Comparative DNA Methylation Analysis of Powdery Mildew Susceptible and Resistant Near-Isogenic Lines in Common Wheat

Lina Pan, Xiaoying Liu, Zhenying Wang*

College of Life Science, Tianjin Normal University, 300387, Tianjin, China
E-mail: wzycell@yahoo.com.cn

Abstract: Powdery mildew is one of the most serious diseases of common wheat, and most studies are focus on screening the new resistance genes, while little attention was paid to the epigenetic profiling for resistance responses. However, DNA methylation is one of the most important epigenetic modifications, which plays a crucial role in the regulation of gene expression during plant growth, development, and the stress responses. In this study, the MSAP method was used to assess cytosine methylation variation in the resistant near-isogenic lines (NILs) and the susceptive parent Jing411. The MSAP profiles indicated that both the total DNA methylation rates and locus-specific demethylation rates in NILs were lower than Jing 411. These results suggest that the DNA methylation level in NILs is reduced by contrast with Jing 411. Moreover, we found the plastid acetyl-CoA carboxylase (Acc-1) gene and the cytosolic acetyl-CoA carboxylase (Acc-2) gene were De-methylated in NILs, it can be supposed that the expression of these genes in NILs may be increaseed. Therefore, we conclude that the DNA methylation level in the resistant NILs is reduced, and the variation of DNA methylation plays a potential role in the resistance responses to Powdery mildew.

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1. Introduction

Powdery mildew is a fungal disease of wheat, caused by Blumenia graminis (DC.) Speer f.sp. tritici, is one of the most serious diseases of common wheat in the world(1). Infection of Powdery mildew results in early leaf blight, reduced photosynthesis, respiration enhanced, and spike rate decreased, the production can be cut from 5% to 10%, and serious losses up to 20% (2). Recently years, powdery mildew has become more seriously in northern China, because of the improvement in irrigation and usage of nitrogenous fertilizer. In 1990s, a pandemic outbreak of powdery mildew across the country resulted in 1.438 million tons losses (1990) and 77 million tons (1991) at two consecutive years, more than 12 million hectares were affected (3). Therefore, powdery mildew has become a major disease of wheat production, to prevent and control powdery mildew, lots of studies are focus on the new resistance genes screening, but the function of epigenetic modification in resistance responses to powdery mildew is still unclear.

Epigenetics is a stable and heritable alternative state which effect on gene expression without changing DNA sequence of promoter or coding regions of genes (4, 5). DNA methylation and covalent modifications of histones is the major epigenetic information which was transmitted from cell to daughter cell and from one generation to next generation (5, 6). The reversible epigenetic modifications play an important role in plant growth, development, Organ differentiation and adapting to changeable environmental stresses to avoid unnecessary excessive genetic rearrangements, which took a great part in maintaining genome stability and controlling the gene expression (7-9). Earlier studies have shown that the regulation of epigenetic modification plays a critical role in flower differentiation (10, 11), vernalization (12, 13) and the expression of light-sensitive genes (14-16). DNA methylation is the most conservative epigenetic modification(4, 17), which is limited to symmetrical CG and CHG sites in the pericentromeric and repetitive regions of plant genomes, and the DNA methylation is also present in the promoter regions of genes(18,19). About 3% to 8% of cytosine residues are methylated in the genomic DNA of vertebrate, and the methylation ratio is up to 5%-30% in plant (20). DNA methylation can inhibit gene expression and plays an important role in maintaining specific patterns of gene expression during plant growth, development (21). There are studies on the phenotypic effects of DNA methylation, for example, some loss of methylation induces a genome-wide transcriptional reactivation of transposable elements, while quadruple mutations in DNA methylases *met1*, *drm1*, *drm2* and *cmt3* results in embryo lethality (22), but the role in defending against diseases is still being investigated.

To examine whether DNA methylation took part in the resistance responses to powdery mildew of wheat, resistant near-isogenic lines (NILs) and the susceptive

parent Jing411 were used for this study. The DNA methylation level of NILs/Jing411 was evaluated by the methylation sensitive dot blot assay (MS-DBA) and methylation-sensitive amplified polymorphism (MSAP) technique. Data arising from this study should provide a new insight for further research on the function of DNA methylation in the resistance responses to powdery mildew of wheat, for example, instead of new resistance genes screening, control the resistant/susceptive genes expression by DNA methylation may improve the defense to the powdery mildew in wheat.

2. Materials and methods

2.1 Plant materials

The resistant near-isogenic lines (NILs) and its recurrent susceptive parent Jing411 were used in this study. And the NILs was constructed from the wheat cultivar Brock as donor of the resistance gene crossed with the susceptive parent Line 15 and Jing411. The wheat cultivar Brock was kindly provided by Dr. Ray Johnson, Line 15 and Jing 411 are fine wheat varieties, which have been used in agricultural production. Line 015 was crossed with Brock to obtain F1 seeds. F1 individuals were artificially inoculated with powdery mildew race No.15. Selected resistant individuals were crossed with Jing 411 seven times and selfed to produce progenies (resistant NILs of Jing 411).

