Gene expression profiles of hybrid necrosis in synthetic hexaploid wheat

Mizuno N[1,2], Takumi S[1]

[1] Graduate school of Agricultural Science, Kobe University. , 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan. [2] Research Fellow of the Japan Society for the Promotion of Science (JSPS)

ABSTRACT

Some abnormal growth phenotypes including hybrid necrosis are often observed in F_1 hybrids (genome constitution, ABD, 2n=21) and synthetic hexaploid wheat lines (AABBDD, 2n=42) which were artificially produced by crossing between tetraploid wheat (AABB, 2n=28) and *Ae. tauschii* (DD, 2n=14). The hybrid necrosis phenotypes are generally divided into type I and type II necrosis. In the hybrid plants showing type I necrosis, cell death occurs gradually from the older tissues. Little information about causal genes of hybrid necrosis has been reported, and the molecular mechanisms of hybrid necrosis are still largely unknown. To compare comprehensively gene expression profiles among synthetic hexaploid wheat lines showing normal growth (WT) and type I necrosis phenotypes, cDNA-AFLP analysis was performed using mRNAs from three synthetic hexaploid wheat lines between the tetraploid wheat cultivar Langdon (Ldn) with *Ae. tauschii* accessions, and their parental lines. In total, 769 AFLP fragments were observed using 55 selective primer sets. Ldn- and *Ae. tauschii*-derived fragments and additional fragments in the synthetic wheat were observed and compared between the WT and type I necrosis lines. However, no significant differences were found in the number of the Ldn- and *Ae. tauschii*-derived fragments the newly appeared between the WT and type I necrosis lines. Few genes specifically expressed in the WT and type I necrosis lines could be identified, which indicated that limited changes of the gene expression patterns might induce the phenotypic difference between the WT and type I necrosis lines.

INTRODUCTION

Common wheat (*Triticum aestivum* L., 2n=6x=42, genome constitution AABBDD) was originated from a spontaneously occurring interspecific hybridization of tetraploid wheat (*T. turgidum*, 2n=4x=28, AABB) and *Aegilops tauschii* Coss. (syn. *Ae. squarrossa* L., 2n=2x=14, DD) followed by chromosome doubling in the triploid hybrid between. So, *Ae. tauschii* is a wild progenitor and D genome donor of common wheat[1,2]. Tetraploid wheat is artificially crossed with pollens of *Ae. tauschii*, and then synthetic hexaploid wheat can be produced through amphidiploidization of the triploid F_1 hybrid. The production of synthetic hexaploid wheat plants reflect the natural process of *T. aestivum* speciation which occurred approximately 8,000 years ago at the coastal region of South Caspian sea in Iran[3-6]. However, it was reported that triploid F_1 hybrids between tetraploid wheat and *Ae. tauschii* showed some abnormal growth phenotypes and germination failure[7-9]. The abnormal growth phenotypes include two types of hybrid necrosis, hybrid virescence, hybrid chlorosis, and hybrid severe dwarf. The hybrid necrosis phenotypes in the F_1 hybrids between tetraploid wheat and *Ae. tauschii* are generally divided into type I and type II necrosis[9]. In the hybrids plants showing the type I necrosis, cell death occurs gradually from older tissues. On the other hand, the hybrid plants showing a type II necrosis grow normally until exposed to low temperature during which the necrotic phenotype and incomplete emergence from the leaf sheath in tillering stage occurs [9]. In accordance with the model by Hybrid necrosis is classified as a postzygotic reproductive barrier. In accordance with the model by Dobzhansky and Muller, hybrid necrosis is controlled by two complementary genes[10]. In *Arabidopsis thaliana* L., hybrid necrosis is also observed in some intraspecific crosses, and the causal genes of hybrid necrosis were recently isolated[11]. However, the biochemical and molecular mechanisms of hybrid necrosis, and the relationship between the causal genes and the speciation are still largely unknown. Few causal genes of hybrid necrosis have been identified in other species including wheat.

In wheat, *Ne1* and *Ne2* loci are well known to control hybrid necrosis in hexaploid and tetraploid wheat[12,13]. These two dominant complementary genes *Ne1* and *Ne2* are located on chromosome arms 5BL and 2BS, respectively[12-14]. Hybrid necrosis occurring in the F_1 hybrids between tetraploid wheat and *Ae. tauschii* is genetically different from the *Ne1*-*Ne2*-induced necrosis.

To elucidate the mechanism of hybrid necrosis in the hybrids between tetraploid wheat and *Ae. tauschii*, gene expression profiles among the hybrid plants showing the types I necrosis and normal growth features were compared.

