spickett CM, Pitt AR, Morrice N, Kolch W. 2006. Proteomic analysis of phosphorylation, oxidation and nitrosylation in signal transduction. *Biochimica. et Biophysica. Acta.* 1764(12):1823-1841.

Stacy RAP, Nordeng TW, Culiáñez-Maciá FA, Aalen R. 1999. The dormancy-related peroxiredoxin anti-oxidant, PER1, is localized to the nucleus of barley embryo and aleurone cells. *Plant J.* 19:1-8.

Steadman KJ, Crawford AD, Gallagher RS. 2003. Dormancy release in Lolium rigidum seeds is a function of thermal after-ripening time and seed water content. *Funct. Plant Biol.* 30:345-352.

Ströher E, Dietz KJ. 2008. The dynamic thiol-disulphide redox proteome of the Arabidopsis thaliana chloroplast as revealed by differential electrophoretic mobility. *Physiologia Plantarum* 133:566-583.

Tammela P, Salo-Väänänen P, Laakso I, Hopia A, Vuorela H, Nygren M. 2005. Tocopherols, tocotrienols and fatty acids as indicators of natural ageing in Pinus sylvestris seeds. *Scandinavian J. Forest Res.* 20:378-384.

Tilley KA, Benjamin RE, Bagorogoza KE, Okot-Kotber BM, Prakash O, Kwen H. 2001. Tyrosine cross-links: molecular basis of gluten structure and function. *J. Agric. Food Chem.* 49:2627-2632.

To A, Valon C, Savino G, Guilleminot J, Devic M, Giraudat J, Parcy F. 2006. A network of local and redundant gene regulation governs *Arabidopsis* seed maturation. *The Plant Cell* 18:1642-1651.

Torada A, Koike M, Ikeguchi S, Tsutsui I. 2008. Mapping of a major locus controlling seed dormancy using backcrossed progenies in wheat (*Triticum aestivum* L.). *Genome* 51:426-432.

Valledor L, Castillejo MA, Lenz C, Rodríguez R, Cañal MJ, Jorrín J. 2008. Proteomic analysis of *Pinus radiata* needles: 2-DE map and protein identification by LC/MS/MS and substitution-tolerant database searching. *J. Proteome Res.* 7:2616-2631.

Vandenabeele S, Van Der Kelen K, Dat J, Gadjev I, Boonefaes T, Morsa S, Rottiers P, Slooten L, Van Montagu M, Zabeau M, et al., 2003. A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco. *Proc. Natl. Acad. Sci.* USA 100:16113-16118.

Veraverbeke WS, Delcour JA. 2002. Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. *Critical Reviews in Food Science and Nutrition* 42:179-208.

Vierstra, RD. 2003. The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. *Trends Plant Sci.* 8:135-142.

von Well E, Fossey A. 1998. A comparative investigation of seed germination, metabolism and seedling growth between two polyploid *Triticum* species. *Euphytica* 101:83-89.

Wahid A, Perveen M, Gelani S, Basra SMA. 2007. Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *J. Plant Physiol.* 164(3):283-94.

Wang G, Wu WW, Zeng W, Chou CL, Shen RF. 2006. Label-free protein quantification using LC-coupled ion trap or FT mass spectrometry: Reproducibility, linearity, and application with complex proteomes. *J. Proteome Res.* 5(5):1214-1223.

Wang R, Bai Y, Tanino K. 2004. Effect of seed size and sub-zero imbibitions-temperature on the thermal time model of winterfat (*Eurotia lanata (Pursh) Moq.*). *Environ. Exp. Bot.* 51:183-187.

WangY, Chiu JF, He QY. 2007. Bioinformatic application in proteomic research on biomarker discovery and drug target validation. *Current Bioinformatics* 2:11-20.

Weiss D, Ori N. 2007. Mechanisms of cross talk between gibberellin and other hormones. *Plant Physiol.* 144:1240-1246.

Wieser H. 2007. Chemistry of gluten proteins. *Food Microbiology* 24:115-119.

Wilkinson MD, McKibbin RS, Bailey PC, Flintham JE, Gale MD, Lenton JR, Holdsworth MJ. 2002. Use of comparative molecular genetics to study pre harvest sprouting in wheat. *Euphytica.* 126:27-33.

Williams KL, Hochstrasser DF. 1997. Introduction to the proteome. In: MR Wilkins, KL Williams, RD Appel, DF Hochstrasser (eds). *Proteome Research: New Frontiers in Functional Genomics.* Springer-Verlag, Berlin, 1-30.

Williams KL.1999. Genomes and proteomes: towards a multidimensional view of biology. *Electrophoresis* 20:678-688.

Wojtyla L, Garnczarska M, Zalewski T, Bednarski W, Ratajczak L, Jurga S. 2006. A comparative study of water distribution, free radical production and activation of antioxidative metabolism in germinating pea seeds. *J. Plant Physiol.* 163:1207-1220.

Wolf C, Hochgräfe F, Kusch H, Albrecht D, Hecker M, Engelmann S. 2008. Proteomic analysis of antioxidant strategies of *Staphylococcus aureus*: diverse responses to different oxidants. *Proteomics* 8:3139-3153.

Wong JH, Balmer Y, Cai N, Tanaka CK, Vensel WH, Hurkman WJ, Buchanan BB. 2003. Unraveling thioredoxin-linked metabolic processes of cereal starchy endosperm using proteomics. *FEBS Lett.* 547:151-156.

Wong JH, Cai N, Balmer Y, Tanaka CK, Vensel WH, Hurkman WJ, Buchanan BB. 2004. Thioredoxin targets of developing wheat seeds identified by complementary proteomic approaches. *Phytochemistry* 65:1629-1640.

Wormuth D, Heiber I, Shaikali J, Kandlbinder A, Baier M, Dietz KJ. 2007. Redox regulation and antioxidative defence in Arabidopsis leaves viewed from a systems biology perspective. *J. Biotechnol.* 129(2):229-48.

Wouters MA, Fan SW, Haworth NL. 2010. Disulfides as redox switches: from molecular mechanisms to functional significance. *Antioxidants and Redox Signalling* 12:53-91.

Wouters MA, Iismaa S, Fan SW, Haworth NL. 2011. Thiol-based redox signalling: Rust never sleeps. *Int. J. Biochem. Cell Biol.* doi:10.1016/j.physletb.2003.10.071

Wu G, Wilen RW, Robertson AJ, Gusta LV. 1999. Isolation, chromosomal localisation, and differential expression of mitochondrial manganese superoxide dismutase and chloroplastic copper/zinc superoxide dismutase genes in wheat. *Plant Physiol.* 120(2):513-520.

Yamaguchi-Shinozaki K, Shinozaki K. 2007. Gene networks involved in drought stress response and tolerance. *J. Exp. Bot.* 58(2):221-227.

Yano H, Kuroda M. 2006. Disulfide proteome yields a detailed understanding of redox regulations: A model study of thioredoxin-linked reactions in seed germination. *Proteomics* 6:294-300.

Yano H, Kuroda S, Buchanan BB. 2002. Disulfide proteome in the analysis of protein function and structure. *Proteomics* 2:1090-96.

Yano H, Wong JH, Cho MJ, Buchanan BB. 2001. Redox changes accompanying the degradation of seed storage proteins in germinating rice. *Plant Cell Physiol.* 42:879-883.

Yano H. 2003. Fluorescent labeling of disulfide proteins on 2D gel for screening allergens: A preliminary study. *Anal. Chem.* 75:4682-4685.

Youn B, Kim SJ, Moinuddin SG, Lee C, Bedgar DL, Harper AR, Davin LB, Lewis NG, Kang C. 2006. Mechanistic and structural studies of apoform, binary, and ternary complexes of the Arabidopsis alkenal double bond reductase At5g16970. *J. Biol. Chem.* 281:40076-40088.

Young TE, Gallie DR. 2000. Regulation of programmed cell death in maize endosperm by abscisic acid. *Plant Mol. Biol.* 42:397-414.

Zhu J, Khan K. 2001. Effects of genotype and environment on glutenin polymers and breadmaking quality. *Cereal Chemi.* 78(2):125-130.

Supplemental Table S1: Raw data for the proteins which displayed systematic expression differences in dormant and non-dormant hybrid genotypes identified by 2DE coupled with LC-MS/MS analysis

Spot No.[a]	Putative Identity[b]	Accession No[c]	MS/MS-MASCOT peptides (m/z, charge state, score)[d]	Cysteine containing peptides (m/z, charge state, score)[e]
1	Phosphoglycerate kinase, cytosolic -18 peptides, 63% cover, score 1231	P12783.1 GI:129916 *Triticum aestivum*	GVTTIIGGGDSVAAVEK, 787.68 (+2), 93 IVPATAIPDGWMGLDVGPDSIK, 1134.31 (+2), 1Ox, 85 LAAALPDGGVLLLENVR, 861.49 (+2), 84 IGVIESLLAK, 521.87 (+2), 80 LASVADLYVNDAFGTAHR, 961.06 (+2), 1Dm, 78	LSELLGLEVVMAPDCIGEEVEK IAA-1223.99 (+2), 1Ox, 103
1	EST CL1Contig602-23 peptides, 39% cover, score 1245 (93% ID to Os02g0621700 [Oryza sativa (japonica cultivar-group)] gi|115447367|ref|NP_001047463.1) Succinyl-CoA ligase, beta-chain, putative	CL1Contig602 – 93	ESGMTLITAEDLDDAAEK, 955.08 (+2), 110 MSLVNEMYFAITLDR, 910.19 (+2), 1Ox, 96 LHGGTPANFLDVGGSASEGQVVEAFK, 863.41 (+3), 95 AILVNIFGGIMK, 646.61 (+2), 1Ox, 78 LNFDDNAAFR, 592.52 (+2), 1Dm, 73	VYLCEK IAA-811.45 (1), 31 LYELFCK IAA-487.18 (+2), 47 TDCTMLEINPLAETADK IAA-969.61 (+2), 1Ox, 91 CDVIASGIVNAAK IAA-659.66 (+2), 93
2	0.19 dimeric alpha-amylase inhibitor [Triticum aestivum]-10 peptides, 95% cover, score 737	AAV39517.1 GI:54778507	DVAAYPDA, 821.40 (+1), 36	SGPWMCYPGYAFK IAA-790.04 (+2), 1Ox, 58 VPALPGCRPVLK IAA-654.22 (+2), 38 LQCNGSQVPEAVLR IAA-786.92 (+2), 2Dm, 80 ECCQQLADISEWCR 3 IAA-928.14 (+2), 86 CGALYSMLDSMYK IAA-770.22 (+2), 85 EHGVQEGQAGTGAFPSCR IAA-944.43 (+2), 115 LTAASITAVCK IAA-567.91 (+2), 82

				LPIVIDASGDGAYVCK IAA-839.37 (+2), 120
3	0.19 dimeric alpha-amylase inhibitor [Triticum aestivum]-9 peptides, 95% cover, score 590	AAV39519.1 GI:54778511	DVAAYPDA, 821.40 (+1), 36	SGPWMCYPGYAFK IAA-790.04 (+2), 1Ox, 58 VPALPGCRPVLK IAA-654.22 (+2), 38 LQCNGSQVPEAVLR IAA-786.92 (+2), 2Dm, 80 ECCQQLADISEWCR 3 IAA-928.14 (+2), 86 CGALYSMLDSMYK IAA-770.22 (+2), 85 EHGVQEGQAGTGAFPSCR IAA-944.43 (+2), 115 LTAASITAVCK IAA-567.91 (+2), 82 LPIVIDASGDGAYVCK IAA-839.37 (+2), 120
4	0.19 dimeric alpha-amylase inhibitor [Triticum aestivum]-10 peptides, 95% cover, score 547	AAV39519.1 GI:54778511	DVAAYPDA, 821.40 (+1), 36	SGPWMCYPGYAFK IAA-790.04 (+2), 1Ox, 58 VPALPGCRPVLK IAA-654.22 (+2), 38 LQCNGSQVPEAVLR IAA-786.92 (+2), 2Dm, 80 ECCQQLADISEWCR 3 IAA-928.14 (+2), 86 CGALYSMLDSMYK IAA-770.22 (+2), 85 EHGVQEGQAGTGAFPSCR IAA-944.43 (+2), 115 LTAASITAVCK IAA-567.91 (+2), 82 LPIVIDASGDGAYVCK IAA-839.37 (+2), 120

5	Dimeric alpha-amylase inhibitor [Triticum aestivum]-3 peptides, 31% cover score 105	AAY42618.1 GI:65993829	none	LQCNGSQVPEAVLR IAA-786.50 (+2), 1Dm, 37 LPIVVDASGDGAYVCK IAA-832.66 (+2), 105
6	Dimeric alpha-amylase inhibitor [Triticum aestivum]-3 peptides, 27% cover, score 102	AAY42618.1 GI:65993829	All contain cysteine	LQCNGSQVPEAVLR IAA-786.71 (+2), 1Dm, 57 EHGVSEGQAGTGAFPSCR IAA-924.78 (+2), 1Dm, 66 LPIVVDASGDGAYVCK IAA-832.23 (+2), 125
7	Endogenous alpha-amylase/subtilisin inhibitor [Triticum aestivum]-15 peptides, 78% cover, score 786	P16347.1 GI:123975	AHGGGLTMAPGHGR, 668.09 (+2), 1Ox, 76 ADANYYVLPANR, 683.84 (+2), 75 YSGAEVHEYK, 592.21 (2), 51 GGAWFLGATEPYHVVVFK, 989.52 (+2), 50 HVITGPVR, 440.49 (+2), 1Dm, 32	CPLFVSQEADGQR IAA-753.99 (+2), 83 AYTTCVQSTEWHIDSELVSGR IAA-1220.01 (+2), 97 LMACGDSCQDLGVFR IAA (C8)-844.85 (+2), 1Ox, 32 2 IAA-864.56 (+2), 126
8	Endogenous alpha-amylase/subtilisin inhibitor [Triticum aestivum]-15 peptides, 80% cover, score 695	P16347.1 GI:123975	AHGGGLTMAPGHGR, 668.31 (+2), 1Ox, 57 GGAWFLGATEPYHVVVFK, 989.44 (+2), 50 ADANYYVLPANR, 684.73 (+2), 1Dm, 49 HVITGPVR, 878.70 (+1), 33 YSGAEVHEYK, 1183.08 (+1), 31	CPLFVSQEADGQR IAA-753.97 (+2), 74 AYTTCVQSTEWHIDSELVSGR IAA-1221.12 (+2), 1Dm, 84 LMACGDSCQDLGVFR IAA (C8)-836.23 (+2), 1Dm, 44 2IAA-873.41 (+2), 1Dm, 1Ox, 91 Bimane-903.03 (+2), 17
9	EST CL1Contig11939-24 peptides, 45% cover, score 1129 (86% ID to Beta-amylase; 1,4-alpha-D-glucan Maltohydrolase [Hordeum vulgare subsp. vulgare] gi|75107132|sp|P82993.1)	CL1Contig11939	SGPELTIEMILQAAQPK, 922.81 (+2), 2Dm, 1Ox, 100 SAVQMYADYMASFR, 820.31 (+2), 94 NIEYLTLGVDDQPLFHGR, 1044.07 (+2), 1Dm, 88 FFVDNGTYLTEQGR, 824.37 (+2), 1Dm, 88 EFLDAGVIVDIEVGLGPAGELR, 1135.36 (+2), 83	LQAIMSFHQCGGNVGDVVNIPIPQWVR IAA-1013.24 (+3), 2Dm, 20 YPSYPQSHGWSFPGIGEFICYDK IAA-1368.42 (+2), 60 HHASLNFTCAEMR IAA-795.53 (+2), 1Ox, 37 EGLNMACENALPR IAA-746.02 (+2), 1Dm, 1Ox, 28 MHANLPHDPCVDPVAPLQR IAA-723.77 (+3), 2Dm, 41

10	EST CL1Contig11939-27 peptides, 49% cover, score 1277 (86% ID to Beta-amylase; 1,4-alpha-D-glucan Maltohydrolase [Hordeum vulgare subsp. vulgare] gi|75107132|sp|P82993.1)	CL1Contig11939	EFLDAGVIVDIEVGLGPAGELR, 1135.23 (+2), 131 SAVQMYADYMASFR, 836.70 (+2), 2Ox, 93 SGPELTIEMILQAAQPK, 922.35 (+2), 1Dm, 1Ox, 87 FFVDNGTYLTEQGR, 824.30 (+2), 1Dm, 87 LSNQLVEGQNYVNFK, 877.95 (+2), 2Dm, 78	LQAIMSFHQCGGNVGDVVNIPIPQWVR IAA-1012.96 (+3), 2Dm, 42 YPSYPQSHGWSFPGIGEFICYDK IAA-912.86 (+3), 1Dm, 49 HHASLNFTCAEMR IAA-795.69 (+2), 1Dm, 1Ox, 42 MHANLPHDPCVDPVAPLQR IAA-1084.39 (+2), 1Dm, 64
11	Beta amylase [Triticum aestivum] -17 peptides, 74% cover, score 917	AAP80614.1 GI:32400764	FFVDNGTYLTEQGR, 824.57 (+2), 1Dm, 98 AAAAMVGHPEWEFPR, 835.00 (+2), 80 FFLAWYSNNLIK, 758.34 (+2), 76 YDPTAYNTILR, 664.36 (+2), 1Dm, 73 EGLNMACENALPR, 745.87 (+2), 1Ox, 69	YPSYPQSHGWSFPGIGEFICYDK IAA-1368.13 (+2), 85 ASLNFTCAEMR IAA-650.64 (+2), 1Dm, 74 EGLNMACENALPR IAA-745.87 (+2), 1Ox, 57
	EST CL1Contig11939-30 peptides, 55% cover, score 1762 (86% ID to Beta-amylase;1,4-alpha-D-glucan Maltohydrolase gi|75107132|sp|P82993.1|AMYB_HORSP)	CL1Contig11939	SAVQMYADYMASFR, 820.58(+2), 108 SGPELTIEMILQAAQPK, 922.20 (+2), 1Dm, 1Ox, 102 NIEYLTLGVDDQPLFHGR, 1044.17 (+2), 101 FFVDNGTYLTEQGR, 824.57 (+2), 1Dm, 98 EFLDAGVIVDIEVGLGPAGELR, 1135.09 (+2), 91	LQAIMSFHQCGGNVGDVVNIPIPQWVR IAA-1012.78 (+3), 1Dm, 22 YPSYPQSHGWSFPGIGEFICYDK IAA-1368.13 (+2), 85 HHASLNFTCAEMR IAA-787.54 (+2), 66 EGLNMACENALPR IAA-745.87 (+2), 1Ox, 69 MHANLPHDPCVDPVAPLQR SH-703.63 (+3), 1Dm, 23 IAA-723.82 (+3), 2Dm, 56
12	Beta amylase [Triticum aestivum] -19 peptides, 71% cover, score 1126	AAP80614.1 GI:32400764	DSEQSSQAMSAPEELVQQVLSAGWR, 1367.77 (+2), 1Dm, 114 VPSHAAEITAGYYNLHDR, 1007.67 (+2), 106 AAAAMVGHPEWEFPR, 835.07 (+2), 95 FFVDNGTYLTEQGR, 824.06 (+2), 94 FFLAWYSNNLIK, 758.67 (+2), 75	YPSYPQSHGWSFPGIGEFICYDK IAA-1368.15 (+2), 82 ASLNFTCAEMR IAA-650.65 (+2), 67 EGLNMACENALPR IAA-746.25 (+2), 1Dm, 1Ox, 74
	EST CL1Contig11939-32 peptides, 58% cover, score 2058 (86% ID to Beta-amylase; 1,4-	CL1Contig11939	EFLDAGVIVDIEVGLGPAGELR, 1135.06 (+2), 135 NIEYLTLGVDDQPLFHGR, 1044.73 (+2), 1Dm, 114 DSEQSSQAMSAPEELVQQVLSAGWR, 1367.77 (+2), 1Dm, 114	LQAIMSFHQCGGNVGDVVNIPIPQWVR IAA-1012.58 (+3), 20 YPSYPQSHGWSFPGIGEFICYDK

	alpha-D-glucan Maltohydrolase gi|75107132|sp|P82993.1|AMYB_HORSP)		LSNQLVEGQNYVNFK, 877.16 (+2), 103 SAVQMYADYMASFR, 828.34 (+2), 1Ox, 101	IAA-1368.15 (+2), 82 HHASLNFTCAEMR IAA-787.28 (+2), 60 EGLNMACENALPR IAA-746.25 (+2), 1Dm, 1Ox, 74 MHANLPHDPCVDPVAPLQR SH-703.52 (+3), 1Dm, 23 IAA-1084.17 (+2), 80
13	Betaine-aldehyde dehydrogenase [Triticum aestivum] – 11 peptides, 35% cover, score 535	AAL05264.1 GI:21747870	QVTEYTSDAPWGWYK, 916.47 (+2), 1Dm, 72 SPIVVFDDVDIDK, 731.68 (+2), 64 VAFTGSYATGQK, 615.76 (+2), 60 ELGEGGIDNYLSIK, 754.78 (+2), 56 EFSTEEEAIELANDTHYGLAGAVISGDR, 999.76 (+3), 1Dm, 55	VAPALAAGCTAVLKPSELASVTCLELGDVCK 3 IAA-1067.91 (+3), 1Dm, 42 VSDPLEEGCR Bimane-648.13 (+2), 17
14	14.5 kDa Heat-shock protein [Triticum monococcum]-6 peptides, 44% cover, score 282	CAM96560.1 GI:186886566	ELPGAYAFVVDMPGLGSGDIK, 1076.77 (+2), 1Ox, 92 FVLPENADMEK, 655.33 (+2), 1Dm, 1Ox, 42 TIQVQVA, 759.01 (+1), 37	none
15	Protein disulfide isomerase 3 precursor [Triticum aestivum]-32 peptides, 66% cover, score 1575	AAK49425.1 GI:13925728	FLIGDIEASQGAFQYFGLK, 1053.24 (+2), 1Dm, 118 LAPILDEAAATLQSEEDVVIAK, 1148.65 (+2), 112 VVVADNVHDVVFK, 721.04 (+2), 95 APEDATYLEDGK, 654.97 (+2), 85 EQVEAGQIVAWLK, 736.00 (+2), 80	NVLIEFYAPWCGHCK 2 IAA-947.79 (+2), 61
16	Small Ran-related GTP-binding protein [Triticum aestivum]-7 peptides, 44% cover, score 250	AAL30396.1 GI:19919694	SNYNFEKPFLYLAR, 880.66 (+2), 1Dm, 44 LVIVGDGGTGK, 1015.94 (+1), 37 NLQYYEISAK, 1229.41 (+1), 30	KYEPTIGVEVHPLDFTTNCGK IAA-803.28 (+3), 1Dm, 55 FYCWDTAGQEK Bimane-770.18 (+2), 1Dm, 27 DGYYIHGQCAIIMFDVTSR IAA-1132.47 (+2), 1Dm, 1Ox, 23 VCENIPIVLCGNK 2IAA-759.35 (+2), 1Dm, 47
17	Manganese superoxide dismutase [Triticum aestivum]-14 peptides, 80% cover, score 822	AAB68035.1 GI:1621627	MNAEGAALQGSGWVWLALDK, 1067.20 (+2), 1Ox, 131 GSNLHPLLGIDVWEHAYYLQYK, 1308.56 (+2), 87 LSVETTPNQDPLVTK, 821.29 (+2), 80 GDASAVVHLQSAIK, 698.35 (+2), 80 LGWAIDEDFGSIEK, 790.56 (+2), 74	none

18	Manganese superoxide dismutase [Triticum aestivum]- 15 peptides, 82% cover, score 922	AAB68035.1 GI:1621627	MNAEGAALQGSGWVWLALDK, 1067.20 (+2), 1Ox, 131 GSNLHPLLGIDVWEHAYYLQYK, 1308.56 (+2), 87 LSVETTPNQDPLVTK, 821.29 (+2), 80 GDASAVVHLQSAIK, 698.35 (+2), 80 LGWAIDEDFGSIEK, 790.56 (+2), 74	none
19	Manganese superoxide dismutase [Triticum aestivum] – 14 peptides, 70% cover, score 818	AAB68035.1 GI:1621627	MNAEGAALQGSGWVWLALDK, 1067.76 (+2), 1Dm, 1OX, 115 ALEQLDAAVSK, 573.04 (+2), 83 GSNLHPLLGIDVWEHAYYLQYK, 1309.17 (+2), 1Dm, 71 LGWAIDEDFGSIEK, 1579.92 (+1), 71 GDASAVVHLQSAIK, 698.61 (+2), 68	none
20	High molecular weight glutenin subunit 1Ax1 [Triticum aestivum] -10 peptides, 18% cover, score 609	CAA43331.1 GI :21743	QPGYYSTSPQQLGQGQPR, 997.06 (+2), 1Dm, 82 YYPTSPQQPGQEQQPR, 952.90 (+2), 1Dm, 75 YYPTSSQQPGQLQQLAQGQQGQQPER, 983.67 (+3), 3Dm, 75 GQQGQQSGQGQQLGQGQQGQQPGQK, 857.34 (+3), 3Dm, 71 QYEQQVVVPPK, 658.25 (+2), 64	DVSPECQPVGGGPVAR IAA-812.97 (+2), 69 AQQLAAQLPAMCR IAA-737.56 (+2), 1Ox, 67
21	Glutenin, high molecular weight subunit 12 [Triticum aestivum]-21% cover, score 542	P08488.1 GI:121452	QVVDQQLAGR, 557.81 (+2), 1Dm, 77 SVAVSQVAR, 458.77 (+2), 64 QGSYYPGQASPQQPGQGQQPGK, 1147.67 (+2), 6Dm, 63 QGYDSPYHVSAEQQAASPMVAK, 794.92 (+3), 2Dm, 1Ox, 56 QYEQTVVPPK, 594.82 (+2), 55	ELQESSLEACR IAA-661.82 (+2), 1Dm, 75 AQQPATQLPTVCR IAA-735.63 (+2), 69
22	Glutenin, high molecular weight subunit 12 [Triticum aestivum]-11 peptides, 21% cover, score 506	P08488.1 GI:121452	WQEPGQGQQWYYPTSLQQPGQGQQIGK, 1041.48 (+3), 4Dm, 77 QVVDQQLAGR, 558.30 (+2), 1Dm, 72 QGSYYPGQASPQQPGQGQQPGK, 1144.98 (+2), 1Dm, 55 QGYDSPYHVSAEQQAASPMVAK, 794.93 (+3), 2Dm, 1Ox, 49 QYEQTVVPPK, 595.62 (+2), 1Dm, 48	ELQESSLEACR IAA-1322.95 (+1), 1Dm, 68 AQQPATQLPTVCR IAA-736.05 (+2), 1Dm, 46
23	Glutenin HMW, subunit 1Dx5' -6 peptides, 32% cover, score 304	ADH04662.1 GI: 296280726	GGSFYPGETTPPQQLQQR, 995.98 (+2), 84 IFWGIPALLK, 579.80 (+2), 58 ELQELQER, 1045.84 (+1), 1Dm, 29	ACQQVMDQQLR IAA-697.43 (+2), 1Dm, 1Ox, 79 DISPECHPVVVSPVAGQYEQQIVVPPK IAA-992.14 (+3), 2Dm, 52

24	EST CL1Contig3986-14 peptides, 25% cover, score 619 (99% ID to triticin [Triticum aestivum] gi|171027826| gb|ACB41346.1)	CL1Contig3986	SQAGLTEYFDEENEQFR, 1031.90 (+2), 107 YGQSQSVQGQSQSQK, 821 36 (+2), 2Dm, 78 SSQLHSSQNIFSGFDVR, 955 70 (+2), 1Dm, 76 EPESYPHTQYEEGQSQAK, 1055.19 (+2), 1Dm, 72 LLAEALGTSGK, 1059.87 (+1), 54	(miscleaved) SQAGLTEYFDEENEQFRCTGVFAIR Bimane-1034.41 (+3), 2Dm, 12 YHNTHGLVYIIQGSGFAGLSFPGCPETFQK IAA-1110.48 (+3), 3Dm, 88
25	EST CL1Contig3975-5 peptides, cover 17%, score 106 (98% ID to low molecular weight glutenin subunit B3-2 [Triticum aestivum] gi|269854581|gb|ACZ51338.1)	CL1Contig3975	none	VFLQQQCSPVAMPQSLAR IAA-1039.92 (+2), 1Dm, 1Ox, 47 SQMLQQSSCHVMQQQCCQQLPQIPQQSR Bimane (C16)-1160.91 (+3), 2Dm, 1Ox, 29 3 IAA-1155.67 (+3), 7Dm, 1Ox, 40

[a]Spot number corresponds to 2D gel in Figure 3.2

[b]Putative names of the identified proteins are listed. Protein digests were analyzed by nano-LC-MS/MS and 'Mascot' search of MS/MS spectra using protein (NCBInr) and EST wheat databases followed by homology identification of EST sequences with BLAST. All identifications met statistical confidence criteria according to 'Mascot' and BLAST scoring schemes. Number of matched peptides, sequence coverage and probability based MOWSE scores are given according to Mascot Search Results. When the only match was the EST, the GenBank accession number of protein to which this EST clone has the most similarity and percentage sequence identity are given in parenthesis. GenBank accession numbers of EST sequences corresponding to particular constructed EST Contigs are given in supplementary Table S4.

[c]GenBank accession numbers of matching proteins or ESTs are given.

[d]At least five (if available) matched peptides from MS/MS analysis and the subsequent Mascot database search are given. The peptides identified are indicated along with their m/z values, charge states and MOWSE scores as given by Mascot. Additional common modifications for matching peptides are indicated: Dm stands for Gln and/or Asn deamidation, and Ox for methionine oxidation.

[e]Peptide sequences with Cys sites of modification including free reduced Cys (SH), Cys-bimane (Bimane), and carbamidomethylated Cys (IAA) .