2.2 Methylation sensitive dot blot assay and ELISA

Genomic DNA was isolated from expanded leaves of individual plants by a modified CTAB method (23). DNA's were dot blotted onto Hybond and cross-linked for 4 mins by exposure to UV radiation. The membrane was blocked (PBS with 0.5% [v/v] Tween-20, 5% BSA) 1h at 37℃ followed by incubation with the 5-Methyl Cytidine antibody [2E5] (ab51552) for 2 hours at RT at 1:1000 dilution in blocking buffer. The Odyssey Infrared Imaging System was used to test the different dots. Genomic DNA was coated on a polystyrene plate for ELISA using 5-Methyl Cytidine antibody [2E5] (ab51552).

2.3 MSAP analysis

MSAP analysis is a modified version of the standard amplified fragment length polymorphism (AFLP) technique (24), by incorporating a pair of isoschizomers, Hpa II/Msp I, which possess differential sensitivity to cytosine methylation at the CCGG sites. 500 ng of genomic DNA was digested by Hpa I-EcoR I or Msp I -EcoR I (New England Biolabs) in a total volume of 25 μl at 37℃ for 2 hours. The reaction was stopped in 80℃ for 20 mins, and then adaptor ligation was carried out by T4 ligase. After pre-amplification, one pair of pre-selective primers and 64 pairs of selective primers were used for amplifications (**Supplementary Table 1**). LI-COR 4300 system was used to calculate the different bands. Silver stained sequencing gel was used to resolve and visualize the amplification products. Only clear and reproducible bands that appeared in two independent PCR amplifications were scored.

2.4 Cloning and sequencing of MSAP products

Bands of interest in the silver-stained MSAP gels were eluted and reamplified with the appropriate selective primer combinations. Sizes of the PCR products were verified by agarose gel electrophoresis, and then cloned into the pMD®18-T Vector (TaKaRa) according to the manufacturer's specifications, and then transformed to the cloned fragments to the E.coli DH5α. DNA sequence analysis was carried out at Beijing Genomics Institute. The Advanced BlastN and BlastX programs at the NCBI website (http://www.ncbi.nlm.nih.gov/) were respectively used for mapping and homology analysis of the cloned DNA sequences that gave quality-reads.

3. Results

3.1 Decrease in the overall relative cytosine methylation level in the NILs versus Jing 411

To evaluate the function of variable DNA methylation in the resistance responses to powdery mildew of wheat, near-isogenic lines (NILs) of Jing 411 were constructed with apparently resistance to powdery mildew. NILs is derived from Jing 411, which has nearly the same genetic background, the different powdery mildew-resistant phenotype of NILs and its recurrent susceptive parent Jing411 may derive from a single gene. However, the resistance response to powdery mildew is complex process, which requires the collaboration of multiple genes, not only one single gene. It can be expected that epigenetic modification such as DNA methylation may play an important role in the resistance response to powdery mildew of wheat. To investigate whether there is any methylation change of wheat infected with powdery mildew, total genomic DNA of NILs and Jing 411 were isolated from expanded leaves of individual plants infected with powdery mildew or not, an antibody against 5-methyl-cytosine was used for the methylation sensitive dot blot assay. It can be seen in **Figure 1A**, the cytosine methylation level was decreased significantly by powdery mildew infection in NILs also in Jing 411. For Jing 411, the DNA methylation level was reduced by 29% (**Figure 1A**, line 1 and line 4), while 28% was reduced in NILs (Figure 1A, line 2 and line 3). However, the DNA methylation level of NILs is slightly lower than Jing 411 without powdery mildew infection (Figure 1A, line 1 and line 2). Meanwhile, the cytosine methylation level is also lower than Jing 411 after infection (Figure 1A, line 3 and line 4). Furthermore, Enzyme-Linked Immunosorbent Assay (ELISA) with anti-5-methyl-cytosine antibody confirmed the above results, the DNA methylation level

in NILs was significantly reduced (23%) in contrast with Jing 411, and there were obviously demethylation in NILs (65%) and Jing411(21%) which were infected with powdery mildew (**Figure 1B**). These results indicated that the DNA methylation level can be reduced by powdery mildew infection, and the cytosine methylation level in NILs is lower than Jing 411, which suggest that there more gene expression in NILs than Jing 411, and there are more gene expressions after infected by powdery mildew.

