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Full Length Research Paper

Proteomics-based dissection of biotic stress responsive proteins in bread wheat (*Triticum aestivum* L.)

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Proteomic techniques that allow the identification and quantification of stress-related proteins, mapping dynamics of their expression and post translational modifications represent an important approach in the research of plant stresses. Biotic stress is one of the major stresses limiting crop productivity and the geographical distribution of many important crops worldwide. Two hundred and seventeen protein spots reproducibly were detected from six gels by using two-dimensional electrophoresis. After tryptic digestion, MALDI-TOF/MS analysis and database searching of some of the identified proteins indicated that the proteins are known to be involved in several biotic stress related functions as disease associate with pathogens. Mass spectrometry analysis allowed the identification of 185 differential expressed proteins with isoforms including well known biotic stress responsive proteins. Keumgang (13%), Jinpum (8%), China-108 (14%), Yeonnon-67 (11%), Norin-61 (22%) and Kantou-107 (32%) were identified as biotic stress responses proteins directly coupled to disease and pathogen infection on wheat. Nevertheless, our studies provides new insights into identification of biotic stress responses protein in disease infected wheat grain by natural condition, the post-translational modification in protein sequences, verify eventual differences among the genotypes in relation to them, and demonstrates the advantages of proteomic analysis.

Key words: Biotic stress, matrix-assisted laser desorption ionization-time of flight, proteomics, post-translational modification, two-dimensional electrophoresis, wheat.

INTRODUCTION

Plants responding to biotic stresses produce several protective compounds and proteins such as pathogenesis related (PR) proteins, directly disease related proteins and other proteins. Biotic is one of the serious stresses affecting plant growth and productivity. A clear perceptive of the molecular mechanisms involved in plants response to biotic stress is of fundamental importance to plant science. Knowledge about these mechanisms is also critical for continued development of rational breeding and transgenic strategies to improve stress tolerance in cereal crops. Proteomic approach has become a poweful tool to study plant responses to biotic and abiotic stress (Veeranagmallaiah et al., 2008). The diseased grains have poor quality and are contaminated with mycotoxins, which are not suitable for consumption by both humans and animals (McMullen et al., 1997). Host resistance is considered the most practical means to control this disease (Martin and Johnston, 1982).

The study of PR-proteins is also important for crop production due to the fact that many plant-derived pathogen related proteins have been identified as members of PR-protein families 2, 3, 4, 5, 8, 10 and 14 (Hoffmann-Sommergruber, 2002). Among these are pathogenesis

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related proteins coded by the host plant that accumulate in response to pathogen infection or other signals related to plant defense responses. Several PR proteins have been characterised at the molecular level and have shown to have antifungal activity *in vitro* (Datta and Muthukrishnan, 1999), and show enzymatic activity such as β-1, 3-glucanase and chitinase (PR2 and PR3, respectively), both involved in the degradation of microbial cell wall structural polysaccharides (Legrand et al., 1987) and PR4 and PR9, characterised by ribonuclease and peroxidase activity, respectively (Caporale et al., 2004). No enzymatic activity has been found up to now for proteins belonging to PR1 and PR5 families; nonetheless several genes belonging to these classes have been over expressed in transgenic plants strengthening the defensive role proposed for the consequent proteins (Liu et al., 1993). Biotic stresses, such as bacterial, fungal, algal and viral diseases, can also cause biotic stress in grain crops. Crops have improved habitat chracteristics against biotic and abiotic stress. At this instant, there is a good amount of work to answer about the types of proteins under- and/or over expressed during a particular or integrative stress, their impacts on cellular metabolism and the location of the proteins. Additionally, microarray studies cannot provide information about either the subcellular localization or the protein post-translational modifications (PTMs) that may be essential for its function, transport and activation (Gygi et al., 1999). PTMs and interactomics, the real thermometer of the proteomics status in a field, still remains a major challenge. In the case of biotic stress, since they interact with other cell proteins, to maintain proteins in their functional conformations, or prevent aggregation of non-native proteins and refold denatured proteins to regain their functional conformation, it is expected that proteins-protein interaction plays a main role in abiotic and biotic-stress proteomics research. Regarding the PTM research, which is indicative of the final players in most abiotic and biotic responses, it is limited to phosphorrylation in Arabidopsis (Rossignol et al., 2006). In the future, we will think about more studies that include metabolomics as an integral part of the systems biology approach to study plant response to a variety of stress conditions. Progress in these directions will lead to the modeling of entire metabolic pathways in the coming years and thus usher in an era of predictive biology. This will represent a giant step for biotechnology, allowing it to contribute significantly to the design of genetic solutions to the ever-present threats of biotic and abiotic stress. Surely, integrating data from transcriptomics, proteomics, and metabolomics will allow for a more precise knowledge of how changes in gene expression lead to changes in metabolism. In our study, we aim to resume the proteins with significant properties, which have been shown to play a critical role against biotic stresses unswervingly, for stress-dependent gene regulation, and contrast these data with those of proteins detected using the two-dimensional gel electrophoresis, mass spectrometry and bioinformatics tools coupled to PTMs by dbPTM in wheat.

MATERIALS AND METHODS

Plant materials

Six genotype of wheat seeds (two Korean: Keumgang, Jinpum; two Chinese: China-108, Yennon-78 and two Japanese: Norin-61, Kantou-107) were used in this study for identification of biotic stress responses proteins by proteomics analysis. The disease free seeds were collected from the field of the National Institute of Crop Science, Suwon, Korea. Disease free wheat seeds were grown in field under low temperature (-20 ~ -10°C) for four months, then slowly increase temperature and naturally exposed up to 28°C until harvesting. Sorting of disease infected plant and grain during grain maturation, naturally was carried out. The seeds harvested for analysis were kept at -20°C until sample preparation.

Sample preparation by KCl solubility method

Osborne (1924) solubility method routinely use to fractionate wheat endosperm proteins takes advantage of the solubility properties of wheat endosperm proteins in KCl, SDS, and acetone with some modifications (Hurkman and Tanaka, 2007). 50 mg of flour was suspended in 200 µl of cold (4°C) KCl buffer (50 mM Tris-HCl, 100 mM KCl, 5 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.8). The suspension was incubated on ice for 5 min with intermittent mixing by vortex including sonication (Sonics and Materials Inc., USA) and centrifugation at 16,000 × *g* for 15 min at 4°C (Hanil Science Industrial Co. Ltd. Korea). The pellet or KCl-insoluble fraction was suspended in 800 µl of sodium dodecyl sulfate (SDS) buffer (2% SDS, 10% glycerol, 50 mM DL-dithiothreitol (DTT), 40 mM Tris-Cl, pH 6.8), incubated for 1 h at room temperature, and insoluble material removed by centrifugation at 16,000 × *g* for 10 min at room temperature. The proteins were precipitated from the SDS buffer by the addition of 4 volume of cold (-20°C) acetone and incubated overnight at -20°C. Following centrifugation, the pellet was rinsed by pipetting cold acetone onto the pellet, centrifuging at 16,000 × g for 10 min at room temperature, and pipetting the acetone off the pellet. The pellet (proteins including gluten) was dried by vacuum centrifugation (BIOTRON Inc., Korea) and solubilized in lysis buffer.

Two-dimensional (2-D) gel electrophoresis

Soluble proteins of whole seed storage were examined by 2-D gel electrophoresis according to the protocol of O`Farrell (1975). Sample solutions (50 µl) were loaded on to the acidic side of the isoelectric focusing gels (IEF) gels for the first dimensional, and anodic and cathodic electrode solutions were filled in the upper and lower electrode chambers, respectively. SDS-polyacrylamide gel electrophoresis (PAGE) in the second dimension (Nihon Eido, Tokyo, Japan) was performed with 12% separation and 5% stacking gels. Protein spots in 2-DE gels were visualized by Coomassie brilliant blue (CBB) R-250 staining (Woo et al. 2002).

Each sample was run three times and the best visualized gels were selected.

In-gel digestion and mass spectrometry analysis

Selected protein spots were excised from preparative loaded gels, stained with CBB (R-250), then washed with 100 µl distilled water. Each gel piece with protein was dehydrated by 25 mM ammonium bicarbonate (ABC) / 50% acetonitrile (ACN) and washed with 10

mM DTT /0.1 M ammonium bicarbonate (ABC). Gel pieces were dried under vacuum centrifugation, rehydrated with 55 mM iodoacetamide (IAA) / 0.1 M ABC for 30 min in dark place. After removing the solution, the gels pieces were vortexed with 100 mM ammonium bicarbonate for 5 min and soaked in acetonitrile (ACN) for dehydration so that the resulting gel pieces would shrink and become an opaque-white color. The gel pieces were then dried under vacuum centrifugation. For tryptic digestion, trypsin solution (4 µl) was added in rehydrated gel particles and incubated for 45 min at 4°C and overlaid with 30 µL of 25 mM ABC (pH 8.0) to keep them immersed throughout digestion. The gel pieces were then incubated overnight at 37°C. After incubation, the solution was spin down and transferred to a 500 µl siliconized tube. The gel particles were suspended in 40 µl ACN / double distilled water (DDW) / trifluoroacetic acid (TFA) (660 µl:330 µl:10 µl) at 3 times and 100% ACN, then vortexed for 30 mins, respectively. The supernatant was dried under vacuum centrifugation for 2 h.