Supplemental Table S2: Raw data for the differentially-labelled proteins with reduced Cys which displayed systematic expression differences in dormant and non-dormant genotypes identified by 2DE coupled with LC-MS/MS analysis

Spot N	Putative Identity[b]	Accession No[c]	MS/MS-MASCOT peptides (m/z, charge state, score)[d]	Cysteine containing peptides (m/z, charge state, score)[e]
26	EST CL1Contig14818-41 peptides, 67% cover, score 2151 (94% ID to Enolase, putative, expressed [Oryza sativa (japonica cultivar-group)] gi|110288667|gb|ABB46862.2)	CL1Contig14818	YGQDATNVGDEGGFAPNIQENK, 1163.35 (+2),2Dm, 110 SGETEDTFIADLAVGLSTGQIK, 1126.50 (+2), 87 IEEELGDAAVYAGLK, 789.34 (+2), 87 QLVLPVPAFNVINGGSHAGNK, 1067.45 (+2), 2Dm, 86 DPTAQTELDNFMVQQLDGTK, 1134.83 (+2), 2Dm, 1Ox, 84	GNPTVEVDVCCSDGTFAR IAA (C11)-963.63 (+2), 76 2 IAA-992.60 (+2), 81 IAA (C11)/Bimane(C10)-1059.62 (+2), 1Dm, 41 NEWGWCK IAA-979.44 (+1), 33 LGANAILAVSLAVCK IAA-750.53 (+2), 83 MTEECGVEVQIVGDDLLVTNPTR IAA-1296.03 (+2), 1Ox, 112 SCNALLLK IAA-918.46 (+1), 25
27	small subunit ADP glucose pyrophosphorylase [Triticum aestivum]-20 peptides, 61% cover, score 1182	AAF61173.1 GI:7340287	IGDNVMIINVDNVQEAAR, 993.87 (+2), 1Ox, 109 HADLNPHANDSVLGIILGGGAGTR, 1178.29 (+2), 108 EQFPGANDFGSEVIPGATSTGMR, 1192.59 (+2), 1Ox, 106 AMMVDTTILGLDDAR, 827.72 (+2), 2Ox, 101 IYVLTQFNSASLNR, 813.39 (+2), 100	LIDIPVSNCLNSNISK IAA-894.28 (+2), 1Dm, 68 Bimane-961.55 (+2), 1Dm, 32 VLDADVTDSVIGEGCVIK IAA-945.51 (+2), 77 Bimane-1012.19 (+2), 58
	EST CL1Contig14818-24 peptides, 50% cover, score 1720 (94% ID to Enolase, putative, expressed [Oryza sativa (japonica cultivar-group)] gi|110288667|gb|ABB46862.2)	CL1Contig14818	DPTAQTELDNFMVQQLDGTK, 1126.19 (+2), 135 YGQDATNVGDEGGFAPNIQENK, 1163.58 (+2), 1Dm, 114 VNQIGSVTESIEAVK, 787.63 (+2), 104 IEEELGDAAVYAGLK, 789.60 (+2), 103 AAVPSGASTGVYEALELR, 896.01 (+2), 97 LAMQEFMILPTGATSFK, 959.06 (+2), 2Ox, 93	GNPTVEVDVCCSDGTFAR 2 IAA-992.56 (+2), 114 SCNALLLK Bimane-526..63 (+2), 42
28	small subunit ADP glucose pyrophosphorylase [Triticum aestivum]-23 peptides, 68% cover, score 1160	AAF61173.1 GI:7340287	IGDNVMIINVDNVQEAAR, 986.41 (+2), 102 HADLNPHANDSVLGIILGGGAGTR, 1178.30 (+2), 1Dm, 99 AMMVDTTILGLDDAR, 827.88 (+2), 2Ox, 96 IYVLTQFNSASLNR, 813.89 (+2), 94 NEGFVEVLAAQQSPDNPDWFQGTADAVR, 1532.07 (+2), 1Dm, 83	VLDADVTDSVIGEGCVIK IAA-945.89 (+2), 79 Bimane-1012.01 (+2), 54
	EST CL1Contig14818- 35 peptides, 64% cover, score 2085 (94% ID to Enolase, putative, expressed [Oryza sativa (japonica cultivar-group)] gi|110288667|gb|ABB46862.2)	CL1Contig14818	YGQDATNVGDEGGFAPNIQENK, 1162.99 (+2), 119 DPTAQTELDNFMVQQLDGTK, 1126.44 (+2), 109 (miscleaved) YGQDATNVGDEGGFAPNIQENKEGLELLK, 1037.18 (+3), 4Dm, 108 SGETEDTFIADLAVGLSTGQIK, 1126.91 (+2), 1Dm, 104 MTEECGVEVQIVGDDLLVTNPTR, 1288.55 (+2), 100	GNPTVEVDVCCSDGTFAR 2 IAA-992.60 (+2), 97 IAA (C11)/Bimane (C10)-1059.10 (+2), 84 2 Bimane-1125.87 (+2), 49 NEWGWCK IAA-979.94 (+1), 35 Bimane-1112.63 (+1), 25

				LGANAILAVSLAVCK Bimane-817.35 (+2), 56 SCNALLLK SH-861.66 (+1), 24 IAA-919.66 (+1), 1Dm, 29 Bimane-1051.17 (+1), 36
29	triosephosphat-isomerase [Triticum aestivum] – 13 peptides, 52% cover, score 713	CAC14917.1 GI:11124572	GGAFTGEVSAEMLVNLGVPWVILGHSER, 981.53 (+3), 1Dm, 1Ox, 91 DWTNVVVAYEPVWAIGTGK, 1053.10 (+2), 78 SLMGESSEFVGEK, 708.33 (+2), 1Ox, 65 VAYALAQGLK, 1033.53 (+1), 51 TNVSPEVAESTR, 1289.62 (+1), 43	LRPEIQVAAQNCWVK SH-878.01 (+2), 62 IAA-907.28 (+2), 1Dm, 84 Bimane-973.50 (+2), 1Dm, 36 VIACVGETLEQR IAA-688.21 (+2), 1Dm, 71 Bimane-754.10 (+2), 49 IIYGGSVTGASCK IAA-1312.69 (+1), 80 Bimane-723.38 (+2), 77
30	triosephosphat-isomerase [Triticum aestivum]- 10 peptides, 51% cover, score 506	CAC14917.1 GI:11124572	VASPAQAQEVHANLR, 796.74 (+2), 1Dm, 81 EAGSTMAVVAEQTK, 719.75 (+2), 1Ox, 61 SLMGESSEFVGEK, 700.76 (+2), 60 TNVSPEVAESTR, 646.34 (+2), 1Dm, 46 DWTNVVVAYEPVWAIGTGK, 1053.58 (+2), 1Dm, 29	LRPEIQVAAQNCWVK IAA-907.34 (+2), 1Dm, 68 VIACVGETLEQR IAA-687.95 (+2), 56 Bimane-755.43 (+2), 1Dm, 38 IIYGGSVTGASCK IAA-656.74 (+2), 29 Bimane-723.70 (+2), 77
31	phosphoglucomutase [Triticum aestivum]-30 peptides, 69% cover, score 1325	CAC85913.1 GI:18076790	YNMGNGGPAPESVTDK, 819.17 (+2), 1Dm, 81 YDYENVDAEAAK, 694.74 (+2), 1Dm, 75 LVTVEDIVLQHWATYGR, 1000.85 (+2), 1Dm, 73 VWVGQDSLLSTPAVSAIIR, 1007.31 (+2), 1Dm, 72 FFVTPSDSVAIIAANAVQSIPYFASGLK, 972.41 (+3), 2Dm, 68	FSFCFDGLHGVAGAYAK IAA-924.26 (+2), 68 Bimane-990.69 (+2), 64 MFVDELGASESSLLNCVPK IAA-1057.24 (+2), 1Dm, 1Ox, 79 Bimane-1123.34 (+2), 1Dm, 1Ox, 79 FFGNLMDAGMCSVCGEESFGTGSDHIR 2 IAA-1000.12 (+3), 1Dm, 1Ox, 56 IAA (C14)/Bimane(C11)-1049.50 (+3), 1Dm, 2Ox, 49 2 Bimane-1088.81 (+3), 1Dm, 1Ox, 47
32	EST CL1Contig13096 – 36 peptides, 55% cover, score 2011 (96% ID to Alanine aminotransferase 2 [Hordeum vulgare] gi|1703227|sp|P52894.1)	CL1Contig13096	NEGLVLLADEVYQENIYADNK, 1205.83 (+2), 108 ALVVINPGNPTGQVLAEENQYDIVK, 1341.12 (+2), 102 DGFPANADDIFLTDGASPGVHLMMQLLIR, 1038.86 (+3), 102 SLGYGEEDLPLVSYQSVSK, 1036.39 (+2), 1Dm, 82 GGYMEITGFSAPVR, 751.10 (+2), 1Ox, 73	CEYAVR Bimane- 930.46 (+1), 19 EVLALCDHPDLLK SH-733.66 (+2), 46 IAA-762.22 (+2), 93 Bimane-828.74 (+2), 46 GYYGECGK Bimane-1066.49 (+1), 17 IASVNLCSNITGQILASLVMNPPK IAA-1278.74 (+2), 1Ox, 126

				LEGITCNK IAA-935.51 (+1), 1Dm, 34 Bimane-1068.55 (+1), 1Dm, 24 AEGAMYLFPQICLPQK IAA-933.76 (+2), 68 Bimane-1008.49 (+2), 1Ox, 33 CTILPQEEK IAA-559.76 (+2), 1Dm, 32
	EST gi|24993241 – 5 peptides, 34% cover, score 337 (80% ID to Phosphofructokinase putative [Oryza sativa] gi|116310015|emb|CAH67040.1)	gi|24993241	HAFFDLSSGSPNPLVAYR, 990.35 (+2), 1Dm, 128 GGFDLNMIVDGIVAR, 797.44 (+2), 1Ox, 81 GFYGADDDHVR, 626.54 (+2), 51	AALVTCGGLCPGLNTVLR 2 IAA-936.73 (+2), 58 IAA (C6)/Bimane(C10)-1003.77 (+2), 1Dm, 39 2 Bimane-1070.26 (+2), 1Dm, 60
33	alcohol dehydrogenase ADH1A [Triticum aestivum]-22 peptides, 69% cover, score 1230	ABL74258.1 GI:119388723	GSTVAIFGLGAVGLAAAEGAR, 944.51 (+2), 133 DHTKPVQQVLAEMTNGGVDR, 738.84 (+3), 3Dm, 1Ox, 97 FITHSVPFSEINK, 760.13 (+2), 69 TDLPNVVEMYMR, 734.61 (+2), 65 IIGVDLNASR, 530.45 (+2), 1Dm, 64	ILFTSLCHTDVYFWEAK IAA-1065.68 (+2), 62 Bimane-1132.45 (+2), 42 SAESNMCDLLR IAA-648.32 (+2), 61 Bimane-715.14 (+2), 49 FSIDGKPIYHFVGTSTFSEYTVMHVGCVAK IAA-1126.50 (+3), 78 VCVLSCGISTGLGASINVAKPPK 2 IAA-1164.88 (+2), 103 IAA (C6)/Bimane (C2)-1232.18 (+2), 1Dm, 62 FGCTEFVNPK IAA-600.30 (+2), 1Dm, 52 Bimane-666.57 (+2), 45
	EST CL1Contig17645-16 peptides, 40% cover, score 726 (95% ID to Formate dehydrogenase, mitochondrial; NAD-dependent formate dehydrogenase; [Hordeum vulgare subsp. vulgare] gi|21263612|sp|Q9ZRI8.1)	CL1Contig17645	FEEDLDAMLPK, 654.62 (+2), 81 IVGVFYQAGEYADK, 781.15 (+2), 1Dm, 73 (miscleaved) GHHYIVTDDKEGLNSELEK, 1092.80 (+2), 71 HIEDMHVLITTPFHPAYVTAER, 865.47 (+3), 1Dm, 1Ox, 57 GEWNVAGIAHR, 605.73 (+2), 57	NPNFVGCVEGALGIR Bimane-868.86 (+2), 68 LKPFNCNLLYHDR IAA-845.71 (+2), 45 GAIMDTQAVADACSSGHIAGYGGDVWFPQPAPK IAA-1131.73 (+3), 2Dm, 1Ox, 68
34	alcohol dehydrogenase ADH1A [Triticum aestivum]-17 peptides, 60% cover, score 893	ABL74258.1 GI:119388723	GSTVAIFGLGAVGLAAAEGAR, 944.78 (+2), 128 DHTKPVQQVLAEMTNGGVDR, 1099.06 (+2), 1Dm, 77 GTFFGNFKPR, 585.98 (+2), 74 AAVAWEAGKPLSMEEVEVAPPQAMEVR, 1457.01 (+2), 1Dm, 1Ox, 73	ILFTSLCHTDVYFWEAK IAA-1065.77 (+2), 23 Bimane-1133.04 (+2), 1Dm, 39 SAESNMCDLLR IAA-1295.84 (+1), 54 Bimane-1444.64 (+1), 1Ox, 17

				FSIDGKPIYHFVGTSTFSEYTVMHVGCVAK IAA-1132.10 (+3), 1Ox, 42 VCVLSCGISTGLGASINVAKPPK 2 IAA-1164.99 (+2), 87 FGCTEFVNPK IAA-600.44 (+2), 1Dm, 22 Bimane-1331.74 (+1), 52
	EST CL1Contig17645- 18 peptides, 47% cover, score 844 (95% ID to Formate dehydrogenase, mitochondrial; NAD-dependent formate dehydrogenase; (Hordeum vulgare subsp. Vulgare) gi|21263612|sp|Q9ZRI8.1)	CL1Contig17645	miscleaved) GHHYIVTDDKEGLNSELEK, 1093.02 (+2), 92 IVGVFYQAGEYADK780.84 (+2), 77 HIEDMHVLITTPFHPAYVTAER, 1297.58 (+2), 1Ox, 70 GEWNVAGIAHR, 605.63 (+2), 67 FEEDLDAMLPK, 662.75 (+2), 1Ox, 65	NPNFVGCVEGALGIR IAA-802.33 (+2), 69 LKPFNCNLLYHDR IAA-845.84 (+2), 52 Bimane-912.70 (+2), 1Dm, 31 CDVIVINTPLTEK IAA-751.90 (+2), 1Dm, 77 Bimane-1634.99 (+1), 60 GAIMDTQAVADACSSGHIAGYGGDVWFPQPAPK IAA-1696.46 (+2), 1Dm, 1Ox, 52
35	glyceraldehyde-3-phosphate dehydrogenase [Triticum aestivum]-24 peptides, 78% cover, score 1396	ABQ81648.1 GI:148508784	GILGYVDEDLVSTDFQGDNR, 1106.90 (+2), 141 FGIVEGLMTTVHAMTATQK, 1018.10 (+2), 97 LVSWYDNEWGYSTR, 888.44 (+2), 94 NPEEIPWAAAGAEYVVESTGVFTDK, 1340.81 (+2), 86 VALQSPDVELVAVNDPFITTDYMTYMFK, 1080.88 (+3), 1Dm, 2Ox, 86	EVAVFGCR IAA-937.53 (+1), 22 DAPMFVCGVNEK IAA-692.04 (+2), 1Ox, 73 SDIDIVSNASCTTNCLAPLAK IAA(C15)-1097.00 (+2), 1Dm, 6 2 IAA-1125.52 (+2), 77 Bimane (C15)-1163.83 (+2), 25 IAA(C15)/Bimane(C11)-1192.39 (+2), 80 2 Bimane-1258.90 (+2), 74
36	glyceraldehyde-3-phosphate dehydrogenase [Triticum aestivum]-26 peptides, 77% cover, score 1497	ABQ81648.1 GI:148508784	FGIVEGLMTTVHAMTATQK, 1018.38 (+2), 155 GILGYVDEDLVSTDFQGDNR, 1107.00 (+2), 131 LVSWYDNEWGYSTR, 888.59 (+2), 100 VPTVDVSVVDLTVR, 749.97 (+2), 97 NPEEIPWAAAGAEYVVESTGVFTDK, 1340.89 (+2), 93	DAPMFVCGVNEK IAA-692.10 (+2), 1Ox, 77 SDIDIVSNASCTTNCLAPLAK IAA (C15)-1098.22 (+2), 1Dm, 18 2 IAA-1126.81 (+2), 1Dm, 38 IAA (C15)/Bimane(C11)-1192.86 (+2), 2Dm, 67 2 Bimane-1259.55 (+2), 1Dm, 90
37	EST CL1Contig6941-30 peptides, 44% cover, score 1734 (90% ID to Pyruvate orthophosphate dikinase 1 [Oryza sativa] gi|75254569|gb|Q6AVA8.1)	CL1Contig6941	MLYEGDWISLNGSTGEVILGK, 1150.26 (+2), 1Dm, 1Ox, 124 IAVDMVNEALVDR, 731.55 (+2), 1Dm, 1Ox, 110 ELCAETGAAEDDVLAR, 860.53 (+2), 106 LYGEFLVNAQGEDVVAGIR, 1025.73 (+2), 102 QIANNVFTNMGK, 677.58 (+2), 1Dm, 1Ox, 98	ELCAETGAAEDDVLAR IAA-861.13 (+2), 1Dm, 92 Bimane-927.55 (+2), 1Dm, 51 VGICGEHGGEPSSVAFFAK IAA-975.53 (+2), 1Dm, 47 Bimane-1042.31 (+2), 1Dm, 55 AGLDYVSCSPFR IAA-686.54 (+2), 64 Bimane-753.18 (+2), 52

38	Phosphoglycerate kinase, cytosolic (Triticum aestivum) – 20 peptides, 61% cover, score 968	P12783.1 GI:129916	(miscleaved) SLVEEDKLELATSLIETAK, 1044.69 (+2), 111 LASVADLYVNDAFGTAHR, 960.68 (+2), 89 LAAALPDGGVLLLENVR, 861.33 (+2), 71 ELDYLVGAVANPK, 1388.79 (+1), 70 IVPATAIPDGWMGLDVGPDSIK, 1126.59 (+2), 56 TFAEALDTTK, 1096.60 (+1), 55	LSELLGLEVVMAPDCIGEEVEK IAA-1224.03 (+2), 1Ox, 65 Bimane- 1290.48 (+2), 1Ox, 51
39	putative fructose 1-,6-biphosphate aldolase [Triticum aestivum] – 13 peptides, 66% cover, score 624	CAD12665.1 GI:18496065	IGATEPSQLSIDQNAQGLAR, 1035.54 (+2), 1Dm, 110 TVPAAVPAIVFLSGGQSEEEATLNLNAMNK, 1536.80 (+2), 1Dm, 101 ALNDQHVLLEGTLLKPNMVTPGSDSK, 1389.73 (+2), 1Dm, 70 ANSEATLGTYK, 1154.90 (+1), 1Dm, 50 KPWNLSFSFGR, 1338.82 (+1), 43	YAIICQENGLVPIVEPEILVDGPHDIDR IAA-1059.01 (+3), 1Dm, 83 Bimane-1104.13 (+3), 2Dm, 78 CAYVTEIVLAACYK 2IAA-1661.87 (+1), 1Dm, 36
40	beta amylase [Triticum aestivum]-16 peptides, 69% cover, score 980	AAP80614.1 GI:32400764	DSEQSSQAMSAPEELVQQVLSAGWR, 1367.36 (+2), 113 VPSHAAEITAGYYNLHDR, 1007.78 (+2), 90 FFVDNGTYLTEQGR, 824.35 (+2), 1Dm, 87 AAAAMVGHPEWEFPR, 835.21 (+2), 73 YDPTAYNTILR, 664.32 (+2), 1Dm, 58	YPSYPQSHGWSFPGIGEFICYDK IAA-912.39 (+3), 93 ASLNFTCAEMR IAA-658.64 (+2), 1Ox, 62 Bimane-717.61 (+2), 1Dm, 49 EGLNMACENALPR SH-709.23 (+2), 29 IAA-738.31 (+2), 1Dm, 64
41	beta amylase [Triticum aestivum]-7 peptides, 37% cover, score 328	AAP80614.1 GI:32400764	FFVDNGTYLTEQGR, 824.77 (+2), 1Dm, 87 FFLAWYSNNLIK, 759.67 (+2), 1Dm, 58 VPSHAAEITAGYYNLHDR, 1008.63 (+2), 1Dm, 53 (miscleaved) AAAAMVGHPEWEFPRDAGQYNDAPQR, 963.30 (+3), 2Dm, 30	YPSYPQSHGWSFPGIGEFICYDK IAA-912.63 (+3), 1Dm, 72 Bimane-956.93 (+3), 1Dm, 61
42	EST gi|49520392-4 peptides, 22% cover, score 97 (91% ID to monomeric alpha-amylase inhibitor [Triticum turgidum subsp. dicoccoides] gi|229615618|gb|ACQ83985.1)	gi|49520392	Low scores	LQCVGSQVPEAVLR SH-749.89 (+2), 57 IAA-778.30 (+2), 69 Bimane-846.29 (+2), 1Dm, 41
43	0.19 dimeric alpha-amylase inhibitor [Triticum aestivum] – 11 peptides, 95% cover, score 692	AAV39519.1 GI:54778511	GVAAYPDA, 763.37 (+1), 33	LPIVIDASGDGAYVCK IAA- 839.54 (+2), 119 Bimane-906.34 (+2), 74 EHGVQEGQAGTGAFPSCR IAA- 944.16 (+2), 107 LQCNGSQVPEAVLR IAA- 786.65 (+2), 1Dm, 87 ECCQQLADISEWCR 3 IAA- 928.62 (+2), 1Dm, 86

				Bimane (C3)/IAA (C13) - 965.45 (+2), 16 CGALYSMLDSMYK IAA – 1538.68 (+1), 75 SGPWMCYPGYAFK IAA – 790.75 (+2), 1Ox, 58 VPALPGCRPVLK IAA – 654.01 (+2), 41 LTAASITAVCK IAA – 1134.64 (+1), 38
44	dimeric alpha-amylase inhibitor [Triticum aestivum] – 11 peptides, 41% cover, score 768	AAY42614.1 GI: 65993731	DVAAYPDA, 821.40 (+1), 48	LQCNGSQVPEAVLR IAA-786.34 (+2), 2Dm, 88 Bimane-852.81 (+2), 1Dm, 81 DCCQQLADISEWCR 2 IAA (C2/C13) - 893.58 (+2), 75 3 IAA- 920.94 (+2), 84 CGALYSMLDSMYK IAA-778.18 (+2), 1Ox, 66 EHGVSEGQAGTGAFPSCR IAA- 923.95 (+2), 104 LTAASITAVCR IAA-581.93 (+2), 84 LPIVVDASGDGAYVCK SH-804.17 (+2), 53 IAA-832.43 (+2), 120 Bimane-899.61 (+2), 1Dm, 90
45	dimeric alpha-amylase inhibitor [Triticum aestivum] – 11 peptides, 41% cover, score 768	AAY42614.1 GI: 65993925	EHGAQEGQAGTGAFPR, 806.75 (+2), 101 DVAAYPDA, 821.34 (+1), 52	LQCNGSQVPEAVLR IAA-786.90 (+2), 2Dm, 87 Bimane-852.88 (+2), 1Dm, 94 DCCQQLAHISEWCR IAA (C13)-583.63 (+3), 46 3 IAA-931.83 (+2), 90 2 IAA (C2,C3)/Bimane (C13)-998.62 (+2), 1Dm, 46 CGALYSMLDSMYK IAA-769.93 (+2), 97 LTAASITAVCR IAA-581.88 (+2), 99 LPIVVDASGDGAYVCK IAA-832.46 (+2), 119 Bimane-889.32 (+2), 88

46	Alpha-amylase/trypsin inhibitor CM3 precursor (Chloroform/methanol-soluble protein CM3) (Triticum aestivum)-9 peptides, 73% cover, score 612	P17314.1 GI:123957	YFIALPVPSQPVDPR, 850.46 (+2), 1Dm, 65 LPEWMTSASIYSPGKPYLAK, 752.50 (+3), 1Ox, 51 EMQWDFVR, 555.67 (+2), 50	DYVLQQTCGTFTPGSK IAA-901.37 (+2), 112 LYCCQELAEISQQCR 3 IAA-979.78 (+2), 91 SGNVGESGLIDLPGCPR IAA-864.40 (+2), 111 Bimane-931.78 (+2), 1Dm, 92 LLVAPGQCNLATIHNVR SH-607.56 (+2), 1Dm, 33 IAA-938.55 (+2), 102 Bimane-670.98 (+3), 2Dm, 14 YCPAVEQPLWI IAA-688.71 (+2), 35
47	EST CL1Contig4351-8 peptides, 23% cover, score 400 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig4351	HMGPGSSIINTTSVNAYK, 947.89 (+2), 1Dm, 1Ox, 87 (miscleaved) GHEDKDAEETLQALR, 857.49 (+2), 1Dm, 85 GNATLLDYTATK, 634.96 (+2), 1Dm, 70 VALVTGGDSGIGR, 1202.08 (+1), 20	ALAGDLGYEENCR IAA-735.31 (+2), 1Dm, 58 VDILVNNAAEQYVRPCITEISEQDLER IAA-1060.76 (+3), 4Dm, 18 Bimane-1104.09 (+3), 2Dm, 65
48	EST CL1Contig4351-24 peptides, 47% cover, score 1310 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig4351	GNATLLDYTATK, 633.99 (+2), 104 HMGPGSSIINTTSVNAYK, 939.32 (+2), 1Dm, 100 VNGVAPGPIWTPLIPASFPEEK, 1160.66 (+2), 1Dm, 84 VALVTGGDSGIGR, 1201.42 (+1), 72 VVEEVANAHGGR, 619.68 (+2), 1Dm, 69	FPPQQQDCQPGK IAA-716.02 (+2), 1Dm, 51 Bimane-782.50 (+2), 1Dm, 48 AVCLCFALEGATVNFTYVK 2 IAA-1083.20 (+2), 1Dm, 62 ALAGDLGYEENCR IAA-734.45 (+2), 80 Bimane-802.34 (+2), 1Dm, 36 VDILVNNAAEQYVRPCITEISEQDLER SH-1040.92 (+3), 2Dm, 66 IAA-1059.21 (+3), 1Dm, 77 Bimane-1103.85 (+3), 2Dm, 78
49	EST CL1Contig3679-20 peptides, 47% cover, score 1086 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig3679	HMGPGSSIINTTSVNAYK, 938.97 (+2), 123 VNGVAPGPIWTPLIPASFPEEK, 1160.47 (+2), 1Dm, 80 GNATLLDYTATK, 1267.89 (+1), 73 TNIFSYFLMAK, 676.73 (+2), 1Dm, 1Ox, 70 VALVTGGDSGIGR, 1202.06 (+1), 64	FPPQQQDCQPGK IAA-706.47 (+2), 2Dm, 29 Bimane-782.68 (+2), 1Dm, 52 AVCLCFALEGATVNFTYVK 2 IAA-1083.13 (+2), 1Dm, 54 ALAGDLGYEENCR IAA-735.28 (+2), 1Dm, 64 VDILVNNAAEQYVRPCITEISEQDLER SH-1041.14 (+3), 2Dm, 40 IAA-1058.92 (+3), 71 Bimane-1103.54 (+3), 1Dm, 100

50	EST CL1Contig3679 – 20 peptides, 48% cover, score 939 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig3679	HMGPGSSIINTTSVNAYK, 947.81 (+2), 2Dm, 1Ox, 102 DAEETLQALR, 1146.70 (+1), 1Dm, 75 VVEEVANAHGGR, 619.12 (+2), 72 GNATLLDYTATK, 634.46 (+2), 71 TNIFSYFLMAK, 668.56 (+2), 1Dm, 65	FPPQQQDCQPGK IAA-715.52 (+2), 1Dm, 39 AVCLCFALEGATVNFTYVK 2 IAA-1082.89 (+2), 1Dm, 40 ALAGDLGYEENCR SH-705.18 (+2), 1Dm, 41 IAA-1467.73 (+1), 51 Bimane-801.17 (+2), 1Dm, 45 VDILVNNAAEQYVRPCITEISEQDLER IAA-1058.57 (+3), 1Dm, 70 Bimane-1103.78 (+3), 3Dm, 71
51	EST CL1Contig3679-7 peptides, 18% cover, score 216 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig3679	(miscleaved) GHEDKDAEETLQALR, 857.05 (+2), 1Dm, 60 HMGPGSSIINTTSVNAYK, 947.65 (+2), 1Dm, 1Ox, 54 VNGVAPGPIWTPLIPASFPEEK, 1161.05 (+2), 1Dm, 44	VDILVNNAAEQYVRPCITEISEQDLER Bimane-1104.26 (+3), 2Dm, 64
52	aspartate aminotransferase [Triticum aestivum] – 21 peptides, 65% cover, score 1127	ABY58643.1 GI:164471780	ALLPFFDSAYQGFASGSLDK, 1068.02 (+2), 1Dm, 105 MFVADGGELLMAQSYAK, 924.81 (+2), 1Dm, 1Ox, 97 QIGMFTFTGLNSDQVAFMR, 1098.75 (+2), 1Dm, 2Ox, 83 LIFGADSPAIQENR, 766.23 (+2), 1Dm, 60 LVIRPMYSNPPLHGASIVATILK, 1254.07 (+2), 1Dm, 1Ox, 57	VATVQCLSGTGSLR IAA-725.17 (+2), 103 Bimane-792.25 (+2), 1Dm, 24 VGALSIVCGSADIAVK IAA-780.56 (+2), 60
	EST CL1Contig9364- 11 peptides, 27% cover, score 536 (99% ID to UDP-D-glucuronate decarboxylase [Hordeum vulgare] gi|50659026|gb|AAT80326.1)	CL1Contig9364	TNVIGTLNMLGLAK, 730.97 (+2), 1Ox, 91 ELINPEVTVTMTENTPDDPR, 1144.23 (+2), 1Ox, 85 NEVIVADNFFTGSK, 771.12 (+2), 63 ILVTGGAGFIGSHLVDK, 842.85 (+2), 49 DGLVLMEDDFR, 655.53 (+2), 46	HDVTEPLLVEVDQIYHLACPASPIFYK IAA-1052.47 (+3), 1Dm, 58 Bimane-1097.18 (+3), 1Dm, 36
53	EST CL1Contig7328-18 peptides, 53% cover, score 928 (89% ID to Malic enzyme, NADP-specific [Oryza sativa] gi|38261493|gb|AAR15892.1)	CL1Contig7328	DIKPTALIGSAGVGQSFTK, 946.65 (+2), 1Dm, 91 AYDLGLASHLPRPK, 769.53 (+2), 80 AIFGSGSPFDPVK, 661.36 (+2), 73 RPIILALSNPTSK, 705.40 (+2), 71 ATHLVFNDDIQGTAAVVLAGLMAGLK, 1321.88 (+2), 1Dm, 1Ox, 66	SECTAEQAYSWSQGR IAA-880.52 (+2), 73 Bimane-947.11 (+2), 89 YAESCMYSPIYR IAA-771.13 (+2), 1Dm, 63
	EST CL1Contig7283- 15 peptides, 49% cover, score 713 (90% ID to Malic enzyme, NADP-specific [Oryza sativa] gi|38261493|gb|AAR15892.1)	CL1Contig7283	SGEEADAAMAGVATGGVEDAYGEDR, 1222.53 (+2), 1Ox, 123 SIQVIVVTDGER, 658.55 (+2), 98 YMFLMDLQER, 682.12 (+2), 1Dm, 1Ox, 54 VLEVLR, 727.51 (+1), 34 GLSFTEK, 781.97 (+1), 31	ATEDQPVTPWAVCIASGHSLLR IAA-1204.89 (+2), 99 Bimane-1271.91 (+2), 1Dm, 74 LMIDNVEELLPVVYTPTVGEACQK IAA-1368.28 (+2), 1Dm, 1Ox, 70 ILGLGDLGCQGMGIPVGK IAA-900.95 (+2), 1Ox, 85