Figure 1

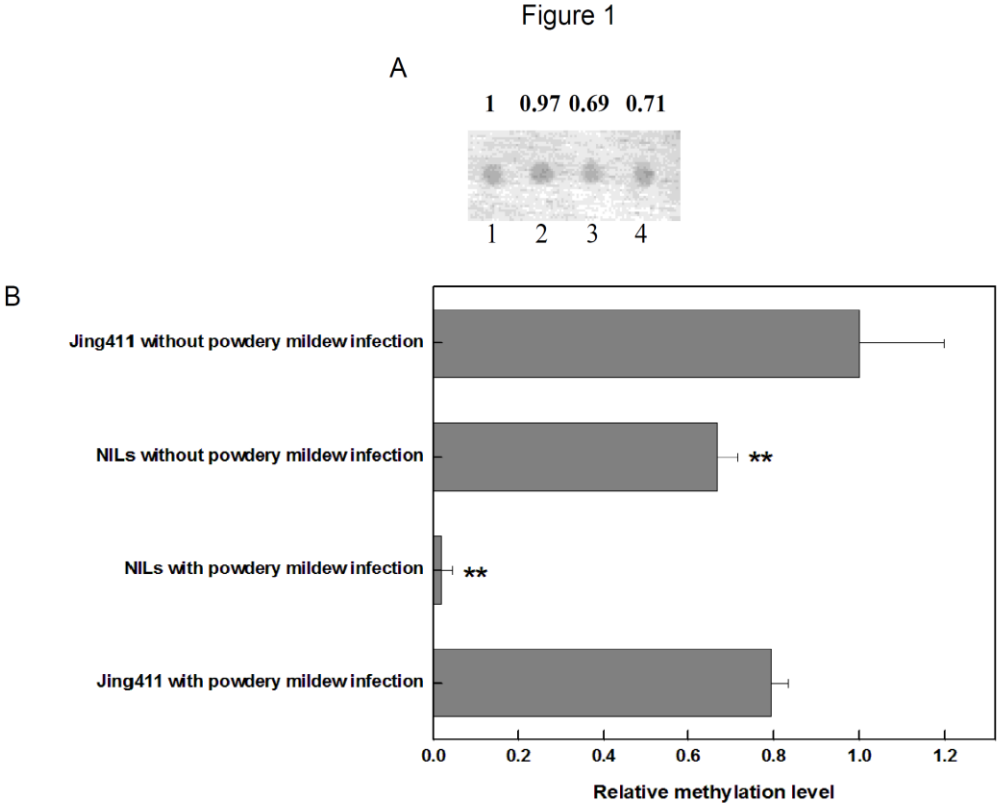

Figure 1. Decrease in the overall relative cytosine methylation level in the NILs versus Jing 411. Genomic DNA was isolated from NILs and Jing 411 with or without powdery mildew infection, Methylation sensitive dot blot assay (A) and ELISA (B) analysis were used to estimate the methylation level. Line1, Jing 411, without powdery mildew infection; Line 2, NILs without powdery mildew infection; Line 3, NILs with powdery mildew infection; Line 4, Jing 411 with powdery mildew infection

To further estimate the function of epigenetic modification in the resistance responses to powdery mildew of wheat, methylation-sensitive amplified polymorphism (MSAP) was used to assess the DNA methylation variation in NILs and Jing 411. In MSAP technique, a pair of isoschizomers Hpa II and Msp I were used to replace Mse I in traditional AFLP (amplified fragment length polymorphism) analysis. Hpa II and Msp I can recognize the same restriction site (5'-CCGG-3'), but have different sensitivity to the methylation states of the cytosines. Hpa II will not recognize or cut if either of the cytosines is fully (double-strand) methylated, whereas, Msp I will not cut if the external cytosine is fully- or hemi- (single-strand) methylated (McClelland et al. 1994). For a given DNA sample, if there is a clearly band in both Hpa I-EcoR I and Msp I-EcoR I digest indicated non-methylated loci. Presence of MSAP fragments in Msp I-EcoR I profiles and absence in Hpa II-EcoR I profiles, suggest that the full methylation of the internal cytosine. The opposite pattern, bands present in Hpa II-EcoR I digestions but absent in Msp I-EcoR I digestions, were characterized as hemi-methylation of the external cytosine (25, 26).

As shown in **Figure 2**, by using 64 pairs of primer combinations, 2891 clear and reproducible bands were detected in susceptible recurrent parent Jing 411, among which there are 139 bands (4.8%) only exist in Jing 411, while there are 2752 bands consist in both Jing 411 and NILs. Similarly, there are 3060 bands detected in resistant NILs, and 308 bands (10.1%) only exist in NILs. The different mapping patterns of Jing 411 and NILs may come from genetic mutation, or the changes of DNA methylation, which alter the recognition capability of isoschizomers Hpa II /Msp I

in CCGG sites. Owing to that both the total bands and the bands which only exist in NILs are increased compare with Jing 411, it is possible that the overall DNA methylation level was reduced in NILs, since isoschizomers Hpa II /Msp I can recognize and cut CCGG sites without methylation, consequently, the bands amplified by PCR were increased.