In MALDI-TOF/MS (AXIMA CFR$^+$ Plus, Shimadzu, Japan) analysis, proteins separated by 2-DE were digested in gels according to the method described by Fukuda et al. (2003). The samples were added in 10 µl (0.1% TFA) for digestion. The digests were desalted with C_{18} Zip Tip (Millipore, Boston) and subjected to analysis by MALDI-TOF Mass spectrometry.

Bioinformatics analysis

The proteins were identified by searching NCBI non-redundant database using the MASCOT program (http://www.matrixscience.com,Matrixscienc,UK). The search parameters allowed for modifications of acetyl (K), carbamidomethyl (C), oxidation (M), propionamide (C) with peptide tolerance (50 ~ 200 ppm). For MS/MS searches, the fragmentation of a selected peptide molecular ion peak is used to identify with a probability of less than 5%.Thus, MS/MS spectra with a MASCOT score higher than the significant score ($p < 0.05$) were assumed to be correct. When more than one peptide sequence was assigned to a spectrum with a significant score, the spectra were manually examined. Sequence length, gene name and also protein functions were identified by searching Swiss-Prot/TrEMBL database using UniProtKB (http://www.uniprot.org).

RESULTS

2D-PAGE based comparision of six wheat grain proteins

According to this method, the separation of protein spots did not seem to be satisfactory at around the neutral (pH 4 - 7) pH range. Therefore, to avoid the overlapping of protein spots and to increase the resolution capacity, we also adopted an IEF gel specific for pH range 3 - 10 in addition to the acidic and the basic pH range. With these methods, we identified more than 250 protein spots among six cultivars by pH 3-10 range gels, which discovered about 45, 32, 38, 40, 26 and 36 protein spots, respectively (Figures 1 and 2).

We established by 2-D gel electrophoresis (2DE) that these spots patterns were highly reproducible for at least three self-determining protein extractions. Using 2-DE gels for pH 3 - 10, we revealed qualitative variations of 35 protein spots in six wheat cultivars (Figure 1). Among them, the protein spots A_{1-11} were only found in Keumgang (Figure 1A) followed by B_{1-5} in Jinpum (Figure 1B), C_{1-2} in China-108 (Figure 1C), D_{1-11} in Yeonnon-67 (Figure 1D), E_{1-2} in Norin-61 (Figure 1E) and F_{1-4} in Kantou-107 (Figure 1F).

Idenification of biotic stress-responsive proteins among six wheat cultivars

Out of 308 biotic stress responsive proteins, 40 proteins were identified in Keumgang followed by 18 24 in Jinpum, 42 in China-108, 34 in Yeonnon-78, 68 in Norin-61, 100 in Kantou-107 (Figure 3). We identified different kinds of biotic stress responses proteins such as jasmonate induced protein (23 kDa), purothionin alpa-1, 2 (13.5 - 14.5 kDa) and gamma-1,2 (5 kDA), thionin like proteins (13 - 14 kDa), alpha-amylase (4.7 - 45.34 kDa), antifungal protein (3.87 - 4.45 kDa), thuamatin like proteins (23 - 24 kDa), antimicrobial protein (4.12 kDa), AP2 transcriptional activator (5.50 kDa), calcium-dependent protein kinase (59.72 kDa), chitinase (10.59 - 36.16 kDa), cyclophilin (10.70 - 22.84 kDa) and cysteine proteinase like (8.70 - 40.78 kDa) in six wheat cultivars (Table 1). Some spots also revealed directly disease resistance responsive protein against wheat, maize, arabidopsis and rice such as disease resistance proteins (8.24 - 155.12 kDa), downy mildew (154.31 kDa), powdery mildew (159.71 kDa), fusarium (65.04 kDa), NBS-LRR disease resistance proteins (2.68 - 71.62 kDa) with putative NBS-LRR type proteins (2.68 - 21.85 kDa), probable disease resistance proteins (43.54 - 131.57 kDa) and putative disease resistance proteins (7.42 - 158.80 kDa), RGA – like proteins (10.95 - 101.93 kDa) among these cultivars. We are also identified PR proteins such as PR1, PR1a, PR 1b, PR4, PR4b, PR5, PR10, PRB1-3 including some precursor (11.18 - 90.60 kDa), germin-like proteins (23 - 24 kDa), kinase R-like proteins (18 - 19 kDa), mosaic virus helicase domain binding protein (14.75 kDa), viral resistance protein (23.62), mal-like protein (90.73 kDa) in our experiment.Some spots were detected biotics stress responsive proteins such as peroxidase 3, 7, 10, 11, 12, 13, 17, 18, 30, 31, 38, 39, 41, 44, 65, 72.73, III-123 (33 - 39 kDa), peroxiredoxin Q, 2B,2F (17.41 - 23.34 kDa), puroindoline A, B (16 kDa), serpin Z1, Z1B, Z2B, Z3, ZX (12.98 - 43.85 kDa), ubiqutin 16, E2 27, E1 1, E2 (3.43 - 110.53 kDa), wheatwin 1, 2 (15 kDa), WRKY 1, 23, 34, 35, 55 (5.52 - 53.97 kDa), WSCI proteinase inhibitor (9.28 kDa) and xylanase inhibitors 801NEW,1,TL-X1 (15.63 - 42.37 kDa) in six wheat cultivars (Table 1).

Post-translational modifications

Post-translational modifications of proteins greatly increase protein complexity and dynamics, co-ordinating the intricate regulation of biological events. The global identification of post translational modifications is a difficult task that is currently accelerated by advances in

