Proteins identification of wheat-rye translocation lines by MALDI-TOF-TOF mass spectrometry and ESI-QTOF/MS

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ABSTRACT

The wheat-rye translocation lines have been agriculturally developed for the crop resistant to powdery mildew, leaf rust, Hessian fly, barley yellow dwarf virus, and drought stress. Chaupon rye contains 2RL chromatin to harbor resistance genes for powdery mildew and leaf rust. In order to identify 2RL chromosome-derived specific proteins, we compared the proteome of 'Coker797' (non-2RL) with those of 'Hamlet' (2RL) and near-isogenic line (NIL) carrying 2RL by 2D-gel electrophoresis and MALDI/ESI-MS. In the leaf proteome, 24 protein spots were clearly increased in 2RL-carrying lines compared to non-2RL line. The specific proteins of 2RL-lines included heat shock protein 70, chaperon protein DnaK, malate dehydrogenase I, and triosephosphate isomerase, which were confirmed in the EST database of NILs. In the root proteome, three protein spots were identified as putative peroxidase, cytoplasmic aldolase, and oxo-phytodienoic acid reductase. These results suggest that defense mechanism-related proteins and enhanced catabolic enzymes play roles of acquiring the resistance to biotic and abiotic stress in wheat-rye translocation lines.

INTRODUCTION

Cultivated ryes (*Secale cereale*) have been known to be more resistant to the pest than wheat (*Triticum aetivum*). Thus, several molecular breeding have been attempted to develop resistant wheat-rye translocation lines. At present, the most common translocation line is the 1BL/1RS[1]. Far from the merits of pest-resistant rye 1R short-chromosome, lines carrying this chromosome display several problems in quality such as reduced gluten strength, dough stickiness, and poor loaf volume of wheat[2]. Another line, Hamlet (PI549276) is derived from a 2BS/2RL wheat-rye translocation resistant to Hessian fly in which Chaupon rye was used as the donor of 2RL chromatin to harbor resistance genes for powdery mildew and leaf rust [3]. The Hamlet-line was obtained by crossing between wheat cultivar ND7532 and rye cultivar Chaupon to translocate the chromosome 2RL (*H21* resistant gene to biotype L of Hessian fly) from rye to wheat. In this study, we performed gel-based proteomics to identify the specifically expressed proteins of leaf and root of 2RL-carrying wheat-rye translocation Hamlet line compared to non-2RL 'Coker797' and near isogenic-line (NIL).

MATERIALS AND METHODS

Plant Materials

A near isogenic-line (NIL) carrying the *H21* gene resistant to biotype L of Hessian fly was developed by backcross introgression (Coker797*4/Hamlet) and repeated selection by verifying the resistance to larvae of biotype L of Hessian fly[4].

Total protein extraction and quantitation

The proteins from leaf and root tissues of Coker797, Hamlet, and NIL lines were extracted by trichloroacetic acid/acetone precipitation method. The leaf and root proteins of the dried powder were solubilized in rehydration buffer containing 9 M urea/2 M thiourea/4% CHAPS. The solubilized proteins were quantitated by a modified Bradford method[5].

2D-PAGE/Image Analysis

Total proteins of 250 μg were electrofocused using 3-10 NL IPG strip gel (GE Healthcare) for 90,000 Vhr in leaf sample and for 12,000 Vhr in root sample. The IPG strips were equilibrated and the isoelectrofocused proteins were separated on 13% SDS-PAGE gel on Ettan Dalt system (GE Healthcare). After the electrophoresis, the 2D-gels were stained with silver staining kit (GE Healthcare). After silver staining, the gels were scanned using scanner (Powerlook III, UMAX) and the 2D-images were analyzed using Progenesis workstation version 2005 (Nonlinear Dynamics). In order assay consistent features of the 2D-gel patterns, we performed at least duplicate experiments per group.