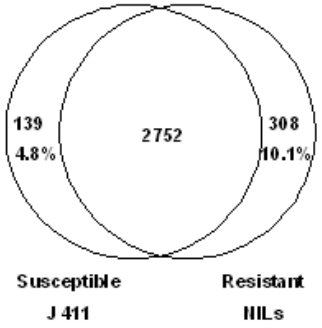

Figure 2. Venn diagram showing the number of bands which were amplified by MSAP in susceptible Jing 411 and resistant NILs

As shown in Table 1, there are 796 among 2891 bands (27.5%) which were fully methylated at the internal C, and 45.2% bands were hemi-methylated at the external C in Jing 411. It was discovered that the total methylation rate in NILs was reduced from 72.7% to 67% in contrast to Jing 411, both the fully methylated at the internal C and hemi-methylated at the external C were reduced, from 27.5% to 25.4% and 45.2% to 41.6% respectively (**Table 1**). To exclude the impact of gene mutation, the 2752 bands which present in both Jing 411 and NILs were counted. The results in **Table 2** indicated that unmethylated bands in NILs were slightly higher than Jing 411 (6.9% increased), both the fully methylated at the internal C and hemi-methylated at the external C frequencies were lower than Jing 411. These data in table 2 are the same as table 1, which suggest that the methylation level of genome DNA in resistant NILs is lower than susceptible Jing 411, and there is no connection with gene mutation.

Table 1 Number of bands amplified by MSAP in NILs and its recurrent parent Jing 411. Genomic DNA was isolated from NILs and Jing 411, and the total DNA methylation level were evaluated by MSAP analysis

Wheat line	Total bands	None-methylated CCGG sites	Methylated CCGG sites Fully methylated sites (internal C) Hpa II (-) /Msp I(+)	Hemi-methylated sites (external C) Hpa II(+)/Msp I(-)	Total
J 411	2891	789 (27.3%)	796 (27.5%)	1306 (45.2%)	2102(72.7%)
NILs	3060	1010 (33%)	776 (25.4%)	1274 (41.6%)	2050 (67%)

Table 2 Number of bands which exist in both NILs and its recurrent parent Jing 411. Genomic DNA was isolated from NILs and Jing 411, and the total DNA methylation level were evaluated by MSAP analysis, while only the bands which exist in both NILs and Jing 411 was calculated

Wheat line	Total bands	None-methylated CCGG sites	Methylated CCGG sites Fully methylated sites (internal C) Hpa II(-)/Msp I(+)	Hemi-methylated sites (external C) Hpa II(+)/Msp I(-)	Total
J 411	2752	782 (28.4%)	756 (27.5%)	1214 (44.1%)	1970(71.6%)
NILs	2752	970 (35.3%)	692 (25.1%)	1090(39.6%)	1782(64.7%)

3.2 The changes of cytosine methylation patterns in NILs compare with Jing 411

In addition to estimate the change of global genomic DNA methylation level, the MSAP profile also allows comparison of cytosine methylation patterns between resistant NILs and susceptible Jing 411 in locus-specific manner. It was found that extensive changes (both demethylation and hypermethylation) in MSAP types presented in NILs (Figure 3). There are bands appeared or disappeared in NILs compared with Jing 411 which were cut by Hpa II-EcoR I or Msp I -EcoR I respectively. Furthermore, some loci showed conversion of the two detectable methylation patterns, such as from the fully methylated at the internal C to the hemi-methylated at the external C (**Figure 3**). As shown in **Table 3**, the MSAP loci can be divided into four major group, group A refers to loci which had no changes in methylation pattern, while group B and C refers to demethylation and hypermethylation loci respectively. And group D refers to the loci that have the fully methylated at the internal C and the hemi-methylated at the external C exchange (Table 3). According to the different cutting pattern, each of the four groups can be divided to some subgroups, as detailed in Table 3. It can be seen that there were 566 (17.7%) demethylation bands detected in NILs, while

only 209 (6.5%) hypermethylation bands existed in NILs (Table 3). These data support the idea that the DNA methylation level in NILs is reduced contrast with Jing 411.

Table 3 cytosine methylation patterns in susceptible Jing 411 and resistant NILs. Genomic DNA was isolated from NILs and Jing 411, and the locus-specific DNA methylation was examined by MSAP analysis

Group	Subgroup	Jing 411		NILs		Number of bands	ratio
		Hpa II	*Msp I*	*Hpa II*	*Msp I*		
No methylation changes	A1	+	+	+	+	712	
（A）	A2	+	–	+	–	1068	2398 (75%)
	A3	–	+	–	+	618	
Demethylation in NILs	B1	+	–	+	+	128	
（B）	B2	–	+	+	+	130	
	B3	–	–	+	+	40	566 (17.7%)
	B4	–	–	+	–	184	
	B5	–	–	–	+	84	
Hypermethylation in	C1	+	+	+	–	14	
NILs（C）	C2	+	+	–	+	56	
	C3	+	+	–	–	7	209 (6.5%)
	C4	+	–	–	–	92	
	C5	–	+	–	–	40	
Methylation exchange	D1	+	–	–	+	18	26 (0.8%)
（D）	D2	–	+	+	–	8	