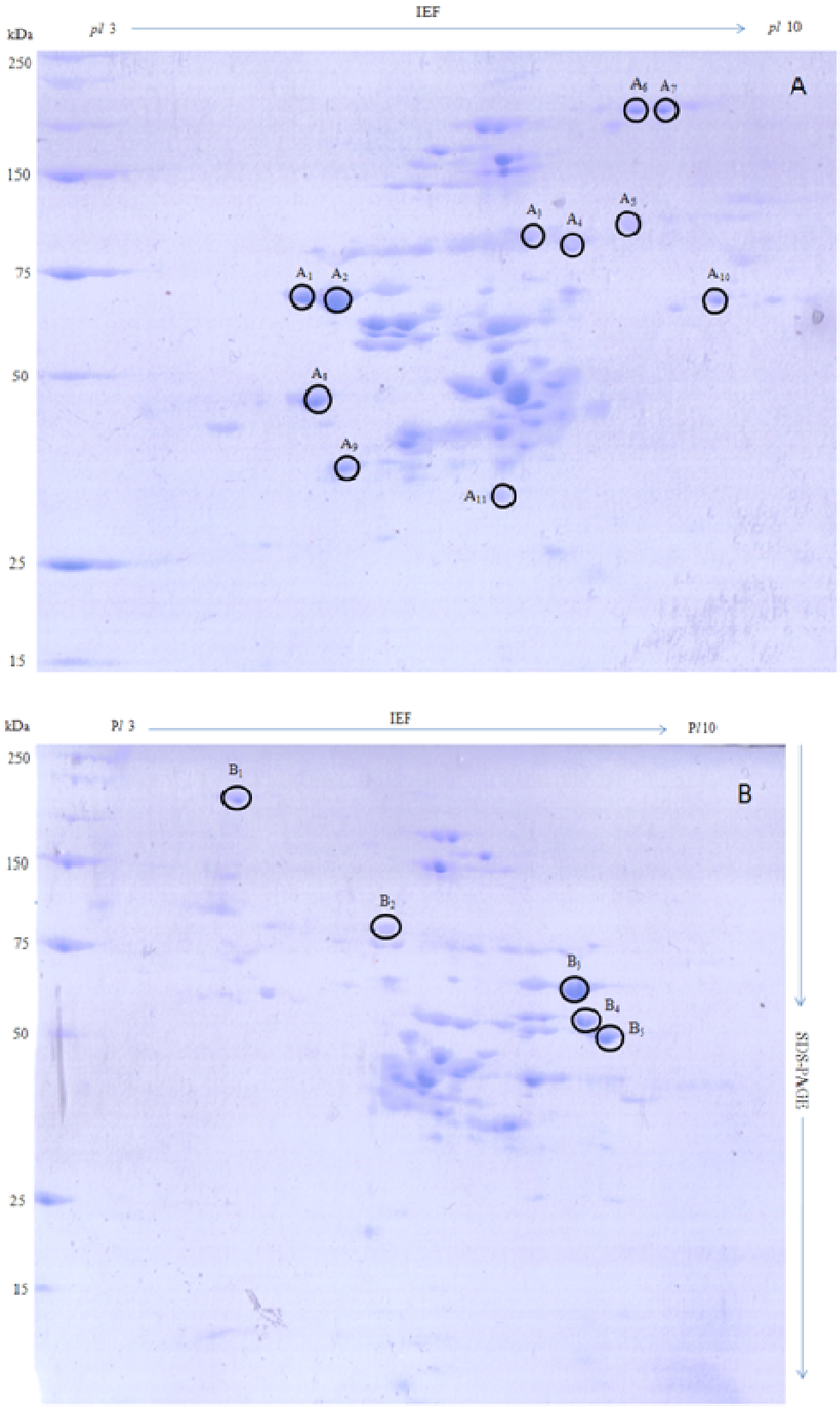

Figure 1. 2D gel analyses of proteins extracted from mature wheat seeds. A, Keumgang; B, Jinpum; C, China-108; D, Yeonnon-78; E, Norin-61; F, Kantou-107. First dimension was performed on IEF with pH 3-10. In the second dimension gels were used and proteins were visualized using coomassie brilliant blue R-250. Circle shows that dissimilar protein spots among wheat cultivars.

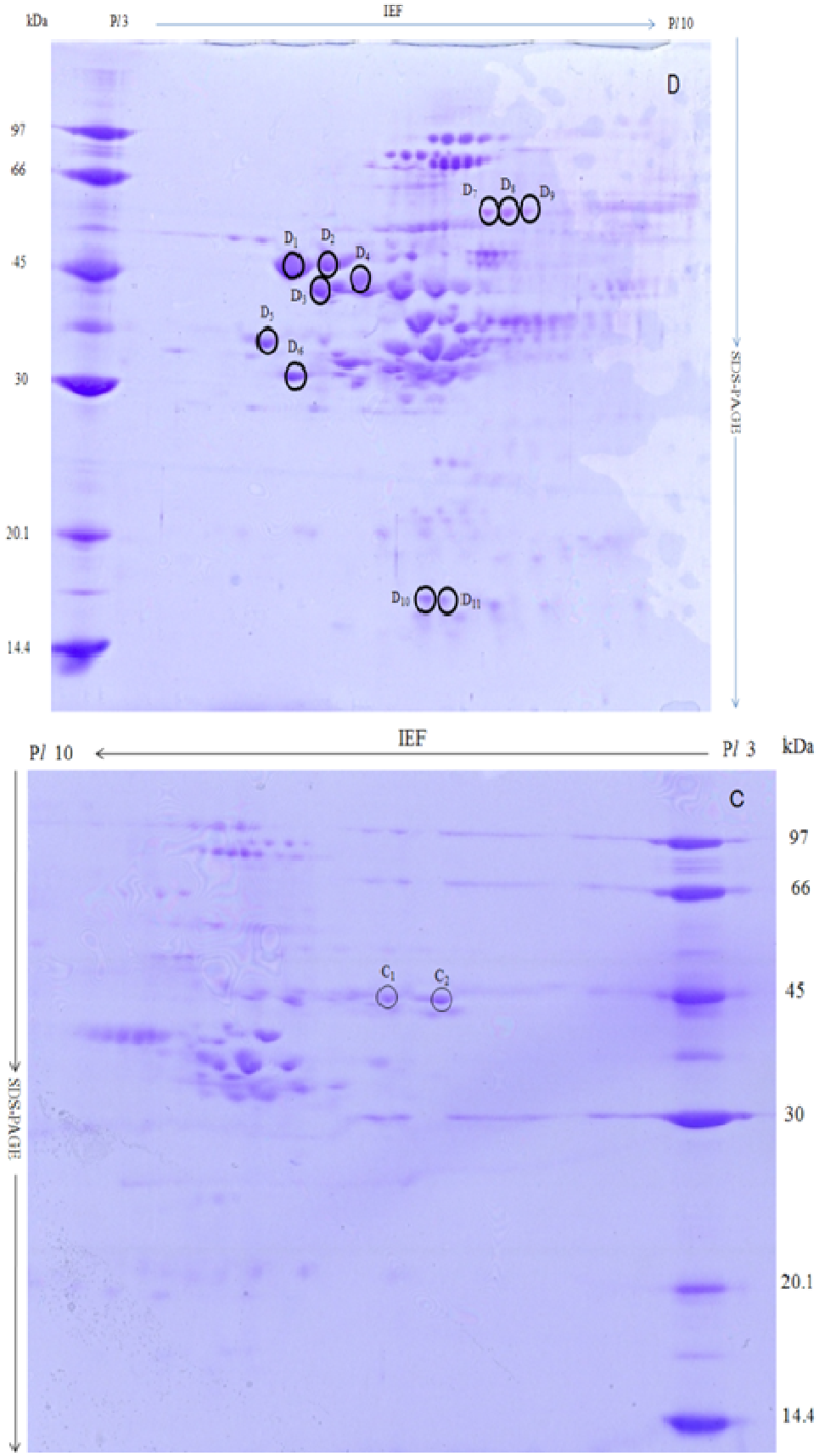

Figure 1. Contd.

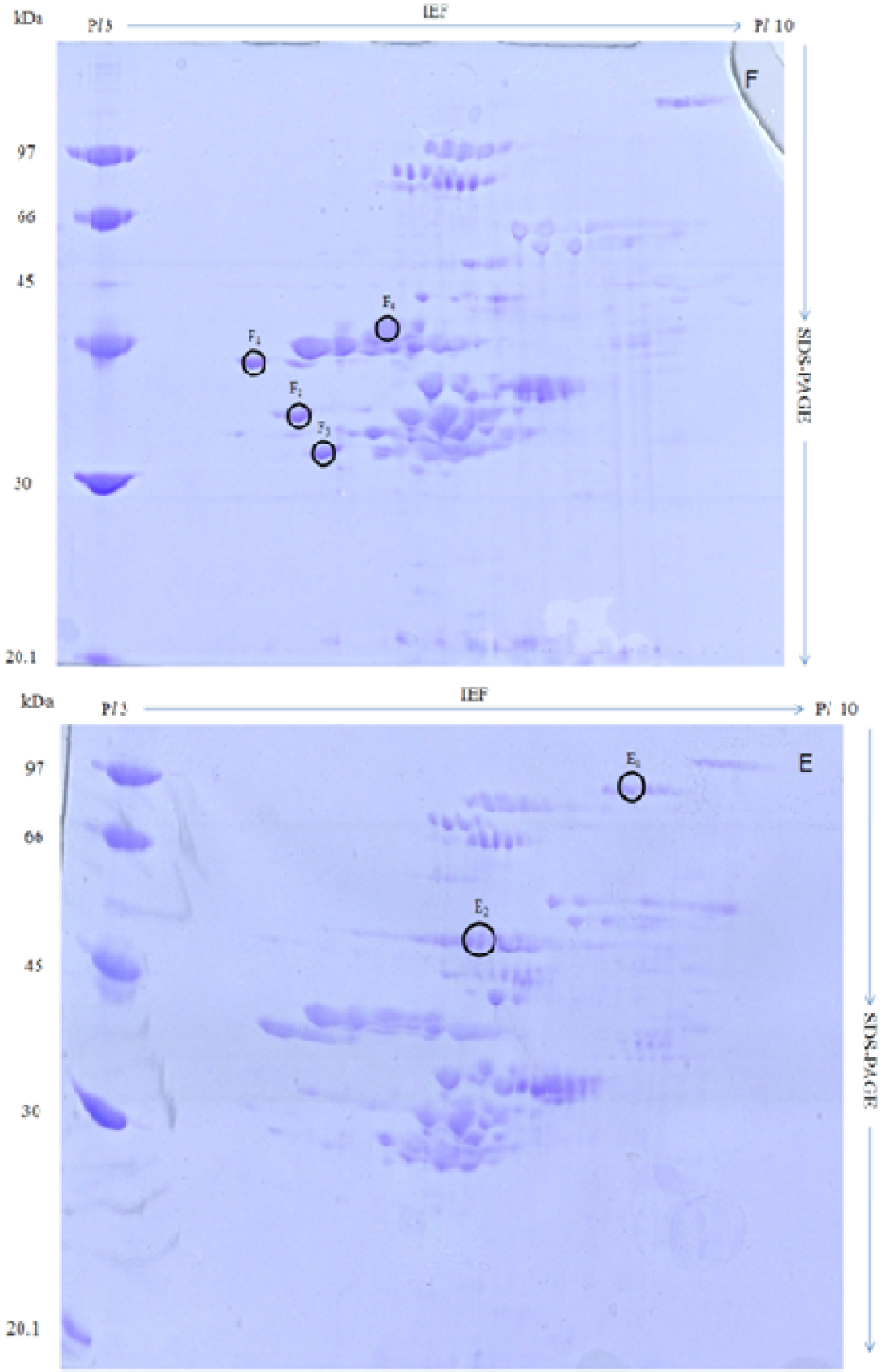

Figure 1. Contd.

