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Expressed Sequence Tags of the Wheat-rye Translocation Line Possessing 2BS/2RL

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ABSTRACT

Hamlet (PI549276) possessing 2RL was obtained by cross between a wheat cultivar ND7532 (Froid/Centurk) and a rye cultivar Chaupon. Chaupon was known to have resistant gene to biotype L of Hessian fly [*Mayetiola destructor* (Say)] larvae. The wheat-rye translocation line (Coker797*4/Hamlet) was also known to be resistant to biotype L of Hessian fly larvae. We analysed a set of 96 ESTs from the wheat-rye translocation line (2BS/2RL). ESTs were classified by various physiological processings, such as primary metabolism, secondary metabolism, transcription, translation, transport, signal transduction, defense, transposable element, and others. Three sequences encoding thioredoxin peroxidase, 26S rRNA, and rubisco small subunits were homologous to registered genes in rye. Although limited number of clones were used to develop ESTs, these clones and their sequence information may be useful for researchers studying general physiology and molecular biology on the translocation line.

Keywords : wheat-rye translocation line, cDNA library, ESTs, 2BS/2RL.

Cultivated rye (*Secale cereal* L.) is one of the major cereal crops for both grain and forage. It is important as a source of pest resistance genes which have been introduced in wheat breeding programs. It is known as a donor of genome (R) in triticale. Wheat-rye translocation and substitution lines have been widely used by breeders in many countries for decades (Baum & Appels, 1991; Rabinovich, 1998). These wheat lines were determined as high productivity, adaptability, and disease and insect resistance (Heun & Friebe, 1990; Friebe et al., 1991; Cakmak et al., 1997).

The most common transloctions present in breeding programs are the 1BL/1RS and the 1AL/1RS. The short arm of chromosome group 1 from rye is known to carry resistance genes of *Sr31, Lr26, Yr9,* and *Pm8* to stem rust (*Puccinia gramins* Pers.), leaf rust (*P. recondita* Rob. ex Desm.), strip rust (*P. striiformis* Westend.), and powdery mildew (*Blumeria gramins* DC.), respectively. It is also known to enhance adaptation, stress tolerance, and yield potential in wheat (Hsam & Zeller, 1997; McIntosh et al., 1993; Reynaldo et al., 1998; Singh et al., 1998). Despite the advantages associated with 1RS translocation, deleterious influences on wheat end-use quality have been identified. The most commonly cited deleterious effects are reduced gluten strength, poor loaf volume, and dough stickiness (Fenn et al., 1994; Seo et al., 1995).

Hamlet (PI549276) developed by the Kansas Agricultural Experiment Station at Kansas State University is the form of a 2BS/2RL wheat-rye translocation and is resistant to Hessian fly [*Mayetiola destructor* (Say)] (Sears et al., 1992). Hamlet is derived from a cross between a susceptible hexaploid wheat 'ND7532' (Froid/Centurk) and a resistant diploid rye 'Chaupon' to Hessian fly. Friebe et al. (1991) concluded that the long arm of rye chromosome 2R carried a gene or gene complex that coded materials acting as antibiosis to Hessian fly larvae.

The long arm of chromosome group 2 from Chaupon rye lacks genes that code seed storage proteins. Only short arm of 2R has *Sec-2* and *Sec-5* loci responsible for a unique family of 75 kDa γ-secalins which does not have analogues in the other cereals (Malyshev et al., 1998). Therefore, the 2BS/2RL wheat-rye translocation line should not affect wheat storage protein because most of wheat storage protein were encoded by the genes located on chromosome groups 1 and 6. Knackstedt et al. (1994) reported that significant differences were not found for flour protein, mixograph mixing tolerance, loaf volume, and crumb grain score between five 2BS/2RL translocation lines and eleven non-translocation lines.

DNA marker for 2RL translocation was identified by DNA polymerase chain reaction (PCR) with primers derived from R173 family of moderately repetitive rye DNA (Lee et al., 1996). Seo et al. (1997) reported a molecular marker associated with Hessian fly resistance gene (*H21*) on 2RL.