54	EST CL1Contig7328-15 peptides, 52% cover, score 627 (89% ID to Malic enzyme, NADP-specific [Oryza sativa] gi|38261493|gb|AAR15892.1)	CL1Contig7328	VLVQFEDFANHNAFTLLEK, 1118.70 (+2), 1Dm, 72 DIKPTALIGSAGVGQSFTK, 945.55 (+2), 68 LFVPAQANNAYIFPGFGLGVVTSGAIR, 1390.67 (+2), 56 ATHLVFNDDIQGTAAVVLAGLMAGLK, 1321.81 (+2), 1Dm, 1Ox, 56 NIWLVDSK, 974.50 (+1), 43	SECTAEQAYSWSQGR IAA-880.27 (+2), 33 Bimane-947.52 (+2), 1Dm, 12 YAESCMYSPIYR IAA-778.79 (+2), 1Ox, 34
	EST CL1Contig7283-12 peptides, 49% cover, score 431 (90% ID to Malic enzyme, NADP-specific [Oryza sativa] gi|38261493|gb|AAR15892.1)	CL1Contig7283	SGEEADAAMAGVATGGVEDAYGEDR, 1222.48 (+2), 1Ox, 78 GLLPPVVLSQELQEK, 825.84 (+2), 54 YMFLMDLQER, 689.54 (+2), 2Ox, 50	
55	EST CL1Contig17645-23 peptides, 58% cover, score 1204 (95% ID to Formate dehydrogenase, mitochondrial gi|21263612|sp|Q9ZRI8.1|FDH_HORVU)	CL1Contig17645	IVGVFYQAGEYADK, 780.18 (+2), 93 GEWNVAGIAHR, 606.14 (+2), 1Dm, 73 FEEDLDAMLPK, 662.54 (+2), 1Ox, 68 HIEDMHVLITTPFHPAYVTAER, 859.86 (+3), 68 YMPNHAMTPHISGTTIDAQLR, 796.20 (+3), 1Dm, 2Ox, 54	NPNFVGCVEGALGIR IAA-803.12 (+2), 1Dm, 52 Bimane-869.10 (+2), 1Dm, 70 LKPFNCNLLYHDR IAA-845.34 (+2), 63 CDVIVINTPLTEK IAA-751.77 (+2), 83 GAIMDTQAVADACSSGHIAGYGGDVWFPQPAPK IAA-1131.01 (+3), 1Ox, 95
56	formate dehydrogenase [Triticum aestivum]-14 peptides, 43% cover, score 858	AAP80655.1 GI:32400847	IVGVFYQAGEYADK, 780.61 (+2), 103 HIEDMHVLITTPFHPAYVTAER,859.71 (+3), 79 GEWNVAGIAHR, 605.31 (+2), 68 EGLNSELEK, 509.58 (+2), 60 NFLPGYQQVVK, 647.06 (+2), 59	NPNFVGCVEGALGIR IAA-802.12 (+2), 87 Bimane-868.61 (+2), 77
	aspartate aminotransferase [Triticum aestivum]-12 peptides, 42% cover, score 684	ABY58643.1 GI:164471780	ALLPFFDSAYQGFASGSLDK, 1067.40 (+2), 86 LIFGADSPAIQENR, 766.03 (+2), 77 EYLPITGLADFNK, 741.20 (+2), 73 LVIRPMYSNPPLHGASIVATILK, 836.35 (+3), 1Ox, 69 MFVADGGELLMAQSYAK, 932.10 (+2), 2Ox, 60	VATVQCLSGTGSLR IAA-725.16 (+2), 114 Bimane-792.04 (+2), 1Dm, 51 VGALSIVCGSADIAVK IAA-780.70 (+2), 28
57	EST CL1Contig210-39 peptides, 48% cover, score 1561 (94% ID to Isocitrate dehydrogenase [Oryza sativa (japonica cultivar-group)] gi|5007084|ref|AAD37809.1)	CL1Contig210	SDYLNTEEFIDAVAAELK, 1014.74 (+2), 113 GGETSTNSIASIFAWTR, 889.93 (+2), 1Dm, 91 TIEAEAAHGTVTR, 678.59 (+2), 91 SKYEAAGIWYEHR, 805.71 (+2), 87	CATITPDEDR IAA-1176.61 (+1), 22 EPIICK IAA-759.50 (+1), 24 LVPGWTKPICIGR IAA-1496.90 (+1), 15 SEGGYVWACK IAA-578.67 (+2), 41 Bimane-645.37 (+2), 26

				NYDGDVQSDFLAQGFGSLGLMTSVLMCPDGK IAA-1119.49 (+3), 2Dm, 2Ox, 85 LEEACVGTVESGK IAA-1378.67 (+1), 55
58	cytosolic malate dehydrogenase [Triticum aestivum]-19 peptides, 71% cover, score 1417	AAT64932.1 GI:49343245	VLVVANPANTNALILK, 825.42 (+2), 117 MDATAQELSEEK, 684.44 (+2), 1Ox, 105 VLVTGAAGQIGYALVPMIAR, 1000.46 (+2), 103 ELVQDDEWLNGEFIATVQQR, 1196.19 (+2), 1Dm, 92 GVMLGADQPVILHMLDIEFAAEALK, 900.08 (+3), 1Ox, 89	GVVATTDVVEACTGVNVAVMVGGFPR IAA-1311.34 (+2), 1Ox, 105 Bimane-919.05 (+3), 1Ox, 68 AQASALEAHAAPNCK IAA-769.84 (+2), 94 Bimane-836.67 (+2), 81 NISCLTR IAA-432.19 (+2), 35 Bimane-498.82 (+2), 45 LSSALSAASSACDHIR IAA-823.53 (+2), 95 Bimane-889.76 (+2), 105
59	cytosolic malate dehydrogenase [Triticum aestivum]- 16 peptides, 61% cover, score 800	AAT64932.1 GI:49343245	VLVVANPANTNALILK, 825.67 (+2), 83 VLVTGAAGQIGYALVPMIAR, 1008.91 (+2), 1Ox, 73 GVMLGADQPVILHMLDIEFAAEALK, 900.56 (+3), 1Dm, 1Ox, 67 NAIIWGNHSSSQYPDVNHATVK, 813.94 (+3), 1Dm, 64 MELIDAAFPLLK, 681.29 (+2), 61	AQASALEAHAAPNCK IAA-770.26 (+2), 81 Bimane-836.78 (+2), 72 LSSALSAASSACDHIR IAA-823.53 (+2), 86 Bimane-890.09 (+2), 82 KLSSALSAASSACDHIR IAA-887.82 (+2), 75 Bimane-636.66 (+3), 1Dm, 60 ALAYSCLA IAA-868.54 (+1), 21 Bimane-1001.56 (+1), 18
60	EST CL1Contig13096-28 peptides, 52% cover, score 1474 (96% ID to Alanine aminotransferase 2 [Hordeum vulgare] gi|1703227|sp|P52894.1|ALA2_HORVU)	CL1Contig13096	NEGLVLLADEVYQENIYADNK, 1205.99 (+2), 122 DGFPANADDIFLTDGASPGVHLMMQLLIR, 1045.14 (+3), 1Dm, 1Ox, 110 SLGYGEEDLPLVSYQSVSK, 1036.04 (+2), 101 GGYMEITGFSAPVR, 750.54 (+2), 1Ox, 92 ALVVINPGNPTGQVLAEENQYDIVK, 1342.33 (+2), 1Dm, 86	EVLALCDHPDLLK SH-733.31 (+2), 14 IAA-761.93 (+2), 88 Bimane-829.13 (+2), 1Dm, 55 GYYGECGK Bimane-1067.28 (+1), 14 IASVNLCSNITGQILASLVMNPPK IAA-1279.75 (+2), 1Dm, 1Ox, 70 Bimane-898.32 (+3), 2Dm, 1Ox, 48 LEGITCNK IAA-935.08 (+1), 1Dm, 34 AEGAMYLFPQICLPQK IAA-941.72 (+2), 1Ox, 58 Bimane-1008.69 (+2), 1Dm, 1Ox, 36 CTILPQEEK Bimane-1250.95 (+1), 29

61	EST CL1Contig13096-34 peptides, 56% cover, score 2137 (96% ID to Alanine aminotransferase 2 gi|1703227|sp|P52894.1|ALA2_HORVU)	CL1Contig13096	NEGLVLLADEVYQENIYADNK, 1205.72 (+2), 143 ALVVINPGNPTGQVLAEENQYDIVK, 1341.26 (+2), 123 DGFPANADDIFLTDGASPGVHLMMQLLIR, 1050.13 (+3), 1Dm, 2Ox, 116 SLGYGEEDLPLVSYQSVSK, 1036.20 (+2), 112 DAIASGIASR, 480.71 (+2), 89	CEYAVR IAA-399.57 (+2), 43 EVLALCDHPDLLK IAA-762.08 (+2), 77 Bimane-828.33 (+2), 59 GYYGECGK IAA-467.33 (+2), 35 IASVNLCSNITGQILASLVMNPPK IAA-1278.88 (+2), 1Ox, 111 LEGITCNK IAA-467.83 (+2), 43 Bimane-534.51 (+2), 41 AEGAMYLFPQICLPQK IAA-942.12 (+2), 1Dm, 1Ox, 57 Bimane-1008.32 (+2), 1Ox, 37 CTILPQEEK IAA-559.60 (+2), 41 Bimane-626.09 (+2), 26
62	Serpin-Z1A [Triticum aestivum] – 26 peptides, 78% cover, score 1506	Q41593.1 GI:75282265	SAASNAAFSPVSLYSALSLLAAGAGSATR, 1356.54 (+2), 119 AAEVTTQVNSWVEK, 781.83 (+2), 1Dm, 107 VSSVFHQAFVEVNEQGTEAAASTAIK, 907.84 (+3), 1Dm, 101 QFFMYILLPEAPGGLSSLAEK,776.58 (+3), 1Ox, 84 GAWTEQFDSYGTK, 745.37 (+2), 84	FANGVFVDASLLLKPSFQEIAVCK IAA-885.81 (+3), 2Dm, 56 CLGLQLPFGDEADFSEMVDSLMPQGLR IAA-1015.19 (+2), 1Dm, 1Ox, 66 Bimane-1065.07 (+3), 1Dm, 2Ox, 46
	EST CL1Contig18337 – 25 peptides, 63% cover, score 1326 (96% ID to Serpin-Z1A [Triticum aestivum] gi|75282265|sp|Q41593.1)	CL1Contig18337	AAEVTTQVNSWVEK, 781.83 (+2), 1Dm, 107 VSSVFHQAFVEVNEQGTEAAASTAIK, 907.84 (+3), 1Dm, 101 QFFMYILLPEAPGGLSSLAEK, 776.58 (+3), 1Ox, 84 GAWTDQFDSYGTK, 738.70 (+2), 73 AETQSVDFQTK, 627.35 (+2), 70	VAFANGVFVDASLLLKPSFQELAVCK IAA-942.44 (+3), 2Dm, 91 Bimane-987.15 (+3), 1Dm, 33 CLGLQLPFGDEADFSEMVDSLMPQGLR IAA-1015.19 (+3), 1Dm, 1Ox, 66 Bimane-1065.07 (+3), 1Dm, 2Ox, 46
63	Serpin-Z1A[Triticum aestivum]-21 peptides, 74% cover, score 1497	Q41593.1 GI:75282265	VSSVFHQAFVEVNEQGTEAAASTAIK, 907.77 (+3), 1Dm, 134 SAASNAAFSPVSLYSALSLLAAGAGSATR, 1356.67 (+2), 127 AAEVTTQVNSWVEK, 781.79 (+2), 1Dm, 100 GAWTEQFDSYGTK, 745.37 (+2), 89 DQLVATLGTGK, 551.84 (+2), 88	FANGVFVDASLLLKPSFQEIAVCK IAA-1328.09 (+2), 1Dm, 26 CLGLQLPFGDEADFSEMVDSLMPQGLR IAA-1529.85 (+2), 1Dm, 2Ox, 68 Bimane-1065.27 (+3), 2Dm, 2Ox, 12
64	Serpin-Z1B (TriaeZ1b) (WSZ1b) (WZS2) (Triticum aestivum)-18 peptides, 67% cover, score 1208	P93693.1 GI:75279910	SAASNAAFSPVSLHSALSLLAAGAGSATR, 1343.52 (+2), 115 VSSVFHQAFVEVNEQGTEAAASTAIK, 908.32 (+3), 2Dm, 99 (miscleaved) YKAETQSVDFQTK, 1544.82 (+1), 83 AAEVTTQVNSWVEK, 781.88 (+2), 1Dm, 81	VAFANGVFVDASLLLKPSFQELAVCK IAA-941.91 (+3), 1Dm, 99 Bimane-986.75 (+3), 1Dm, 41

			GAWTDQFDSYGTK, 1475.65 (+1), 76	CLGLQLPFSDEADFSEMVDSPMPQGLR IAA-1520.52 (+2), 108
65	Serpin-Z2B [Triticum aestivum]-17 peptides, 55% cover, score 1172	P93692.1 GI:75279909	AFVEVNETGTEAAATTIAK, 961.99 (+2), 139 LSAEPEFLEQHIPR, 833.40 (+2), 106 AAEVTAQVNSWVEK, 766.35 (+2), 106 DILPAGSIDNTTR, 686.57 (+2), 87 ISLGIEASDLLK, 629.87 (+2), 83	VAFANGVFVDASLQLKPSFQELAVCK IAA-947.39 (+3), 1Dm, 104 Bimane-992.33 (+3), 2Dm, 72
66	Serpin-Z1C; TriaeZ1c;WSZ1c [Triticum aestivum] - 20 peptides, 64% cover, score 1239	Q9ST58.1 GI:75313848	VSSVFHQAFVEVNEQGTEAAASTAIK, 908.05 (+3), 2Dm, 109 SAASNAVFSPVSLHVALSLLAAGAGSATR, 1363.65 (+2), 1Dm, 108 ISFETEASDLLK, 676.85 (+2), 88 AAEVATQVNSWVEK, 766.40 (+2), 84 ADTQSVDFQTK, 620.76 (+2), 1Dm, 77	CLGLQLPFSNEADFSEMVDSPMAHGLR IAA-1008.73 (+3), 2Dm, 66 Bimane-1063.39 (+3), 2Dm, 2Ox, 68
67	EST CL1Contig10503-10 peptides, 28% cover, score 516 (98% 20S Proteasome subunit alpha type-7-A Oryza sativa gi|75131289|ref|Q6YT00.1)	CL1Contig10503	QLEEEEIDEYVAEIEAEK, 1084.43 (+2), 1Dm, 88 LTVEDPVTVEYITR, 818.75 (+2), 1Dm, 79 AITVFSPDGHLFQVEYALEAVR, 1232.59 (+2), 1Dm, 58 ALLEVVESGGK, 1102.10 (+1), 53 GVDTVVLGVEK, 1115.88 (+1), 50	IASLDTHIALACAGLK Bimane-894.86 (+2), 1Dm, 49
68	EST CL1Contig9579-34 peptides, 37% cover, score 2060 (96% ID to Cell division control protein 48, Oryza sativa gi|108706222|ref|ABF94017.1)	CL1Contig9579	AVANETGAFFFLINGPEIMSK, 1136.89 (+2), 1Ox, 112 MDIIDLEDETIDAEILNSMAVTNDHFK, 1042.56 (+3), 1Dm, 2Ox, 101 IVSQLLTLMDGLK, 723.97 (+2), 1Ox, 100 TALTTSNPSALR, 616.58 (+2), 96 LDQLIYIPLPDVESR, 886.97 (+2), 1Dm, 94	LGDVVSVHQCPDVK IAA-777.66 (+2), 1Dm, 59 Bimane-843.77 (+2), 24 GVLFYGPPGCGK IAA-627.21 (+2), 1Dm, 51 AIANECQANFISIK IAA-790.09 (+2), 77 Bimane-857.20 (+2), 1Dm, 16 GSAPCVLFFDELDSIATQR IAA-1063.89 (+2), 35 YTQGFSGADITEICQR IAA-923.75 (+2), 77 Bimane-990.08 (+2), 75
69	27K protein [Triticum aestivum]-7 peptides, 53% cover, score 265	BAC76688.1 GI:30793446	DGLLDAANLTLVPYGNAVVR, 1037.08 (+2), 1Dm, 63 GHNLSLEYGR, 573.48 (+2), 53 QTAELVPPHQFVPWVVVDGKPLYNDYGNFK, 1154.34 (+3), 2Dm, 41	VHVAIYYESLCPYSVR Bimane-1046.00 (+2), 1Dm, 52 QGLDPKPVTECYK IAA-768.36 (+2), 28 GYPLLEACR IAA-1078.64 (+1), 23
70	27K protein [Triticum aestivum]-8 peptides, 59% cover, score 373	BAC76688.1 GI:30793446	QTAELVPPHQFVPWVVVDGKPLYNDYGNFK, 1153.57 (+3), 1Dm, 76 GHNLSLEYGR, 573.53 (+2), 52 DGLLDAANLTLVPYGNAVVR, 1037.21 (+2), 1Dm, 36	VHVAIYYESLCPYSVR IAA-979.32 (+2), 1Dm, 53 Bimane-1045.60 (+2), 77 VHLGFIYCVSDLVLK IAA-882.45 (+2), 75 QGLDPKPVTECYK IAA-768.16 (+2), 1Dm, 18

				GYPLLEACR IAA-1078.64 (+1), 30
71	EST CL1Contig17267-11 peptides, 48% cover, score 499 (88% ID to 27k protein [Triticum aestivum] gi|290350670|dbj|BAI78303.1)	CL1Contig17267	DGLLDAADLTLVPYGNAK, 923.64 (+2), 81 GHNLSLEYGR, 573.58 (+2), 54 FVTNHLLK, 486.59 (+2), 45 QTAELVPPHQFVPWVVVDGKPLYNDYGK, 1066.52 (+3), 35	VHVAIYYESLCPYSAR Bimane-1031.15 (+2), 72 VHLGFIYCVSDLVMK IAA-899.84 (+2), 1Ox, 57 EWESCFQK IAA-557.56 (+2), 25 QGLDPKPVTECYK IAA-512.70 (+3), 1Dm, 30 FEAYICK IAA-465.83 (+2), 38 GYPLLEACR IAA-539.78 (+2), 33
72	EST CL1Contig17267- 12 peptides, 49% cover, score 543 (89% ID to 27K protein [Triticum aestivum] gi|30793446|dbj|BAC76688.1)	CL1Contig17267	DGLLDAADLTLVPYGNAK, 923.81 (+2), 104 GHNLSLEYGR, 573.44 (+2), 59 QTAELVPPHQFVPWVVVDGKPLYNDYGK, 1066.93 (+3), 2Dm, 48	VHVAIYYESLCPYSAR IAA-964.67 (+2), 87 Bimane-1030.92 (+2), 86 VHLGFIYCVSDLVMK IAA-891.22 (+2), 75 EWESCFQK, IAA-1114.17 (+1), 29 QGLDPKPVTECYK IAA-768.10 (+2), 41 FEAYICK IAA-930.62 (+1), 26 GYPLLEACR 1078.89 (+1), 28
73	1-Cys peroxiredoxin PER1; Thioredoxin Peroxidase [Triticum aestivum] – 16 peptides, 69% cover, score 813	Q6W8Q2.1 GI:75324900	PGLTIGDTVPNLELDSTHGK, 1032.81 (+2), 89 MFPQGFETADLPSK, 792.50 (+2), 1Ox, 82 VTYPIMADPDR, 647.51 (+2), 1Ox, 54 DIEAYKPGSK, 1107.50 (+1), 47	LLGISCDDVQSHK IAA-736.62 (+2), 86 Bimane-802.95 (+2), 67 LSFLYPSCTGR IAA-651.03 (+2), 42 Bimane-718.01 (+2), 1Dm, 27 VATPANWNPGECVVIAPGVSDDEAK IAA-1298.80 (+2), 66

74	dehydroascorbate reductase [Triticum aestivum]-15 peptides, 85% cover, score 782	AAL71854.1 GI:28192421	WIADSDVITQVIEEK, 873.71 (+2), 1Dm, 95 ALVDELQALEEHLK, 804.71 (+2), 94 AHGPYINGANISAVDLSLAPK, 1055.20 (+2), 1Dm, 86 LYHLQVALEHFK, 1497.83 (+1), 70 YPTPSLVTPAEYASVGSK, 934.22 (+2), 63	AAVGHPDTLGDCPFSQR SH-886.04 (+2), 55 IAA-915.06 (+2), 1Dm, 59 Bimane-981.13 (+2), 60
75	EST CL1Contig4569-19 peptides, 37% cover, score 1016 (90% ID to hypothetical protein OsJ_29519 [Oryza sativa Japonica Group] gi|222641664|gb|EEE69796.1)	CL1Contig4569	ILLGGSAIESEGNFVQPTIVEISPAAPVVR,1532.93 (+2), 1Dm, 104 GAPTTPLITIAMTK, 715.65 (+2), 1Ox, 88 LLLHESIYQTFLDQLVEVYK, 1226.92 (+2), 1Dm, 82 QEHQFLAELGLAPR, 804.96 (+2), 80 GTLLGPLHTTASK, 648.38 (+2), 77	ILPEGIGEVQEIIDMCDYAVGLSR IAA-1348.52 (+2), 1Dm, 1Ox, 57 Bimane-1414.57 (+2), 1Dm, 1Ox, 57 CLLELSGNNAIIVMDDADIPLAVR IAA-1315.27 (+2), 1Dm, 1Ox, 72 Bimane-1382.10 (+2), 1Dm, 1Ox, 41
			ILLGGSAIESEGNFVQPTIVEISPAAPVVR, 1532.93 (+2), 1Dm, 104 EAIEINNSVPQGLSSSIFTR, 1081.67 (+2), 84 LLLHESIYQTFLDQLVEVYK, 1226.92 (+2), 1Dm, 82 GTLLGPLHTTASK, 648.36 (+2), 77 SVLFAAVGTAGQR, 639.71 (+2), 1Dm, 69	CLLELSGNNAIIVMDDADIPLAVR IAA-1315.28 (+2), 1Dm, 1Ox, 72 Bimane-1382.10 (+2), 1Dm, 1Ox, 41 WIGPHGSDCGIVNVNIPTNGAEIGGAFGGEK IAA-1042.33 (+3), 1Dm, 79 Bimane-1087.27 (+3), 2Dm, 80 ATCTINYGSELPLAQGINFG IAA-1064.38 (+2), 1Dm, 57
76	EST CL183Contig1-12 peptide, 40% cover, score 620 (86% ID to Embryo-specific protein [Oryza sativa Japonica Group] gi|4105692|ref|AAD02496.1)	CL183Contig1	GDALPLGLPQIMMALTR, 906.75 (+2), 1Ox, 85 AYMSGPAHGIHPLANAAGK, 940.20 (+2), 1Ox, 73 GGVLFMPGVPGVVER, 765.41 (+2), 1Ox, 63 EVDLPAANTGAR, 1214.25 (+1), 55 TVHFWQVDR, 1188.37 (+1), 44	QVEAHHFCAHLNEDVR IAA-982.56 (+2), 1Dm, 52 Bimane-1049.15 (+2), 1Dm, 30 QCLIFDGPEAGAR IAA-717.28 (+2), 78
77	EST CL183Contig1-14 peptides, 38% cover, score 646 (86% ID to Embryo-specific protein [Oryza sativa Japonica Group] gi|4105692|ref|AAD02496.1)	CL183Contig1	AYMSGPAHGIHPLANAAGK, 940.62 (+2), 1Dm, 1Ox, 82 EVDLPAANTGAR, 1213.64 (+1), 73 GDALPLGLPQIMMALTR, 907.17 (+2), 1Ox, 70 GGMLFMPGVPGVVER, 789.87 (+2), 2Ox, 49 (miscleaved) KFSVSFQK, 970.58 (+1), 48	QVEAHHFCAHLNEDVR IAA-982.45 (+2), 1Dm, 40 Bimane-1048.09 (+2), 1Dm, 46 QCLVFDGPDAGAR IAA-703.45 (+2), 72 Bimane-769.85 (+2), 77 QDLADCVEK Bimane-606.60 (+2), 1Dm, 45
78	EST CL1Contig3675-14 peptides, 18% cover, score 480 (58% ID to hypothetical protein Zea mays gi|228310|ref|1802402A)	CL1Contig3675	AFLQPSHHDADEIAFVR, 977.23 (+2), 1Dm, 66 EGDVIVIPAGSIVYSANTHR, 1050.57 (+2), 1Dm, 57 (miscleaved) GGGGSGSEKEDIQPR, 738.13 (+2), 1Dm, 40 GEGEIYEASEEQIR, 806.13 (+2), 1Dm, 37 EGEGVLVLLR, 1084.99 (+1), 29	ESFCIR IAA-811.82 (+1), 24 FHQITGDQCHHLR Bimane-892.04 (+2), 1Dm, 48
79	globulin 3 [Triticum aestivum]-8 peptides, 16% cover. Score 389	ACJ65514.1 GI:215398470	DQQDEGFVAGPEQQQEHER, 1115.40 (+2), 2Dm, 81 GSAFVVPPGHPVVEIASSR, 953.57 (+2), 72 EVQEVFR, 906.30 (+1), 46	GSSNLQVVCFEINAER SH-884.30 (+2), 1Dm, 56 IAA-912.86 (+2), 2Dm, 94 Bimane-979.26 (+2), 1Dm, 70

80	globulin 3 [Triticum aestivum]-29 peptides, 54% cover, score 1260	ACJ65514.1 GI:215398470	AFVVPGLTDADGVGYVAQGEGVLTVIENGEK, 1035.85 (+3), 1Dm, 101 QGDVIVAPAGSIMHLANTDGR, 1069.63 (+2), 1Dm, 1Ox, 95 VAVANITPGSMTAPYLNTQSFK, 1164.44 (+2), 1Dm, 1Ox, 81 GSAFVVPPGHPVVEIASSR, 953.26 (+2), 74 FQYFSAKPLLASLSK, 850.97 (+2), 1Dm, 67	LAVVLEGEGEVEIVCPHLGR IAA-1088.97 (+2), 62 Bimane-1155.47 (+2), 19 GSSNLQVVCFEINAER IAA-912.14 (+2), 1Dm, 104 Bimane-978.60 (+2), 78
81	globulin 3 [Triticum aestivum] – 9 peptides, 19% cover, score 453	ACJ65514.1 GI:215398470	DQQDEGFVAGPEQQQEHER, 1114.25 (+2), 78 GSAFVVPPGHPVVEIASSR, 953.51 (+2), 72 (miscleaved) GSGSESEEEQDQQRYETVR, 1107.98 (+2), 1Dm, 53 EVQEVFR, 907.58 (+1), 1Dm, 49	GSSNLQVVCFEINAER SH-884.12 (+2), 1Dm, 85 IAA-912.59 (+2), 1Dm, 117 Bimane-978.63 (+2), 1Dm, 82
82	globulin 3 [Triticum aestivum]-6 peptides, 11% cover, score 348	ACJ65514.1 GI:215398470	DQQDEGFVAGPEQQQEHER, 1114.87 (+2), 1Dm, 73 GSAFVVPPGHPVVEIASSR, 953.75 (+2), 39 EVQEVFR, 906.53 (+1), 36	GSSNLQVVCFEINAER IAA-912.12 (+2), 110 Bimane-978.62 (+2), 77
83	EST gi|39568029 – 7 peptides, 23% cover, score 400 (92% ID to globulin 3 [Triticum aestivum] gi|215398470|gb|ACJ65514.1)	gi|39568029	DQQDEGFVAGPEQQQEHER, 1114.42 (+2), 1Dm, 82 GSAFVVPPGHPVVEIASSR, 953.80 (+2), 65 LDDPAQELAFGRPAR, 829.36 (+2), 1Dm, 39 EVQEVFR, 906.69 (+1), 35 VWLAGR, 701.68 (+1), 25	GSSNLQVVCFEINAER IAA-911.88 (+2), 97 Bimane-978.81 (+2), 83

[a]Spot numbers correspond to 2D gels in Figure 4. Proteins were found in different solubility fractions as indicated: the aqueous buffer fraction of whole seed protein extracts (A) followed by the SDS-soluble Fraction (SF), or total SDS-soluble whole seed protein extracts (TS).

[b]Putative names of the identified proteins are listed. Protein digests were analyzed by nano-LC-MS/MS and 'Mascot' search of MS/MS spectra using protein (NCBInr) and EST wheat databases followed by homology identification of EST sequences with BLAST. All identifications met statistical confidence criteria according to 'Mascot' and BLAST scoring schemes. Number of matched peptides, sequence coverage and probability based MOWSE scores are given according to Mascot Search Results. When the only match was the EST, the GenBank accession number of protein to which this EST clone has the most similarity and percentage sequence identity are given in parenthesis. GenBank accession numbers of EST sequences corresponding to particular constructed EST Contigs are given in supplementary Table S4.

[c]GenBank accession numbers of matching proteins or ESTs are given.

[d]At least five (if available) matched peptides from MS/MS analysis and the subsequent Mascot database search are given. The peptides identified are indicated along with their m/z values, charge states and MOWSE scores as given by Mascot. Additional common modifications for matching peptides are indicated: Dm stands for Gln and/or Asn deamidation, and Ox for methionine oxidation.

[e]Peptide sequences with Cys sites of modification including free reduced Cys (SH), Cys-bimane (Bimane), and carbamidomethylated Cys (IAA) .