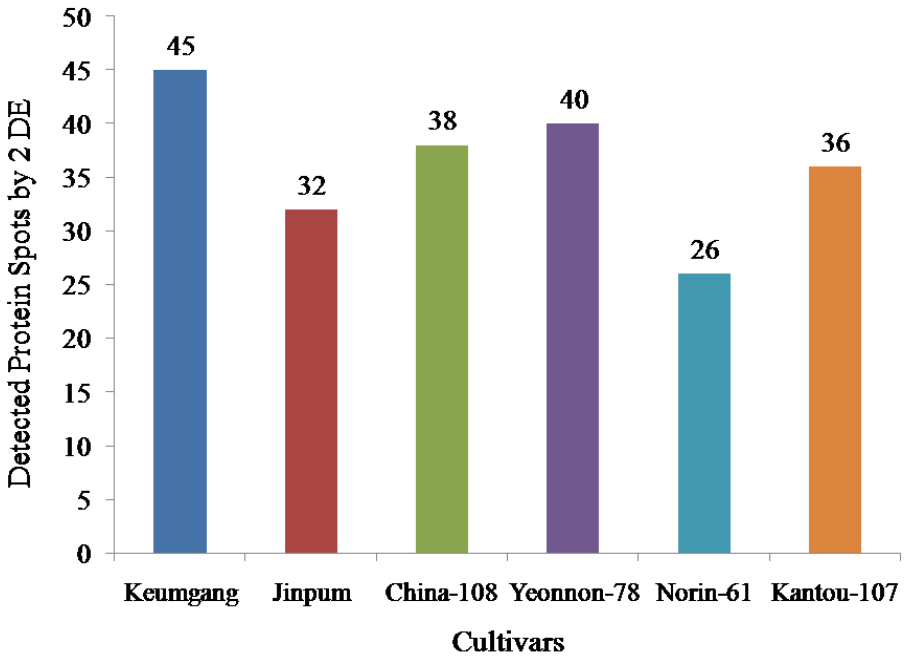

Figure 2. Distribution of total detected protein spots by two-dimensional electrophoresis in six wheat cultivars.

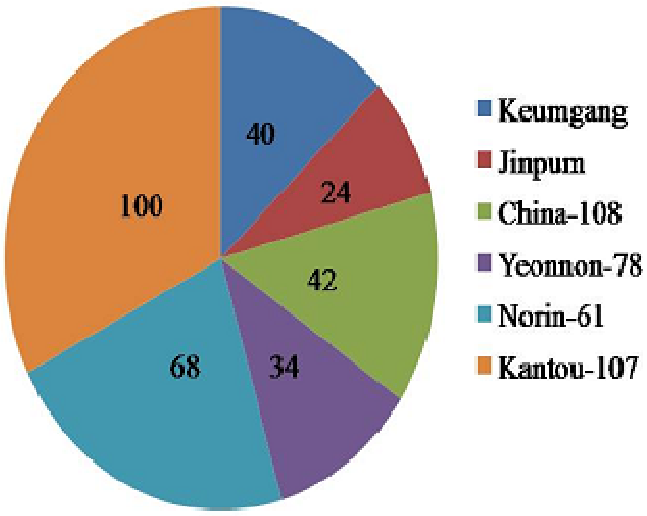

Figure 3. Functional distribution of the total identified biotic stress responsive proteins among six wheat cultivars

proteomics techniques. Presently, numerous techniques have rapidly been developed and applied to the global identifications of PTMs and their modifications site. We used dbPTM methods for PTMs and cleavage site prediction. For instance, pathogenesis related proteins (Wheatwin 1 and 2) is responsible for fungal disease like wheat scab (*Fusarium* spp.). We identified clevage site (22 number amino acid) in wheatwin 1 protein sequences (AATAQQATN) by using dbPTM database (http://dbptm.mbc.nctu.edu.tw/). In our opinion, this protein will be changed by phosphorylation (Pyrrolidone carboxylic acid) for changing the cell signaling (Figure 4), which is possible to control the *Fusarium* like biotic stress.

DISCUSSION

The research of biotic stresses also took advantage of proteomic techniques in previous years. The most frequent technique is the identification of differentially expressed spots on 2-DE by mass spectrometry like in the other fields of plant biology. Compared with DNA- or mRNA-based methods, proteomics has been proved to be more effective to screen disease resistance-related proteins and uncover resistance mechanisms by displaying changes in protein expression patterns. Until now, proteomic approaches have been successfully used in discovering the resistance mechanisms in maize against kernel rot caused by *Fusarium* or *Aspergillus* (Chen et al., 2004, Chen and Chen, 2002, Sonia et al., 2004), while little has been reported on maize leaf fungal disease. Wheat grain proteins have been studied using 2-DE composed of the first dimension of IEF over the two pH range of pH 4-7 and pH 6 - 11 and the second dimension of SDS-PAGE (Woo et al., 2002). These results by two-dimensional electrophoresis strongly indicate that these identified proteins are cultivar specific and show the difference among these cultivars (Yahata et al., 2005). 2-DE developed about 30 years ago is still the most frequently used method to investigate differential protein

Table 1. List of identified biotic stress-responsive proteins in six wheat cultivars including name of gene.

Protein Description	M*r* (kDa)	P*I*	Species	Accession number	Gene name	Cultivar Specific SC (%)					
						K^a	K^b	C^a	C^b	J^a	J^b
23 kDa jasmonate –induced protein	22.83	5.92	*Hordeum vulgure*	JI23_HORVU	-	-	-	-	-	-	9
Alpha-1-purothionin	13.51	4.86	*Triticum aestivum*	THN1_WHEAT	THI1.1	27	-	8	-	-	32
Alpha-2-purothionin	14.54	5.13	*Triticum aestivum*	THN2_WHEAT	THI1.2	-	-	28	56	-	-
Alpha-amylase AMY3	45.34	8.01	*Triticum aestivum*	AMY3_WHEAT	AMY1.1	-	53	17	-	-	-
Alpha-amylase inhibitor 0.19	13.32	6.66	*Triticum aestivum*	IAA1_WHEAT	-	-	9	-	-	-	-
Alpha-amylase inhibitor 0.53	13.17	5.23	*Triticum aestivum*	IAA5_WHEAT	-	36		33	28	-	-
Alpha-amylase inhibitor WDAI-3	4.79	7.57	*Triticum aestivum*	IAA3_WHEAT	IHA-B1-2	-	11	11	-	-	11
Alpha-amylase/trypsin inhibitor CM2	15.44	6.86	*Triticum aestivum*	IAAC2_WHEAT	-	-	-	-	-	-	10
Antifungal protein AX1	5.08	8.21	*Beta vulgaris*	AX1_BETVU	-	-	-	-	-	-	65
Antifungal protein R	4.45	9.50	*Hordeum vulgare*	THHR_HORVU	-	-	-	-	-	54	-
Antifungal protein S	3.87	8.23	*Hordeum vulgare*	THHS_HORVU	-	-	-	67	68	81	-
Antimicrobial peptide MBP-1	4.12	11.35	*Zea mays*	MBP1_MAIZE	-	-	-	-	-	-	39
AP2 transcriptional activator	5.50	8.04	*Triticum turgidum* subsp. durum	gi|67937814	DRF1	-	-	-	52	-	
Calcium-dependent protein kinase	59.72	6.20	*Triticum aestivum*	gi|164472660	CPK1C	-	15	18	-	39	27
Chitinase	10.59	8.54	*Triticum aestivum*	gi|1160277	ChiA 0.1	-	26	-	-	50	20
Chitinase (EC 3.2.1.14) precursor, basic	36.16	7.81	*Arabidopsis thaliana*	B45511	-	-	-	-	-	24	-
Chitinase 1	27.05	8.67	*Triticum aestivum*	Q8W429_WHEAT	Chi 1	37	-	-	-	-	-
Chitinase II precursor	24.71	5.00	*Triticum aestivum*	gi|4741842	Cht2	25	28	28	-	-	-
Cyclophilin	13.58	9.19	*Triticum aestivum*	gi|82547214	CYP23-d	26	33	27	22	77	39
Cyclophilin 2	18.31	8.61	*Oryza sativa*	Q40674_ORYSA	-	-	-	-	-	-	28
Cyclophilin A	10.70	6.31	*Triticum aestivum*	gi|42493201	CYP18-3	17	-	-	-	-	-
Cyclophilin A-3	18.37	8.53	*Triticum aestivum*	Q93W25_WHEAT	CyP3	30	-	-	-	65	-
Cyclophilin B-B	22.84	9.58	*Triticum aestivum*	gi|194339233	-	-	-	31	-	-	23
Cyclophilin B-D	22.82	9.58	*Aegilops tauschii*	gi|194339243	-	-	-	-	-	65	-
Cystatin WC-4	15.77	9.13	*Triticum aestivum*	Q2XNE8_WHEAT	-	-	-	26	50	-	-
Cysteine proteinase	40.78	6.80	*Triticum aestivum*	gi|109119897	-	14	-	24	26	28	7
Cysteine proteinase inhibitor 12	27.25	6.07	*Oryza sativa* subsp. japonica	CYT12_ORYSJ	Os01g0270100	-	-	-	-	-	23
Cysteine-rich antifungal protein 1	8.70	8.48	*Arabidopsis thaliana*	AFP1_ARATH	-	-	-	-	-	-	17
Disease resistance gene analog	17.51	5.22	*Zea mays*	Q9ZTI7_MAIZE	-	-	-	-	-	-	16
Disease resistance gene analog PIC19	10.77	4.88	*Zea mays*	gi|3982630	-	-	-	-	-	34	14
Disease resistance protein	21.19	6.71	*Arabidopsis thaliana*	Q19FJ1_ARATH	At4g14370	-	-	20	-	29	17