Figure 3

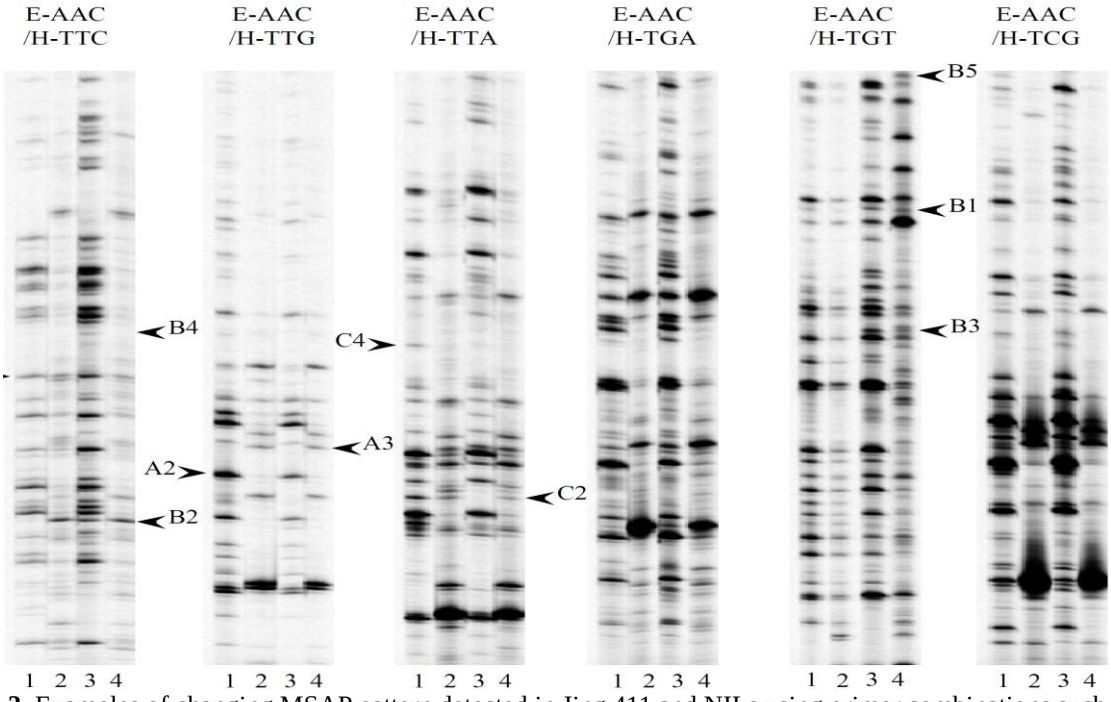

Figure 3. Examples of changing MSAP pattern detected in Jing 411 and NILs using primer combinations such as E-AAC/H-TTC or E-AAC/H-TTG etc. Line 1 and 2 refers to digestion with *Hpa II- EcoR I* and *Msp I- EcoR I* respectively in Jing 411, while line 3 and 4 were cutting with *Hpa II- EcoR I* and *Msp I- EcoR I* respectively in NILs. Asterisks such as B4, A2 refers to the subgroups which were described in table 3

3.3 Mapping and homology analysis of the DNA sequences which gave quality bands

Since there are two rounds of PCR amplifications in the MSAP technique, it is necessary to rule out possible PCR artifacts as a cause for the observed differential methylation patterns in NILs versus Jing

411. To validate the methylation changes revealed by MSAP in NILs versus Jing 411, we selected genomic DNA which is cut by Hpa II-EcoR I or Msp I-EcoR I as probes for Southern blot analysis using isolated MSAP bands, which represented several different MSAP patterns (Figure 3, Table 3). As a result, there are 37 bands screened by Southern blot hybridization, of which 18 bands are Demethylation in NILs (data not shown). According to the different cutting pattern (Table 3), these bands can be divided to four classes, belong to subgroup B2 (5 bands), B3 (2 bands), B4 (10 bands) and B5 (1 band) (**Supplementary Table 2**). In contrast, there are 19 bands hypermethylation in NILs among them, belong to subgroup C1 (1 band), C2 (2 bands), C4 (3 bands) and C5 (13 bands) respectively (**Supplementary Table 3**).

To further investigate the probable function of these bands in powdery mildew infection, the 37 bands were cloned and sequenced. Based on Blastn analysis at the NCBI website (http://blast.ncbi.nlm.nih.gov), there are 12 clones are mapped to Triticum aestivum, the others are mapped to Triticum urartu, Triticum monococcum or similar to Hordeum vulgare, Oryza sativa, Zea mays et al. As shown in Supplementary Table 2, bands B2-1 was mapped to Triticum urartu clone BAC 059G16 plastid acetyl-CoA carboxylase (Acc-1) gene, while bands B2-3 was mapped to Triticum turgidum subsp. durum clone BAC 1053F12+1054I5 cytosolic acetyl-CoA carboxylase (Acc-2) gene. The 2 bands are demethylated in NILs versa Jing 411, suggest that the expression of Acc-1 or Acc-2 genes were increased in NILs. It can be supposed that acetyl-CoA carboxylase may play a role in powdery mildew infection. Because of the rich genetic information of wheat, and there are not completely sequenced, more bands of which belong to Triticum aestivum BAC library, others mapped to similar species, the effects of these bands are difficult to study. Along with the further study of the wheat genome, we believed that more function can be found of these bands.