Supplemental Table S3: Raw data for the redox-sensitive proteins with reduced cysteine residue differences in dormant and non-dormant hybrid genotypes identified by 2DE coupled with LC-MS/MS analysis

Spot No.[a]	Putative Identity[b]	Accession No[c]	MS/MS-MASCOT peptides (m/z, charge state, score)[d]	Cysteine containing peptides (m/z, charge state, score)[e]
84	CL1Contig14818- 36 peptides, 65% cover, score 2430 (94% ID to Enolase, putative, expressed [Oryza sativa (japonica cultivar-group)] gi|110288667|gb|ABB46862.2)	CL1Contig14818	SGETEDTFIADLAVGLSTGQIK, 1126.57 (+2), 115 AAVPSGASTGVYEALELR, 895.97 (+2), 105 QLVLPVPAFNVINGGSHAGNK, 1067.00 (+2), 1Dm, 99 VNQIGSVTESIEAVK, 787.50 (+2), 98	GNPTVEVDVCCSDGTFAR 2 IAA-992.76 (+2), 1Dm, 90 Bimane (C11)/IAA (C10)-1059.14 (+2), 88 NEWGWCK IAA-979.41 (+1), 35 LGANAILAVSLAVCK IAA-750.46 (+2), 114 MTEECGVEVQIVGDDLLVTNPTR IAA-1296.03 (+2), 1Ox, 119 SCNALLLK IAA-919.58 (+1), 1Dm, 25
85		CAZ76054.1 GI: 253783729	GIMGYVEEDLVSTDFVGDSR, 1103.01 (+2), 1Ox, 126 NPEEIPWGEAGADYVVESTGVFTDK, 1356.17 (+2), 1Dm, 95 IINDNFGIIEGLMTTVHAITATQK, 1300.61 (+2), 1Dm, 84 VPTVDVSVVDLTVR, 749.87 (+2), 75 DAPMFVVGVNEDK, 710.66 (+2), 74	2 IAA-1251.18 (+2), 2Dm, 127 IAA (C17)/Bimane (C13)-1317.50 (+2), 2Dm, 82 2 Bimane-1383.32 (+2), 2Dm, 45
86	CL1Contig11939 – 23 peptides, 44% cover, score 1216 (86% ID to Beta-amylase Precursor, 1,4-alpha-D-glucan Maltohydrolase, Hordeum vulgare subsp. Spontaneum gi|75107132|sp|P82993.1)	CL1Contig11939	NIEYLTLGVDDQPLFHGR, 1045.04 (+2), 2Dm, 113 FFVDNGTYLTEQGR, 1647.84 (+1), 1Dm, 95 SGPELTIEMILQAAQPK, 921.83 (+2), 1Ox, 81 SAVQMYADYMASFR, 836.70 (+2), 2Ox, 80 LSNQLVEGQNYVNFK, 877.40 (+2), 1Dm, 75	YPSYPQSHGWSFPGIGEFICYDK IAA-913.15 (+3), 1Dm, 67 Bimane-957.36 (+3), 1Dm, 44 HHASLNFTCAEMR IAA-787.81 (+2), 43 Bimane-854.39 (+2), 1Dm, 35 MHANLPHDPCVDPVAPLQR IAA-1084.44 (+2), 63
87	0.19 dimeric alpha-amylase inhibitor [Triticum aestivum]-15 peptides, 96% cover, score 777	AAV39517.1 GI:54778507	DVAAYPDA, 820.40 (+1), 42	SGPWMCYPGYAFK IAA-1579.77 (+2), 1Ox, 33 VPALPGCRPVLK IAA-654.17 (+2), 44 LQCNGSQVPEAVLR IAA-786.33 (+2), 1Dm, 93 ECCQQLADISEWCR 3 IAA-928.20 (+2), 81 IAA(C13)/Bimane(C3)-965.03 (+2), 1Dm, 15 CGALYSMLDSMYK IAA-1538.64 (+1), 60

				EHGVQEGQAGTGAFPSCR IAA-1888.04 (+1), 87 LTAASITAVCK IAA-568.08 (+2), 37 LPIVIDASGDGAYVCK IAA-839.50 (+2), 100 Bimane-906.69 (+2), 1Dm, 25
88	Alpha-amylase/trypsin inhibitor CM3; Chloroform/methanol-soluble protein CM3, [Triticum aestivum] – 9 peptides, 69% cover, score 598	P17314.1 GI:123957	YFIALPVPSQPVDPR, 850.05 (+2), 71 LPEWMTSASIYSPGKPYLAK, 746.86 (+3), 64 EMQWDFVR, 564.06 (+2), 1Dm, 1Ox, 55	TNLLPHCR IAA-1011.51 (+1), 1Dm, 20 DYVLQQTCGTFTPGSK IAA-901.77 (+2), 1Dm, 112 LYCCQELAEISQQCR 2 IAA (C4, C14)-951.12 (+2), 62 3 IAA- 980.20 (+2), 1Dm, 79 Bimane (C4)/2 IAA (C3, C14)-1045.94 (+2), 11 SGNVGESGLIDLPGCPR IAA-864.66 (+2), 105 LLVAPGQCNLATIHNVR SH-910.67 (+2), 1Dm, 86 IAA-938.36 (+2), 97
89	granule-bound starch synthase precursor [Triticum aestivum] – 29 peptides, 63% cover, score 1373	AAD26156.1 GI:4588609	LTGITGIVNGMDVSEWDPTK, 1067.77 (+2), 1Dm, 111 IYGPDAGTDYEDNQQR, 921.57 (+2), 110 FNAPLAHQMMAGADVLAVTSR, 1100.72 (+2), 90 FLAVNYDITTALEGK, 827.89 (+2), 81 TGGLGDVLGGLPPAMAANGHR, 981.76 (+2), 1Dm, 79	VFVDHPCFLEK IAA-696.77 (+2), 1Dm, 25 FSLLCQAALEVPR IAA-1503.83 (+1), 39 Bimane-819.37 (+2), 43 VAFCIHNISYQGR IAA-783.25 (+2), 51 Bimane-849.71 (+2), 49 GCELDNIMR IAA-1107.56 (+1), 21 FEPCGLIQLQGMR Bimane-849.42 (+2), 1Ox, 35 YGTPCACASTGGLVDTIVEGK 2IAA-1078.80 (+2), 71 IAA (C7)/Bimane (C5)-1145.88 (+2), 1Dm, 23 2 Bimane-1211.89 (+2), 42 LSVDCNVVEPADVK Bimane-839.75 (+2), 41 (miscleaved) LSVDCNVVEPADVKK IAA-836.89 (+2), 63 Bimane-909.90 (+2), 33
90	EST CL1339Contig2-14 peptides, 33% cover, score 692	CL1339Contig2	VLTTFGIFSVNQGR, 770.63 (+2), 1Dm, 84 VIIIDVVIGSGVSR, 713.84 (+2), 80 LLVGDYNQSPIVR, 737.79 (+2), 1Dm, 73	LFLDPSYVTALCGIAEWFTDER IAA-1302.45 (+2), 40

	(56% ID to Putative o-methyltransferase ZRP4,Oryza sativa, AAL31646.1, GI:16924034)		EMQVLYDVFMMR, 805.54 (+2), 3Ox, 72 DEVLLK, 716.50 (+1), 49	TLFEVAHGCTR IAA-646.11 (+2), 46 Bimane-712.46 (+2), 39 WVMCLWQDEDAVK SH-820.53 (+2), 1Dm, 1Ox, 15 IAA-848.68 (+2), 1Dm, 1Ox, 54 Bimane-907.17 (+2), 52
91	EST CL1Contig18963-13 peptides, 36% cover, score 807 (73% ID to Thiamine biosynthetic enzyme [Oryza sativa (japonica cultivar-group)] gi|32352138|ref|BAC78562.1)	CL1Contig18963	TGMEALDTNVSEDAIVGLTR, 1054.95 (+2), 1Dm, 1Ox, 124 SVSPGGSGWLGSQLFSAMVVR, 1070.19 (+2), 1Dm, 1Ox, 96 EVVPGMIVAGIEVAEIDGPQR, 1090.19 (+2), 94 LFNGVVVEDLVVK, 717.08 (+2), 1Dm, 67 HAALFTSTVLSR, 652.09 (+2), 67	IVVSSCGHEGLFSANGK SH-854.16 (+2), 1Dm, 59 IAA-881.73 (+2), 1Dm, 93 Bimane-948.36 (+2), 1Dm, 41 MCPTFGATIISGQK IAA-764.44 (+2), 1Dm, 1Ox, 69 Bimane-830.95 (+2), 1Dm, 1Ox, 68
92	EST CL1Contig17645-19 peptides, 49% cover, score 981 (95% ID to Formate dehydrogenase, mitochondrial; NAD-dependent formate dehydrogenase; (Hordeum vulgare subsp. Vulgare) gi|21263612|sp|Q9ZRI8.1)	CL1Contig17645	(miscleaved) GHHYIVTDDKEGLNSELEK, 1093.04 (+2), 1Dm, 79 IVGVFYQAGEYADK, 781.29 (+2), 1Dm, 75 HIEDMHVLITTPFHPAYVTAER, 1298.01 (+2), 1Dm, 1Ox, 64 GEWNVAGIAHR, 605.75 (+2), 63 YMPNHAMTPHISGTTIDAQLR, 1194.00 (+2), 1Dm, 2Ox, 55	NPNFVGCVEGALGIR IAA-802.15 (+2), 69 Bimane-868.89 (+2), 75 LKPFNCNLLYHDR SH-817.42 (+2), 1Dm, 25 IAA-845.81 (+2), 62 Bimane-912.80 (+2), 1Dm, 23 CDVIVINTPLTEK IAA-1501.84 (+1), 69 Bimane-1634.88 (+1), 74 GAIMDTQAVADACSSGHIAGYGGDVWFPQPAPK IAA-1126.45 (+3), 1Dm, 79
93	cytosolic malate dehydrogenase [Triticum aestivum]-21 peptides, 71% cover, score 1369	AAT64932.1 GI:49343245	VLVVANPANTNALILK, 825.72 (+2), 92 VLVTGAAGQIGYALVPMIAR, 1001.32 (+2), 1Dm, 88 ELVQDDEWLNGEFIATVQQR, 1196.22 (+2), 1Dm, 81 MDATAQELSEEK, 676.13 (+2), 80 NAIIWGNHSSSQYPDVNHATVK, 1219.62 (+2), 78	GVVATTDVVEACTGVNVAVMVGGFPR IAA-1311.20 (+2), 1Ox, 116 AQASALEAHAAPNCK IAA-769.87 (+2), 88 NISCLTR SH-805.67 (+1), 27 IAA-864.14 (+1), 32 LSSALSAASSACDHIR IAA-823.54 (+2), 109 Bimane-889.23 (+2), 14 ALAYSCLA IAA-868.02 (+1), 28 Bimane-1001.30 (+1), 19
94	cytosolic malate dehydrogenase [Triticum aestivum]-12 peptides, 55% cover, score 639	AAT64932.1 GI:49343245	VLVVANPANTNALILK, 826.09 (+2), 1Dm, 83 MDATAQELSEEK, 684.64 (+2), 1Ox, 78 NAIIWGNHSSSQYPDVNHATVK, 813.69 (+3), 72 VLVTGAAGQIGYALVPMIAR, 1008.78 (+2), 1Ox, 71 MELIDAAFPLLK, 689.03 (+2), 1Ox, 68	AQASALEAHAAPNCK IAA-770.27 (+2), 85 Bimane-836.99 (+2), 1Dm, 41 LSSALSAASSACDHIR IAA-823.53 (+2), 95

				Bimane-889.76 (+2), 105 ALAYSCLA IAA-868.38 (+1), 28
	EST CL1Contig4238-15 peptides, 38% cover, score 1069 (93% ID to 60S acidic ribosomal protein P0 [Oryza sativa Indica Group] gi|77552928|gb|ABA95724.1)	CL1Contig4238	FASGVSMVASLSLAISYPTMAAAPHMFLNAYK, 1132.45 (+3), 3Ox, 114 FAVAAPAAAASGGAAAAAPK, 821.15 (+2), 114 NVLAVALETDYSYDHADK, 1012.59 (+2), 110 DFLELSNLLVGNVGLIFTK, 1047.03 (+2), 96 VLIAVADNVGSK, 593.20 (+2), 90	LCQLLEEYTK IAA-649.04 (+2), 62 Bimane-715.59 (+2), 70
95	EST CL1Contig11606 – 18 peptides, 38% cover, score 1016 (84% ID to Serine hydroxymethyltransferase, mitochondrial precursor, putative, expressed [Oryza sativa (japonica cultivar-group)] gi|108862549|gb|ABA97575.2)	CL1Contig11606	TGIELIASENFTSLAVMEALGSPLTNK, 1411.98 (+2), 1Ox, 109 IMGLDLPSGGHLTHGYYTAGGK, 1131.33 (+2), 1Ox, 86 VSSDTGYVDYDR, 688.94 (+2), 82 NAVFGDSSALAPGGVR, 1517.76 (+1), 1Dm, 82 ISATSIYFSSLPYK, 789.31 (+2), 79	YYGGNEVIDEVEELCR IAA-973.73 (+2), 1Dm, 98 Bimane-1039.85 (+2), 84
96	EST CL1Contig13096-33 peptides, 52% cover, score 1776 (96% ID to Alanine aminotransferase 2 (Hordeum vulgare subsp. Vulgare) gi|1703227|sp|P52894.1)	CL1Contig13096	NEGLVLLADEVYQENIYADNK, 1205.99 (+2), 121 ALVVINPGNPTGQVLAEENQYDIVK, 1342.24 (+2), 2Dm, 107 DGFPANADDIFLTDGASPGVHLMMQLLIR, 1050.16 (+3), 1Dm, 2Ox, 103 LLESTGIVVVPGSGFGQVPGTWHFR, 881.33 (+3), 1Dm, 81 SLGYGEEDLPLVSYQSVSK, 1036.52 (+2), 1Dm, 80	CEYAVR Bimane-930.69 (+1), 22 EVLALCDHPDLLK IAA-762.21 (+2), 83 Bimane-828.95 (+2), 1Dm, 42 IASVNLCSNITGQILASLVMNPPK IAA-1278.99 (+2), 1Ox, 108 LEGITCNK IAA-935.65 (+1), 1Dm, 32 Bimane-1067.81 (+1), 24 AEGAMYLFPQICLPQK IAA-933.98 (+2), 1Dm, 72 Bimane-1008.47 (+2), 1Ox, 39 CTILPQEEK IAA-559.53 (+2), 37 Bimane-1250.79 (+1), 29
97	Serpin-Z2A; TriaeZ2a;WSZ2a (Triticum aestivum)-18 peptides, 67% cover, score 1237	Q9ST57.1 GI:75313847	TFVEVNETGTEAAAATIAK, 962.0 (+2), 136 LHALAEQVVQFVLADASYADSPR, 1250.88 (+2), 112 DQLVATLGEGEAER, 744.41 (+2), 93 AAEVTAQVNSWVEK, 766.43 (+2), 92 DILPAGSISNTTR, 1345.66 (+1), 1Dm, 91	VTFANGVFVDASLPLKPSFQELAVCK IAA-947.23 (+3), 2Dm, 88 Bimane-991.57 (+3), 2Dm, 59
	serpin 3 [Triticum aestivum]- 17 peptides, 64% cover, score 1134	ACN59485.1 GI:224589270	VSSVFHQAFVEVNEQGTEAAASTAIK, 907.67 (+3), 1Dm, 100 SAASNAAFSPVSLHSALSLLAAGAGSATR, 1344.18 (+2), 1Dm, 99 AAEVTTQVNSWVEK, 781.80 (+2), 1Dm, 93 GAWTDQFDSYGTK, 738.24 (+2), 81 AETQSVDFQTK, 627.34 (+2), 80	VAFANGVFVDASLLLKPSFQELAVCK IAA-942.22 (+3), 1Dm, 97 Bimane-987.11 (+3), 1Dm, 70 CLGLQLPFSDEADFSEMVDSPMPQGLR SH-1005.76 (+3), 1Dm, 2Ox, 43 IAA-1536.48 (+2), 2Ox, 60

				Bimane-1069.20 (+3), 1Dm, 2Ox, 72
98	Serpin-Z2A (TriaeZ2a) (WSZ2a) (Triticum aestivum) – 14 peptides, 49% cover, score 869	Q9ST57.1 GI:75313847	LHALAEQVVQFVLADASYADSPR, 1250.94 (+2), 117 DQLVATLGEGEAER, 744.90 (+2), 1Dm, 101 TFVEVNETGTEAAAATIAK, 962.42 (+2), 89 AAEVTAQVNSWVEK, 766.94 (+2), 1Dm, 82 DILPAGSISNTTR, 673.29 (+2), 77	VTFANGVFVDASLPLKPSFQELAVCK IAA-946.43 (+3), 1Dm, 81 Bimane-991.02 (+3), 1Dm, 73
99	HSP70 [Triticum aestivum] – 33 peptides, 61% cover, score 1907	AAB99745.1 GI:2827002	IINEPTAAAIAYGLDK, 830.88 (+2), 1Dm, 119 SINPDEAVAYGASVQAAILSGEGNEK, 1295.87 (+2), 111 EQVFSTYSDNQPGVLIQVYEGER, 1330.22 (+2), 1Dm, 100 TTPSYVAFTDTER, 744.37 (+2), 97 NQVAMNPTNTVFDAK, 833.86 (+2), 1Dm, 1Ox, 85	GEGPAIGIDLGTTYSCVGVWQHDR IAA- 863.91 (+3), 1Dm, 62 Bimane-908.13 (+3), 1Dm, 44 ELEGICNPIIAK IAA-679.67 (+2), 1Dm, 50 Bimane-745.69 (+2), 50
100	EST CL1Contig3486-12 peptides, 35% cover, score 583 (92% ID to 2-alkenal reductase [Hordeum vulgare subsp. vulgare] gi|62765876|gb|AAX99161.1)	CL1Contig3486	MSGNDEPSHVPDFVQGEVLTTLGVSK, 920.89 (+3), 1Dm, 1Ox, 88 MEGFIVTDHYGSYR, 838.09 (+2), 68 YVTGFPAEDDMELVPATAR, 1049.48 (+2), 1Ox, 60 FEEEMAGYLK, 608.49 (+2), 38 FGFDDAFNYK, 1223.49 (+1), 34	NLYLSCDPYLR IAA-707.64 (+2), 42 Bimane-774.09 (+2), 1Dm, 35 ISGCYVVGSAGSDEK IAA-764.75 (+2), 73 Bimane-831.75 (+2), 23 VSVCGLISQYNLEQSEGVR IAA-1069.78 (+2), 2Dm, 56 Bimane-1136.71 (+2), 1Dm, 61 NLFCIITK IAA-1008.53 (+1), 19
101	EST CL1Contig5198- 13 peptides, 49% cover, score 873 (92% ID to aldehyde dehydrogenase family 7 member A1 [Zea mays] gi|226531366|ref|NP_001149126.1)	CL1Contig5198	ILLGGSAIESEGNFVQPTIVEISPAAPVVR, 1022.21 (+3), 1Dm, 117 SVLFAAVGTAGQR, 638.87 (+2), 97 LLLHESIYQTFLDQLVEVYK, 1226.60 (+2), 83 EAIEINNSVPQGLSSSIFTR, 1082.62 (+2), 2Dm, 83 GTLLGPLHTTASK, 648.41 (+2), 51	CLLELSGNNAIIVMDDADIPLAVR IAA-1307.35 (+2), 1Dm, 102 WIGPHGSDCGIVNVNIPTNGAEIGGAFGGEK IAA-1042.58 (+3), 2Dm, 63 Bimane-1086.84 (+3), 2Dm, 53 ATCTINYGSELPLAQGINFG IAA-1063.42 (+2), 74
102	EST CL1Contig9410 – 22 peptides, 40% cover, score 1057 (93% ID to globulin 3, Triticum aestivum, gi:215398470 ACJ65514.1)	CL1Contig9410	QGDVIVAPAGSIMHLANTDGR, 1062.02 (+2), 1Dm, 95 VAVANITPGSMTAPYLNTQSFK, 1163.93 (+2), 1Ox, 78 GSAFVVPPGHPVVEIASSR, 953.86 (+2), 72 SFVVPGLTDADGVGYVAQGEGVLTVIENGER, 1575.38 (+2), 1Dm, 71 EASEGGQGHHWPLPPFR, 951.83 (+2), 65	LAVVLEGEGEVQIVCPHLGQDSER IAA – 1318.03 (+2), 45 GSSNLQVVCFEINAER IAA- 912.34 (+2), 80 Bimane- 978.87 (+2), 72
103	EST gi|39568029 – 8 peptides, 28% cover, score 386 (92% ID to globulin 3 [Triticum aestivum] gi|215398470|gb|ACJ65514.1)	gi|39568029	DQQDEGFVAGPEQQQEHER, 114.46 (+2), 1Dm, 81 GSAFVVPPGHPVVEIASSR, 953.54 (+2), 46 LDDPAQELAFGRPAR, 828.50 (+2), 28 EVQEVFR, 906.44 (+1), 35	GSSNLQVVCFEINAER IAA-911.90 (+2), 92 Bimane-979.34 (+2), 1Dm, 25
104	EST gi|39568029 – 7 peptides, 21% cover, score 318	gi|39568029	DQQDEGFVAGPEQQQEHER, 114.46 (+2), 1Dm, 81 GSAFVVPPGHPVVEIASSR, 953.54 (+2), 46 LDDPAQELAFGRPAR, 828.50 (+2), 28	GSSNLQVVCFEINAER IAA-912.73 (+2), 1Dm, 75 Bimane-978.34 (+2), 52

	(92% ID to globulin 3 [Triticum aestivum] gi|215398470|gb|ACJ65514.1)		EVQEVFR, 906.44 (+1), 35	
105	EST CL1Contig9406-18 peptides, 61% cover, score 1039 (84% ID to r40g2 protein [Oryza sativa Indica Group] gi|34394517|gb|BAC83804.1)	CL1Contig9406	LVPFNPAYEDASVLWTESK, 1083.43 (+2), 118 LVPYNPAYQDESVLWTESR, 1134.27 (+2), 93 MVNNIYLNFDAFHGDK, 958.40 (+2), 1Dm, 1Ox, 80 DGEVVLAPVNPK, 1237.79 (+1), 68 DQAPAASGPNQIFK, 723.17 (+2), 1Dm, 61	ASEDYCLAVR IAA-592.77 (+2), 60 Bimane-659.25 (+2), 31 NGTVCLAPTNPR IAA-650.89 (+2), 1Dm, 34 Bimane-717.39 (+2), 1Dm, 29
106	EST CL1097Contig3 – 6 peptides, 23% cover, score 284 (77% ID to Lipoprotein-like protein [Oryza sativa (japonica cultivar-group)] gi|14209547|ref|BAB56043.1)	CL1097Contig3	(miscleaved) KIFESLPAEEQK, 710.14 (+2), 60 LPLGAPALMVSPQGER, 826.83 (+2), 1Dm, 1Ox, 55 DGAVRPDLVR, 549.59 (+2), 49 TGLWANPWVPSMLGK, 837.67 (+2), 1Dm, 1Ox, 39 YSFSTEELK, 1103.51 (+1), 26	LNQDVLQCPVYDSDDK IAA-955.18 (+2), 71 Bimane-1021.73 (+2), 55

[a]Spot number corresponds to 2D gels in Figure 4.

[b]Putative names of the identified proteins are listed. Protein digests were analyzed by nano-LC-MS/MS and 'Mascot' search of MS/MS spectra using protein (NCBInr) and EST wheat databases followed by homology identification of EST sequences with BLAST. All identifications met statistical confidence criteria according to 'Mascot' and BLAST scoring schemes. Number of matched peptides, sequence coverage and probability based MOWSE scores are given according to Mascot Search Results. When the only match was the EST, the GenBank accession number of protein to which this EST clone has the most similarity and percentage sequence identity are given in parenthesis. GenBank accession numbers of EST sequences corresponding to particular constructed EST Contigs are given in supplementary Table S3.

[c]GenBank accession numbers of matching proteins or ESTs are given.

[d]At least five (if available) matched peptides from MS/MS analysis and the subsequent Mascot database search are given. The peptides identified are indicated along with their m/z values, charge states and MOWSE scores as given by Mascot. Additional common modifications for matching peptides are indicated: Dm stands for Gln and/or Asn deamidation, and Ox for methionine oxidation.

[e]Peptide sequences with Cys sites of modification including free reduced Cys (SH), Cys-bimane (Bimane), and carbamidomethylated Cys (IAA) .

APPENDIX IV

Supplemental Table S4: Accession numbers of corresponding EST sequences combined in Contigs

Spot No.[a]	Putative Identity[b]	Contig No	Accession No[c]
1	EST CL1Contig602-23 peptides, 39% cover, score 1245 (93% ID to Succinyl-CoA ligase, beta-chain, *Oryza sativa* gi|115447367)	CL1Contig602	gi|32128985; gb|BT009434.1| gi|50402569; gb|AY622377.1| gi|241990722; dbj|AK330878.1| gi|300681517; emb|FN564434.1| gi|300681419; emb|FN564427.1| gi|194239068; emb|AM932684.1| gi|300681466; emb|FN564430.1|
9	EST CL1Contig11939-24 peptides, 45% cover, score 1129 (86% ID to Beta-amylase; 1,4-alpha-D-glucan Maltohydrolase [Hordeum vulgare subsp. vulgare] gi|75107132|sp|P82993.1)	CL1Contig11939	gi|1771781; emb|X98504.1| gi|4138595; emb|Y16242.1| gi|32400763; gb|AF470353.1| gi|241986995; dbj|AK334256.1| gi|241985242; dbj|AK332502.1| gi|241989148; dbj|AK336127.1| gi|300681509; emb|FN564433.1|
10	EST CL1Contig11939-27 peptides, 49% cover, score 1277 (86% ID to Beta-amylase; 1,4-alpha-D-glucan Maltohydrolase [Hordeum vulgare subsp. vulgare] gi|75107132|sp|P82993.1)	CL1Contig11939	gi|1771781; emb|X98504.1| gi|4138595; emb|Y16242.1| gi|32400763; gb|AF470353.1| gi|241986995; dbj|AK334256.1| gi|241985242; dbj|AK332502.1| gi|241989148; dbj|AK336127.1| gi|300681509; emb|FN564433.1|
12	EST CL1Contig11939-32 peptides, 58% cover, score 2058 (86% ID to Beta-amylase; 1,4-alpha-D-glucan Maltohydrolase gi|75107132|sp|P82993.1|AMYB_HORSP)	CL1Contig11939	gi|1771781; emb|X98504.1| gi|4138595; emb|Y16242.1| gi|32400763; gb|AF470353.1| gi|241986995; dbj|AK334256.1| gi|241985242; dbj|AK332502.1| gi|241989148; dbj|AK336127.1| gi|300681509; emb|FN564433.1|
24	EST CL1Contig3986-14 peptides, 25% cover, score 619 (99% ID to triticin [Triticum aestivum] gi|171027826|gb|ACB41346.1|)	CL1Contig3986	gi|171027812; gb|EU482412.1| gi|386166; gb|S62630.1|S62630 gi|171027825; gb|EU482413.1| gi|171027836; gb|EU482414.1| gi|147744619; gb|EF489470.1| gi|241985493; dbj|AK332754.1| gi|241983669; dbj|AK331611.1| gi|171027862; gb|EU482416.1| gi|215398467; gb|FJ439134.1| gi|241990597; dbj|AK330754.1| gi|300681466; emb|FN564430.1|
25	EST CL1Contig3975-5 peptides, cover 17%, score 106 (98% ID to low molecular weight glutenin subunit B3-2 [Triticum aestivum] gi|269854581|gb|ACZ51338.1)	CL1Contig3975	gi|298160879; dbj|AB567685.1| gi|169666916; gb|EU369705.1| gi|169666914; gb|EU369704.1| gi|56480739; gb|AY831784.1| gi|112790695; gb|DQ822596.1| gi|169666952; gb|EU369723.1| gi|169666950; gb|EU369722.1| gi|169666948; gb|EU369721.1| gi|56480771; gb|AY831800.1| gi|269854580; gb|FJ907551.1| gi|51870701; dbj|AB164416.1| gi|217426776; gb|FJ447463.1|

gi|169666926; gb|EU369710.1|
gi|169666924; gb|EU369709.1|
gi|169666922; gb|EU369708.1|
gi|169666920; gb|EU369707.1|
gi|169666918; gb|EU369706.1|
gi|169666912; gb|EU369703.1|
gi|169666910; gb|EU369702.1|
gi|169666908; gb|EU369701.1|
gi|169666906; gb|EU369700.1|
gi|169666904; gb|EU369699.1|
gi|164470653; gb|EU189088.1|
gi|109240247; dbj|AB262661.1|
gi|44885909; dbj|AB119007.1|
gi|47607141; gb|AY608418.1|
gi|262070689; gb|FJ755316.1|
gi|262070699; gb|FJ755323.1|
gi|17425179; dbj|AB062859.1|
gi|17425187; dbj|AB062863.1|
gi|17425169; dbj|AB062854.1|
gi|298354337; dbj|AB567727.1|
gi|17425177; dbj|AB062858.1|
gi|17425171; dbj|AB062855.1|
gi|17425167; dbj|AB062853.1|
gi|85815963; gb|DQ357057.1|
gi|17425173; dbj|AB062856.1|
gi|4090272; emb|Y17845.1|
gi|17425185; dbj|AB062862.1|
gi|17425189; dbj|AB062864.1|
gi|47607145; gb|AY608420.1|
gi|85815965; gb|DQ357058.1|
gi|17425175; dbj|AB062857.1|
gi|262070676; gb|FJ755309.1|
gi|169666942; gb|EU369718.1|
gi|169666934; gb|EU369714.1|
gi|169666938; gb|EU369716.1|
gi|169666930; gb|EU369712.1|
gi|164470661; gb|EU189092.1|
gi|169666936; gb|EU369715.1|
gi|169666928; gb|EU369711.1|
gi|17425183; dbj|AB062861.1|
gi|164470667; gb|EU189095.1|
gi|269854582; gb|FJ907552.1|
gi|169666932; gb|EU369713.1|
gi|45477538; gb|AY542898.1|
gi|169666940; gb|EU369717.1|
gi|44885907; dbj|AB119006.1|
gi|51870699; dbj|AB164415.1|
gi|260594487; gb|GQ892576.1|
gi|62737749; gb|AY994362.1|
gi|109727209; gb|DQ657349.1|
gi|1857649; gb|U86025.1|TAU86025
gi|260594505; gb|GQ892588.1|
gi|223007037; gb|FJ615310.1|
gi|223007035; gb|FJ615309.1|
gi|123204243; gb|EF190882.1|
gi|85815959; gb|DQ357055.1|
gi|85815957; gb|DQ357054.1|
gi|1857653; gb|U86027.1|TAU86027
gi|21772; emb|X13306.1|
gi|112790692; gb|DQ822594.1|
gi|9967352; dbj|AB007764.2|
gi|123204250; gb|EF190884.1|
gi|78059070; gb|DQ146396.1|
gi|123204247; gb|EF190883.1|
gi|17425181; dbj|AB062860.1|
gi|223007039; gb|FJ615311.1|
gi|262070697; gb|FJ755322.1|
gi|262070687; gb|FJ755315.1|
gi|194718432; gb|EU863824.1|
gi|194718428; gb|EU863822.1|
gi|163860187; gb|EU329426.1|
gi|56480845; gb|AY831837.1|
gi|56480859; gb|AY831844.1|
gi|56480777; gb|AY831803.1|

			gi|56480891; gb|AY831860.1| gi|183229591; gb|EU571726.1| gi|205364155; gb|FJ172533.1| gi|56480827; gb|AY831828.1| gi|56480805; gb|AY831817.1| gi|56480849; gb|AY831839.1| gi|56480821; gb|AY831825.1| gi|56480781; gb|AY831805.1| gi|164470665; gb|EU189094.1| gi|85815961; gb|DQ357056.1| gi|56480889; gb|AY831859.1| gi|56480887; gb|AY831858.1| gi|56480885; gb|AY831857.1| gi|56480883; gb|AY831856.1|
23	EST CL1Contig14818-40 peptides, 66% cover, score 2681 (94% ID to Enolase, putative, expressed [Oryza sativa (japonica cultivar-group)] gi|110288667|gb|ABB46862.2)	CL1Contig14818	gi|241983960; dbj|AK331902.1| gi|241983596; dbj|AK331538.1| gi|241988158; dbj|AK335415.1| gi|300681434; emb|FN564428.1|
27	EST CL1Contig14818-24 peptides, 50% cover, score 1720 (94% ID to Enolase, putative, expressed [Oryza sativa (japonica cultivar-group)] gi|110288667|gb|ABB46862.2)	CL1Contig14818	gi|241983960; dbj|AK331902.1| gi|241983596; dbj|AK331538.1| gi|241988158; dbj|AK335415.1| gi|300681434; emb|FN564428.1|
28	EST CL1Contig14818- 35 peptides, 64% cover, score 2085 (94% ID to Enolase, putative, expressed [*Oryza sativa* (japonica cultivar-group)] gi|110288667|gb|ABB46862.2)	CL1Contig14818	gi|241983960; dbj|AK331902.1| gi|241983596; dbj|AK331538.1| gi|241988158; dbj|AK335415.1| gi|300681434; emb|FN564428.1|
37	EST CL1Contig6941-17 peptides, 32% cover, score 1059 (90% ID to Pyruvate, orthophosphate dikinase 1 [*Oryza sativa* Indica Group] gi|75254569)	CL1Contig6941	gi|241986082; dbj|AK333343.1| gi|32400837; gb|AF475130.1| gi|241983545; dbj|AK331486.1| gi|300681509; emb|FN564433.1|
47	EST CL1Contig4351-8 peptides, 23% cover, score 400 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig4351	gi|241986787; dbj|AK334048.1| gi|241989692; dbj|AK330233.1| gi|241989131; dbj|AK336110.1| gi|241988664; dbj|AK335921.1| gi|112784980; gb|DQ862061.1| gi|241989137; dbj|AK336116.1| gi|241983646; dbj|AK331588.1| gi|241989791; dbj|AK330332.1| gi|241989140; dbj|AK336119.1| gi|241988596; dbj|AK335853.1| gi|241990858; dbj|AK331014.1| gi|241986212; dbj|AK333473.1| gi|241985599; dbj|AK332860.1| gi|241985804; dbj|AK333065.1| gi|300681572; emb|FN645450.1| gi|300681517; emb|FN564434.1| gi|241990840; dbj|AK330996.1| gi|241985125; dbj|AK332385.1| gi|241983246; dbj|AK331186.1|