Single-pass sequencing of cDNAs randomly selected from a library of genes helps to quickly identify functions of expressed genes, to understand

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the complexity of gene expression, and to offer a complementary approach for biochemical and genetic analysis. In the plant kingdom, partial cDNA sequencing had been performed to generate expressed sequence tags (ESTs) in *Arabidopsis* (Cooke et al., 1996), rice (Uchimya et al., 1992; Sasaki et al., 1994), maize (Keith et al., 1993), *Citrus* (Hisada et al., 1997), *Brassica napus* (Park et al., 1993), *Brassica campestris* (Lim et al., 1996), and *Medicago truncatula* (Covitz et al., 1998). It may be possible to compile a large number of genes from many different species by using the approach of EST analysis.

In this study, we describe the collection of ESTs from the wheat-rye translocation line (2BS/2RL), which is resistant to Hessian fly and has desirable agronomic characteristics.

MATERIALS AND METHODS

Plant materials and cDNA library construction

The wheat-rye translocation line (Coker 797*4/ Hamlet) selected for resistance to biotype L of Hessian fly larvae was grown in pots at 25/18℃ (day/night). Total RNA was isolated from the 4-week old leaves using a commercially available Trizol reagent (Gibco BRL). Poly$(A)^+$ RNA was separated from total RNA using PolyATract mRNA isolation system (Promega). The cDNA library was constructed from 5 μg of poly$(A)^+$ RNA using ZAP-cDNA Gigapack Ⅲ cloning kit (Stratagene). The primary library represents approximately 0.9×10^6 recombinants. The cDNA clones were excised as pBluscript SK (+/-) phagemids (Stratagene) in the bacterial host SOLA strain according to the mass excision protocol supplied by Stratagene.

Nucleotide sequencing

Single clones were randomly selected and amplified in 5 ml culture of LB medium containing 50 μg/ml amplicillin for 12~16 hours. Excised phagemid for sequencing reaction was extracted by alkaline lysis method (Sambrook et al., 1989). The size of inserted cDNA was estimated by 1.0% agarose gel electrophoresis after digestion with *EcoR* I and *Xho* I. Template cDNA was amplified with T3 promoter primer using the BigDye terminator cycle sequencing ready reaction kit (Perkin Elmer). Electrophoresis was performed on the ABI PRISM 310 Genetic Analyzer (Perkin Elmer).

Data analysis

Analyzed sequences were edited manually by removing vector and ambiguous sequences. Nucleotide sequences obtained from approximately 200~500 base pairs of each clone were used for database search. Each sequences were translated in all six reading frames and compared with the non-redundant database at the National Center for Biotechnology Information (NCBI) using the BLASTX program. Sequences that did not match with those in the protein database were compared with non-redundant database using the BLASTN program. The remaining unidentified ESTs were compared with dbEST using the BLASTN program.

RESULTS

In order to generate young seedling ESTs of wheat-rye translocation line (2BS/2RL) which is resistant to biotype L of Hessian fly and has desirable agronomic traits, we constructed a cDNA library from mRNA of 4-week old leaf tissues. Plaque forming unit (pfu) of the primary library was 0.9×10^6. The average size of inserted cDNA was approximately 1.0 kb. The nucleotide sequences obtained by single-pass sequencing were mainly 200~500 base pairs.

Among the 96 ESTs generated from the cDNA library, 59 clones showed homology with amino acid sequences of registered genes. A summary of putative identification of ESTs matched with amino acid sequences are shown in Table 1. Among the rest of 37 ESTs, 6 ESTs were shown to have homology with nucleotide sequences at non-redundant database (Table 2) and 6 of them had homology with nucleotide sequences at dbEST. Twenty five ESTs did not have homology with any known sequences.

Sixty two ESTs obtained by single pass sequencing were homologus to genes found in plants. Three clones (WRC61, WRC69, and WRC71) showed homology to genes, which were encoding thioredoxin peroxidase, 26S rRNA, and rubisco small subunits, registered in *Secale cereale.* A few sequences had homology with genes identified in animal and microorganisms such as *Homo sapiens, Schizosaccaromyces pombe, Escherichia coli,* and *Caenorhabditis elegans.*

The functional classification of ESTs represented physiological processing, such as primary metabolism, secondary metabolism, transcription, translation, transport, signal transduction, defense, transposable element, and others (Table 3). Thirty-three clones were related to primary metabolism involved photosynthesis, photorespiration, and glycolysis. Those clones were identified as they contain genes encoding 2-oxoglutarate/malate translocator, chlorophyll a/b binding protein, chlorophyll a/b binding protein CP29 precursor, dTDT-glucose 4-6-dehydrogenase, ferredoxin-NADP redutase, ferredoxin precursor, glycer-

Table 1. Summary of putative identification of genes searched homology with BLASTX program.