MS analysis/Bioinformatics

Altered protein spots with more than 50% spot intensity were cut into fine slices with a razor blade, then transferred to Eppendorf tubes, and subjected to in-gel trypsin digestion according to the previous protocol[6]. All mass spectra were acquired at a reflection mode by MALDI-TOF MS (4700 Proteomics Analyzer, Applied Biosystems) and nano-LC Q-TOF MS (Premier II, Micromass). Since the genomic database of wheat cultivar is not sufficient, *de novo* internal sequencing were conducted using MS/MS fragmentation analysis and homology search against nr-BLAST database. The protein identification was performed by MASCOT (Matrixscience) and further searched by the expressed sequence tag (EST) database of wheat-rye translocation lines[7].

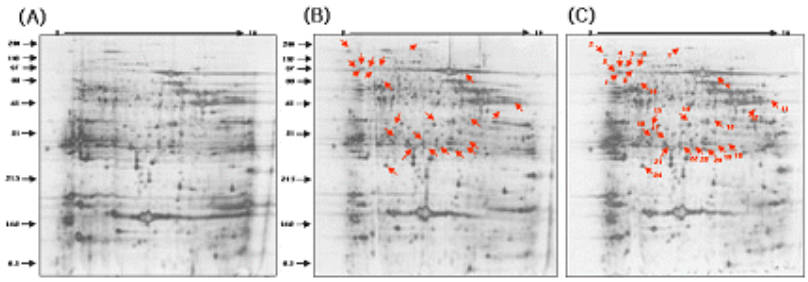

Figure 1. The silver-stained 2D-gel images of leaf -Coker797 (A), Hamlet (B), and NIL (C).

Table 1. The catalogue of the leaf proteins expressed specifically in 2RL-carryling Hamlet and Near-isogenic Line

Spot No.	Accession No.	identified protein	Organism	MW(kDa)/ pI	MS	Sequence Cover. (%)	EST -DB
1	BAD34660	methionine synthase	*Hordeum vulgare subsp. vulgare*	84452/5.68	ESI	5	-
2	AAF23074	heat shock protein 70	*Triticum aestivum*	39680/4.56	ESI	18	Yes
	ABA97211	chaperone protein DnaK	*Oryza sativa* (japonica cultivar-group)	74041/5.11	ESI	15	Yes
3						26	-
4	NP114266	ATP synthase CF1 beta chain	*Triticum aestivum*	53824/5.06	ESI	56	-
5						24	-
6	ABA92225	expressed protein	*Oryza sativa* (japonica cultivar-group)	8638/5.21	MALDI	47	-
7	NP114266	ATP synthase CF1 alpha subunit	*Triticum aestivum*	53824/5.06	ESI	22	-
8	AAD41663	resistance protein	*Oryza sativa*	19146/8.57	MALDI	48	-
9	CAD54448	ribulose 1,5-bisphosphate carboxylase large subunit	*Haworthia vittata*	49166/6.43	ESI	4	-
10	AAP55143	plastid-lipid associated protein, putative	*Oryza sativa (*japonica cultivar-group)	40016/4.42	ESI	2	-
11	AAU11110	ribulose-1,5-bisphosphate carboxylase /oxygenase large subunit	*Psathyrostachys fragilis subsp. Seca*	53064/6.44	ESI	8	-
12	CAA59228	NADPH dehydrogenase	*Hordeum vulgare*	42122/9.25	ESI	12	-
	CAA44032	rbcL	*Aegilops crassa*	46871/6.46	ESI	4	-
	CAB43994	malate dehydrogenase 1	*Brassica napus*	37708/7.59	ESI	10	Yes
13	CAA44027	rbcL	*Triticum aestivum*	46973/6.60	MALDI	39	-
14						41	
15	CAD30025	ferredoxin-NADP(H) oxidoreductase	*Triticum aestivum*	40206/6.92	MALDI	38	-
16	CAC14917	triosephosphat-isomerase	*Triticum aestivum*	26786/5.38	MALDI	37	Yes
17						44	
18	CAA44027	rbcL	*Triticum aestivum*	46973/6.60	MALDI	39	-
19	AAK72543	ribulose-1,5-biphosphate carboxylase	*Wolffia arrhiza*	49590/6.32	MALDI	37	-
20	CAA44027	rbcL	*Triticum aestivum*	46973/6.60	MALDI	37	-
21	CAC83406	ribulose-1,5-biphosphate-carboxylase	*Moraea namaquamontana*	49179/6.46	MALDI	34	-
22	NP114256	ATP synthase CF1 alpha subunit	*Triticum aestivum*	55261/6.11	MALDI	4	-
23	NP114267	ribulose-1,5-bisphosphate carboxylase /oxygenase large subunit	*Triticum aestivum*	52817/6.22	MALDI	35	-
24	NP114256	ATP synthase CF1 alpha subunit	*Triticum aestivum*	55261/6.11	MALDI	4	-