48	EST CL1Contig4351-24 peptides, 47% cover, score 1310 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig4351	gi|241986787; dbj|AK334048.1| gi|241989692; dbj|AK330233.1| gi|241989131; dbj|AK336110.1| gi|241988664; dbj|AK335921.1| gi|112784980; gb|DQ862061.1| gi|241989137; dbj|AK336116.1| gi|241983646; dbj|AK331588.1| gi|241989791; dbj|AK330332.1| gi|241989140; dbj|AK336119.1| gi|241988596; dbj|AK335853.1| gi|241990858; dbj|AK331014.1| gi|241986212; dbj|AK333473.1| gi|241985599; dbj|AK332860.1| gi|241985804; dbj|AK333065.1| gi|300681572; emb|FN645450.1| gi|300681517; emb|FN564434.1| gi|241990840; dbj|AK330996.1| gi|241985125; dbj|AK332385.1| gi|241983246; dbj|AK331186.1|
49	EST CL1Contig3679-20 peptides, 47% cover, score 1086 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig3679	gi|241986787; dbj|AK334048.1| gi|241989692; dbj|AK330233.1| gi|241989131; dbj|AK336110.1| gi|241988664; dbj|AK335921.1| gi|112784980; gb|DQ862061.1| gi|241989137; dbj|AK336116.1| gi|241983646; dbj|AK331588.1| gi|241989791; dbj|AK330332.1| gi|241989140; dbj|AK336119.1| gi|241988596; dbj|AK335853.1| gi|241990858; dbj|AK331014.1| gi|241986212; dbj|AK333473.1| gi|241985599; dbj|AK332860.1| gi|241985804; dbj|AK333065.1| gi|300681572; emb|FN645450.1| gi|300681517; emb|FN564434.1| gi|241990840; dbj|AK330996.1| gi|241985125; dbj|AK332385.1| gi|241983246; dbj|AK331186.1|
50	EST CL1Contig3679 – 20 peptides, 48% cover, score 939 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig3679	gi|241986787; dbj|AK334048.1| gi|241989692; dbj|AK330233.1| gi|241989131; dbj|AK336110.1| gi|241988664; dbj|AK335921.1| gi|112784980; gb|DQ862061.1| gi|241989137; dbj|AK336116.1| gi|241983646; dbj|AK331588.1| gi|241989791; dbj|AK330332.1| gi|241989140; dbj|AK336119.1| gi|241988596; dbj|AK335853.1| gi|241990858; dbj|AK331014.1| gi|241986212; dbj|AK333473.1| gi|241985599; dbj|AK332860.1| gi|241985804; dbj|AK333065.1| gi|300681572; emb|FN645450.1| gi|300681517; emb|FN564434.1| gi|241990840; dbj|AK330996.1| gi|241985125; dbj|AK332385.1| gi|241983246; dbj|AK331186.1|
51	EST CL1Contig3679-7 peptides, 18%	CL1Contig3679	gi|241986787; dbj|AK334048.1| gi|241989692; dbj|AK330233.1|

	cover, score 216 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)		gi|241989131; dbj|AK336110.1| gi|241988664; dbj|AK335921.1| gi|112784980; gb|DQ862061.1| gi|241989137; dbj|AK336116.1| gi|241983646; dbj|AK331588.1| gi|241989791; dbj|AK330332.1| gi|241989140; dbj|AK336119.1| gi|241988596; dbj|AK335853.1| gi|241990858; dbj|AK331014.1| gi|241986212; dbj|AK333473.1| gi|241985599; dbj|AK332860.1| gi|241985804; dbj|AK333065.1| gi|300681572; emb|FN645450.1| gi|300681517; emb|FN564434.1| gi|241990840; dbj|AK330996.1| gi|241985125; dbj|AK332385.1| gi|241983246; dbj|AK331186.1|
52	EST CL1Contig9364- 11 peptides, 27% cover, score 536 (99% ID to UDP-D-glucuronate decarboxylase [Hordeum vulgare] gi|50659026|gb|AAT80326.1)	CL1Contig9364	gi|241988969; dbj|AK330191.1| gi|241988441; dbj|AK335699.1| gi|283806364; dbj|AB539590.1| gi|32401370; gb|AF542190.1| gi|32128595; gb|BT009044.1| gi|241986807; dbj|AK334068.1| gi|241985019; dbj|AK332279.1| gi|241988516; dbj|AK335774.1| gi|59805043; gb|AY771357.1| gi|241983202; dbj|AK331142.1| gi|241987675; dbj|AK334932.1| gi|32128677; gb|BT009126.1| gi|241987699; dbj|AK334956.1| gi|241990485; dbj|AK330642.1| gi|45331148; gb|AY555573.1| gi|56182356; gb|AY707920.1| gi|56182350; gb|AY707917.1| gi|241984007; dbj|AK331949.1| gi|56182348; gb|AY707916.1| gi|56182352; gb|AY707918.1| gi|241984962; dbj|AK332222.1| gi|241986776; dbj|AK334037.1| gi|241990864; dbj|AK331020.1| gi|56182354; gb|AY707919.1| gi|241983545; dbj|AK331486.1| gi|90902166; gb|DQ449508.1| gi|241986129; dbj|AK333390.1| gi|241989694; dbj|AK330235.1| gi|34597579; gb|AY373831.1| gi|241984877; dbj|AK332137.1| gi|42517095; dbj|AB162139.1|
53	EST CL1Contig7328-18 peptides, 53% cover, score 1090 (89% ID to hypothetical protein OsJ_03416 [Oryza sativa Japonica Group] gi|125571984|gb|EAZ13499.1)	CL1Contig7328	gi|241987616; dbj|AK334873.1| gi|241985107; dbj|AK332367.1| gi|162957174; gb|EU170134.1| gi|161019483; gb|EU082065.1| gi|241986484; dbj|AK333745.1| gi|241983357; dbj|AK331297.1| gi|241988469; dbj|AK335727.1| gi|82907750; gb|DQ247872.1| gi|241983663; dbj|AK331605.1|
53	EST CL1Contig7283-14 peptides, 45% cover, score 650 (90% ID to Os01g0743500 [Oryza sativa Japonica Group] gi|115439879|ref|NP_001044219.1)	CL1Contig7283	gi|241987616; dbj|AK334873.1| gi|241985107; dbj|AK332367.1| gi|162957174; gb|EU170134.1| gi|161019483; gb|EU082065.1| gi|241986484; dbj|AK333745.1| gi|241983357; dbj|AK331297.1| gi|241988469; dbj|AK335727.1| gi|82907750; gb|DQ247872.1| gi|241983663; dbj|AK331605.1|

54	EST CL1Contig7328-15 peptides, 52% cover, score 627 (89% ID to Malic enzyme, NADP-specific [Oryza sativa] gi|38261493|gb|AAR15892.1)	CL1Contig7328	gi|241987616; dbj|AK334873.1| gi|241985107; dbj|AK332367.1| gi|162957174; gb|EU170134.1| gi|161019483; gb|EU082065.1| gi|241986484; dbj|AK333745.1| gi|241983357; dbj|AK331297.1| gi|241988469; dbj|AK335727.1| gi|82907750; gb|DQ247872.1| gi|241983663; dbj|AK331605.1|
54	EST CL1Contig7283-12 peptides, 49% cover, score 431 (90% ID to Malic enzyme, NADP-specific [Oryza sativa] gi|38261493|gb|AAR15892.1)	CL1Contig7283	gi|241987616; dbj|AK334873.1| gi|241985107; dbj|AK332367.1| gi|162957174; gb|EU170134.1| gi|161019483; gb|EU082065.1| gi|241986484; dbj|AK333745.1| gi|241983357; dbj|AK331297.1| gi|241988469; dbj|AK335727.1| gi|82907750; gb|DQ247872.1| gi|241983663; dbj|AK331605.1|
55	EST CL1Contig17645-23 peptides, 58% cover, score 1204 (95% ID to Formate dehydrogenase, mitochondrial gi|21263612|sp|Q9ZRI8.1|FDH_HORVU)	CL1Contig17645	gi|241985345; dbj|AK332605.1| gi|32400846; gb|AF479036.1| gi|241986702; dbj|AK333963.1| gi|241989299; dbj|AK336277.1| gi|241987753; dbj|AK335010.1| gi|241983624; dbj|AK331566.1| gi|241984748; dbj|AK332008.1| gi|241990661; dbj|AK330818.1| gi|241988514; dbj|AK335772.1| gi|32129048; gb|BT009497.1| gi|188038099; gb|EU660902.1| gi|300681497; emb|FN564431.1| gi|300681434; emb|FN564428.1|
33	EST CL1Contig17645-20 peptides, 50% cover, score 706 (95% ID to Formate dehydrogenase, mitochondrial [Hordeum vulgare subsp. vulgare] gi|21263612|sp|Q9ZRI8.1|FDH_HORVU)	CL1Contig17645	gi|241985345; dbj|AK332605.1| gi|32400846; gb|AF479036.1| gi|241986702; dbj|AK333963.1| gi|241989299; dbj|AK336277.1| gi|241987753; dbj|AK335010.1| gi|241983624; dbj|AK331566.1| gi|241984748; dbj|AK332008.1| gi|241990661; dbj|AK330818.1| gi|241988514; dbj|AK335772.1| gi|32129048; gb|BT009497.1| gi|188038099; gb|EU660902.1| gi|300681497;emb|FN564431.1| gi|300681434; emb|FN564428.1|
34	EST CL1Contig17645- 18 peptides, 47% cover, score 844 (95% ID to Formate dehydrogenase, mitochondrial; NAD-dependent formate dehydrogenase; (Hordeum vulgare subsp. Vulgare) gi|21263612|sp|Q9ZRI8.1)	CL1Contig17645	gi|241985345; dbj|AK332605.1| gi|32400846; gb|AF479036.1| gi|241986702; dbj|AK333963.1| gi|241989299; dbj|AK336277.1| gi|241987753; dbj|AK335010.1| gi|241983624; dbj|AK331566.1| gi|241984748; dbj|AK332008.1| gi|241990661; dbj|AK330818.1| gi|241988514; dbj|AK335772.1| gi|32129048; gb|BT009497.1| gi|188038099; gb|EU660902.1| gi|300681497;emb|FN564431.1| gi|300681434; emb|FN564428.1|

57	EST CL1Contig210-39 peptides, 48% cover, score 1561 (94% ID to Isocitrate dehydrogenase, NADP-specific [*Oryza sativa* (japonica cultivar-group)] gi|5007084)	CL1Contig210	gi|241986316; dbj|AK333577.1| gi|241983778; dbj|AK331720.1| gi|32128766; gb|BT009215.1| gi|32128665; gb|BT009114.1| gi|300681517; emb|FN564434.1| gi|241988360; dbj|AK335618.1|
32	EST CL1Contig13096 – 36 peptides, 55% cover, score 2011 (96% ID to Alanine aminotransferase 2 [Hordeum vulgare] gi|1703227|sp|P52894.1)	CL1Contig13096	gi|241984017; dbj|AK331959.1| gi|241983623; dbj|AK331565.1| gi|241986301; dbj|AK333562.1| gi|241986482; dbj|AK333743.1| gi|40641598; emb|AJ606028.1| gi|241986314; dbj|AK333575.1| gi|1173637; gb|U35779.1|TAU35779 gi|1173635; gb|U35778.1|TAU35778 gi|241985554; dbj|AK332815.1| gi|241986245; dbj|AK333506.1| gi|32129055; gb|BT009504.1| gi|241985738; dbj|AK332999.1| gi|1244715; gb|U42336.1|TAU42336
60	EST CL1Contig13096-28 peptides, 52% cover, score 1474 (96% ID to Alanine aminotransferase 2 [Hordeum vulgare] gi|1703227|sp|P52894.1|ALA2_HORVU)	CL1Contig13096	gi|241984017; dbj|AK331959.1| gi|241983623; dbj|AK331565.1| gi|241986301; dbj|AK333562.1| gi|241986482; dbj|AK333743.1| gi|40641598; emb|AJ606028.1| gi|241986314; dbj|AK333575.1| gi|1173637; gb|U35779.1|TAU35779 gi|1173635; gb|U35778.1|TAU35778 gi|241985554; dbj|AK332815.1| gi|241986245; dbj|AK333506.1| gi|32129055; gb|BT009504.1| gi|241985738; dbj|AK332999.1| gi|1244715; gb|U42336.1|TAU42336
61	EST CL1Contig13096-34 peptides, 56% cover, score 2137 (96% ID to Alanine aminotransferase 2 gi|1703227|sp|P52894.1|ALA2_HORVU)	CL1Contig13096	gi|241984017; dbj|AK331959.1| gi|241983623; dbj|AK331565.1| gi|241986301; dbj|AK333562.1| gi|241986482; dbj|AK333743.1| gi|40641598; emb|AJ606028.1| gi|241986314; dbj|AK333575.1| gi|1173637; gb|U35779.1|TAU35779 gi|1173635; gb|U35778.1|TAU35778 gi|241985554; dbj|AK332815.1| gi|241986245; dbj|AK333506.1| gi|32129055; gb|BT009504.1| gi|241985738; dbj|AK332999.1| gi|1244715; gb|U42336.1|TAU42336
75	EST CL1Contig4569-19 peptides, 37% cover, score 1016 (90% ID to Aldehyde dehydrogenase [*Oryza sativa* Japonica Group] gi|11995457	CL1Contig4569	gi|241990676; dbj|AK330832.1| gi|300087068; gb|HM138374.1| gi|241985527; dbj|AK332788.1| gi|241983355; dbj|AK331295.1| gi|241987397; dbj|AK334654.1| gi|21747869; gb|AY050316.1| gi|82469903; gb|AF521191.2| gi|241984995; dbj|AK332255.1| gi|30144413; gb|AY255673.1| gi|21780313; gb|AF521190.1| gi|300681434; emb|FN564428.1| gi|241985538; dbj|AK332799.1| gi|18449342; gb|AF467542.1| gi|241986646; dbj|AK333907.1| gi|241983803; dbj|AK331745.1|

75	EST CL1Contig5198-16 peptides, 52% cover, score 985 (92% ID to aldehyde dehydrogenase family 7 member A1 [*Zea mays*] gi|226531366|ref|NP_001149126.1	CL1Contig5198	gi|241990676; dbj|AK330832.1| gi|300087068; gb|HM138374.1| gi|241983355; dbj|AK331295.1| gi|241985527; dbj|AK332788.1| gi|241987397; dbj|AK334654.1| gi|21747869; gb|AY050316.1| gi|241984995; dbj|AK332255.1| gi|30144413; gb|AY255673.1| gi|82469903; gb|AF521191.2| gi|21780313; gb|AF521190.1| gi|300681434; emb|FN564428.1| gi|241985538; dbj|AK332799.1| gi|18449342; gb|AF467542.1| gi|241986646; dbj|AK333907.1| gi|241983803; dbj|AK331745.1| gi|219819648; gb|FJ517258.1| gi|289186868; gb|GU385904.1| gi|223703121; gb|FJ643535.1| gi|223703119; gb|FJ643534.1| gi|94962614; gb|DQ517494.1| gi|94962612; gb|DQ517493.1| gi|7801373; emb|AJ400713.1| gi|223470530; gb|FJ640559.1| gi|223470528; gb|FJ640558.1|
62	EST CL1Contig18337 – 25 peptides, 63% cover, score 1326 (96% ID to Serpin-Z1A [Triticum aestivum] gi|75282265|sp|Q41593.1)	CL1Contig18337	gi|871550; emb|Z49890.1| gi|1885349; emb|Y11485.1| gi|224589265; gb|FJ705436.1| gi|224589269; gb|FJ705438.1| gi|5734503; emb|AJ245878.1| gi|5734505; emb|AJ245879.1| gi|1885345; emb|Y11486.1| gi|224589267; gb|FJ705437.1| gi|224589271; gb|FJ705439.1| gi|241987877; dbj|AK335134.1| gi|300681509; emb|FN564433.1| gi|15982657; gb|AY049041.1|
67	EST CL1Contig10503-10 peptides, 28% cover, score 516 (98% ID to 20S Proteasome subunit alpha type-7-A [*Oryza sativa*] gi| 75131289)	CL1Contig10503	gi|241985249; dbj|AK332509.1| gi|241989039; dbj|AK336018.1| gi|241985014; dbj|AK332274.1| gi|241987078; dbj|AK334335.1| gi|241984949; dbj|AK332209.1| gi|241983840; dbj|AK331782.1| gi|241986007; dbj|AK333268.1| gi|52548239; gb|AY736119.1| gi|241984863; dbj|AK332123.1| gi|300681466; emb|FN564430.1| gi|241985452; dbj|AK332713.1| gi|218963621; gb|FJ555239.1| gi|241986248; dbj|AK333509.1| gi|300681497; emb|FN564431.1| gi|241986288; dbj|AK333549.1|
68	EST CL1Contig9579-34 peptides, 37% cover, score 2060 (96% ID to Cell division control protein 48[*Oryza sativa*] gi| 108706222)	CL1Contig9579	gi|241983896; dbj|AK331838.1| gi|241987451; dbj|AK334708.1| gi|160548120; gb|EU267938.1| gi|160548116; gb|EU267934.1| gi|241986569; dbj|AK333830.1| gi|241986336; dbj|AK333597.1| gi|32128694; gb|BT009143.1| gi|241985408; dbj|AK332668.1| gi|241989713; dbj|AK330254.1| gi|241985148; dbj|AK332408.1| gi|241985845; dbj|AK333106.1| gi|241983508; dbj|AK331448.1|

			gi|118767196; gb|DQ923395.1| gi|241990502; dbj|AK330659.1| gi|241989161; dbj|AK336140.1| gi|241986539; dbj|AK333800.1| gi|241983931; dbj|AK331873.1| gi|241986530; dbj|AK333791.1| gi|241984763; dbj|AK332023.1| gi|241983287; dbj|AK331227.1| gi|241987450; dbj|AK334707.1| gi|241983821; dbj|AK331763.1| gi|241985531; dbj|AK332792.1| gi|241990850; dbj|AK331006.1| gi|32400783; gb|AF475102.1| gi|241987467; dbj|AK334724.1| gi|241986505; dbj|AK333766.1| gi|241987660; dbj|AK334917.1| gi|32129008; gb|BT009457.1| gi|32400817; gb|AF475120.1| gi|32129011; gb|BT009460.1| gi|109450898; emb|CT009586.1| gi|61656783; emb|CR626929.1| gi|241986691; dbj|AK333952.1| gi|212007820; gb|EU835981.1| gi|109450892; emb|CT009585.1| gi|61656788; emb|CR626930.1| gi|212007811; gb|EU835980.1| gi|109450933; emb|CT009735.1| gi|61656803; emb|CR626934.1| gi|241983369; dbj|AK331309.1| gi|212007832; gb|EU835982.1| gi|241985776; dbj|AK333037.1| gi|241987741; dbj|AK334998.1| gi|241985375; dbj|AK332635.1| gi|32400852; gb|AF479039.1| gi|15148394; gb|AF234648.1|AF234648 gi|241988933; dbj|AK330155.1| gi|32400878; gb|AF479052.1| gi|241990697; dbj|AK330853.1| gi|241986449; dbj|AK333710.1| gi|4558483; gb|AF097363.1| gi|11561807; gb|AF083344.2|AF083344 gi|241990812; dbj|AK330968.1| gi|6013195; gb|AF174433.1| gi|42541822; gb|AY494984.1| gi|300681568; emb|FN564435.1| gi|241988466; dbj|AK335724.1| gi|241989181; dbj|AK336160.1| gi|300681466; emb|FN564430.1| gi|164472655; gb|EU181183.1| gi|220683794; gb|FJ185035.1| gi|224365600; gb|FJ436983.1| gi|219814401; gb|FJ436985.1| gi|219814397; gb|FJ436984.1| gi|241990267; dbj|AK330609.1| gi|241985045; dbj|AK332305.1| gi|241990527; dbj|AK330684.1| gi|32128479; gb|BT008928.1| gi|115392207; gb|DQ984669.1| gi|241990274; dbj|AK330616.1|
73	EST CL1Contig17267- 10 peptides, 45% cover, score 484 (88% ID to 27K thioredoxin family protein [*Triticum aestivum*] gi|290350670|dbj|BAI78303.1)	CL1Contig17267	gi|290350669; dbj|AB518868.1| gi|30793445; dbj|AB085212.1| gi|283480514; emb|FN393742.1| gi|241985969; dbj|AK333230.1| gi|300681452; emb|FN564429.1| gi|300681509; emb|FN564433.1| gi|296280021; gb|GU230856.1| gi|241985793; dbj|AK333054.1|
71	EST CL1Contig17267-11 peptides, 48% cover, score 499	CL1Contig17267	gi|290350669; dbj|AB518868.1| gi|30793445; dbj|AB085212.1| gi|283480514; emb|FN393742.1|

	(88% ID to 27K thioredoxin family protein [*Triticum aestivum*] gi|290350670|dbj|BAI78303.1)		gi|241985969; dbj|AK333230.1| gi|300681452; emb|FN564429.1| gi|300681509; emb|FN564433.1| gi|296280021; gb|GU230856.1| gi|241985793; dbj|AK333054.1|
72	EST CL1Contig17267- 12 peptides, 49% cover, score 543 (89% ID to 27K protein [Triticum aestivum] gi|30793446|dbj|BAC76688.1)	CL1Contig17267	gi|290350669; dbj|AB518868.1| gi|30793445; dbj|AB085212.1| gi|283480514; emb|FN393742.1| gi|241985969; dbj|AK333230.1| gi|300681452; emb|FN564429.1| gi|300681509; emb|FN564433.1| gi|241985793; dbj|AK333054.1| gi|296280021; gb|GU230856.1|
76	EST CL183Contig1-12 peptide, 40% cover, score 620 (86% ID to Embryo-specific Protein [*Oryza sativa* Japonica Group] gi|4105692)	CL183Contig1	gi|241989016; dbj|AK335995.1| gi|241985025; dbj|AK332285.1| gi|227937391; gb|FJ883562.1| gi|300681517; emb|FN564434.1|
77	EST CL183Contig3- 14 peptides, 38% cover, score 646 (87% ID Embryo-specific Protein [*Oryza sativa* Japonica Group] gi|4105692)	CL183Contig3	gi|241989016; dbj|AK335995.1| gi|241985025; dbj|AK332285.1| gi|227937391; gb|FJ883562.1| gi|300681517; emb|FN564434.1|
78	EST CL1Contig3675-14 peptides, 18% cover, score 480 (58% ID to Globulin 2 (11S globulin, legumin) [*Zea mays*] gi|228310)	CL1Contig3675	gi|170695; gb|M81719.1|WHTGBL1A gi|215398469; gb|FJ439135.1| gi|215398467; gb|FJ439134.1| gi|215398471; gb|FJ439136.1| gi|241986013; dbj|AK333274.1| gi|241985493; dbj|AK332754.1| gi|5869972; emb|AJ237942.1| gi|241985929; dbj|AK333190.1|
26	EST CL1Contig14818-41 peptides, 67% cover, score 2151 (94% ID to Enolase, putative, expressed [Oryza sativa (japonica cultivar-group)] gi|110288667|gb|ABB46862.2)	CL1Contig14818	gi|241983960; dbj|AK331902.1| gi|241983596; dbj|AK331538.1| gi|241988158; dbj|AK335415.1| gi|300681434; emb|FN564428.1|
84	EST CL1Contig14818- 36 peptides, 65% cover, score 2430 (94% ID to Enolase, putative, expressed [Oryza sativa (japonica cultivar-group)] gi|110288667|gb|ABB46862.2)	CL1Contig14818	gi|241983960; dbj|AK331902.1| gi|241983596; dbj|AK331538.1| gi|241988158; dbj|AK335415.1| gi|300681434; emb|FN564428.1|
37	EST CL1Contig6941-17 peptides, 32% cover, score 1059 (90% ID to Pyruvate, orthophosphate dikinase 1 [*Oryza sativa* Indica Group] gi|75254569)	CL1Contig6941	gi|241986082; dbj|AK333343.1| gi|32400837; gb|AF475130.1| gi|241983545; dbj|AK331486.1| gi|300681509; emb|FN564433.1|
86	EST	CL1Contig11939	gi|1771781; emb|X98504.1|

	CL1Contig11939 – 23 peptides, 44% cover, score 1216 (86% ID to Beta-amylase Precursor, 1,4-alpha-D-glucan Maltohydrolase, Hordeum vulgare subsp. Spontaneum gi|75107132|sp|P82993.1)		gi|4138595; emb|Y16242.1| gi|32400763; gb|AF470353.1| gi|241986995; dbj|AK334256.1| gi|241985242; dbj|AK332502.1| gi|241989148; dbj|AK336127.1| gi|300681509; emb|FN564433.1|
50	EST CL1Contig3679 – 20 peptides, 48% cover, score 939 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig3679	gi|241986787; dbj|AK334048.1| gi|241989692; dbj|AK330233.1| gi|241989131; dbj|AK336110.1| gi|241988664; dbj|AK335921.1| gi|112784980; gb|DQ862061.1| gi|241989137; dbj|AK336116.1| gi|241983646; dbj|AK331588.1| gi|241989791; dbj|AK330332.1| gi|241989140; dbj|AK336119.1| gi|241988596; dbj|AK335853.1| gi|241990858; dbj|AK331014.1| gi|241986212; dbj|AK333473.1| gi|241985599; dbj|AK332860.1| gi|241985804; dbj|AK333065.1| gi|300681572; emb|FN645450.1| gi|300681517; emb|FN564434.1| gi|241990840; dbj|AK330996.1| gi|241985125; dbj|AK332385.1| gi|241983246; dbj|AK331186.1|
49	EST CL1Contig3679-11 peptides, 33% cover, score 531 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212)	CL1Contig3679	gi|241986787; dbj|AK334048.1| gi|241989692; dbj|AK330233.1| gi|241989131; dbj|AK336110.1| gi|241988664; dbj|AK335921.1| gi|112784980; gb|DQ862061.1| gi|241989137; dbj|AK336116.1| gi|241983646; dbj|AK331588.1| gi|241989791; dbj|AK330332.1| gi|241989140; dbj|AK336119.1| gi|241988596; dbj|AK335853.1| gi|241990858; dbj|AK331014.1| gi|241986212; dbj|AK333473.1| gi|241985599; dbj|AK332860.1| gi|241985804; dbj|AK333065.1| gi|300681572; emb|FN645450.1| gi|300681517; emb|FN564434.1| gi|241990840; dbj|AK330996.1| gi|241985125; dbj|AK332385.1| gi|241983246; dbj|AK331186.1|
48	EST CL1Contig4351-24 peptides, 47% cover, score 1310 (97% ID to glucose and ribitol dehydrogenase homolog - barley gi|7431022|pir||T06212	CL1Contig4351	gi|241986787; dbj|AK334048.1| gi|241989692; dbj|AK330233.1| gi|241989131; dbj|AK336110.1| gi|241988664; dbj|AK335921.1| gi|112784980; gb|DQ862061.1| gi|241989137; dbj|AK336116.1| gi|241983646; dbj|AK331588.1| gi|241989791; dbj|AK330332.1| gi|241989140; dbj|AK336119.1| gi|241988596; dbj|AK335853.1| gi|241990858; dbj|AK331014.1| gi|241986212; dbj|AK333473.1| gi|241985599; dbj|AK332860.1| gi|241985804; dbj|AK333065.1| gi|300681572; emb|FN645450.1| gi|300681517; emb|FN564434.1| gi|241990840; dbj|AK330996.1| gi|241985125; dbj|AK332385.1| gi|241983246; dbj|AK331186.1|