Clone No.	Putative Identification	Size (Kbp)	Score†	ID† (%)	PS† (%)	Organism
WRB48	1-aminocyclopropane-1-carboxylate oxidase	1.3	348	81	93	*Sorghum bicolor*
WRC63	2-oxoglutarate/malate translocator	0.5	262	90	94	*Panicum miliaceum*
WRB39	4-nitrophenylphosphatase	1.2	184	58	76	*Schizosaccharomyces pombe*
WRB22	Auxin transport protein REH1	0.5	145	96	96	*Oryza sativa*
WRB74	Blue-light photoreceptor	2.0	501	83	90	*Oryza sativa*
WRB26	C-4 sterol methyl oxidase	1.0	479	61	80	*Arabidopsis thaliana*
WRB23	Calcium-dependent ser/thr protein kinase	0.8	282	75	88	*Arabidopsis thaliana*
WRB82	Catalase 1	2.0	369	100	100	*Triticum aestivum*
WRB30	Chlorophyll a/b binding protein	1.0	269	88	93	*Triticum aestivum*
WRB24	Chlorophyll a/b binding protein(cab-11)	0.5	146	85	96	*Lycopersicon esculentum*
WRB83	Chlorophyll a/b binding protein(cab-11)	0.4	435	85	95	*Lycopersicon esculentum*
WRC62	Chlorophyll a/b binding protein precursor	1.0	373	93	94	*Oryza sativa*
WRB8	Chlorophyll a/b binding protein of LHC Ⅱ type Ⅰ	1.1	491	90	94	*Triticum aestivum*
WRB14	Chlorophyll a/b binding protein of LHC Ⅱ type Ⅰ	1.1	293	77	82	*Triticum aestivum*
WRB35	Chlorophyll a/b binding protein of LHC Ⅱ type Ⅰ	0.9	278	86	89	*Triticum aestivum*
WRB50	Chlorophyll a/b binding protein of LHC Ⅱ type Ⅰ	1.0	575	90	93	*Triticum aestivum*
WRB37	Chlorophyll a/b binding protein CP29 precursor	0.8	565	100	100	*Hordeum vulgare*
WRB52	Chlorophyll a/b binding protein CP29 precursor	0.7	352	82	82	*Hordeum vulgare*
WRC17	Chloroplast 50S ribosomal protein L31	0.7	269	64	84	*Medicago sativa*
WRB64	Chloroplast triose phosphate translocator precursor	0.9	360	87	96	*Zea mays*
WRB40	Curved DNA-binding protein	0.5	485	98	98	*Escherichia coli*
WRC45	Copia-like transposable element	0.8	501	88	94	*Arabidopsis thaliana*
WRB33	dTDP-glucose 4-6-dehydratase	1.0	607	96	99	*Arabidopsis thaliana*
WRC18	Ferredoxin-NADP reductase (FNR)	1.3	173	37	45	*Oryza sativa*
WRC74	Ferredoxin precursor	0.3	188	97	97	*Triticum aestivum*
WRB28	Glyceradehyde 3-phosphate dehydrogenase, cytosolic	0.7	600	98	100	*Hordeum vulgare*
WRC51	Hypothetical protein	0.9	234	61	68	*Arabidopsis thaliana*
WRC19	Lipid transfer protein Cw(21)	0.9	276	79	92	*Hordeum vulgare*
WRB73	Methyltransferases	1.2	133	56	73	*Caenorhabditis elegans*
WRB87	MYB-like protein isolog	0.5	180	55	74	*Arabidopsis thaliana*
WRB6	NADP-dependent glyceradehyde-3-phosphate dehydrogenase	1.8	448	85	92	*Zea mays*
WRB76	NADP-dependent oxidoreductase P1	0.8	257	60	80	*Arabidopsis thaliana*
WRB2	Phenylalanine tRNA synthetase	0.9	439	64	77	*Homo sapiens*
WRC3	Phosphatase like protein	1.0	144	54	76	*Arabidopsis thaliana*
WRC11	Photosystem Ⅰ antenna protein	1.0	318	73	73	*Hordeum vulgare*
WRB34	Photosystem Ⅰ reaction centre subunit Ⅹ	0.6	467	95	100	*Hordeum vulgare*
WRC2	Photosystem Ⅰ reation centre subunit Ⅹ precursor (PSI-K)	0.8	364	93	97	*Hordeum vulgare*
WRB90	Photosystem Ⅰ reaction centre subunit (PSI-L)	0.6	413	95	96	*Hordeum vulgare*
WRB81	Photosystem Ⅱ 10 kDa polypeptide	0.7	348	81	92	*Oryza sativa*
WRB18	Photosystem Ⅱ 10 kDa polypetide precursor	0.4	197	48	67	*Solanum tuberosum*
WRC64	Photosystem Ⅱ oxygen-evolving complex protein Ⅰ	0.9	544	93	98	*Oryza sativa*
WRC68	Precursor of the oxygen evolving complex 17 kDa protein	0.8	225	59	68	*Zea mays*
WRC39	Protein translation factor SUI Ⅰ homolg (GOS2 protien)	0.4	124	96	100	*Zea mays*
WRB80	Protein translation factor SUI Ⅰ homolg (GOS2 protien)	0.8	377	87	95	*Oryza sativa*
WRB36	Pyruvate kinase	1.5	465	73	85	*Ricinus communis*
WRB15	Putative protein	0.8	240	60	80	*Arabidopsis thaliana*
WRC27	RAS-related protein RAB7	0.9	274	98	100	*Pennisetum ciliare*
WRC75	Ribulose-bisphosphate carboxylase	0.6	196	90	90	*Triticum aestivum*
WRB7	Ribulose-bisphosphate carboxylase	0.8	342	75	76	*Triticum aestivum*
WRB9	Ribulose 1,5-bisphosphate carboxylase activase	1.6	443	96	98	*Hordeum vulgare*
WRB12	Ribulose 1,5-bisphosphate carboxylase activase	0.9	374	98	98	*Hordeum vulgare*
WRB90	Ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit	0.8	413	95	96	*Hordeum vulgare*
WRB41	Rubulose bisphosphate carboxylase small chain	0.6	407	98	98	*Aegilops sequarrosa*
WRC71	Ribulose bisphosphate carboxylase small chain precursor	0.7	361	98	100	*Secale cereale*
WRB17	SAH7 protein	0.7	263	52	69	*Arabidopsis thaliana*
WRC77	Serine/threonine kinase	2.0	179	66	82	*Sorghum bicolor*
WRB75	Transketolase 2	0.8	397	82	93	*Capsicum annuum*
WRB31	Unknown protein	1.6	272	89	94	*Arabidopsis thaliana*
WRC94	Unknown protein	0.6	295	77	86	*Arabidopsis thaliana*