Taken together, our studies shown that the DNA methylation level was reduced by powdery mildew infection, and the DNA methylation level in resistant NILs was lower than its recurrent susceptive parent Jing411. These data support that the variation of DNA methylation may play an important role in the resistance responses to Powdery mildew

4. Discussion

Powdery mildew is one of the most serious diseases of wheat in the world, which is caused by fungal pathogens. To reduce the damage, the use of genetically modified (GM) wheat lines with enhanced resistance to powdery mildew is being explored as an alternative to the use of chemical fungicides. Therefore, little attention was paid on the epigenetic profiling for resistance responses against powdery mildew.

In this study, we focused on the function of the variable epigenetic modification especially DNA methylation in the resistance responses to powdery mildew of wheat, while the resistant near-isogenic lines (NILs) and its recurrent susceptive parent were used for MSAP analysis. The NILs was created by crossing Block (donor line, which is Powdery mildew resistant) with recurrent parent Jing 411 and the resistance to Powdery mildew is used as a selection marker. The resulting NILs have nearly the same genetic background compared with Jing 411. The Powdery mildew resistant difference may come from a gene, which is a potential Powdery mildew gene. However, the organism is a relatively complex system, the difference of single gene may affect the global genes expression, and the changes can be reflected in the epigenetic level such as DNA methylation. Hypermethylation typically occurs at CpG islands in the promoter region and is associated with gene inactivation. It is shown in this study, the total DNA methylation level of NILs is lower than Jing 411 by 6.9% (**Table 2**), suggest that there are more genes expression in NILs compared with Jing 411. Furthermore, the global genes expression changes especially up-regulated of the metabolism associated genes (Supplementary Table 2) are resulting by the probable resistant genes in NILs. It can be supposed that the enhanced metabolic was responsible for the resistance response of powdery mildew.

Furthermore, three fragments linked to the resistance of Powdery mildew were screened by AFLP in NILs (data not shown), and a series of demethylation or hypermethylation genes were cloned in our study (Supplementary Table 2, Supplementary Table 3), they may be regulated by the fragments linked genes screened by AFLP in NILs. While, the methylation level of acetyl-CoA carboxylase is reduced obviously in NILs, means that the incensement of resistance to Powdery mildew is associated with the metabolism level. However, due to the huge genome of wheat and the limited of the sequence data, there are more experiments need to be done to integrate the results of our study (MSAP) with the AFLP and cDNA AFLP results. And lots of work should be done to elucidate the function of DNA methylation in the resistance responses against powdery mildew.

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***Corresponding author:**
Dr. Zhenying Wang,
College of Life Science, Tianjin Normal University, 393 Binshui West Street, Xiqing District, 300387, Tianjin, China.
E-mail: wzycell@yahoo.com.cn

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Supplementary Table 1

Adaptors, pre-selective and selective primers used in this work

Adapters	
*Eco*R I-sense	5'-CTCGTAGACTGCGTACC-3'
*Eco*R I-antisense	5'-AATTGGTACGCAGTC-3'
Hpa II/Msp I-sense	5'-GATCATGAGTCCTGCT-3'
Hpa II/Msp I-antisense	5'-CGAGCAGGACTCATGA-3'
Pre-selective primers	
E-A-sensxe	5'-GACTGCGTACCAATTCA-3'
H/M-antisense	5'-ATCATGAGTCCTGCTCGG-3'
Selective primers	
E-AAC	5'-GACTGCGTACCAATTCAAC-3'
E-AAG	5'-GACTGCGTACCAATTCAAG-3'
E-ACA	5'-GACTGCGTACCAATTCACA-3'
E-ACT	5'-GACTGCGTACCAATTCACT-3'
E-ACC	5'-GACTGCGTACCAATTCACC-3'
E-ACG	5'-GACTGCGTACCAATTCACG-3'
E-AGC	5'-GACTGCGTACCAATTCAGC-3'
E-AGG	5'-GACTGCGTACCAATTCAGG-3'
H/M-TCT	5'-ATCATGAGTCCTGCTCGGTCT-3'
H/M-TCG	5'-ATCATGAGTCCTGCTCGGTCG-3'
H/M-TCC	5'-ATCATGAGTCCTGCTCGGTCC-3'
H/M-TTC	5'-ATCATGAGTCCTGCTCGGTTC-3'
H/M-TTG	5'-ATCATGAGTCCTGCTCGGTTG-3'
H/M-TTA	5'-ATCATGAGTCCTGCTCGGTTA-3'
H/M-TGA	5'-ATCATGAGTCCTGCTCGGTGA-3'
H/M-TGT	5'-ATCATGAGTCCTGCTCGGTGT-3'