90	EST CL1339Contig2-14 peptides, 33% cover, score 692 (56% ID to Putative o-methyltransferase ZRP4,*Oryza sativa*, AAL31646.1, gi|16924034)	CL1339Contig2	gi|241986577; dbj|AK333838.1| gi|241990665; dbj|AK330822.1| gi|241988967; dbj|AK330189.1| gi|241989773; dbj|AK330314.1| gi|241985820; dbj|AK333081.1| gi|241988539; dbj|AK335796.1| gi|4098237; gb|U76384.1|TAU76384 gi|241985953; dbj|AK333214.1| gi|194239078; emb|AM932687.1| gi|32129122; gb|BT009571.1| gi|32128910; gb|BT009359.1| gi|145693799; gb|EF423612.1| gi|145693795; gb|EF423610.1| gi|145321006; gb|EF413031.1| gi|241985647; dbj|AK332908.1| gi|77818927; gb|DQ223971.1| gi|145693797; gb|EF423611.1| gi|32128934; gb|BT009383.1| gi|33323467; gb|AF502287.1| gi|30385245; gb|AY226581.1| gi|241987848; dbj|AK335105.1| gi|241987593; dbj|AK334850.1|
91	EST CL1Contig18963-13 peptides, 36% cover, score 807 (73% ID to Thiamine biosynthetic enzyme [Oryza sativa (japonica cultivar-group)] gi|32352138)	CL1Contig18963	gi|32128858; gb|BT009307.1| gi|32128563; gb|BT009012.1| gi|241983870; dbj|AK331812.1|
53	EST CL1Contig7328-18 peptides, 53% cover, score 1090 (89% ID to Malic enzyme, NADP-specific [*Oryza sativa* Japonica Group] gi|38261493)	CL1Contig7328	gi|241987616; dbj|AK334873.1| gi|241985107; dbj|AK332367.1| gi|162957174; gb|EU170134.1| gi|161019483; gb|EU082065.1| gi|241986484; dbj|AK333745.1| gi|241983357; dbj|AK331297.1| gi|241988469; dbj|AK335727.1| gi|82907750; gb|DQ247872.1| gi|241983663; dbj|AK331605.1|
53	EST CL1Contig7283-14 peptides, 45% cover, score 650 (90% ID to Malic enzyme, NADP-specific [*Oryza sativa* Japonica Group] gi| 38261493)	CL1Contig7283	gi|241987616; dbj|AK334873.1| gi|241985107; dbj|AK332367.1| gi|162957174; gb|EU170134.1| gi|161019483; gb|EU082065.1| gi|241986484; dbj|AK333745.1| gi|241983357; dbj|AK331297.1| gi|241988469; dbj|AK335727.1| gi|82907750; gb|DQ247872.1| gi|241983663; dbj|AK331605.1|
55	EST CL1Contig17645-23 peptides, 58% cover, score 1204 (95% ID to Formate dehydrogenase, mitochondrial gi|21263612|sp|Q9ZRI8.1|FDH_HORVU)	CL1Contig17645	gi|241985345; dbj|AK332605.1| gi|32400846; gb|AF479036.1| gi|241986702; dbj|AK333963.1| gi|241989299; dbj|AK336277.1| gi|241987753; dbj|AK335010.1| gi|241983624; dbj|AK331566.1| gi|241984748; dbj|AK332008.1| gi|241990661; dbj|AK330818.1| gi|241988514; dbj|AK335772.1| gi|32129048; gb|BT009497.1| gi|188038099; gb|EU660902.1| gi|300681497; emb|FN564431.1| gi|300681434; emb|FN564428.1|
92	EST CL1Contig17645-19 peptides,	CL1Contig17645	gi|241985345; dbj|AK332605.1| gi|32400846; gb|AF479036.1|

	49% cover, score 981 (95% ID to Formate dehydrogenase, mitochondrial; NAD-dependent formate dehydrogenase; (Hordeum vulgare subsp. Vulgare) gi|21263612|sp|Q9ZRI8.1)		gi|241986702; dbj|AK333963.1| gi|241989299; dbj|AK336277.1| gi|241987753; dbj|AK335010.1| gi|241983624; dbj|AK331566.1| gi|241984748; dbj|AK332008.1| gi|241990661; dbj|AK330818.1| gi|241988514; dbj|AK335772.1| gi|32129048; gb|BT009497.1| gi|188038099; gb|EU660902.1| gi|300681497; emb|FN564431.1| gi|300681434; emb|FN564428.1|
101	EST CL1Contig5198- 13 peptides, 49% cover, score 873 (92% ID to aldehyde dehydrogenase [*Oryza sativa*] gi|11995457)	CL1Contig5198	gi|241990676; dbj|AK330832.1| gi|300087068; gb|HM138374.1| gi|241985527; dbj|AK332788.1| gi|241983355; dbj|AK331295.1| gi|241987397; dbj|AK334654.1| gi|21747869; gb|AY050316.1| gi|82469903; gb|AF521191.2| gi|241984995; dbj|AK332255.1| gi|30144413; gb|AY255673.1| gi|21780313; gb|AF521190.1| gi|300681434; emb|FN564428.1| gi|241985538; dbj|AK332799.1| gi|18449342; gb|AF467542.1| gi|241986646; dbj|AK333907.1| gi|241983803; dbj|AK331745.1|
75	EST CL1Contig5198-16 peptides, 52% cover, score 985 (92% ID to aldehyde dehydrogenase [*Oryza sativa*] gi|11995457)	CL1Contig5198	gi|241990676; dbj|AK330832.1| gi|300087068; gb|HM138374.1| gi|241985527; dbj|AK332788.1| gi|241983355; dbj|AK331295.1| gi|241987397; dbj|AK334654.1| gi|21747869; gb|AY050316.1| gi|82469903; gb|AF521191.2| gi|241984995; dbj|AK332255.1| gi|30144413; gb|AY255673.1| gi|21780313; gb|AF521190.1| gi|300681434; emb|FN564428.1| gi|241985538; dbj|AK332799.1| gi|18449342; gb|AF467542.1| gi|241986646; dbj|AK333907.1| gi|241983803; dbj|AK331745.1|
95	EST CL1Contig11606 – 18 peptides, 38% cover, score 1016 (84% ID to Serine hydroxymethyltransferase, mitochondrial precursor, putative, expressed [Oryza sativa (japonica cultivar-group)] gi|108862549|gb|ABA97575.2)	CL1Contig11606	gi|241983137; dbj|AK331077.1| gi|32128823; gb|BT009272.1| gi|241989861; dbj|AK330402.1| gi|300681517; emb|FN564434.1|
32	EST CL1Contig13096-22 peptides, 45% cover, score 1091 (96% ID to Alanine aminotransferase 2 [Hordeum vulgare] gi|1703227|sp|P52894.1|ALA2_HORVU)	CL1Contig13096	gi|241984017; dbj|AK331959.1| gi|241983623; dbj|AK331565.1| gi|241986301; dbj|AK333562.1| gi|241986482; dbj|AK333743.1| gi|40641598; emb|AJ606028.1| gi|40641598; dbj|AK333575.1| gi|1173637; gb|U35779.1|TAU35779 gi|1173635; gb|U35778.1|TAU35778 gi|241985554; dbj|AK332815.1| gi|241986245; dbj|AK333506.1| gi|32129055; gb|BT009504.1| gi|241985738; dbj|AK332999.1| gi|1244715; gb|U42336.1|TAU42336
60	EST	CL1Contig13096	gi|241984017; dbj|AK331959.1|

	CL1Contig13096-28 peptides, 52% cover, score 1474 (96% ID to Alanine aminotransferase 2 [Hordeum vulgare] gi|1703227|sp|P52894.1|ALA2_HORVU)		gi|241983623; dbj|AK331565.1| gi|241986301; dbj|AK333562.1| gi|241986482; dbj|AK333743.1| gi|40641598; emb|AJ606028.1| gi|40641598; dbj|AK333575.1| gi|1173637; gb|U35779.1|TAU35779 gi|1173635; gb|U35778.1|TAU35778 gi|241985554; dbj|AK332815.1| gi|241986245; dbj|AK333506.1| gi|32129055; gb|BT009504.1| gi|241985738; dbj|AK332999.1| gi|1244715; gb|U42336.1|TAU42336
61	EST CL1Contig13096-31 peptides, 53% cover, score 1655 (96% ID to alanine aminotransferase 2 [Hordeum vulgare subsp. vulgare] gi|1703227|sp|P52894.1|ALA2_HORVU)	CL1Contig13096	gi|241984017; dbj|AK331959.1| gi|241983623; dbj|AK331565.1| gi|241986301; dbj|AK333562.1| gi|241986482; dbj|AK333743.1| gi|40641598; emb|AJ606028.1| gi|40641598; dbj|AK333575.1| gi|1173637; gb|U35779.1|TAU35779 gi|1173635; gb|U35778.1|TAU35778 gi|241985554; dbj|AK332815.1| gi|241986245; dbj|AK333506.1| gi|32129055; gb|BT009504.1| gi|241985738; dbj|AK332999.1| gi|1244715; gb|U42336.1|TAU42336
96	EST CL1Contig13096-33 peptides, 52% cover, score 1776 (96% ID to Alanine aminotransferase 2 (Hordeum vulgare subsp. Vulgare) gi|1703227|sp|P52894.1)	CL1Contig13096	gi|241984017; dbj|AK331959.1| gi|241983623; dbj|AK331565.1| gi|241986301; dbj|AK333562.1| gi|241986482; dbj|AK333743.1| gi|40641598; emb|AJ606028.1| gi|40641598; dbj|AK333575.1| gi|1173637; gb|U35779.1|TAU35779 gi|1173635; gb|U35778.1|TAU35778 gi|241985554; dbj|AK332815.1| gi|241986245; dbj|AK333506.1| gi|32129055; gb|BT009504.1| gi|241985738; dbj|AK332999.1| gi|1244715; gb|U42336.1|TAU42336
68	EST CL1Contig9579-34 peptides, 37% cover, score 2060 (96% ID to Cell division control protein 48[*Oryza sativa*] gi| 108706222)	CL1Contig9579	gi|241983896; dbj|AK331838.1| gi|241987451; dbj|AK334708.1| gi|160548120; gb|EU267938.1| gi|160548116; gb|EU267934.1| gi|241986569; dbj|AK333830.1| gi|241986336; dbj|AK333597.1| gi|32128694; gb|BT009143.1| gi|241985408; dbj|AK332668.1| gi|241989713; dbj|AK330254.1| gi|241985148; dbj|AK332408.1| gi|241985845; dbj|AK333106.1| gi|241983508; dbj|AK331448.1| gi|118767196; gb|DQ923395.1| gi|241990502; dbj|AK330659.1| gi|241989161; dbj|AK336140.1| gi|241986539; dbj|AK333800.1| gi|241983931; dbj|AK331873.1| gi|241986530; dbj|AK333791.1| gi|241984763; dbj|AK332023.1| gi|241983287; dbj|AK331227.1| gi|241987450; dbj|AK334707.1| gi|241983821; dbj|AK331763.1| gi|241985531; dbj|AK332792.1| gi|241985531; dbj|AK331006.1| gi|32400783; gb|AF475102.1| gi|241987467; dbj|AK334724.1| gi|241986505; dbj|AK333766.1| gi|241987660; dbj|AK334917.1| gi|32129008; gb|BT009457.1| gi|32400817; gb|AF475120.1|

			gi|32129011; gb|BT009460.1|
			gi|109450898; emb|CT009586.1|
			gi|61656783; emb|CR626929.1|
			gi|241986691; dbj|AK333952.1|
			gi|212007820; gb|EU835981.1|
			gi|109450892; emb|CT009585.1|
			gi|61656788; emb|CR626930.1|
			gi|212007811; gb|EU835980.1|
			gi|109450933; emb|CT009735.1|
			gi|61656803; emb|CR626934.1|
			gi|241983369; dbj|AK331309.1|
			gi|212007832; gb|EU835982.1|
			gi|241985776; dbj|AK333037.1|
			gi|241987741; dbj|AK334998.1|
			gi|241985375; dbj|AK332635.1|
			gi|32400852; gb|AF479039.1|
			gi|15148394; gb|AF234648.1|AF234648
			gi|241988933; dbj|AK330155.1|
			gi|32400878; gb|AF479052.1|
			gi|241990697; dbj|AK330853.1|
			gi|241986449; dbj|AK333710.1|
			gi|4558483; gb|AF097363.1|
			gi|11561807; gb|AF083344.2|AF083344
			gi|241990812; dbj|AK330968.1|
			gi|6013195; gb|AF174433.1|
			gi|42541822; gb|AY494984.1|
			gi|300681568; emb|FN564435.1|
			gi|241988466; dbj|AK335724.1|
			gi|241989181; dbj|AK336160.1|
			gi|300681466; emb|FN564430.1|
			gi|164472655; gb|EU181183.1|
			gi|220683794; gb|FJ185035.1|
			gi|224365600; gb|FJ436983.1|
			gi|219814401; gb|FJ436985.1|
			gi|219814397; gb|FJ436984.1|
			gi|241990267; dbj|AK330609.1|
			gi|241985045; dbj|AK332305.1|
			gi|241990527; dbj|AK330684.1|
			gi|32128479; gb|BT008928.1|
			gi|115392207; gb|DQ984669.1|
			gi|241990274; dbj|AK330616.1|
94	EST CL1Contig4238-15 peptides, 38% cover, score 1069 (93% ID to 60S acidic ribosomal protein P0, putative [*Oryza sativa* Indica Group] gi| 108706222)	CL1Contig4238	gi|241983896; dbj|AK331838.1|
			gi|241987451; dbj|AK334708.1|
			gi|160548120; gb|EU267938.1|
			gi|160548116; gb|EU267934.1|
			gi|241986569; dbj|AK333830.1|
			gi|241986336; dbj|AK333597.1|
			gi|32128694; gb|BT009143.1|
			gi|241985408; dbj|AK332668.1|
			gi|241989713; dbj|AK330254.1|
			gi|241985148; dbj|AK332408.1|
			gi|241985845; dbj|AK333106.1|
			gi|241983508; dbj|AK331448.1|
			gi|118767196; gb|DQ923395.1|
			gi|241990502; dbj|AK330659.1|
			gi|241989161; dbj|AK336140.1|
			gi|241986539; dbj|AK333800.1|
			gi|241983931; dbj|AK331873.1|
			gi|241986530; dbj|AK333791.1|
			gi|241984763; dbj|AK332023.1|
			gi|241983287; dbj|AK331227.1|
			gi|241987450; dbj|AK334707.1|
			gi|241983821; dbj|AK331763.1|
			gi|241985531; dbj|AK332792.1|
			gi|241985531; dbj|AK331006.1|
			gi|32400783; gb|AF475102.1|
			gi|241987467; dbj|AK334724.1|
			gi|241986505; dbj|AK333766.1|
			gi|241987660; dbj|AK334917.1|
			gi|32129008; gb|BT009457.1|
			gi|32400817; gb|AF475120.1|

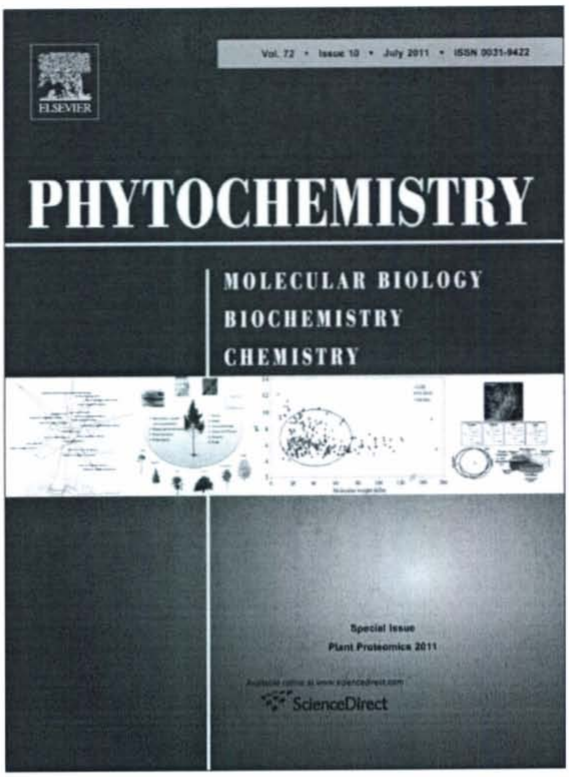
			gi|32129011; gb|BT009460.1| gi|109450898; emb|CT009586.1| gi|61656783; emb|CR626929.1| gi|241986691; dbj|AK333952.1| gi|212007820; gb|EU835981.1| gi|109450892; emb|CT009585.1| gi|61656788; emb|CR626930.1| gi|212007811; gb|EU835980.1| gi|109450933; emb|CT009735.1| gi|61656803; emb|CR626934.1| gi|241983369; dbj|AK331309.1| gi|212007832; gb|EU835982.1| gi|241985776; dbj|AK333037.1| gi|241987741; dbj|AK334998.1| gi|241985375; dbj|AK332635.1| gi|32400852; gb|AF479039.1| gi|15148394; gb|AF234648.1|AF234648 gi|241988933; dbj|AK330155.1| gi|32400878; gb|AF479052.1| gi|241990697; dbj|AK330853.1| gi|241986449; dbj|AK333710.1| gi|4558483; gb|AF097363.1| gi|11561807; gb|AF083344.2|AF083344 gi|241990812; dbj|AK330968.1| gi|6013195; gb|AF174433.1| gi|42541822; gb|AY494984.1| gi|300681568; emb|FN564435.1| gi|241988466; dbj|AK335724.1| gi|241989181; dbj|AK336160.1| gi|300681466; emb|FN564430.1| gi|164472655; gb|EU181183.1| gi|220683794; gb|FJ185035.1| gi|224365600; gb|FJ436983.1| gi|219814401; gb|FJ436985.1| gi|219814397; gb|FJ436984.1| gi|241990267; dbj|AK330609.1| gi|241985045; dbj|AK332305.1| gi|241990527; dbj|AK330684.1| gi|32128479; gb|BT008928.1| gi|115392207; gb|DQ984669.1| gi|241990274; dbj|AK330616.1|
73	EST CL1Contig17267- 10 peptides, 45% cover, score 484 (89% ID to 27K protein [Triticum aestivum] gi|30793446|dbj|BAC76688.1)	CL1Contig17267	gi|290350669; dbj|AB518868.1| gi|30793445; dbj|AB085212.1| gi|283480514; emb|FN393742.1| gi|241985969; dbj|AK333230.1| gi|300681452; emb|FN564429.1| gi|300681509; emb|FN564433.1| gi|241985793; dbj|AK333054.1| gi|296280021; gb|GU230856.1|
100	EST CL1Contig3486-12 peptides, 35% cover, score 583 (92% ID to 2-alkenal reductase [Hordeum vulgare subsp. vulgare] gi|62765876|gb|AAX99161.1)	CL1Contig3486	gi|241985549; dbj|AK332810.1| gi|241987354; dbj|AK334611.1| gi|32128990; gb|BT009439.1| gi|241990119; dbj|AK330461.1| gi|241987988; dbj|AK335245.1| gi|241984786; dbj|AK332046.1| gi|115349933; gb|DQ872398.1|
76	EST CL183Contig1-12 peptide, 40% cover, score 620 (86% ID to Embryo-specific Protein [*Oryza sativa* Japonica Group] gi|4105692)	CL183Contig1	gi|300681419; emb|FN564427.1| gi|241989016; dbj|AK335995.1| gi|241985025; dbj|AK332285.1| gi|227937391; gb|FJ883562.1| gi|300681517; emb|FN564434.1|
77	EST CL183Contig3- 14 peptides, 38% cover, score 646 (87% ID to Embryo-specific Protein [*Oryza sativa* Japonica Group]	CL183Contig3	gi|241989016; dbj|AK335995.1| gi|241985025; dbj|AK332285.1| gi|227937391; gb|FJ883562.1| gi|300681517; emb|FN564434.1|

	gi|4105692)		
102	EST CL1Contig9410 – 22 peptides, 40% cover, score 1057 (93% ID to globulin 3, [Triticum aestivum] gi|215398470)	CL1Contig9410	gi|215398469; gb|FJ439135.1| gi|170695; gb|M81719.1|WHTGBL1A gi|215398467; gb|FJ439134.1| gi|215398471; gb|FJ439136.1| gi|241986013; dbj|AK333274.1| gi|171027812; gb|EU482412.1| gi|386166; gb|S62630.1|S62630 gi|147744619; gb|EF489470.1| gi|300681517; emb|FN564434.1|
105	EST CL1Contig9406-18 peptides, 61% cover, score 1039 (84% ID to r40g2 protein [*Oryza sativa* Indica Group] gi|34394517)	CL1Contig9406	gi|241985661; dbj|AK332922.1| gi|66277463; gb|DQ022951.1| gi|241985389; dbj|AK332649.1| gi|300681568; emb|FN564435.1| gi|299109310; emb|FN564426.1| gi|300681452; emb|FN564429.1| gi|300681466; emb|FN564430.1| gi|300681502; emb|FN564432.1|
106	EST CL1097Contig3 – 6 peptides, 23% cover, score 284 (77% ID to Lipoprotein-like protein [*Oryza sativa* (japonica cultivar-group)] gi|14209547)	CL1097Contig3	gi|241989016; dbj|AK335995.1| gi|241986981; dbj|AK334242.1|

[a]Spot number corresponds to 2D gel in Figures 5-7.

[b]Putative names of the identified proteins are listed. Protein digests were analyzed by nano-LC-MS/MS and 'Mascot' search of MS/MS spectra using protein (NCBInr) and EST wheat databases followed by homology identification of EST sequences with BLAST. Number of matched peptides, sequence coverage and probability based MOWSE scores are given according to Mascot Search Results. GenBank accession numbers of EST sequences corresponding to particular constructed EST Contigs are given.

[c]GenBank accession numbers of ESTs are given.



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Thiol redox-sensitive seed proteome in dormant and non-dormant hybrid genotypes of wheat

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ABSTRACT

The thiol redox-sensitive and the total proteome in harvest-ripe grains of closely related genotypes of wheat (*Triticum aestivum* L.), with either a dormant or a non-dormant phenotype, were investigated using hybrid lines of spring wheat double haploid population segregating transgressively, to gain further insight into seed dormancy controlling events. Redox signalling by reactive oxygen species has been shown to play a role in seed dormancy alleviation. Thiol-disulfide proteins are of particular importance in the context of redox-dependent regulation as a central and flexible mechanism to control metabolic and developmental activities of the cells. Here we describe functional proteomic profiling of reversible oxidoreductive changes and characterize *in vivo* intrinsic reactivity of cysteine residues using thiol-specific fluorescent labelling, solubility-based protein fractionation, two-dimensional electrophoresis, and mass spectrometry analysis in conjunction with wheat EST sequence libraries. Quantitative differences between genotypes were found for 106 spots containing 64 unique proteins. Forty seven unique proteins displayed distinctive abundance pattern, and among them 31 proteins contained 78 unique redox active cysteines. Seventeen unique proteins with 19 reactive modified cysteines were found to have differential post-translational thiol redox modification. The results provide an insight into the alteration of thiol-redox profiles in proteins that function in major processes in seeds and include groups of redox- and stress-responsive, genetic information processing and cell cycle control, transport and storage proteins, enzymes of carbohydrate metabolism, proteases and their inhibitors.

1. Introduction

Pre-harvest sprouting (PHS) in many wheat cultivars, due to low harvest dormancy, causes reduced grain quality and therefore serious economic losses to wheat growers (Finch-Savage and Leubner-Metzger, 2006). Thus, a defined level of seed dormancy is an essential component of seed quality. While the dormancy condition is often thought of as a quiescent state, it is in fact a dynamic state in which cell metabolism is active, but growth processes are repressed (Bewley, 1997). The length of the dormancy period is under genetic and environmental control, and engineering it is of considerable economic importance. PHS resistance is a complex trait that is determined by genotype together with a number of other factors: stage of maturity; environmental conditions during grain ripening; spike, plant and crop morphology; biotic and abiotic stress. Genetic variation for seed dormancy exists in wheat (Fofana et al., 2008). One of the embryo factors related to the level of seed dormancy was suggested to be sensitivity to phytohormone abscisic acid (ABA) (Gubler et al., 2005). In wheat, no increase in ABA content is associated with the induction of dormancy but there is strong evidence that changes in ABA responsiveness are related to the maintenance of dormancy. On the other hand, it has been demonstrated that dormancy genes are tightly linked to seed coat colour as determined by dominant R alleles (Flintham, 2000). This association between PHS tolerance and red pigmentation is likely due to a pleiotropic effect of the genes controlling grain colour, rendering PHS resistance breeding more complex in white wheat (Fofana et al., 2008). The successful introgression of dormancy genes from red wheat resulted in the development of white wheat with a significant level of dormancy and tolerance to PHS (McCaig and DePauw, 1992). Furthermore, white dormant varieties showed low germinability when ripened under dry experimental conditions but could not impose sufficient dormancy if ripened in wet environments. Although a number of molecular markers for dormancy

Abbreviations: ABA, abscisic acid; AER, 2-alkenal reductase; DH, dehydrogenase; DHAR, dehydroascorbate reductase; ESP, embryo-specific protein; GA, gibberellic acid; LC-MS/MS, liquid chromatography tandem mass spectrometry; mBBr, monobromobimane; PHS, pre-harvest sprouting; Prx, peroxiredoxin; ROS, reactive oxygen species; Trx, thioredoxin; 2-DE, two-dimensional electrophoresis; 2-D IEF PAGE, two-dimensional isoelectric focusing polyacrylamide gel electrophoresis.

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quantitative trait loci (QTLs) have been identified (Mares et al., 2005; Mori et al., 2005), the corresponding genes involved in the regulation of this process have not been cloned (Torada et al., 2008). Understanding of the processes that occur during wheat seed dormancy control, the biomarker proteins and molecular signalling mechanisms involved will contribute to the targeted breeding of wheat varieties with storage proteins better protected against uncontrolled proteolysis caused by sprouting and will help to design novel sprouting resistance strategies during wheat and other crops production.

Redox signalling by reactive oxygen species (ROS) has been shown to play a role in seed dormancy alleviation. Germinating seeds of plant species from different taxonomic backgrounds produce ROS, including H_2O_2, O_2^-, and hydroxyl radicals, as well as reactive nitrogen species, such as nitric oxide, which are key components of signalling networks regulating growth, development, and pathogen defence (Caliskan and Cuming, 1998; Wojtyla et al., 2006; Oracz et al., 2009; Kranner et al., 2010). Rehydration of desiccated tissue is associated with increased intracellular ROS formation due to the restarting of metabolism. In addition, ROS may be generated extracellularly by NADPH oxidase or peroxidases as part of an oxidative burst during rehydration (Colville and Kranner, 2010). ROS production in desiccated seeds would mainly result from non-enzymatic mechanisms, such as those of Amadori and Maillard, and lipid peroxidation favoured at very low moisture content (El-Maarouf-Bouteau and Bailly, 2008). Insufficient antioxidant control allows the accumulation of ROS thus leading to oxidative damage of a wide range of biomolecules and ultimately to necroses and cell death, contributing to seed deterioration (Kranner et al., 2010). Proteins scavenge an estimated 50–75% of reactive radicals and can retain stable markers of oxidative damage in the form of irreversible protein modifications such as carbonylation of a number of amino acids – particularly Arg, His, Lys, Pro, Thr, and Trp, formation of 3-nitroTyr and di-Tyr, protein–protein cross-linking, and a multistage oxidation of Cys thiols (Davies et al., 1999; Møller et al., 2007). Seeds generally activate their antioxidant systems on rehydration during the early stage of germination (De Gara et al., 1997; Tommasi et al., 2001; Müller et al., 2010). The ability of seeds to germinate was linked to the accumulation of a critical level of H_2O_2, since this molecule was produced at a higher level in non-dormant imbibed seeds than in dormant imbibed seeds (Oracz et al., 2007; Bailly et al., 2008). The evidence has been provided using ROS-producing and -scavenging chemicals for a key role of ROS in seed germination and dormancy alleviation (Oracz et al., 2009). However, the precise mechanisms by which ROS affect seed dormancy status and germination potential remain to be elucidated. It is also likely that ROS (Kwak et al., 2006; Finkelstein et al., 2008; Bailly et al., 2008) and/or antioxidative pathways interact with the phytohormones in controlling dormancy. Dynamic redox changes were recently found in the redox-sensitive proteome upon wheat seed dormancy release in the hard red spring-type wheat genotype RL4137 (*Triticum aestivum* L.) (Bykova et al., 2011). It was shown that during after-ripening an accumulation of ROS modifies redox status and downstream events, as well as altering protein function through oxidative modifications, which in turn might reduce levels of the dormancy-inducing hormone ABA and/or block ABA signalling, while stimulating the production of the germination-inducing hormones gibberellins (GAs) and GA signalling. Redox modification of the regulatory sulfhydryl/disulfide groups in proteins is both monitored and induced by cells, and mediates dynamic signalling integral to defence responses to environmental change. As cysteine often has catalytic or structural roles in proteins, such modifications profoundly influence protein function (Wouters et al., 2010). In cereal seeds, the regulatory sulfhydryl/disulfide bond system alters the structure and activity of proteins controlling an array of events in seed germination such as mobilization of storage proteins, activation of proteases, transcription, cell division, radical scavenging and detoxification (Montrichard et al., 2009). In addition to its functional and structural roles, cysteine is subject to several forms of oxidative post-translational modification, including reversible sulfenation (SOH), irreversible sulfination (SO_2H) or sulfonation (SO_3H), nitrosylation (SNO), disulfide formation and glutathionylation, which furnish it with the ability to serve as a regulatory switch on proteins that is responsive to the cellular redox state (Reddie and Carroll, 2008). Owing to their intrinsically high nucleophilicity and amino acid side-chain reactivity, cysteines are especially important as functional 'hotspots' in the proteome.

In this study, a differential proteomic analysis of six hybrid lines of spring wheat (*Triticum aestivum* L.) double haploid population, derived from the cross 8021-V2 (high PHS resistance, white seed coat; DePauw et al., 1992) and AC Karma (low PHS resistance, white seed coat; Knox et al., 1995) segregating transgressively for dormancy phenotype, and two parent genotypes, was used to gain further insight into biochemical mechanisms underlying dormancy controlling events. Here we characterize the proteome expressed in wheat lines varying in dormancy properties in order to determine the functional protein network and metabolic regulation associated with dormancy and germination potential. The reactive landscape of the proteome, redox active proteins with specific modification sites that undergo reversible cysteine oxidation under physiological conditions and responding differentially in dormant and non-dormant seed protein extracts, is probed by a thiol-redox proteomics approach.

2. Results and discussion

2.1. Monitoring phenotypic trait for germination resistance and differential protein expression

PHS resistance was assessed in twelve hybrid and two parent lines using germination index (Fig. 1). Previous studies have demonstrated that dormant and non-dormant fixed lines can be clearly

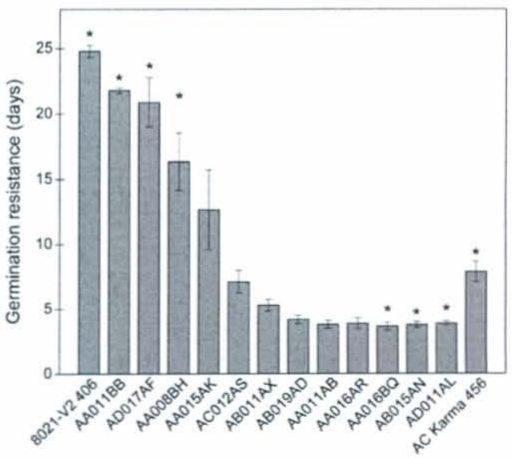

Fig. 1. Germination resistance of kernels from 12 hybrid lines of spring wheat (*Triticum aestivum* L.) double haploid population, derived from the cross 94C15/9014 × 8021-V2 (high PHS resistance, white seed coat) and AC Karma (low PHS resistance) segregating transgressively for superior levels of grain dormancy. Dormant and non-dormant parents were used as standards. Mean germination resistance values ± standard error are shown. The lines with values indicated by asterisk (*) were chosen for further redox proteomics analysis.

differentiated when grown under controlled environmental conditions and, in addition, achieve similar levels of grain dormancy to field-grown plants (Nyachiro et al., 2002; Clarke et al., 2005; Hickey et al., 2010). In our germination resistance experiments, four hybrid lines and the parent standard 8021-V2 showed consistently very high levels of grain dormancy. Six hybrid lines displayed germination characteristics that resulted in a level of dormancy even slightly lower than non-dormant donor parent AC Karma. The spring wheat hybrid lines used in this study are part of a larger population of 380 doubled haploid lines from the cross AC Karma/SC8021V2 previously characterized for expression of dormancy. There was significant bidirectional transgressive segregation in both the glasshouse and field environments (Clarke et al., 2005). Three hybrid lines (BB, AF, BH) with one parent line (8021-V2) showing highest PHS resistance, and three susceptible hybrid lines (AL, AN, BQ) with one parent line (AC Karma), a total of 8 lines (Fig. 1), were chosen as phenotypic extremes for proteomic analysis.