Table 1. Contd.

Protein Description	Mr (kDa)	PI	Species	Accession number	Gene name	Cultivar Specific SC (%)					
						K[a]	K[b]	C[a]	C[b]	J[a]	J[b]
Disease resistance protein	20.59	5.16	*Arabidopsis thaliana*	Q19GU3_ARATH	-	-	-	-	-	-	11
Disease resistance protein	24.03	9.78	*Arabidopsis thaliana*	Q19HS6_ARATH	-	-	-	-	-	-	44
Disease resistance protein	23.82	9.80	*Arabidopsis thaliana*	gi|104645544	-	-	-	-	-	-	33
Disease resistance protein	38.00	8.49	*Arabidopsis thaliana*	gi|104646408	-	-	-	-	-	-	11
Disease resistance protein (TIR class), putative	72.48	8.28	*Arabidopsis thaliana*	gi|30686220	F16G20_210	-	-	-	12	20	-
Disease resistance protein (TIR-NBS-LRR)	155.12	6.35	*Arabidopsis thaliana*	gi|42569093	-	-	-	-	17	17	-
Disease resistance protein RPP13	97.20	6.14	*Arabidopsis thaliana*	RPP13_ARATH	RPP13	-	-	-	-	7	-
Disease resistance protein RPP1-WsA	135.76	6.23	*Arabidopsis thaliana*	T52346	-	-	-	-	19	-	-
Disease resistance protein-like	77.19	6.30	*Arabidopsis thaliana*	Q8RWB2_ARATH	-	-	-	-	-	-	8
Disease resistance response protein 206	9.32	5.76	*Oryza sativa*	gi|149392571	-	-	-	-	-	-	22
Disease resistance response protein 39	8.24	6.00	*Pisum sativum*	DR39_PEA	PI39	-	-	-	-	-	18
Disease resistance response protein Pi49	16.73	4.94	*Pisum sativum*	DRR3_PEA	DRR49A	-	-	-	-	11	-
Disease resistance RPP5 like protein	21.36	7.66	*Arabidopsis thaliana*	gi|110739135	At4g16880	-	-	-	20	-	22
Disease resistance-like protein	12.19	9.30	*Glycine max*	Q9M5V7_SOYBN	-	-	-	18	-	64	23
Disease resistance-like protein GS6-1	15.57	8.53	*Glycine max*	gi|22037383	-	-	-	-	-	-	53
Disease resistance-like protein KR5	26.80	6.13	*Glycine max*	Q7XYS7_SOYBN	-	-	-	-	-	-	23
Disease resistance-responsive family protein	20.41	8.65	*Arabidopsis thaliana*	gi|15226465	-	-	-	-	-	-	8
Downy mildew resistance protein RPP5	154.31	5.12	*Arabidopsis thaliana*	gi|6449046	-	-	-	-	-	-	20
Fusarium resistance protein I2C-5-like	65.04	9.06	*Oryza sativa*	Q5JMA3_ORYSA	P0690B02.12	-	-	-	21	-	-
Gamma-1-purothionin	5.23	9.49	*Triticum aestivum*	THG1_WHEAT	-	44	-	44	-	-	44
Gamma-2-purothionin	5.14	9.12	*Triticum aestivum*	THG2_WHEAT	-	46	46	-	-	-	46
Germin-like protein 12-2	24.66	5.91	*Oryza sativa* subsp. japonica	GL122_ORYSJ	Os12g0154800	-	-	-	-	-	11
Germin-like protein 1-3	23.61	8.45	*Oryza sativa* subsp. japonica	GL13_ORYSJ	GER8	-	-	-	-	11	16
Germin-like protein 5-1	23.83	7.01	*Oryza sativa* subsp. japonica	GL51_ORYSJ	Os05g0197200	-	-	-	-	-	15
Kinase R-like protein	18.16	7.12	*Triticum aestivum*	Q8W1G3_WHEAT	-	16	15	23	32	6	-
Kinase R-like protein	19.15	5.39	*Aegilops tauschii*	Q8VWL6_AEGTA		-	-	-	-	-	23
LRR-like protein	20.00	11.07	*Oryza sativa*	Q6J656_ORYSA	-	-	-	-	-	-	15
Mal-like protein	90.73	6.24	*Triticum aestivum*	Q8H6G1_WHEAT	-	16	-	14	6	26	-

Table 1. Contd.

Protein Description	Mr (kDa)	PI	Species	Accession number	Gene name	Cultivar Specific SC (%)					
						K[a]	K[b]	C[a]	C[b]	J[a]	J[b]
Mosaic virus helicase domain binding protein	14.75	8.78	*Triticum aestivum*	gi|32400853	-	-	35	-	-	-	-
NBS-LRR	17.18	8.65	*Oryza rufipogon*	Q2VBV5_ORYRU	RGA	-	-	-	32	28	25
NBS-LRR disease resistance protein	13.07	8.60	*Oryza sativa*	Q6K906_ORYSA	OJ1568_B05.21	-	-	-	15	34	18
NBS-LRR disease resistance protein homologue	20.62	8.74	*Hordeum vulgare*	gi|28555853	-	-	-	-	-	-	38
NBS-LRR protein kinase	8.86	5.67	*Triticum aestivum*	Q45QF9_WHEAT	-	-	-	-	-	-	51
NBS-LRR resistance protein RGH1-like	71.62	5.89	*Oryza sativa*	Q8LJ95_ORYSA	P0691E06.16	-	-	-	14	12	14
NBS-LRR type resistance protein	9.88	7.00	*Triticum aestivum*	Q8LK47_9POAL	-	-	8	-	26	31	56
NBS-LRR type RGA	2.68	8.45	*Triticum aestivum*	Q3S9M9_WHEAT	-	-	-	75	-	25	16
NBS-type putative resistance protein	11.29	5.63	*Glycine max*	Q947F3_SOYBN	-	-	-	-	-	-	45
Pathogenesis-related 1b	17.63	8.52	*Triticum monoccocum*	gi|73921468	-	18	-	31	-	-	-
Pathogenesis-related homeodomain protein	90.60	4.86	*Arabidopsis thaliana*	PRH_ARATH	PRH	-	-	-	-	13	-
Pathogenesis-related maize seed protein	18.43	8.92	*Zea mays* subsp. parviglumis	Q2XXD2_ZEAMP	-	-	-	-	-	13	24
Pathogenesis-related protein 1	11.18	6.88	*Triticum aestivum*	gi|83031480	-	-	-	24	-	29	28
Pathogenesis-related protein 10	16.73	4.94	*Pisum sativum*	T06527	DRR49A	-	-	8	-	14	-
Pathogenesis-related protein 1a	17.42	8.19	*Hordeum vulgare*	S37166	-	-	-	-	-	16	-
Pathogenesis-related protein 4	13.08	7.00	*Triticum aestivum*	gi|6002595	PR4	35	-	-	-	-	-
Pathogenesis-related protein 4b	16.46	4.61	*Oryza sativa*	Q6T5J8_ORYSA	-	-	-	-	-	18	-
Pathogenesis-related protein 5	25.23	4.75	*Arabidopsis thaliana*	PR5_ARATH	At1g75040	-	-	17	-	-	-
Pathogenesis-related protein homolog F14M19.60	21.36	9.02	*Arabidopsis thaliana*	T04232	-	-	-	-	-	16	-
Pathogenesis-related protein PRB1-3	17.68	8.93	*Hordeum vulgare*	PR13_HORVU	-	-	-	-	-	14	-
Pathogenesis-related protein precursor	15.48	6.70	*Triticum aestivum*	Q6PWL9_WHEAT	PR4f-a	-	-	-	28	53	-
Peroxidase	32.36	8.37	*Triticum aestivum*	PER1_WHEAT	-	-	20	27	-	24	-
Peroxidase (EC 1.11.1.7)	37.24	6.10	*Oryza sativa*	Q7XSU7_ORYSA	-	-	-	-	-	-	10
Peroxidase 10	38.00	6.17	*Arabidopsis thaliana*	PER10_ARATH	PER10	-	-	-	-	17	-
Peroxidase 11	37.28	5.19	*Arabidopsis thaliana*	PER11_ARATH	PER11	-	-	-	-	15	-
Peroxidase 12	39.53	8.58	*Arabidopsis thaliana*	PER12_ARATH	PER12	-	-	-	-	22	-
Peroxidase 13	34.744	4.95	*Arabidopsis thaliana*	PER13_ARATH	PER13	-	-	-	-	-	23
Peroxidase 17	36.64	5.06	*Arabidopsis thaliana*	PER17_ARATH	PER17	-	-	-	-	24	-
Peroxidase 18	35.61	5.21	*Arabidopsis thaliana*	PER18_ARATH	PER18	-	-	-	-	-	23
Peroxidase 3	34.88	8.74	*Arabidopsis thaliana*	PER3_ARATH	PER3	-	-	-	-	11	-
Peroxidase 30	35.76	9.71	*Arabidopsis thaliana*	PER30_ARATH	PER30	-	-	-	-	-	10