MATERIALS AND METHODS

Synthetic hexaploid wheat lines used in this study were generated from F_1 hybrids between between tetraploid wheat cultivar Langdon (Ldn) and *Ae. tauschii* accessions[15]. Three synthetic wheat lines and their parental Ldn and for *Ae. tasuchii* accessions, KU-2059, KU-2159 and KU-2828, were used for the cDNA-AFLP analysis. Synthetic wheat lines from KU-2828 showed the type I necrosis. Selfed-seed progeny (F_2) could be obtained from the hybrid necrosis F_1 plants, although the harvested seed number was quite low. In this study, the phenotype of the triploid hybrids and the synthetic hexaploid wheat lines showing normal growth features is represented as a wild type (WT). Total RNA was isolated from seedling leaves. The mRNA was purified by the polyATract mRNA isolation system (Promega,

Table 1. Sequence of universal and selective primers used in the cDNA-AFLP analysis.

Primer name	Sequence (5'-3')
EcoRI universal primer	GACTGCGTACCAATTC
EcoRI selective primers	GACTGCGTACCAATTCNNN[a])
MseI universal primer	GATGAGTCCTGAGTAA
MseI selective primers	GATGAGTCCTGAGTAANNN

[a] N means the single nucleotide either A, C, G or T

USA). First strand cDNA was synthesized using a first strand cDNA synthesis kit (TOYOBO, Japan), the second strand cDNA was synthesized with DNA polymerase I and RNase H. The second strand cDNA samples were used for AFLP amplification reactions by AFLP® Core Reagent Kit (Invitrogen, Tokyo, Japan). The digested and ligated cDNA samples were pre-amplified with *Eco*RI and *Mse*I universal primers followed by a second amplification with selective primer sets (Table 2). The AFLP products were fractionated by electrophoresis through 13% non-denaturing polyacrylamide gels and visualized by the silver staining method[11]. Specific bands were recovered from polyacrylamide gels, and then amplified using the *Eco*RI and *Mse*I selective primers for direct sequencing.

RESULTS AND DISCUSSION

To compare comprehensively the gene expression profiles between WT and type I necrosis lines, cDNA-AFLP analysis was performed using RNA from the synthetic hexaploid wheat lines and their parental lines. Totally 769 AFLP fragments were detected using the 55 selective primer sets. Out of the 769 fragments, 119 fragments commonly appeared in all lines, the three synthetic hexaploid wheat lines, Ldn and the three *Ae. tauschii* accessions (Fig. 1A). 22 fragments were detected only in Ldn. 53 fragments were found only in at least one line of the three synthetic wheat lines: in other words, these 53 fragments were specific to the synthetic wheat. 196 fragments appeared in at least one of the three *Ae. tauschii* accessions. 108, 202 and 14 fragments were detected as the common fragments between at least one synthetic hexaploid wheat line and at least one *Ae. tauschii* accession, Ldn and at least one synthetic hexaploid wheat line, Ldn and at least one *Ae. tauschii* accession, respectively. The numbers for the absent and present in hexaploid wheat lines were summarized in Figure 1B and compared between WT and type I necrosis lines. Significant differences were found in the number of the Ldn-derived fragments absent in the synthetic hexaploid wheat between WT and type I necrosis lines. On the other hand, the numbers of *Ae. tauschii*-derived fragments absent in the hexaploid wheat lines did not differ significantly between WT and type I necrosis lines. No significant differences were observed in the total number of fragments absent in the hexaploid wheats and the number of additional fragments in WT and type I necrosis lines.

To clarify the molecular nature of the following three types of the cDNA-AFLP fragments, the AFLPfragments were directly sequenced, and their