Three groups of proteins with differential fluorescence and protein staining characteristics could be revealed during the two-dimensional isoelectric focusing polyacrylamide gel electrophoresis (2-D IEF PAGE) comparison of the fluorescence pattern with the total protein pattern (Supporting Information Tables S1–S3). A number of protein spots with systematic differences between dormant and non-dormant genotypes were detected in the stained image but were absent in the fluorescence image indicating that these proteins do not contain any labelled Cys residues. Secondly, many protein spots were observed in the fluorescence image as well as in the stained gel, and thirdly, a few protein spots gave high fluorescent signal but were either absent or faint in the total protein image, indicating a low abundant highly labelled Cys-containing protein in this position. The fluorescence to protein ratio is a reflection of the number of labelled/reduced Cys residues in the protein sequence, and of protein expression level. Therefore, three types of differences were observed in our experiments: one type of response was due to differences in protein expression for unlabelled proteins detected by protein staining only (Supporting Information Table S1) or for protein spots with about the same level of Cys reduction/oxidation (no change in the fluorescence to protein ratio, Supporting Information Table S2); the second type of response was due to changes in both reduction/oxidation of Cys and protein expression level, where the fluorescent and protein signals showed differences but the fluorescence to protein ratio was not necessarily different between the genotypes (overlapping proteins in Supporting Information Tables S2 and S3); and the third type of response was due to the reduction/oxidation of Cys residues without prominent changes in the protein expression level (increase/decrease in fluorescence to protein ratio, Supporting Information Table S3). The thiol modification ratio was used to reflect changes in Cys redox state.

2.2. Identification of wheat proteins and assignment of modified cysteine thiols

The strategy for protein identification was based on considerable bioinformatic resources and an extensive database that is available because of the recognized economic importance of wheat. A transcriptomics resource for wheat was essential for achieving a high rate of successful protein identification by LC-MS/MS (Supporting Information Tables S1–S7). Although publicly available EST databases are rich sources of comparative sequence information, they have grown exponentially in recent years and now represent the largest collection of genetic sequences. The major drawbacks of using EST databases in high throughput functional proteomic analyses are their large size and redundancy that make both automatic Mascot searches and results interpretation very inefficient. In our approach, we created a custom database where contig sequences were constructed from wheat EST entries to reduce the size of the database (Fig. 2, Supplementary Table S7). In addition to 51% of spots matched to *Triticum aestivum* protein sequences (54 spots), 40% of spots could be uniquely identified from the wheat EST database (42 spots), 9% could be identified from both approaches (10 spots) and in most cases sets of peptides unique to protein or EST sequences were used. These findings demonstrate the utility of EST databases for proteomic profiling in plants and provide a strong argument for their use in organisms without completely sequenced genomes. Specific labelling of reduced free Cys with monobromobimane (mBBr) helped to identify and distinguish redox responding proteins even from a mixture of different proteins in one spot (Supplementary Table S5). The 2D gel approach often results in overlapping of proteins with very close p*I* values or shifted p*I* due to post-translational modifications. Several spots contained more than one protein with identified bimane-Cys residues and for these proteins the quantitative fluorescence to protein ratio is tentative only (spots 27, 28, 32, 33, 34, 52, 56 in Supporting Information Table S2, and spot 94 in Table S3). Many proteins were represented by more than one isoform, 11 proteins had two, and one protein had three isoforms. To globally characterize Cys functionality in native proteomes based on reactivity profiling we adopted a strategy for differential alkylation with either mBBr during protein extraction or with iodoacetamide prior to in-gel digestion. This

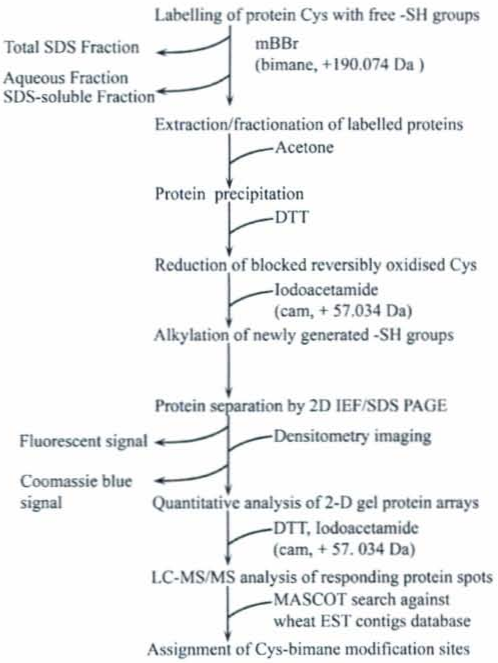

Fig. 2. Thiol redox-sensitive proteome in dormant and non-dormant genotypes of wheat were followed by fluorescent mBBr *in situ* labelling of protein cysteines with free SH groups in native seed protein extracts. After labelling, proteins were either solubilized into a total protein extract or fractionated into aqueous and SDS-soluble extracts. In addition to the bimane derivatized protein cysteines, unlabelled cysteines remained oxidised. Proteins with remaining reversibly oxidised cysteines, disulfide bonds and mixed disulfides, were completely reduced with DTT and alkylated with iodoacetamide (carbamidomethylation, CAM) prior to separation by 2D IEF/SDS PAGE as well as prior to in-gel digestion of individual protein spots.

allowed us to discriminate redox active from inactive cysteines and detect Cys residues with mixed redox modifications (Fig. 2). Therefore, in mass spectrometry (MS) analysis, all Cys residues differentially reduced at the time of protein extraction, had a covalent bimane modification (bimane, +190.074 Da monoisotopic mass), whereas non-labelled (oxidised) Cys were distinguished by carbamidomethylation (cam, +57.034 Da monoisotopic mass) modification (Fig. 2). Metastable decomposition product ions containing free cysteine (Supplementary Tables S5 and S6), a result of partial photolytic fragmentation of bimane-modified peptides was also observed during MS analysis (Petrotchenko et al., 2006). In collision induced dissociation (CID) tandem MS (MS/MS) spectra fragment y- and b-type ions provided an easily interpretable peptide sequence information with covalently bound bimane-Cys remaining intact in peptide fragmentation product ions. The presence of all three modified forms bimane-Cys, cam-Cys and Cys-SH increased confidence in the peptide sequencing and in the assignment of Cys modification sites (Supplementary Tables S5 and S6).

A total of 97 redox modified Cys were detected in 93 peptides from 64 unique proteins responding differentially in dormant and non-dormant closely related wheat genotypes. The identified cysteines with known functional roles perform important catalytic and/or regulatory functions for their parent proteins, or correspond to sites for glutathionylation, nitrosylation and disulfide formation, and therefore offer points of protein control by oxidative stress pathways.

2.3. Protein abundance differences in dormant and non-dormant lines

A set of proteins displayed quantitative differences in abundance between dry and/or imbibed for 24 h seeds of dormant and non-dormant genotypes. Among the spots that discriminated

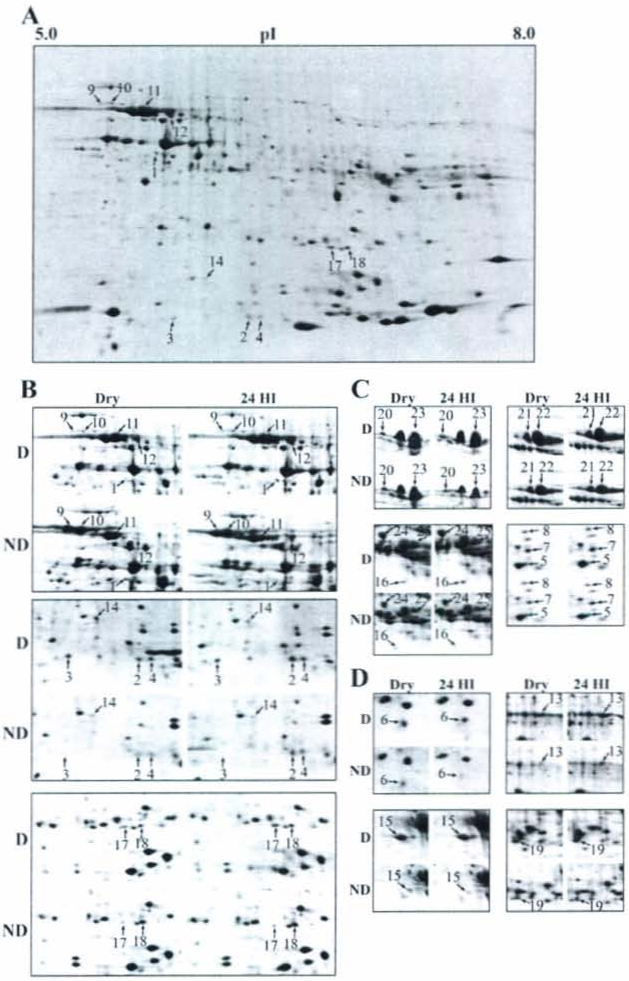

Fig. 3. Differential protein expression in mature dry and imbibed seeds of dormant and non-dormant closely related genotypes. Reference 2-DE map of aqueous protein fraction (A) extracted from dry seed material of dormant (AD017AF) hybrid line. Close-up of protein expression differences in aqueous (B), total SDS-soluble (C) protein extracts, and SDS-soluble protein fraction (D) between dry and imbibed for 24 h, 24 HI, seeds of representative dormant, D, (AD017AF) and non-dormant, ND, (AD011AL) hybrid lines. Identified protein spots with systematic abundance differences between dormant and non-dormant genotypes are marked with arrows and numbers.

the genotypes, sixteen unlabelled proteins in 25 spots were found in the protein expression maps (Supporting Information Table S1, Fig. 3A–D). Among these were one enzyme of glycolysis/gluconeogenesis (spot 1) and one TCA-cycle enzyme (in the same spot 1), four enzymes of starch metabolism (spots 2–12, Fig. 3B–D), one enzyme of amino acid metabolism (spot 13, Fig. 3D), two proteins involved in protein folding (spot 14 in Fig. 3B and spot 15 in Fig. 3D), one component of intracellular protein transport and signal transduction system (spot 16, Fig. 3C), one enzyme involved in antioxidative defence response (spots 17, 18 in Fig. 3B and spot 19 in Fig. 3C), and five storage proteins (spots 20–25, Fig. 3C). Most of these proteins (14 proteins in 22 spots) were found to be more abundant in dry seeds of dormant lines. Three proteins phosphoglycerate kinase, beta-chain of succinyl-CoA ligase, and one of the isoforms of beta amylase showed higher expression level in dry seeds of non-dormant versus dormant lines. Three identified proteins from dormant lines, mitochondrial manganese superoxide dismutase (SOD), small Ran-related GTP-binding protein, and one of the beta amylase isoforms displayed significantly increased protein abundance ratio in response to 24 h of imbibition (Supporting Information Table S1). Interestingly, two spots from aqueous fraction containing SOD protein were differentially expressed, with spot 17 higher and spot 18 slightly lower in abundance in dormant lines (Fig. 3B), indicating possible post-translational modification variants.

Ran GTPase is involved in diverse biological functions, such as nuclear transport, spindle formation during mitosis, DNA replication, and cell division. The functions of Ran signalling in nuclear transport and mitotic progression are well conserved in plants and animals and are essential for viability in every tested organism. This protein was recently demonstrated to be a direct target for $^{\cdot}NO_2$-induced cellular molecular response (Heo and Campbell, 2006; Heo, 2008). The oxidative stress-induced perturbation of the Ran-mediated nuclear import suggests that the unique structural redox architecture of Ran could be a factor in the regulation of cell signal transduction pathways associated with this protein.

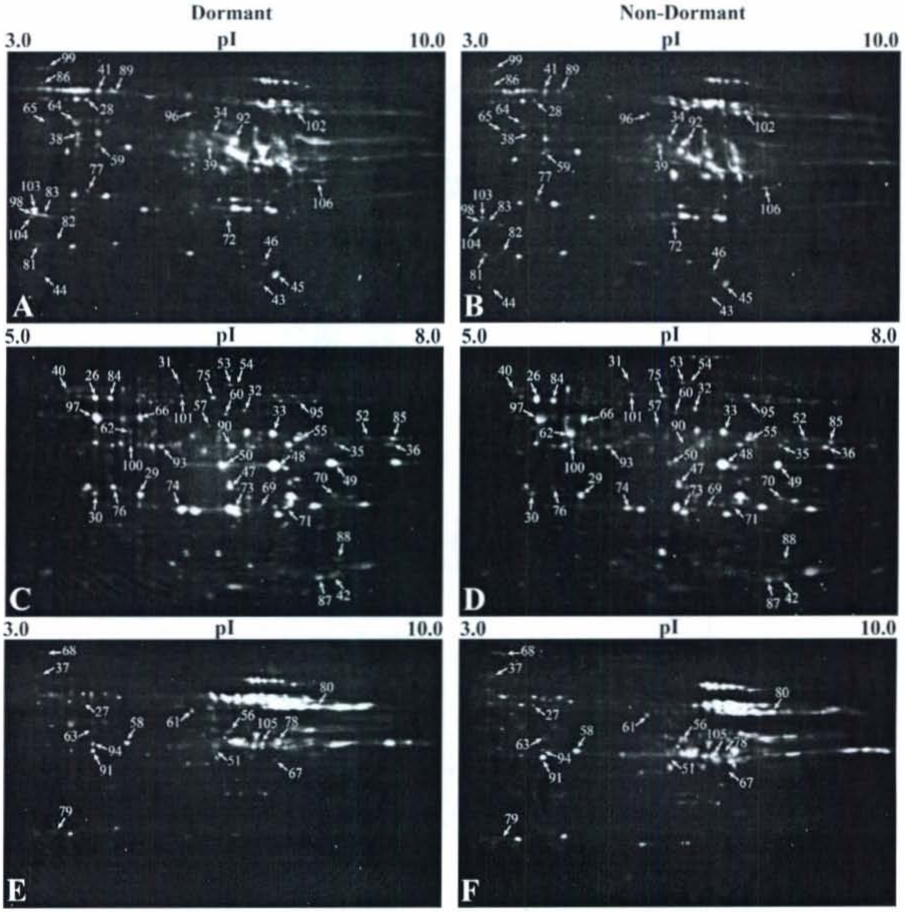

Fig. 4. Differential protein thiol reduction in mature dry seeds of dormant (A, C, E) and non-dormant (B, D, F) closely related genotypes. Representative 2-DE UV images of Cys-bimane fluorescently labelled total SDS-soluble proteins in the pH range 3–10 NL (A and B), aqueous protein extracts in the pH range 5–8 (C and D), and SDS-soluble protein fraction in the pH range 3–10 NL (E and F) from dry seed material of dormant (AD017AF) and non-dormant (AD011AL) hybrid lines. Proteins indicated by arrows and numbers were analysed by LC-MS/MS (Supporting Information Tables S2 and S3). More complete raw data sets for spot identifications are given in Supplementary Tables S4 and S5.

A strong, negative correlation was found between germination capacity and ROS, such as superoxide radical and hydrogen peroxide, as well as with lipid hydroxyperoxides (Bailly et al., 2008). After-ripening of dormant sunflower (*Helianthus annuus* L.) seeds entailed a progressive accumulation of ROS, namely superoxide anions and hydrogen peroxide, in cells of embryonic axes. This accumulation occurred concomitantly with lipid peroxidation and oxidation (carbonylation) of specific embryo proteins (Oracz et al., 2007, 2009). Manganese SOD is involved in superoxide radical detoxification in the mitochondrial matrix. In mitochondria, ROS production is generally caused by an over-reduction of the electron transport chain. In the course of seed development and germination energy-demanding processes relying on respiratory metabolism, and seed mitochondria are exposed to water stress, desiccation, imbibitions, and hypoxic conditions. A number of proteins oxidatively damaged by carbonylation (including SOD) have been identified in the mitochondrial matrix, and the level of protein carbonylation was higher in the mitochondria than in other organelles such as chloroplasts and peroxisomes (Møller et al., 2007). Noticeably, SOD qualifies for both ROS-detoxifying enzymes and enzymes that produce ROS as part of their normal catalytic cycle. Elevated expression level of SOD in highly dormant wheat lines firstly can play a protective role against oxidative stress damage during seed aging or desiccation, and secondly can be involved in regulation of a delicate balance between production and scavenging in ROS homeostasis for perception of environmental factors by seeds during dormancy maintenance.

2.4. Differential expression profiling of the dormancy-related thiol redox-sensitive proteome

A number of labelled thiol redox active proteins displayed differential expression in dormant compared to non-dormant genotypes (Supporting Information Table S2, Fig. 4). Redox responding proteins from the functional groups of carbohydrate metabolism, starch and sucrose metabolism, biosynthesis of secondary metabolites, energy and amino acid metabolism, genetic information processing and cell cycle, antioxidative defence and storage proteins were found in all three solubility fractions total SDS, aqueous and SDS-soluble of the whole seed protein extracts (Fig. 4A–F, Supporting Information Tables S2 and S5). Storage globulins as intact proteins and fragments were found in the total protein extract and in the SDS-soluble fraction (Fig. 4A, B, E and F). A set of 16 proteins identified in 25 spots displayed increased relative protein abundance ratio in dormant lines, and upon imbibition their abundance ratio either decreased or remained at the same level (Supporting Information Table S2). These represented the enzymes of glycolysis/gluconeogenesis metabolism, including triosephosphate isomerase (spot 30) and phosphoglucomutase (spot 31), alcohol dehydrogenase (DH) (spot 33), cytosolic phosphoglycerate kinase (spot 38), four alpha-amylase inhibitors (spots 42–46), glucose and ribitol DH (spots 47–51), formate DH (spots 33, 55), and protein fragments of two storage proteins 7S and 11S globulins (spots 78, 81–83). Another group of 8 proteins was more abundant after 24 h of imbibition in dormant as compared to non-dormant seeds, including one isoform of beta amylase (spots 40, 41), NADP-malic enzyme (in two spots 53 and 54), serpin Z1B (spot 64), one proteinase subunit 20S proteasome alpha type-7-A (spot 67), dehydroascorbate reductase (DHAR) (spot 74), aldehyde dehydrogenase (spot 75), embryo-specific protein (spot 77), and globulin 3 full length (spot 80) and as a fragment (spot 79).

A group of 13 proteins in 19 spots had lower relative protein abundance ratio in dry dormant seed protein extracts, and they were more expressed in non-dormant seeds. These were two enzymes of carbohydrate metabolism enolase (three spots 26, 27, and 28 in all three solubility fractions, Fig. 3) and pyruvate orthophosphate dikinase 1 (spot 37), one enzyme of starch and sucrose metabolism ADP-glucose pyrophosphorylase small subunit (spot 27), one enzyme of secondary metabolism UDP-D-glucuronate decarboxylase (spot 52), cytosolic malate DH (spots 58, 59), two enzymes of amino acid metabolism alanine aminotransferase (spots 32, 61) and aspartate aminotransferase (spots 52, 56), three serine protease inhibitors serpin-Z1A (spots 62, 63), serpin-Z2B (spot 65), and serpin-Z1C (spot 66), cell division control protein 48 (spot 68), two enzymes of antioxidative defence response 27 K thioredoxin family protein (spots 69) and aldehyde DH (spot 75). Upon imbibition of seeds from non-dormant lines a significant increase in expression level could be detected for glyceraldehyde-3-phosphate DH (spots 35, 36), NADP-specific isocitrate DH (spot 57), and serpin Z1C (spot 66). A moderate increase was observed for triosephosphate isomerase (spot 29) and phosphofructokinase (spot 32).

Some identified proteins showed differential expression in aqueous and SDS-based protein fractions indicating possible variation in their solubility. Among these were alcohol DH (spots 33, 34), mitochondrial formate DH (spots 55, 33, 56, 34), alanine aminotransferase (spots 32, 60, 61), and 27 K thioredoxin family protein (spots 71, 72). Other proteins were identified in several spots that displayed variation in protein abundance indicating possible differences in post-translational protein modification between dormant and non-dormant protein extracts. These were triosephosphate isomerase (spots 29, 30), alanine aminotransferase (spots 32, 60), and 27 K thioredoxin family protein (spots 69, 70).

2.5. Protein thiol redox modification in hybrid dormancy lines

A comparative analysis of fluorescently cysteine labelled and protein stained two-dimensional electrophoresis (2-DE) maps identified a set of 36 proteins with quantitative thiol modification differences between dormant and non-dormant genotypes (Supporting Information Table S3, Fig. 4, Supplementary Table S6). Seventeen unique proteins with 19 reactive modified cysteines were found to have differential post-translational thiol redox modification without differences in protein expression level. Among these, a significantly higher thiol modification ratio in dry seeds from dormant lines was found in O-methyltransferase ZRP4 (spot 90), serpin Z-2A (spot 98), globulin 3 full length (spot 102) and fragments (in spots 103, 104), and lipoprotein-like protein (spot 106). The most abundant in aqueous extracts of imbibed dormant seeds with prominent response were proteinaceous inhibitors of -amylases (spots 87, 88). The ~~cereal-type -amylase~~ inhibitor proteins contain five disulfide bonds and are known for their action on -amylases from birds, Baci~~lli, mammals and~~ insects. Four unique Cys residues in seven peptides (Supporting Information Tables S2 and S3) were found to be reduced in three isoforms of dimeric alpha-amylase inhibitor (spots 43–45 in Fig 4A, 87 in Fig 4B). All identified -amylase inhibitor proteins ~~were~~ shown to be putative Trx h-reducible disulfide targets in wheat and barley (Wong et al., 2004; Maeda et al., 2005). The disulfide bonds were proposed to be essential for the activity of some -amylase inhibitor proteins ~~*in vitro* (Kobrehel et al., 1991)~~. Our findings suggest that the redox mobilization of defence proteins has a specific role in the high dormancy genotypes.

Globulin storage proteins legumins (11S globulin 2) and vicilins (7S globulin 3) were highly reduced in dry dormant seeds (Supporting Information Tables S2 and S3, Fig. 4). In addition, a characteristic pattern of reduced globulin 3 fragments of two sizes about 35 kDa and 15 kDa was present in dry dormant seed protein extracts (spots 81–83, 103, 104 in Fig. 4A, Supporting Information Tables S2 and S3). Both storage proteins are synthesized as precursors that undergo molecular maturation by limited proteolysis before deposition. Similar proteinases catalyse the proteolytic

processing of these proteins and contribute to their complete breakdown during germination and seedling growth (Shutov et al., 2003). Therefore, the pattern of well-defined partial proteolytic fragments may reflect a transient synthesis or activation of one or more proteases at this stage of development. Trx was shown to reduce the major storage proteins of wheat seeds *in vitro* (Montrichard et al., 2009; Wong et al., 2004). In a recent study on the effects of endogenous and recombinant Trx induction on rice bran proteins, it was demonstrated that the activation of a cysteine protease was accompanied by unfolding of its substrate, the embryo-specific protein (ESP) (Yano and Kuroda, 2006). Similar to ESP, globulins in wheat seeds are considered to have a folded, possibly protease-resistant structure before imbibition. Meanwhile, it has been suggested that Trx is inactive before imbibition, and seed proteins are degraded in specific stages of germination through the presence of a Trx-dependent mobilization mechanism. Thus it is likely that Trx fine-tunes the complete degradation of globulin 3 storage protein in non-dormant wheat seeds during germination. This finding suggests that in dormant seeds globulins undergo a change (e.g. partial proteolysis) that renders them amenable to digestion following reduction by Trx.

A set of thiol redox-modified proteins without differences in protein expression level was more reduced in non-dormant seeds. These represented one isoform of beta-amylase (spot 86), granule-bound starch synthase (spot 89), alanine aminotransferase (spot 96), and HSP70 (spot 99). Among proteins reduced upon imbibition in non-dormant seeds were glyceraldehyde-3-phosphate DH (spot 85), thiamine biosynthetic enzyme (91), cytosolic malate DH (spot 93), mitochondrial serine hydroxymethyltransferase (spot 95), serpin-Z2A (spot 97), 2-alkenal reductase (spot 100), aldehyde DH (spot 101), and unknown function r40g2 protein (spot 105).

2.6. Impact of dormancy genotypes on functional thiol-redox proteome

Four antioxidative defence-responsive proteins with modified thiols displayed higher protein abundance level in dormant versus non-dormant seed protein extracts. Three proteins, 1-Cys peroxiredoxin (Prx), DHAR, and aldehyde DH showed elevated expression upon 24 h of imbibition. The thioredoxin family 27 K protein was found in four protein spots 69–72 containing two identified isoforms (Supporting Information Table S2, Fig. 4) with spots 70 and 71 more abundant and spots 69 and 72 less abundant in dry dormant seeds, indicating possible post-translational modification variants. 1-Cys Prx, 2-alkenal reductase, and aldehyde DH also showed a decrease in thiol reduction level in response to imbibition (Supporting Information Table S3, Fig. 4).

Prxs are ROS-scavenging enzymes that provide cells with highly efficient machinery for detoxifying H_2O_2, alkyl hydroperoxides, and OH· and were shown to prevent radical attack of lipids, enzymes, and DNA *in vitro*. Moreover, Prxs were also reported to function as redox sensors, linking the redox signalling and ROS networks of cells (Dietz, 2008). Prxs are widely distributed and can be divided into 1-Cys and 2-Cys Prx groups based on the number of conserved cysteine residues in their catalytic cycle. 1-Cys Prx is almost exclusively expressed in seeds, the protein accumulates in the nucleus of aleurone and embryo cells (Stacy et al., 1999) and exhibits antioxidant activity rather than dormancy-related function (Haslekås et al., 2003). When coupled to a thiol-reducing system, 1-Cys Prxs display peroxidase activity. Reduction of 1-Cys Prxs by Trx h was previously observed in wheat (Montrichard et al., 2009) and, in barley seeds, Trx h significantly reduced cysteine residues identical to Cys^{72} and Cys^{147} (Hägglund et al., 2008). It was proposed that the antioxidant function of 1-Cys Prx resulted from the protection of nuclear DNA in seed cells suffering oxidative stress. Our findings demonstrate that 1-Cys Prx expression level was higher in dormant dry and imbibed seeds, whereas the redox state was moderately decreased in imbibed dormant compared to imbibed non-dormant wheat seeds (Supporting Information Tables S2 and S3).

Antioxidant strategies are used by seeds to protect themselves against oxidative stress damage during desiccation and rehydration. One of the enzymes that maintains the redox status and regenerates antioxidants in their active form in the ascorbate–glutathione cycle is DHAR. Interaction between DHAR and Trx h was previously demonstrated in *A. thaliana*, wheat and barley (for review Montrichard et al., 2009). A recent quantitative proteomic study revealed that Cys^{19}, proposed catalytic residue of DHAR enzyme (Dixon et al., 2002), was extensively reduced (over 60%) by Trx h (Hägglund et al., 2008). Hence the reduction of DHAR by Trx h is likely to modulate its function. The conserved Cys^{19} forms mixed disulfides with glutathione GSSG, which preserve the enzyme from being inactivated. Such *S*-glutathionylation of enzymes protects essential cysteinyl residues from irreversible oxidation to the sulfinic acid and sulfonic acid derivatives during redox stress (Dixon et al., 2002). We found an increase in abundance of reduced DHAR form in imbibed seeds of dormant white-grained wheat genotypes versus imbibed seeds of non-dormant genotypes and this could possibly indicate that the enzyme was present in a more active state.

A moderately decreased thiol modification ratio in imbibed dormant seeds was observed for 2-alkenal reductase (spot 100 in Supporting Information Table S3, Fig. 4). Degradation of lipid peroxides leads to the formation of cytotoxic 2-alkenals and oxenes, collectively designated reactive carbonyls. The NADPH-dependent oxidoreductase 2-alkenal reductase (AER) catalyzes the reduction of the α,β-unsaturated bond of reactive carbonyls (Youn et al., 2006). Specific and irreversible protein carbonylation during seed after-ripening has been recently associated with oxidative attack and dormancy alleviation (Oracz et al., 2007). The putative AER proteins from *Triticum* shares 65% identity with At5g16970, one of 11 homologues in *Arabidopsis thaliana* (Youn et al., 2006). The identified labelled conserved Cys^{183}, Cys^{256} and Cys^{275} are located in the nucleotide binding domain of the folded At5g16970. The precise role of the Cys residues for AER function has to be further elucidated.

A major group of identified thiol redox active proteins is involved in carbohydrate, energy and amino acid metabolism. Enzymes of carbohydrate and energy metabolism were redox responding to genetic dormancy variation, including many DHs whose activity is dependent on NAD^+ or $NADP^+$ (Supporting Information Table S2). Maintaining a highly reducing internal environment in plant cells is made possible by interactions between the major soluble non-protein redox couples (NAD^+, $NADP^+$, glutathione and ascorbate). In addition, therefore, to key roles in primary and secondary metabolism, these compounds are at the center of the complex network of reactions surrounding ROS generation and control. Although their physiological functions are not limited to stress conditions, the status of each of them can influence stress responses. Local perturbation of this NAD(P)-dependent buffering system is likely an important process in the transmission of ROS signals (Noctor, 2006). Redox thiol change is one of the biochemical mechanisms through which the status of redox couples could be sensed. Many metabolic proteins identified in this study have been linked to Trx (Montrichard et al., 2009; Wong et al., 2004; Marchand et al., 2006).