Table 1. Contd.

Protein Description	M*r* (kDa)	P*I*	Species	Accession number	Gene name	Cultivar Specific SC (%)					
						K[a]	K[b]	C[a]	C[b]	J[a]	J[b]
Peroxidase 31	35.28	9.22	*Arabidopsis thaliana*	PER31_ARATH	PER31	-	-	-	-	-	20
Peroxidase 38	38.06	7.55	*Arabidopsis thaliana*	PER38_ARATH	PER38	-	-	-	-	-	14
peroxidase 39	35.35	7.59	*Zea mays*	gi|226493663	PER39	-	-	-	-	21	7
Peroxidase 41	36.17	8.51	*Arabidopsis thaliana*	PER41_ARATH	PER41	-	-	-	-	-	11
Peroxidase 44	33.78	10.00	*Arabidopsis thaliana*	PER44_ARATH	PER44	-	-	-	-	-	9
Peroxidase 65	36.99	6.75	*Arabidopsis thaliana*	PER65_ARATH	PER65	-	-	-	-	9	-
Peroxidase 7	38.31	6.45	*Arabidopsis thaliana*	PER7_ARATH	PER7	-	-	-	-	13	-
Peroxidase 72	37.40	8.74	*Arabidopsis thaliana*	PER72_ARATH	PER72	-	-	-	-	-	17
Peroxidase 73	35.90	9.44	*Arabidopsis thaliana*	PER73_ARATH	PER73	-	-	-	-	-	23
Peroxidase III-123 precursor	34.66	8.37	*Oryza sativa*	Q5U1H0_ORYSA	-	-	-	-	-	24	-
Peroxiredoxin Q, chloroplastic	23.34	9.72	*Triticum aestivum*	PRXQ_WHEAT	PRX1	29	16	-	-	16	-
Peroxiredoxin-2B	17.41	5.17	*Arabidopsis thaliana*	PRX2B_ARATH	PRXIIB	-	-	-	-	-	26
Peroxiredoxin-2F, mitochondrial	21.43	8.99	*Arabidopsis thaliana*	PRX2F_ARATH	PRXIIF	-	-	-	-	-	16
Plant disease resistance polyprotein-like	63.72	9.12	*Oryza sativa*	Q5VPA9_ORYSA	-	-	-	-	-	-	25
Powdery mildew resistance protein PM3A	159.71	6.14	*Triticum aestivum*	Q3B9Y4_WHEAT	Pm3	15	-	-	-	-	50
PPR protein-like protein	87.77	6.51	*Oryza sativa*	Q6YW98_ORYSA	-	-	-	-	-	-	18
PR10-like protein	13.63	5.17	*Glycine max*	Q8LJU1_SOYBN	-	-	-	-	-	-	28
PRA1 family protein F4	20.98	8.15	*Arabidopsis thaliana*	PR1F4_ARATH	PRA1F4	-	-	-	-	-	22
Probable calcium-binding protein CML30	22.68	4.20	*Arabidopsis thaliana*	CML30_ARATH	CML30	-	-	-	-	-	20
Probable disease resistance protein At1g58602	131.57	6.11	*Arabidopsis thaliana*	DRL9_ARATH	At1g58602	-	-	-	-	-	3
Probable disease resistance protein At4g19060	43.54	5.59	*Arabidopsis thaliana*	DRL26_ARATH	At4g19060	-	-	-	-	-	24
Probable disease resistance protein At5g43730	96.20	5.68	*Arabidopsis thaliana*	DRL32_ARATH	At5g43730	-	-	6	-	-	-
Probable protein kinase	39.90	6.95	*Arabidopsis thaliana*	T02181	-	-	-	-	-	25	13
Puroindoline -A	16.27	8.34	*Triticum turgidum.*	Q56UP4_9POAL	Pina-D1	25	8	24	11	-	20
Puroindoline-B	16.78	9.06	*Triticum aestivum*	PUIB_WHEAT	PINB	31	29	20	38	27	-
Purothionin A-1	14.61	4.94	*Triticum aestivum*	THNB_WHEAT	THI1.3	18	6	-	-	-	
Putative Cen-like protein, FDR1	16.55	9.39	*Triticum aestivum*	gi|40644758		27	-	-	-	-	-
Putative disease resistance protein	7.42	6.02	*Arabidopsis thaliana*	Q8GWQ5_ARATH	At5g46480/K11I1_7	-	-	9	18	-	-
Putative disease resistance protein	11.06	5.29	*Oryza sativa*	gi|18071378	-	-	-	-	-	-	37

Table 1. Contd.