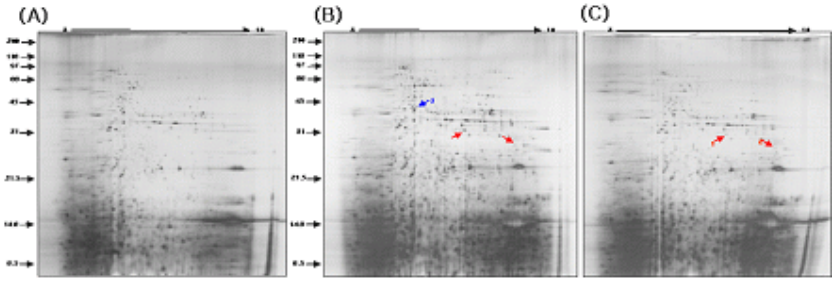

Figure 2. The silver-stained 2D-gel images of root -Coker797 (A), Hamlet (B), and NIL (C).

Table 2. The catalogue of the root proteins expressed specifically in 2RL-carryling Hamlet and Near-isogenic Line.

Spot No.	Accession No.	identified protein	Organism	MW(kDa)/pI	Sequence Coverage (%)	EST-DB
1	CAB79456	putative peroxidase	*Arabidopsis thaliana*	34846/10.05	4	-
2	BAA02729	cytoplasmic aldolase	*Oryza sativa*	38695/6.56	3	-
3	CAD89604	oxo-phytodienoic acid reductase	*Oryza sativa(japonica cultivar-group)*	42438/5.79	4	-
	BAD06519	hypothetical protein	*Pisum sativum*	40922/5.52	5	-

RESULTS AND DISCUSSION

In the leaf proteomic analysis, 24 protein spots were clearly increased in 2RL-carrying lines compared to non-2RL line (Figure 1). From the selected spots, 27 proteins in total were putatively identified by tandem mass spectrometry, which corresponded to 18 unique proteins. The spot number 2, 11, and 12 included two different proteins in single spot. Twelve protein spots were identified against non-wheat organisms due to the incomplete wheat genomic database. Interestingly, heat shock protein 70, chaperon protein DnaK, malate dehydrogenase I, and triosephosphate isomerase were confirmed in the EST database of NILs cDNA library. Thus, these suggest that up-regulation of heat shock proteins and metabolic enzymes are involved in the acquired resistance of wheat-rye translocation to the biotic and abiotic stress. In additions, methionine synthase, ATP synthase CF1 α/β chain, Rubisco large subunit, NADPH dehydrogenase, ferredoxin-NADP(H) oxidoreductase, resistance protein, and plastid lipid-associated proteins were exclusively identified in 2RL-lines by proteomic approach. Four protein spots out of 9 spots assigned as Rubisco large subunit were solely identified by *T. aestivum* and previous wheat leaf proteomic data[8]. Most of leaf proteins in this study did not match with previous leaf proteomic data. However, methionine synthase, HSP70, DnaK and triosephosphate isomerase, were commonly identified as wheat root proteome[9]. By the root proteome analysis of 2RL-lines, three known protein spots were identified as putative peroxidase, cytoplasmic aldolase, and oxo-phytodienoic acid reductase.

In particular, the hydrogen peroxide and peroxidase are known to play a key role in the defense response of plants to the pathogen[10]. Interestingly, the spot number 3 was exclusively identified in Hamlet as oxo-phytodienoic acid reductase and hypothetical protein. In summary, this proteomic study of wheat-rye translocation lines will give helpful clues to solve the defense mechanism of plants against pathogens and provide useful information to select marker-based selection for crop development.

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