One protein involved in protein degradation 20S proteasome subunit alpha 7A had higher expression level in dormant than in non-dormant seeds, and also contained redox active thiol (Supporting Information Table S2, Fig. 4). In plants protein-processing and degradation genes regulate many cellular events leading to development and division, and degrade unwanted or inhibitor proteins during cellular processes (Vierstra, 2003). A number of

proteasome subunits were shown to be potential Trx targets. Trx was shown to enhance proteolysis in cereals by its ability to reductively activate proteases and increase the solubility and proteolytic susceptibility of storage proteins on the one hand, and inactivate protease inhibitors on the other (Montrichard et al., 2009). High-level expression of this protein during imbibition of dormant seeds may affect a rapid degradation of dormancy-alleviating or germination-inducing proteins. Serine proteinase inhibitors of chymotrypsin-like enzymes displayed differential expression and thiol-reduction pattern in dormant and non-dormant genotypes. Six distinct serpins were previously identified in grains of hexaploid bread wheat but their physiological functions have not been completely elucidated (Østergaard et al., 2000). In our study, serpin-Z1B was more abundant in dormant seeds with significantly increased expression level upon imbibition, whereas serpin-Z1A, serpin-Z2B and serpin-Z1C were more abundant in non-dormant seeds (Supporting Information Table S2, Fig. 4). Two isoforms serpin-Z1A and serpin-Z1C showed elevated thiol reduction level in dormant seeds upon imbibition, and one isoform serpin-Z2A was found to be significantly more reduced in dry and imbibed dormant seeds without systematic differences in protein expression between dormant and non-dormant genotypes (Supporting Information Table S3, Fig. 4). Other two isoforms serpin-Z2A and serpin-Z1B displayed higher thiol reduction level in non-dormant seeds. This possibly indicates differential functional role of serpin isoform variants. Plant serpins are likely to use their irreversible mechanism in the inhibition of endogenous and exogenous proteinases capable of breaking down seed storage prolamins, such as digestive proteinases of insect pests or fungal pathogens (Østergaard et al., 2000). The role of oxidative changes in serpins during dormancy control remains to be investigated.

3. Concluding remarks

The results demonstrate that harvest-ripe grains of closely related genotypes of wheat with either a dormant or a non-dormant phenotype, differentially express many proteins in the metabolism, genetic and environmental information processing, antioxidative defence response and storage proteins (Fig. 5). We demonstrate that in non-dormant seeds, thiol redox changes in proteins were associated with conversion to an active state, thereby facilitating the mobilization of nitrogen and carbon for germination and developing seedling. In dormant seeds, there was a biochemical shift in the accumulation of proteins from those active in biosynthesis and metabolism to those with roles in storage and protection against biotic and abiotic stresses. We observed in imbibed dormant seeds higher abundance of antioxidant proteins and enzymes important for redox control, ROS scavenging and detoxification. The proteomic data obtained provide evidence for an increased capacity of potent antioxidant machinery in seeds of high non-deep physiological dormancy genotypes, which could be coupled with their ability to rapidly regenerate antioxidant systems upon rehydration cycles for dormancy maintenance. Approximately 83% of the proteins identified in this study, 44 out of 53 redox active, have been shown *in vitro* to be potential or established Trx targets in land plants (Montrichard et al., 2009). Nine redox sensitive proteins were not previously reported as potential Trx targets (shown in bold in Supporting Information Tables S2 and S3). The results presented here support the hypothesis that antioxidative defence mechanisms could be involved in imposing dormancy. This study is a further step toward a more comprehensive analysis of the genetic and biochemical endodormancy control in wheat. In order to identify low abundance proteins alternative gel-free mass spectrometry-based quantitative approaches in conjunction with different liquid chromatography methods will need to be employed. Further research in this area will be informative for analysing the effect of the environmental conditions on dormancy controlling events to provide molecular fingerprints for dormancy and PHS resistance.

4. Experimental

4.1. Plant material, seed sampling procedure and germination assay

Hybrid lines of spring wheat (*Triticum aestivum* L.) double haploid population, derived from the cross 94C15/9014 = 8021-V2 (high PHS resistance, white seed coat) and AC Karma (low PHS resistance) segregating transgressively were used throughout. Two parent and 12 hybrid lines from Agriculture and Agri-Food Canada breeding program based in Semiarid Prairie Agricultural Research Centre were used as sources of white-grained dormancy in this study. Plants were grown in a climate-controlled greenhouse at 15 °C with 16 h day-light at 50% relative humidity. The temperature was raised to 18 °C at 8 weeks after seeding and to 21 °C at 10 weeks after seeding. Natural light was supplemented with 100 W high-pressure sodium lamps. During watering, fertilizer (Plant-Prod 20–20–20, 500 ml of 15 g/L per pot per day, 1 plant per pot) was applied. Heads on the primary and secondary tiller were harvested at physiological maturity, the stage of critical importance for imposing maximum dormancy (Nyachiro et al.,

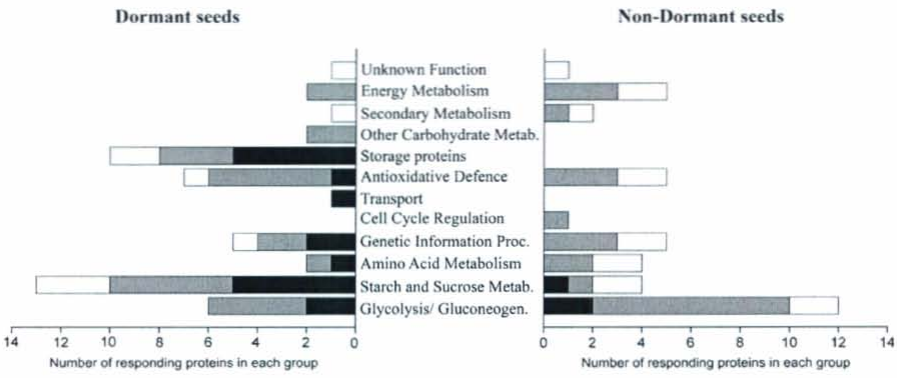

Fig. 5. Functional distribution of unlabelled differentially expressed (black bars), thiol-labelled differentially expressed (grey bars) and thiol redox-sensitive (white bars) seed proteome in dormant and non-dormant hybrid genotypes of wheat.

2002). The harvested heads were immediately dried in a forced air oven at 35 °C for 5 days to decrease the grain moisture content to about 12% and immediately stored at −20 °C until further use. The dormancy level retained was assessed using a germination test as follows. Seeds were placed crease facing down into a Petri dish with filter paper hydrated with 2.5% oxine benzoate fungicide, and incubated at 15 °C and 40% relative humidity in the dark for 21 days. Plates were examined daily, and seeds with radicals and signs of visible pericarp rupture were counted as germinated and removed. On day 21, ungerminated seeds were treated for 1 h with 0.5 mM GA_3 in water, and daily counts were continued for another 5 days to test seed viability. A germination resistance index (GR) was calculated as described (Gordon, 1971); this calculates time at which half the seed germinated.

4.2. Labelling of proteins with mBBr, extraction and fractionation

Proteins were extracted from dry intact seeds and after 24 h of imbibition. The thiolyte monobromobimane (mBBr, Calbiochem, San Diego, CA) labelling of redox reactive available –SH groups was performed simultaneously with protein extraction (Fig. 2, labelling scheme). For total SDS-soluble protein extraction, 20 seeds (600–800 mg FW) were ground in liquid nitrogen to a fine powder with 7 mL 63 mM Tris–HCl, pH 6.8 buffer containing 2% (w/v) SDS, 0.25 mM mBBr (100 mM stock solution in ACN) and protease inhibitor cocktail added directly to the pre-chilled mortar (Rhazi et al., 2003). Samples were vortexed for 30 min at room temperature and incubated at 60 °C for 2 h with occasional vortexing. After centrifugation at 16,000g for 30 min at 22 °C, the supernatants were collected, aliquoted, stored at −80 °C and used further as the total protein extracts. For aqueous protein extracts, 20 seeds were ground in liquid nitrogen to a fine powder with 5 mM Tris–HCl, pH 7.5 buffer containing 1 mM $CaCl_2$, 0.25 mM mBBr, and protease inhibitor cocktail, using a ratio 1 g sample/7 mL buffer. The mixture was transferred to a tube and vortexed for 30 min at 4 °C followed by centrifugation at 16,000g for 25 min at 4 °C. The collected supernatant was used as a fraction containing water soluble proteins. The pellet was resuspended with 5 mM Tris–HCl, pH 7.5 containing 50% (v/v) propan-1-ol, 0.25 mM mBBr and protease inhibitor cocktail, vortexed for 1 h at room temperature and centrifuged again at 16,000g for 25 min at 20 °C. The supernatant was collected and the pellet was washed in the same buffer but containing 70% (v/v) propan-1-ol without label. After centrifugation the supernatant was combined with the 50% propan-1-ol supernatant. The propan-1-ol soluble fraction was discarded due to the lack of detectable differences in gliadin and glutenin storage proteins. The pellet was resuspended with 63 mM Tris–HCl, pH 6.8 containing 2% SDS and protease inhibitor cocktail, without label, and vortexed for 30 min at room temperature. The protein extraction was continued at 60 °C for 2 h with occasional vortexing followed by centrifugation at 16,000g for 30 min at 20 °C. Supernatant was collected and used as SDS-soluble protein fraction. Protein concentrations were determined using a Bradford dye-binding assay (Bio-Rad Laboratories) with BSA as a standard, and fractions were aliquoted and stored at −80 °C.

4.3. Two-dimensional gel electrophoresis

Prior to 2-D PAGE separation, proteins were precipitated and washed with 8x sample volume of ice-cold acetone at a final concentration of 80% (v/v) acetone and 0.07% (w/v) DTT. For the fractions containing SDS, samples were first diluted with 2% (w/v) CHAPS solution to give a dilution of SDS in the sample from 2% to 0.25% (w/v) with a ratio of 8:1 of CHAPS to SDS, and vortexed occasionally for 1 h before being precipitated and washed as described above. The final protein precipitates were dried under nitrogen gas and stored at −80 °C.

For 2-D IEF PAGE, proteins (800 μg) in dried acetone powder were dissolved in 500 μl of IEF rehydration solution containing in the case of water soluble protein fraction 7 M urea, 2 M thiourea, 50 mM dithiothreitol (DTT), 4% (w/v) CHAPS, 1% (w/v) ampholyte (Biolyte 3–10, Bio-Rad Laboratory, Mississauga, ON, Canada), and 0.002% (w/v) bromophenol blue. The SDS-soluble total protein fractions were solubilized in 5 M urea, 2 M thiourea, 50 mM DTT, 2% (w/v) CHAPS, 2% (w/v) sulfobetaine 3–10 (SB 3–10), 1% (w/v) ampholyte, and 0.002% (w/v) bromophenol blue. Proteins were centrifuged at 100,000g for 30 min prior to rehydration of IPG strips overnight. IEF was conducted with the Ettan IPGphor II system and Manifold tray (GE Healthcare, Piscataway, NJ, USA) using 24-cm ReadyStrip IPG strips according to the manufacturer's instructions (Berkelman and Stenstedt, 1998). The strips were focused for a total of 100 kVh. The second dimension Tris–glycine SDS PAGE was carried out using gradient 10–20% acrylamide gels with 4% stacking gels as described (Laemmli, 1970) using an Ettan DALT six (GE Healthcare, Piscataway, NJ, USA) apparatus. Analysis of different *pI* ranges for 2D gels demonstrated that the optimal resolution and coverage of proteins from the SDS-soluble extracts were obtained from IPG strips with non-linear pH gradient 3–10 and for the aqueous fraction with linear pH gradient range 5–8.

4.4. Visualization of protein thiol modifications and image analysis

After electrophoresis, gels were fixed in 12.5% (w/v) TCA for 2 h, transferred to 40% (v/v) methanol, 10% (v/v) acetic acid for 4 h or overnight to reduce the background. The fluorescence images of mBBr-derivatized proteins were visualized with a UV light source (365 nm). As the reaction between mBBr and –SH groups is stoichiometric, rapid and complete (O'Keefe, 1994), the intensity of fluorescent spots and bands is proportional to the number of available –SH groups in the protein. The extent of protein reduction was quantified using densitometry by video imaging with Quantity One software (Bio-Rad). The total protein content was further detected by staining overnight with 0.15% (w/v) Coomassie Blue R250 in 50% (v/v) ethanol, 7% (v/v) acetic acid; destained for 1 h with 25% (v/v) ethanol, 7% (v/v) acetic acid. Normalized conditions were used throughout with respect to protein extraction, labelling and loading on the gels, fluorescent signal detection and Coomassie staining visualization.

The fluorescence images and the images of the total protein pattern were analysed for the normalized spot intensities. The protein extractions/fractionations were replicated two times (biological replicates), and 2–3 gel run replicates per extracted protein sample were performed. The ratio of fluorescence intensity to protein level was determined by dividing the intensity of a protein spot on the fluorescence image by the intensity of the corresponding Coomassie-stained protein spot. The fluorescence to protein ratio is a reflection of the number of labelled/reduced Cys residues in the protein sequence, and of protein expression level. For the same protein spots of replicate gel images, a mean ratio of the protein abundance (Supporting Information Tables S1 and S2) or the ratio of fluorescence signal intensity (Supporting Information Table S2), or fluorescence/protein ratio (Supporting Information Table S3) was calculated and included if the standard deviation was less than 30%. In order to evaluate the redox state of thiols in proteins from dormant dry seeds and after imbibition, the thiol modification ratio of a protein spot (Supporting Information Table S3) was calculated by dividing the mean fluorescence/protein ratio of the dormant samples by the mean fluorescence/protein ratio of the non-dormant samples (Wolf et al., 2008).

4.5. Statistical analysis

Results were analysed with Origin data analysis and graphing software v8.0.63.988 (OriginLab Corporation, Northampton, MA, USA). Only protein spots that showed consistent differences in two biological replicates were considered for quantitative and statistical analysis, thus preventing the assignment of normalized volume values to missing spots. Another issue related to proteomics data is the correlation between spot normalized volume and spot variance, described in some proteomic studies (Valledor et al., 2008), which means that the higher the mean intensity of a spot, the higher the variance, this being explained by a scale phenomenon related to data acquisition (Gustafsson et al., 2004). To reduce this variance-mean dependence between different spot intensities and sample sets, spots were considered to be variable if they showed statistically significant quantitative differences according to the one-away ANOVA test. *P*-values of 0.05 or below were calculated for the majority of protein abundance ratios, fluorescence signal intensity and thiol modification ratios of 1.5 or higher. Therefore, redox thiol modification for a specific protein spot was considered to be significantly increased, when the thiol modification ratio was $\geqslant 1.5$, or significantly decreased, when the thiol modification ratio was $\leqslant 0.67$. Only protein spots with *P*-value of 0.05 or below are discussed in the manuscript. In the analysis of variance, it is assumed that different samples have equal variances, which is commonly called homogeneity of variance. The Levene test and Brown–Forsythe test as part of the one-way ANOVA algorithms were used to verify the assumption. The ratios with low variance (high statistically significant homogeneity) are highlighted in bold (Supporting Information Tables S1–S3).

4.6. LC-MS/MS identification of mBBr labelled proteins

Protein spots were excised from 2-D gels, washed with 100 mM NH_4HCO_3, reduced with 10 mM DTT for 45 min at 56 °C, alkylated with 55 mM iodoacetamide for 30 min in the dark at room temperature, washed again with 100 mM NH_4HCO_3, and digested overnight at 37 °C with modified trypsin (Promega, sequencing-grade). Tryptic peptides were extracted from the gel as previously described (Rampitsch et al., 2006). Automated nano-flow LC-MS/MS analysis of peptide digests was performed using a linear ion trap Finnigan LTQ (Thermo Finnigan, San Jose, CA, USA) mass spectrometer connected on-line with nano-HPLC (Dionex UltiMate™ 3000) essentially as previously described (Bykova et al., 2011). Briefly, chromatographic separation was accomplished with a 17 cm reversed-phase nano-column (75 μm ID, 360 μm OD; packed in-house with Vydac C18, 5 μm bead and 300 Å pore size resin) with flow rate delivered at 250 nl/min and peptide elution using a linear gradient of 4–40% (v/v) ACN for 40 min, followed by a short gradient 40–80% (v/v) ACN for 3 min, and 80% (v/v) ACN for 2 min in 1% (v/v) formic acid, 0.5% (v/v) acetic acid. The analytical column was directly connected to a distally coated, fused silica emitter (New Objective, Cambridge, MA, USA) (360 μm OD/20 μm ID/10 μm tip ID) biased to 1.8 kV. The mass spectrometer was operated in the positive ion mode with source temperature 200 °C, and was tuned in nano-spray mode using 10 μM (Glu)-Fibrinopeptide B (GluFib) singly charged ion at *m/z* 1552.67. Data-dependent analysis was employed with one MS *m/z* range 450–2000 and MS/MS of five most abundant ions in each cycle, 20 s dynamic exclusion.

4.7. Database searching, protein sequence analysis and bimane-Cys assignment

The LC-MS/MS data were interpreted using MASCOT v. 2.1.01 search engine (Matrix Science, UK) first with the NCBInr protein database (Viridiplantae taxonomy, 6573034 sequences) followed by querying 1 361 178 wheat EST sequences (in-house database). The Finnigan Xcalibur (LTQ) raw data were converted into the DTA format and used for protein identification and modification screening.

The bimane-Cys was incorporated into MASCOT and used as variable modification for automated analysis. The following parameters were used for database search: (1) trypsin as digestion enzyme with maximum one missed cleavage; (2) monoisotopic peptide masses were used; (3) the peptide mass tolerance at 1 Da; and the fragment ions mass tolerance at 0.5 Da for LTQ nano-flow LC-MS/MS; (4) variable modifications bimane (C), carbamidomethyl (C), deamidation (NQ), oxidation (M); (5) peptide charge states +1, +2 and +3. The bimane modification was added to the MASCOT list of variable modifications with elemental composition C(10) H(10) N(2) O(2) and mass average/monoisotopic 190.2016/190.0742 for covalent bimane adduct to Cys residue. Only *Triticum aestivum* protein and EST complementary sequence matches were used for identification of proteins and their post translational modifications. A single peptide probability of identification MOWSE score greater than 52–55 indicated identity using the EST database. Peptide matches indicating identity or extensive homology were considered for confident protein identification. Providing that the protein had at least 1–3 confidently identified peptides, peptide matches with bimane-Cys modification were manually verified using the GPMAW 7.0 (Lighthouse Data, Odense, Denmark) software.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2010.12.021.

References

Bailly, C., El-Maarouf-Bouteau, H., Corbineau, F., 2008. From intracellular signalling networks to cell death: the dual role of reactive oxygen species in seed physiology. C.R. Biol. 331, 806–814.

Berkelman, T., Stenstedt, T., 1998. 2-D Electrophoresis using Immobilized pH Gradients: Principles and Methods. Amersham Pharmacia Biotech, Piscataway, NJ. pp. 17–46.

Bewley, J.D., 1997. Seed germination and dormancy. Plant Cell 9, 1055–1066.

Bykova, N.V., Hoehn, B., Rampitsch, C., Banks, T., Stebbing, J.-A., Fan, T., Knox, R., 2011. Redox-sensitive proteome and antioxidant strategies in wheat seed dormancy control. Proteomics, doi:10.1002/pmic.200900810.

Caliskan, M., Cuming, A.C., 1998. Spatial specificity of H_2O_2-generating oxalate oxidase gene expression during wheat embryo germination. Plant J. 15, 165–171.

Clarke, F.R., Knox, R.E., DePauw, R.M., 2005. Expression of dormancy in a spring wheat cross grown in field and controlled environment conditions. Euphytica 143, 297–300.

Colville, L., Kranner, I., 2010. Desiccation tolerant plants as model systems to study redox regulation of protein thiols. Plant Growth Regul. 62, 241–255.

Davies, M.J., Fu, S., Wang, H., Dean, R.T., 1999. Stable markers of oxidant damage to proteins and their application in study of human disease. Free Radic. Biol. Med. 27, 1151–1161.

DePauw, R.M., McCaig, T.N., Clarke, J.M., McLeod, J.G., Knox, R.E., Fernandez, M.R., 1992. Registration of sprouting-resistant white-kerneled wheat germplasms SC8019R1 and SC8021V2. Crop Sci. 32, 838.

Dietz, K.-J., 2008. Redox signal integration: from stimulus to networks and genes. Physiol. Plant. 133, 459–468.

Dixon, D.P., Davis, B.G., Edwards, R., 2002. Functional divergence in the glutathione transferase superfamily in plants. Identification of two classes with putative functions in redox homeostasis in *Arabidopsis thaliana*. J. Biol. Chem. 277, 30859–30869.

De Gara, L., de Pinto, M.C., Arrigoni, O., 1997. Ascorbate synthesis and ascorbate peroxidase activity during the early stage of wheat germination. Physiol. Plant. 100, 894–900.
El-Maarouf-Bouteau, H., Bailly, C., 2008. Oxidative signalling in seed germination and dormancy. Plant Signal Behav. 3, 175–182.
Finch-Savage, W.E., Leubner-Metzger, G., 2006. Seed dormancy and the control of germination. New Phytol. 171, 501–523.
Finkelstein, R., Reeves, W., Ariizumi, T., Steber, C., 2008. Molecular aspects of seed dormancy. Annu. Rev. Plant Biol. 59, 387–415.
Flintham, J.E., 2000. Different genetic components control coat-imposed and embryo-imposed dormancy in wheat. Seed. Sci. Res. 10, 43–50.
Fofana, B., Humphreys, G., Rasul, G., Cloutier, S., Somers, D., 2008. Assessment of molecular diversity at QTLs for preharvest sprouting resistance in wheat using microsatellite markers. Genome 51, 375–386.
Gordon, A.G., 1971. The germination resistance test – a new test for measuring germination quality of cereals. Can. J. Plant Sci. 51, 181–183.
Gubler, F., Millar, A.A., Jacobsen, J.V., 2005. Dormancy release, ABA and pre-harvest sprouting. Curr. Opin. Plant Biol. 8, 183–187.
Gustafsson, J.S., Robert, C., Glasbey, C.A., Blomberg, A., Rudemo, M., 2004. Statistical exploration of variation in quantitative two-dimensional gel electrophoresis data. Proteomics 4, 3791–3799.
Haslekås, C., Viken, M.K., Grini, P.E., Nygaard, V., Nordgard, S.H., Meza, T.J., Aalen, R.B., 2003. Seed 1-cysteine peroxiredoxin antioxidants are not involved in dormancy, but contribute to inhibition of germination during stress. Plant Physiol. 133, 1148–1157.
Hägglund, P., Bunkenborg, J., Maeda, K., Svensson, B., 2008. Identification of thioredoxin disulfide targets using a quantitative proteomics approach based on isotope-coded affinity tags. J. Proteome Res. 7, 5270–5276.
Heo, J., Campbell, S.L., 2006. Ras regulation by reactive oxygen and nitrogen species. Biochemistry 45, 2200–2210.
Heo, J., 2008. Redox regulation of Ran GTPase. Biochem. Biophys. Res. Commun. 376, 568–572.
Hickey, L.T., Dieters, M.J., DeLacy, I.H., Christopher, M.J., Kravchuk, O.Y., Banks, P.M., 2010. Screening for grain dormancy in segregating generations of dormant × non-dormant crosses in white-grained wheat (*Triticum aestivum* L.). Euphytica 172, 183–195.
Knox, R.E., DePauw, R.M., McCaig, T.N., Clarke, J.M., McLeod, J.G., Fernandez, M.R., 1995. AC Karma white spring wheat. Can. J. Plant Sci. 75, 899–901.
Kobrehel, K., Yee, B.C., Buchanan, B.B., 1991. Role of the NADP/thioredoxin system in the reduction of alpha-amylase and trypsin inhibitor proteins. J. Biol. Chem. 266, 16135–16140.
Kranner, I., Minibayeva, F.V., Beckett, R.P., Seal, C.E., 2010. What is stress? Concepts, definitions and applications in seed science. New Phytol. 188, 655–673.
Kwak, J.M., Nguyen, V., Schroeder, J.I., 2006. The role of reactive oxygen species in hormonal responses. Plant Physiol. 141, 323–329.
Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.
Maeda, K., Finnie, C., Svensson, B., 2005. Identification of thioredoxin h-reducible disulphides in proteomes by differential labelling of cysteines: insight into recognition and regulation of proteins in barley seeds by thioredoxin h. Proteomics 5, 1634–1644.
Marchand, C., Le Maréchal, P., Meyer, Y., Decottignies, P., 2006. Comparative proteomic approaches for the isolation of proteins interacting with thioredoxin. Proteomics 6, 6528–6537.
Mares, D., Mrva, K., Cheong, J., Williams, K., Watson, B., Storlie, E., Sutherland, M., Zou, Y., 2005. A QTL located on chromosome 4A associated with dormancy in white- and red-grained wheats of diverse origin. Theor. Appl. Genet. 111, 1357–1364.
McCaig, T.N., Depauw, R.M., 1992. Breeding for pre-harvest sprouting tolerance in white seed colour spring wheat. Crop Sci. 32, 19–23.
Montrichard, F., Alkhalfioui, F., Yano, H., Vensel, W.H., Hurkman, W.J., Buchanan, B.B., 2009. Thioredoxin targets in plants: the first 30 years. J. Proteomics 72, 452–474.
Mori, M., Uchino, N., Chono, M., Kato, K., Miura, H., 2005. Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect. Theor. Appl. Genet. 110, 1315–1323.
Møller, I.M., Jensen, P.E., Hansson, A., 2007. Oxidative modifications to cellular components in plants. Ann. Rev. Plant Biol. 58, 459–481.
Müller, K., Job, C., Belghazi, M., Job, D., Leubner-Metzger, G., 2010. Proteomics reveal tissue-specific features of the cress (*Lepidium sativum* L.) endosperm cap proteome and its hormone-induced changes during seed germination. Proteomics 10, 406–416.
Noctor, G., 2006. Metabolic signalling in defence and stress: the central roles of soluble redox couples. Plant Cell Environ. 29, 409–425.
Nyachiro, J.M., Clarke, F.R., DePauw, R.M., Knox, R.E., Armstrong, K.C., 2002. Temperature effects on seed germination and expression of seed dormancy in wheat. Euphytica 126, 123–127.
Oracz, K., El-Maarouf Bouteau, H., Farrant, J.M., Cooper, K., Belghazi, M., Job, C., Job, D., Corbineau, F., Bailly, C., 2007. ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. Plant J. 50, 452–465.
Oracz, K., El-Maarouf-Bouteau, H., Kranner, I., Bogatek, R., Corbineau, F., Bailly, C., 2009. The mechanisms involved in seed dormancy alleviation by hydrogen cyanide unravel the role of reactive oxygen species as key factors of cellular signalling during germination. Plant Physiol. 150, 494–505.
O'Keefe, D.O., 1994. Quantitative electrophoretic analysis of proteins labeled with monobromobimane. Anal. Biochem. 222, 86–94.
Østergaard, H., Rasmussen, S.K., Roberts, T.H., Hejgaard, J., 2000. Inhibitory serpins from wheat grain with reactive centers resembling glutamine-rich repeats of prolamin storage proteins. Cloning and characterization of five major molecular forms. J. Biol. Chem. 275, 33272–33279.
Petrotchenko, E.V., Pasek, D., Elms, P., Dokholyan, N.V., Meissner, G., Borchers, C.H., 2006. Combining fluorescence detection and mass spectrometric analysis for comprehensive and quantitative analysis of redox-sensitive cysteines in native membrane proteins. Anal. Chem. 78, 7959–7966.
Rampitsch, C., Bykova, N.V., McCallum, B., Beimcik, E., Ens, W., 2006. Analysis of the wheat and *Puccinia triticina* (leaf rust) proteomes during a susceptible host–pathogen interaction. Proteomics 6, 1897–1907.
Rhazi, L., Cazalis, R., Aussenac, T., 2003. Sulfhydryl-disulfide changes in storage proteins of developing wheat grain: influence on the SDS-unextractable glutenin polymer formation. J. Cereal. Sci. 38, 3–13.
Reddie, K.G., Carroll, K.S., 2008. Expanding the functional diversity of proteins through cysteine oxidation. Curr. Opin. Chem. Biol. 12, 746–754.
Stacy, R.A.P., Nordeng, T.W., Culiáñez-Maciá, F.A., Aalen, R., 1999. The dormancy-related peroxiredoxin anti-oxidant, PER1, is localized to the nucleus of barley embryo and aleurone cells. Plant J. 19, 1–8.
Shutov, A.D., Bäumlein, H., Blattner, F.R., Müntz, K., 2003. Storage and mobilization as antagonistic functional constraints on seed storage globulin evolution. J. Exp. Bot. 54, 1645–1654.
Tommasi, F., Paciolla, C., de Pinto, M.C., De Gara, L., 2001. A comparative study of glutathione and ascorbate metabolism during germination of *Pinus pinea* L. Seeds. J. Exp. Bot. 52, 1647–1654.
Torada, A., Koike, M., Ikeguchi, S., Tsutsui, I., 2008. Mapping of a major locus controlling seed dormancy using backcrossed progenies in wheat (*Triticum aestivum* L.). Genome 51, 426–432.
Valledor, L., Castillejo, M.A., Lenz, C., Rodríguez, R., Cañal, M.J., Jorrín, J., 2008. Proteomic analysis of *Pinus radiata* needles: 2-DE map and protein identification by LC/MS/MS and substitution-tolerant database searching. J. Proteome Res. 7, 2616–2631.
Vierstra, R.D., 2003. The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. Trends Plant Sci. 8, 135–142.
Wojtyla, Ł., Garnczarska, M., Zalewski, T., Bednarski, W., Ratajczak, L., Jurga, S., 2006. A comparative study of water distribution, free radical production and activation of antioxidative metabolism in germinating pea seeds. J. Plant Physiol. 163, 1207–1220.
Wolf, C., Hochgräfe, F., Kusch, H., Albrecht, D., Hecker, M., Engelmann, S., 2008. Proteomic analysis of antioxidant strategies of *Staphylococcus aureus*: diverse responses to different oxidants. Proteomics 8, 3139–3153.
Wong, J.H., Cai, N., Balmer, Y., Tanaka, C.K., Vensel, W.H., Hurkman, W.J., Buchanan, B.B., 2004. Thioredoxin targets of developing wheat seeds identified by complementary proteomic approaches. Phytochemistry 65, 1629–1640.
Wouters, M.A., Fan, S.W., Haworth, N.L., 2010. Disulfides as redox switches: from molecular mechanisms to functional significance. Antioxid. Redox Signal. 12, 53–91.
Yano, H., Kuroda, M., 2006. Disulfide proteome yields a detailed understanding of redox regulations: a model study of thioredoxin-linked reactions in seed germination. Proteomics 6, 294–300.
Youn, B., Kim, S.J., Moinuddin, S.G., Lee, C., Bedgar, D.L., Harper, A.R., Davin, L.B., Lewis, N.G., Kang, C., 2006. Mechanistic and structural studies of apoform, binary, and ternary complexes of the Arabidopsis alkenal double bond reductase At5g16970. J. Biol. Chem. 281, 40076–40088.

Thiol redox-sensitive seed proteome in dormant and non-dormant hybrid genotypes of wheat

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The thiol redox-sensitive and the total proteome in closely related dormant and non-dormant genotypes of wheat were monitored by 2D-gel electrophoresis, fluorescent cysteine labelling, and mass spectrometry analysis.