Protein Description	M*r* (kDa)	P*I*	Species	Accession number	Gene name	Cultivar Specific SC (%)					
						K[a]	K[b]	C[a]	C[b]	J[a]	J[b]
Putative disease resistance protein At1g59780	103.56	6.98	*Arabidopsis thaliana*	DRL13_ARATH	At1g59780	-	-	-	-	10	-
Putative disease resistance protein At1g61190	111.46	6.25	*Arabidopsis thaliana*	DRL16_ARATH	At1g61190	-	-	-	10	11	-
Putative disease resistance protein At3g14460	158.80	5.57	*Arabidopsis thaliana*	DRL21_ARATH	At3g14460	-	-	-	-	13	-
Putative disease resistance protein At3g15700	42.32	8.90	*Arabidopsis thaliana*	DRL22_ARATH	At3g15700	-	-	-	-	-	14
Putative disease resistance protein At5g47280	70.03	5.32	*Arabidopsis thaliana*	DRL39_ARATH	At5g47280	-	-	-	-	12	-
Putative disease resistance protein RPM1	82.32	6.63	*Oryza sativa*	Q5VRS9_ORYSA	-	-	-	-	-	21	-
Putative disease resistance RPP13-like protein 1	121.34	8.29	*Arabidopsis thaliana*	R13L1_ARATH	RPPL1	-	-	-	-	-	12
Putative F-box/LRR-repeat protein At3g44810	50.97	6.02	*Arabidopsis thaliana*	FBL52_ARATH	At3g44810 PE	-	-	-	-	16	-
Putative germin-like protein 2-2	23.64	6.49	*Oryza sativa* subsp. japonica	GL22_ORYSJ	Os02g0491700	-	-	-	-	4	-
Putative microtubule-associated protein	13.60	9.13	*Triticum aestivum*	Q70AJ0_WHEAT	p0700D12.21	24	-	-	-	-	-
Putative NBS-LRR disease resistance protein	20.51	5.41	*Arabidopsis lyrata*	gi|207339352	-	-	-	-	-	-	27
Putative NBS-LRR protein	21.85	6.00	*Triticum aestivum*	Q70AJ8_WHEAT	rgas-L8	28	-	32	-	-	-
Putative NBS-LRR resistance protein	2.68	6.92	*Triticum aestivum*	Q3YL69_WHEAT	-	79	58	-	-	-	-
Putative pathogenesis related protein	27.12	5.45	*Oryza sativa*	Q9XHX6_ORYSA	-	-	-	-	-	35	48
Putative receptor-like protein kinase RLPK4	6.40	7.16	*Glycine max*	Q8VWW5_SOYBN	-	-	-	-	-	-	22
Putative RGA protein 567B-3.2	98.02	*5.54*	*Aegilops tauschii*	Q84QH1_AEGTA	-	-	9	-	-	-	-
Putative sucrose synthase type 1	6.11	9.69	*Triticum aestivum*	gi|6491853	SS1	48	-	-	-	-	-
Putative WD-repeat protein	20.08	8.56	*Triticum aestivum*	gi|40644810	-	37	-	-	-	-	-
Putative wheat powder tolerance-related protein	7.78	4.91	*Triticum monococcum*	Q2VQ36_TRIMO	-	72	36	-	-	-	-
Quinone reductase 2	21.73	5.95	*Triticum monococcum*	gi|58500257	-	-	-	35	-	33	-
Receptor-like kinase with LRR repeats	18.49	4.55	*Triticum aestivum*	Q70AH8_WHEAT	p0703B11.26	28	-	11	11	19	43
Resistance gene analog PIC28	10.95	4.62	*Aegilops tauschii*	Q9SEF0_AEGTA	PIC28	-	-	27	-	12	-
Resistance protein	20.14	9.26	*Triticum aestivum*	Q9ZSZ4_WHEAT	RGA1	-	6	-	-	-	-
Resistance protein CAN_RGA1	101.93	5.76	*Triticum aestivum*	gi|33302329	-	13	-	-	-	-	-
Resistance protein RGA2	103.88	5.85	*Triticum urartu*	gi|195975992	rga2	15	14	-	-	-	-
Resistance protein RPP13	24.03	6.06	*Arabidopsis thaliana*	Q570U2_ARATH	At1g59218	-	-	16	-	-	-
Rga2 protein	17.92	6.12	*Triticum monococcum*	Q8L4I8_TRIMO	rga2	21	-	-	35	42	-
Serpin-related	12.98	4.77	*Arabidopsis thaliana*	Q4PSX8_ARATH	-	-	-	-	-	-	22
Serpin-Z1	42.96	5.23	*Arabidopsis thaliana*	SPZ1_ARATH	At1g64030	-	-	-	-	-	5
Serpin-Z1B	43.00	5.44	*Triticum aestivum*	SPZ1B_WHEAT	-	-	-	-	12	-	-
Serpin-Z2B	43.85	6.24	*Oryza sativa* subsp. japonica	SPZ2B_ORYSJ	Os11g0239200	-	-	-	-	-	16
Serpin-Z3	43.19	5.50	*Arabidopsis thaliana*	SPZ3_ARATH	At2g26390	-	-	-	-	16	-
Serpin-ZX	42.92	6.77	*Hordeum vulgare*	SPZX_HORVU	PAZX	-	-	-	-	-	13
SGT1B (enhanced downy mildew 1b); protein binding	39.73	5.03	*Arabidopsis thaliana*	gi|15237122	-	-	-	-	-	5	-
S-receptor kinase 13-10	23.22	4.96	*Arabidopsis lyrata*	Q84XJ4_ARALY	-	-	-	-	-	-	20

Table 1. Contd.

Thaumatin-like protein	23.58	7.85	*Triticum aestivum*	Q8S4P7_WHEAT	-	17	-	-	-	-	-
Thaumatin-like protein	25.89	8.84	*Arabidopsis thaliana*	TLPH_ARATH	At1g18250	-	-	-	-	13	-
Thaumatin-like protein TLP5	24.90	6.04	*Hordeum vulgare*	Q5MBN2_HORVU	-	-	-	-	-	-	9
Thionin BTH7	14.66	7.33	*Hordeum vulgare*	THN7_HORVU	-	-	-	-	-	-	64
Thionin-2.1	14.34	8.48	*Arabidopsis thaliana*	THN21_ARATH	THI2.1	-	-	-	-	-	22
Translationally-controlled tumor protein homolog	18.79	4.55	*Triticum aestivum*	TCTP_WHEAT	TCTP	-	-	28	-	-	26
Type 1 non specific lipid transfer protein precursor	11.13	9.35	*Triticum aestivum*	Q2PCC2_WHEAT	ltp9.2c	44	-	-	15	-	32
Type 2 non specific lipid transfer protein precursor	9.73	8.71	*Triticum aestivum*	Q2PCC5_WHEAT	-	-	-	-	-	17	-
Type-5 thionin	13.73	4.41	*Triticum aestivum*	THN5_WHEAT	TTHV	43	-	25	-	8	14
Ubiquitin	8.52	6.56	*Triticum aestivum*	UBIQ_WHEAT	-	39	7	23	-		48
Ubiquitin carboxyl-terminal hydrolase 16	110.53	6.73	*Arabidopsis thaliana*	UBP16_ARATH	UBP16	-	-	-	-	11	-
Ubiquitin carrier protein E2 27	21.24	5.00	*Arabidopsis thaliana*	UBC27_ARATH	UBC27	-	-	-	-	-	6
Ubiquitin conjugating enzyme E2	12.55	4.93	*Arabidopsis thaliana*	Q42045_ARATH	-	-	-	-	-	48	-
Ubiquitin-activating enzyme E1 1	116.93	5.16	*Triticum aestivum*	UBE11_WHEAT	UBA1	18	-	5	16	48	-
Ubiquitin-conjugating enzyme, E2	3.43	8.42	*Triticum aestivum*	Q9S9I4_WHEAT	-	-	-	-	-	-	93
Viral resistance protein	23.62	6.71	*Arabidopsis thaliana*	Q570X7_ARATH	At1g58842	-	-	-	16	-	-
Wheat powder tolerance-related protein	7.81	5.55	*Triticum aestivum*	gi|33114231	-	-	-	-	-	-	61
Wheatwin-1	15.62	7.57	*Triticum aestivum*	WHW1_WHEAT	PR4A	28	24	33	30	-	13
Wheatwin-2	15.85	8.18	*Triticum aestivum*	WHW2_WHEAT	PR4B	14	-	31	34	-	31
WRKY family transcription factor	39.74	6.03	*Arabidopsis thaliana*	gi|18417879	-	-	-	-	-	24	22
WRKY transcription factor 1	53.97	6.43	*Arabidopsis thaliana*	WRKY1_ARATH	WRKY1	-	-	-	-	-	22
WRKY transcription factor 34	27.93	8.29	*Hordeum vulgare*	gi|112145313	-	-	-	-	-	-	5
WRKY transcription factor 55	5.52	9.78	*Oryza sativa*	Q6IEM6_ORYSA	-	-	-	-	-	-	41
WRKY23 transcription factor	23.74	9.94	*Triticum aestivum*	gi|189172047	-	-	-	18	-	-	-
WRKY35 transcription factor	6.00	9.00	*Triticum aestivum*	gi|189172053	-	-	42	-	-	-	-
WSCI proteinase inhibitor	9.28	6.06	*Triticum aestivum*	Q4TZQ0_WHEAT	-	22	-	38	52	-	-
Xylanase inhibitor 801NEW	42.37	9.14	*Triticum aestivum*	gi|156186253	-	23	-	-	-	-	-
Xylanase inhibitor protein 1	33.25	8.66	*Triticum aestivum*	XIP1_WHEAT	XIPI	12	-	13	23	-	-
Xylanase inhibitor TL-XI precursor	15.63	*8.38*	*Triticum aestivum*	gi|110836641	tlxi	-	-	30	17	-	-

Criteria: M*r*, Mass range; P*I*, iso-electric point; K^a, Keumgang; K^b, Jinpum; C^a, China-108; C^b, Yeonnon-78; J^a, Norin-61; J^b, Kantou-107; SC, sequence coverage.

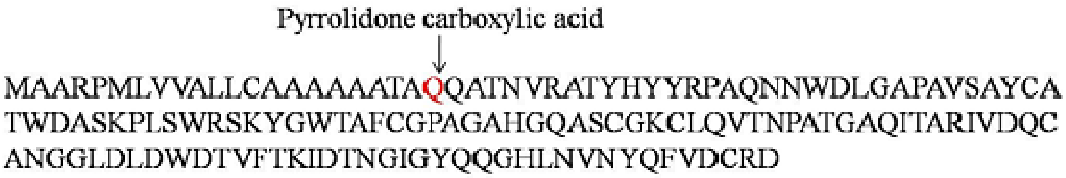

Figure 4. Prediction the PTM cleavage site by dbPTM in Wheatwin 1 protein sequence.

abundance in largescale proteomics experiments on crude protein mixtures (O'Farrell, 1975). These results would confirm previous works describing methyl jasmonate (MeJA) and its free acid jasmonic acid (JA) collectively reffered to as jasmonates, are important cellular regulators in volved in diverse developmental process, such as seed germonation, root growth, fertility, seed ripening and senescence. In addition jasmonate acivate plant defense mechanisms in response to insect driven wounding and various pathogens (Creelman and Mullet, 1997).