† Score, ID (identities), and PS (positive identities) were provided by BLASTX program.

Table 2. Summary of putative identification of genes searched homology with BLASTN program.

Clone No.	Putative Identification	Size (Kbp)	Score †	ID † (%)	PS † (%)	Organism
WRC5	Chlorate/nitrate transfer (CHL1)	1.0	289	69	69	*Arabidopsis thaliana*
WRC61	Heat shock protein 16.9C (hsp16.9C)	0.6	573	93	93	*Triticum aestivum*
WRC69	Hordeum vulgare partial mRNA	0.8	1329	96	96	*Hordeum vulgare*
WRB89	Poly(A)-binding protein	1.0	350	83	83	*Triticum aestivum*
WRC20	Rye 26S rRNA 3′ end and 18S rRNA, 5′ end	1.5	1264	94	94	*Secale cereale*
WRB19	Thioredoxin peroxidase (TPx1)	0.3	724	99	99	*Secale cereale*

† Score, ID (identities), and PS (positive identities) were provided by BLASTN program.

Table 3. Number of clones classified by their function.

Category	Number of clones
Genes identified	65
Primary metabolism	33
Secondary metabolism	2
Transcription	3
Translation	5
Transport	5
Signal transduction	3
Defense	4
Transposable element	1
Miscellaneous	9
Genes matched dbEST	6
Genes unidentified	25
Total	96

adehyde 3-phosphate dehydrogenase, photosystem I antenna protein, photosystem I reaction centre subunit (PSI-L), photosystem II 10 kDa polypeptide, photosystem II oxygen-evolving complex protein I, pyruvate kinase, rubisco, rubisco activase, rubisco small subunit, and transketolase. The abundant ESTs are chlorophyll a/b binding protein and rubisco small subunit encoding genes whose activities are controlled by light. Two clones related to the seconary metabolism were found, which encode C-4 sterol metyl oxidase and 1-aminocyclopropane-1-caoxylase oxidase. These two enzyme were known to participate in sterol metabolism (Bramley, 1997) and enthylene biosynthesis (Kende & Zeevaart, 1997), respectively.