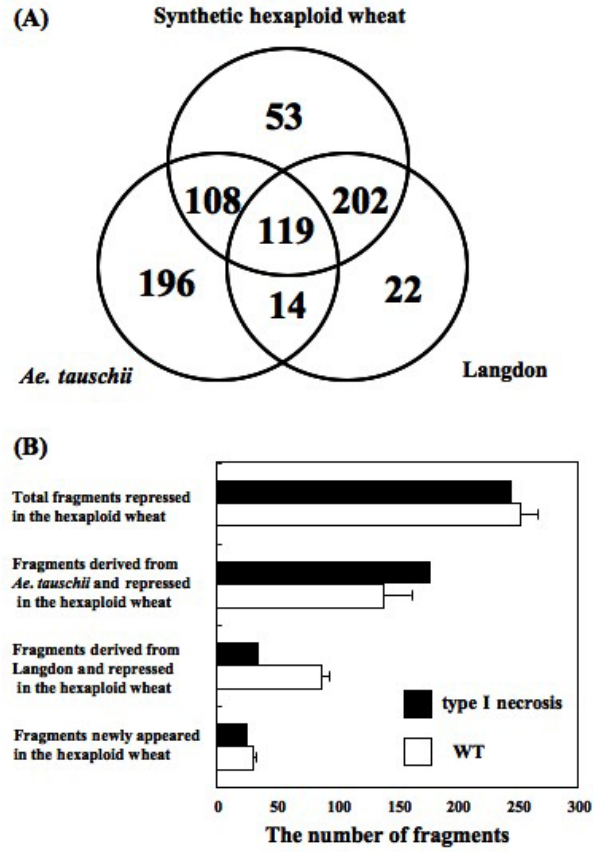

Fig. 1. Summary of the cDNA-AFLP analysis. (A) Classification of the cDNA-AFLP fragments in the synthetic hexaploid wheat, Ldn and *Ae. tauschii* lines. Overlapping areas indicate common bands among the synthetic hexaploid wheat and their parents. (B) Comparison of the number of absent and additional fragments in the synthetic hexaploid wheat lines showing WT and type I necrosis.

nucleotide sequences were analyzed by the BLAST search (Table 2). The analyzed types of the cDNA-AFLP fragments were detected only in Ldn and type I necrosis line, only in WT lines and only in type I necrosis line. The nucleotide sequences of *f-3* and *7-f-2* fragments, which were detected only in Ldn and type I necrosis line, showed high homology to barley PSII 10kD protein and rice acid phosphatase-like protein genes, respectively. Both nucleotide sequence of the *6-4-2* and *6-4-3* fragments, which were detected only in type I necrosis line, were homologous to a methionine synthase gene. The other fragments showed no homology to the previously reported genes of identified function. To confirm the cDNA-AFLP result, RT-PCR analysis was performed using the fragment-specific primers. However, the RT-RCR results unfortunately disagreed with the cDNA-AFLP results, which might be due to PCR amplification without distinguishing between the three homoeologues (Fig. 2). These results indicate that small changes of gene expression patterns might affect the phenotypic difference between WT and type I necrosis lines. Further detailed characterization of

the type I necrosis as well as the type II necrosis will be required to understand the birth of bread wheat.

Table 2. Summary of the BLAST analysis of transcripts identified in the cDNA-AFLP analysis.

Name	Putative function	E-value
Only in Langdon and the type I necrosis lines		
f-3	PSII 10kD protein	6e-78
7-f-2	acid phosphatase-like protein	0.0
1-1-1	Unknown	-
1-1-2	Unknown	-
3-f-1	Unknown	-
3-f-2	Unknown	-
3-f-3	Unknown	-
4-6-1	Unknown	-
4-6-2	Unknown	-
7-f-4	Unknown	-
Only in the WT lines		
6-1-2	Unknown	-
7-f-6	Unknown	-
Only in the type I necrosis lines		
6-4-2	methionine synthase	9.9E-14
6-4-3	methionine synthase	4.1E-16

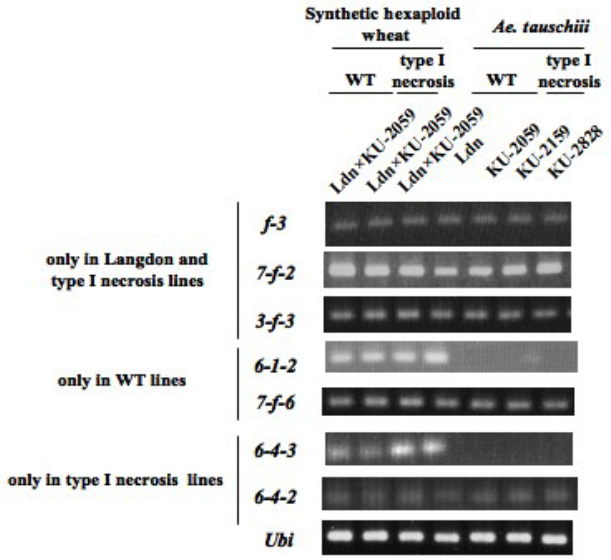

Fig. 2. Expression profiles of genes identified by the cDNA-AFLP analysis in seedlings of the synthetic hexaploid wheat lines showing WT and type I necrosis lines and their parental lines. Comparison of transcripts accumulation levels between WT and type I necrosis lines. *Ubi* was used as an internal control.

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