Thionin contains different purothionin such as alpha-1, 2 and gamma-1, 2. Thionins are homologous Cys-rich proteins of about 5 kDa that have been isolated from different tissues in a wide range of plant taxa and are active against plant pathogens both *in vitro* and *in vivo* (Carmona et al., 1993). Gamma-1-purothionin showed a higher structural analogy with scorpion toxins and against insect defensins which also present the cystine-stabilized alphahelical (CSH) motif (Bruix et al., 1993) and Gamma-2-purothionin inhibits protein translation in cell-free systems resulting in the exhibited the plant toxins for pathogen (Colilla et al. 1990). Alpha-amylase inhibits WDAI-0.19 and WDAI-0.53, which is attractive candidates for the control of seed weevils, as these insects are highly dependent on strach as an energy source (Wang et al., 2008), and WDAI-3 is a homodimeric protein against alpha-amylase from human saliva and from the insect Tenbrio molitor, but inactive against that from pig pancreas or against trypsin (Sanchez-Monge et al., 1989). Antifungal activity has been associated with two immunoc-hemically distinct proteins, the proteins are homologous with thaumatin- and pathogenesis-related proteins of the PR5 family.These proteins have intensely sweet properties of thaumatin, multiple unrelated defense functions against insect and fungal pests can now be associated with the family of thaumatin-homologous proteins (Hejgaard et al., 1991). Anti-microbial proteins (MBP-1) inhibits spore germination or hyphal elongation of several plant pathogenic fungi, including two seed pathogens of maize (*Fusarium moniliforme* Sheld. and *Fusarium graminearum* (*Gibberella zeae* (Schw.) Petsch), and several bacteria, including a bacterial pathogen of maize (*Clavibacter michiganense* ssp. nebraskense) Duvick et al. (1992). TaERF3 has an ERF/AP2 domain (Sakuma et al., 2002), and might be mainly involved in the active defence response to *B. graminis* at an earlier stage through salicylic acid (SA) signalling, and to *F. graminearum* and *Rhizoctonia cerealis* at a later stage through the ethylene/jasmonic acid signalling pathways (Zhang et al 2007). Wheat calcium-dependent protein kinase (CDPK) genes were found to respond to various biotic and abiotic stimuli, including cold, hydrogen peroxide (H_2O_2), salt, drought, powdery mildew (*B. graminis* tritici, BGT), as well as phytohormones abscisic acid (ABA) and gibberellic acid (GA) (Li et al., 2008). Chitinases are important components of plant defense in response to attack by pathogens as *F. graminearum* (Li et al., 2001). Cyclophilin was found to be necessary for host-pathogen recognition in *Arabidopsis thaliana* (Coaker et al., 2005), cyclophilin-like protein was differentially regulated as like as one of the identified NBS-LRR genes (Fofana et al., 2007). These results would confirm preceding works describing genotype-specific disease resistance in plants depending on the expression of complementary avirulence (AVR) genes in the pathogen and resistance (R) genes in the host (Bent, 1996). Plant pathogenic (R) genes have evolved specific recognition capabilities in defense against pathogens. (Recognition of *Peronospora parasitica*) RPP1-WsA, RPP1-WsB, and RPP1-WsC encoded functional products of NBS-LRR (nucleotide binding site-leucine-rich repeat) R protein class. They possess a TIR (Toll, interleukin-1, resistance) domain that is characteristic of certain other NBS-LRR–type R proteins, but in addition, they have unique hydrophilic or hydrophobic N termini. Together, the three *RPP1* genes account for the spectrum of resistance previously assigned to the *RPP1* region and thus comprise a complex *R* locus. *RPP* genes at this locus are subject to the same selective forces that have been demonstrated for structurally different LRR-type *R* genes (Botella et al., 1998), and *Fusarium* resistance protein in rice (Sasaki et al., 2002). Dilbirligi and Gill (2003) have reported many RGA sequences in wheat for identifing disease resistance gene which is similar to RGA protein in our experiment. Plant disease resistance genes operate at the earliest steps of pathogen perception. The Arabidopsis *RPP5* gene specifying resistance to the downy mildew pathogen, *P. parasitica*, was positionally cloned to encode a putative nucleotide binding site and leucine-rich repeats, and its product exhibits striking structural similarity to the plant resistance gene (Parker et al., 1997). At the Powdery mildew 3 loci in hexaploid wheat; *Triticum aestivum*, 10 alleles conferring race-specific resistance to powdery mildew (*B. graminis* sp. tritici) are known (Srichumpa et al., 2005).

Among these are PR proteins coded by the host plant that accumulates in response to pathogen infection or other signals related to plant defense responses. Several PR proteins have been characterised at the molecular level and shown to have antifungal activity *in vitro* (Datta and Muthukrishnan, 1999). Several PR proteins show enzymatic activity such as β-1,3-glucanase and chitinase (PR2 and PR3, respectively); both are involved in the degradation of microbial cell wall structural polysaccharides (Legrand et al., 1987) and PR4 and PR9, characterised by ribonuclease and peroxidase activity, respectively (Caporale et al., 2004). No enzymatic activity has been found up to now for proteins belonging to PR1 and PR5 families, nevertheless several genes belonging to these classes have been over expressed in transgenic plants strengthening the defensive role proposed for the corresponding proteins (Liu et al., 1993). Ascorbate peroxidase, peroxidase and glutathione (GSH)-dependent dehydroascorbate reductase accumulate early in grain fill. SGT1, a component of R-gene triggered disease resistance, and serpin, a serine protease inhibitor, are also present and may protect the developing grain against various pathogens (Wong et al., 2004). Pathogen resistance proteins present at this stage include serpin, chitinase, which hydrolyzes the structural carbohydrate of fungal cell walls, barwin/PR-4 protein (Wheatwin-1, 2), which is induced by fungal pathogens and binds chitin, and xylanase inhibitor protein, which inhibits a fungal enzyme that degrades plant cell walls (Hurkman et al., 2009). The *in vitro* toxicity of wheat ns-LTP associated with alteration in permeability of fungal membranes act as antimicrobial and antifungal (Sun et al., 2008), and Ubiquitin-activating enzyme E1 1 plays role as antiviral and antioxidant (Schulman and Harper, 2009). PINA and PINB acts as a membranotoxin, probably through its antibacterial and antifungal activities, contributing to the defense mechanism of the plant against predators (Capparelli et al., 2005). WRKY genes seem to play important regulatory roles in plants under abiotic and biotic stresses, and flowering plants which have the largest WRKY family are dominant over non-flowering plants in their distribution; WRKY genes might be essential for much of the enhanced adaptability of flowering plants to the environment (Dong et al., 2003). Moreover, a number of WRKY genes from different phylogenetic groups may be activated by the same physiological or environmental stimulus, such as bacterial pathogen attack (Chen and Chen, 2002) and viral pathogen attack (Dong et al., 2003).

Post translational modifications are covalent processes modifying the primary structure of proteins in a sequence-specific way that includes the reversible addition and removal of functional groups by phosphorylation, acylation, glycosylation, nitration, and ubiquitination (Mann and Jensen, 2003). These modifications induce structural changes in protein, and modulate the activities, sub cellular localization, stability, and interactions with proteins and other molecules. PTMs of proteins thus largely increase protein complexity and dynamics, resulting in the intricate regulation of biological events (Kwon et al., 2006). Protein phosphorylation plays a crucial role in pathogen response, for example, plant-pathogen interactions, gene expression, and defense signaling, in plants (Xing et al., 2002).

In conclusion, the proteomic analysis is a very useful tool for providing complex information about differences in the plant proteome during abiotic and biotic stresses. In the study, with the 2-DE system established, proteins that may be related to biotic stress were identified during mature seed in wheat. For instance, wheatwin 1 and 2 is responsible for wheat scab disease (Caruso et al., 1999), which was in physiologically and morphologically healthy mature seeds. Wheat growers can not identify, which is disease free seeds during cultivation. After sowing in the field, it drastically shows in wheat ears before maturation. We provide some functionally biotic stress proteins in mature wheat grains. We need to change the proteins and sub-cellular level by post-translation modifications for finally controling the biotic stress in wheat. Our results possesses great promise as it is supported by advanced proteomics technologies, in particular, developments in the strategies for protein detection and isolation, and to introduce biotic stress tolerance cultivars.

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Abbreviations

PR, Pathogenesis related; **PTMs,** posttrans-lational modifications; **EDTA,** ethylenediaminetetraacetic acid; **SDS-PAGE,** sodium dodecyl sulfate polyacrylamide gel electrophoresis; **CBB,** Coomassie brilliant blue; **ACN,** acetonitrile; **2DE,** dimensional gel electrophoresis; **IEF,** isoelectric focusing gels; **MeJA,** methyl jasmonate; **JA,** jasmonic acid; **CSH,** cystine-stabilized alpha-helical; **SA,** salicylic acid; **CDPK,** calcium-dependent protein kinase; **ABA,** abscisic acid; **GA,** gibberellic acid.

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