Several putative genes associated with transcrption or translation were identified. These genes were related to encode curved DNA-binding protein, MYB-ike protein isolog, poly(A)-binding protein, phenylalanine tRNA synthetase, protein translation factor SUI 1 homolog (GOS2 protein), 26 rRNA, and chloroplast 50S ribosomal protein L31. In the previous studies, the most expressed gene is ribosomal protein which was related to protein synthesis and procesing (Uchimiya et al., 1992, Lim et al., 1996, Covitz et al., 1998). However, only one clone represented ribosomal protein in this study.

In order to response to stimuli, the plant cell has several receptors for signal transduction. The role of receptors amplifies signal and then alters gene expression as a response. Plant protein kinases found in plant cell are related to signal transduction and involved in many aspects of cellular regulation and metabolism (Stone & Walker, 1995). It is known that eukaryotic protein kinases phosphorylate serine and/or threonine or tyrosine (Stone & Walker, 1995). Genes encoding receptor such as blue-light photorecetor belonged to one type of photoreceptors which were present in plant was found in the cDNA library. Calcium-dependent ser/thr protein and ser/thr kinase, which were involved in protein kinase that phosphorylated ser and/or thr were also identified in the cDNA library.

We found putative genes related to transport of specific molecules such as auxin, lipid, and others. These genes encode auxin transport protein REH 1, chloroplast triose phosphate translocator precursor, lipid transfer protein, chlorate/nitrate transport, and RAS-related protein RAB7.

We expected to isolate many genes that were related to defense mechanism because wheat-rye translocation line (2BS/2RL) was resistant to Hessian fly and other pathogens such as powdery mildew, leaf rust, etc. Four clones were homologus to genes associated with defense mechanism, such as genes for NADP-dependent oxidoredutase P1, thioredoxin peroxidase, catalase and heat shock protein 16.9C. NADP-dependent oxidoredutase and thioredoxin peroxidase were known to be involved in defense against oxidative stress (Chae et al., 1994; Babiychuk et al., 1995).

We detected a *copia*-like transposable element that was associated with retrotransposons. Retrotransposons with the presence of long terminal repeat include Ty elements of *Saccharmyces cerevisiae* and *copia*-like element of *Drosophila*. This clone was homologus to putative *copia*-like transposable element of *Arabidopsis thaliana* and was expected to one type of retrotranspon families.

DISCUSSION

The wheat-rye translocated germplasm (2BS/2RL) used in this research was known to be resistant to Hessian fly as was found in Hamlet (Seo et al., 1997). Long arm of 2R was known to be possessed resistance genes for powdery mildew and leaf rust (data not shown). Therefore, 2RL is expected to possess several agriculturally advantageous genes. If we identify and clone these genes, we can develop molecular markers that related to agronomic traits. The cloned genes could also directly used in breeding programs for screening plants for favorable genes.

Although 2RL in the form of wheat-rye translocation didn't show detrimental effects on wheat the end-use quality (Knackstedt et al., 1994), it would be more efficient to use genes for agronomic characteristics rather than using the whole arm. In the previous studies on 2RL, PCR-based marker system was mainly developed for identification of chromosomal translocation (Lee et al., 1996) and for screening of resistant plant of Hessian fly (Seo et al., 1997). However, studies on the expressed genes in wheat-rye translocation lines were very rare. Therefore, further study to focus on identifying 2RL specific genes would be required.

The objective of this study is to analyse expressed genes in young seedling of translocation line (2BS/-2RL). Although genes directly related to resistance to Hessian fly were not found in this study, three sequences encoding thioredoxin peroxidase, 26S rRNA, and rubisco small subunits were homologous to registered genes in rye. Further study would be necessary to prove that genes were expected to be located on 2RL.

Although limited number of clones were used for analyzing ESTs, these clones and their sequence information may be useful for researchers studying general physiology and molecular biology on the translocation line. If large-scale ESTs are performed, this data will be also useful in mapping of translocation line.

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