Supplementary Table 2. Demethylation bands in NILs. The demethylation bands of NILs the silver-stained MSAP gels were eluted and sequenced. The clones were mapped by Blastn analysis at the NCBI website

subgroup	Name	Length	Accession	Description	Max score	Total score	Query coverage	Evalue	Max ident
B2	B2-1	250bp	EU660896.1	Triticum urartu clone BAC 059G16 plastid acetyl-CoA carboxylase (Acc-1) gene, complete cds; nuclear gene for plastid product	313	313	77%	3e-82	95%
	B2-2	284bp	CR626927.1	Bacteroides fragilis NCTC 9343, complete genome	48.2	48.2	9%	0.024	100 %
	B2-3	349bp	AM932680.1	Triticum aestivum 3B chromosome, clone BAC TA3B54F7	484	484	91%	1e-133	93%
			AM932684.1	Triticum aestivum 3B chromosome, clone BAC TA3B95C9	318	318	91%	1e-83	81%
			EU660894.1	Triticum turgidum subsp. durum clone BAC 1053F12+1054I5 cytosolic acetyl-CoA carboxylase (Acc-2) and putative amino acid permease genes, complete cds	300	300	92%	3e-78	80%
	B2-4	201bp	BC136217.1	Xenopus tropicalis calcium/calmodulin-dependent protein kinase (CaM kinase) II beta, mRNA (cDNA clone MGC:122855 IMAGE:7664687), complete cds	44.6	44.6	15%	0.20	90%
	B2-5	201bp	BC136217.1	Xenopus tropicalis calcium/calmodulin-dependent protein kinase (CaM kinase) II beta, mRNA (cDNA clone MGC:122855 IMAGE:7664687), complete cds	44.6	44.6	15%	0.20	90%
B3	B3-1	201bp	AP004693.3	Oryza sativa Japonica Group genomic DNA, chromosome 8, PAC clone:P0461F06	55.4	55.4	24%	1e-04	84%
	B3-2	220bp	AK333719.1	Triticum aestivum cDNA, clone: WT008_K11, cultivar: Chinese Spring	324	324	85%	2e-85	98%

B4	B4-1	300bp	AC137621.2	Oryza sativa Japonica Group chromosome 5 clone OSJNBb0111O13, complete sequence	120	120	46%	5e-24	80%
	B4-2	153bp	AL645643.7	Mouse DNA sequence from clone RP23-253F13 on chromosome 13 Contains part of the Gmds gene for GDP-mannose 4, 6-dehydratase, complete sequence	44.6	44.6	18%	0.14	93%
	B4-3	316bp	AY146588.1	Triticum monococcum BAC clone 453N11, complete sequence	118	197	33%	2e-23	84%
	B4-4	247bp	AP004789.3	Oryza sativa Japonica Group genomic DNA, chromosome 2, PAC clone:P0476C12	181	181	63%	1e-42	85%
	B4-5	114bp	AC104708.2	Oryza sativa Japonica Group chromosome 5 clone OJ1174_H11, complete sequence	84.2	164	66%	1e-13	86%
			NM_001062044.1	Oryza sativa (japonica cultivar-group) Os05g0405600 (Os05g0405600) mRNA, complete cds	80.6	80.6	58%	1e-12	86%
	B4-6	334bp	AC190403.1	Mus musculus BAC clone RP23-78F5 from chromosome 14, complete sequence	44.6	44.6	14%	0.35	80%
	B4-7	298bp	GU358609.1	Citrus sunki x Poncirus trifoloata AFLP marker E+AG/M+CAA mRNA sequence	46.4	46.4	8%	0.088	100 %
	B4-8	286bp	AP006723.2	Oryza sativa Japonica Group genomic DNA, chromosome 8, BAC clone:B1203H11	123	383	73%	4e-25	78%
	B4-9	116bp	AK287755.1	Oryza sativa Japonica Group cDNA, clone: J065159G15, full insert sequence	66.2	66.2	63%	3e-08	79%
	B4-10	194bp	M77475.1	Hordeum vulgare subsp. vulgare seed imbibition protein (Sip1) gene, complete cds	221	221	84%	1e-54	90%
B5	B5-1	227bp	EU835981.1	Triticum aestivum clone BAC 1551N13, complete sequence	181	181	77%	1e-42	82%

Supplementary Table 3. Hypermethylation bands in NILs. The hypermethylation bands of NILs the silver-stained MSAP gels were eluted and sequenced. The clones were mapped by Blastn analysis at the NCBI website

subgroup	Name	Length	Accession	Description	Max score	Total score	Query coverage	Evalue	Max ident
C1	C1-1	130bp	DQ245674.1	Zea mays clone 19112 mRNA sequence	60.8	60.8	36%	1e-06	87%
C2	C2-1	284bp	EF486291.1	Triticum aestivum clone Tri-MS-17 retrotransposon-like sequence	417	417	90%	1e-113	96%
			EF486290.1	Triticum aestivum clone Tri-MS-16 retrotransposon-like sequence	417	417	90%	1e-113	96%
			EF486289.1	Triticum aestivum clone Tri-MS-15 retrotransposon-like sequence	403	403	90%	3e-109	94%
	C2-2	218bp	EU660893.1	Triticum urartu clone BAC 252P12+402A6 cytosolic acetyl-CoA carboxylase (Acc-2) and putative amino acid permease genes, complete cds	334	694	88%	9e-89	98%
C4	C4-1	217bp	FJ040859.1	Penaeus monodon nuclear autoantigenic sperm protein (NASP) mRNA, complete cds	48.2	48.2	16%	0.018	91%
	C4-2	206bp	EF192063.1	Trichosanthes dioica clone TDM09 unknown mRNA	41.0	41.0	19%	2.4	82%
	C4-3	111bp	DQ147744.1	Apis mellifera AFLP marker a78.155 genomic sequence	42.8	42.8	25%	0.32	92%

C5	C5-1	236bp	EU835198.1	Triticum turgidum subsp. dicoccoides clones BAC 391M13 and BAC 1144M20 genomic sequence	304	1144	88%	2e-79	92%
			AM932684.1	Triticum aestivum 3B chromosome, clone BAC TA3B95C9	268	491	88%	1e-68	88%
	C5-2	125bp	AK335824.1	Triticum aestivum cDNA, clone: WT013_N21, cultivar: Chinese Spring	46.4	46.4	32%	0.031	85%
	C5-3	116bp	AK287755.1	Oryza sativa Japonica Group cDNA, clone: J065159G15, full insert sequence	68.0	68.0	66%	9e-09	79%
	C5-4	179bp	AM932684.1	Triticum aestivum 3B chromosome, clone BAC TA3B95C9	196	411	84%	4e-47	93%
	C5-5	130bp	EU938389.1	Secale cereale cultivar JNK 2F2R-1500 subregion genomic sequence	55.4	55.4	38%	6e-05	84%
	C5-6	130bp	AC091233.8	Oryza sativa chromosome 3 BAC OSJNBa0053G10 genomic sequence, complete sequence	59.0	59.0	73%	5e-06	73%
	C5-7	298bp	EU130887.1	Pratylenchus vulnus isolate CA92 clone 1 28S ribosomal RNA gene, partial sequence	46.4	46.4	8%	0.089	100%
	C5-8	137bp	EU827249.1	Fusarium virguliforme AFLP fragment Fvgly13 mRNA sequence	46.4	46.4	27%	0.035	86%
	C5-9	163bp	AF326781.1	Triticum monococcum actin (ACT-1) gene, partial cds; putative chromosome condensation factor (CCF), putative resistance protein (RGA-2), putative resistance protein (RGA2) and putative nodulin-like-like protein (NLL) gene, complete cds; and retrotransposons Josephine, Angela-2, Angela-4, Heidi, Greti, Angela-3, Fatima, Erika-1, Angela-6, Angela-5, Barbara, Isabelle, Erika-2, and Claudia	212	212	82%	5e-52	94%
			AY663391.1	Triticum turgidum cultivar Langdon clone BAC 1156G16, complete sequence	203	203	82%	3e-49	93%
			EU660896.1	Triticum urartu clone BAC 059G16 plastid acetyl-CoA carboxylase (Acc-1) gene, complete cds; nuclear gene for plastid product	196	327	83%	4e-47	91%
	C5-10	202bp	AF488415.1	Triticum monococcum chromosome 7Am BAC 5K14 complete sequence	257	257	92%	2e-65	90%
			FN564430.1	Triticum aestivum chromosome 3B-specific BAC library, contig ctg0464b	201	887	92%	1e-48	85%
	C5-11	99bp	GU817319.1	Triticum aestivum clone BAC_2383A24 chromosome 3B, complete sequence	122	211	77%	4e-25	96%
			FN564426.1	Triticum aestivum chromosome 3B-specific BAC library, contig	118	308	75%	5e-24	94%

			ctg0005b					
C5-12	317bp	FN564429.1	Triticum aestivum chromosome 3B-specific BAC library, contig ctg0382b	437	1107	91%	2e-119	93%
		FN564431.1	Triticum aestivum chromosome 3B-specific BAC library, contig ctg0528b	338	550	91%	1e-89	86%
		AY368673.1	Triticum turgidum HMW-glutenin locus, complete sequence	304	304	90%	3e-79	83%
C5-13	204bp	FR823384.1	Neospora caninum Liverpool complete genome, chromosome IV	44.6	44.6	18%	0.24	89%

6/9/2013