African Journal of Biotechnology Vol. 10(46), pp. 9253-9263, 22 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.298
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Parental epigenetic difference in DNA methylation-level may play contrasting roles for different agronomic traits related to yield heterosis in maize

Wei Yang[1], Xiaoming Yu[1], Weiguang Yang[2] and Bao Liu[1]*

[1]Key Laboratory of Molecular Epigenetic of MOE, Northeast Normal University, Changchun, Jilin 130024, P. R. China.
[2]College of Agronomy, Jilin Agricultural University, Changchun, Jilin 130118, P. R. China.

Accepted 16 June, 2011

Although, maize is the crop wherein heterosis or hybrid vigor has been exploited to nearly the fullest extent, the molecular and genetic basis underlying this remarkable biological phenomenon remains largely an enigma.To further explore the issue from an epigenetic perspective, we sought to probe for possible relationships between the parental epigenetic difference in the form of DNA methylation revealed by the epigenetic marker MSAP, among a set of 11 maize inbred lines and heterosis in four agronomic traits manifested by a set of 30 F1 hybrids resulting from a half-diallel crossing among the inbred lines. We found that a specific type of DNA methylation-level difference, that is, relative CHG (H denotes A, C or T) methylation levels at the 5'-CCGG-3' sites exhibits a statistically significant negative correlation with heterosis in the number of rows per ear (NRE) and a positive correlation with the number of kernels per row (NKR), whereas, no correlation was detected between any of the DNA methylation-level differences and the rest two studied traits, 100-kernel weight (HKW) and kernel weight per ear (KWE). In a sharp contrast, parental genetic distance revealed by the genetic marker AFLP did not show any correlation with heterosis for any of the four studied agronomic traits. Our results suggest that parental epigenetic difference in particular types of DNA methylation-level difference plays some significant roles in the manifestation of heterosis of specific traits in maize, but the effects can be in opposite directions, and hence, offsetting each other and cumulating to cryptic effects on yield, itself.

Key words: DNA methylation, epigenetics, heterosis, agronomic traits, maize.

INTRODUCTION

Hybrid vigor or heterosis refers to the superior performance in one or more traits of crossbreds (F1 hybrids) relative to their inbred parents. This superiority can be related to increase in body-size, growth-rate and enhanced yield and its underlying components (Birchler et al., 2003).

Maize (*Zea mays* L.) is a leading crop wherein heterosis has been exploited to nearly the fullest extent, but the underlying molecular and genetic bases remain largely unknown (Birchler et al., 2003; Hochholdinger and Hoecker, 2007; Liu and Tollenaar, 2009; Soengas et al., 2003). In recent years, great efforts have been made to search for molecular markers that enable the categorization of the maize germplasm into distinct heterotic groups, such that heterosis can be used more efficiently and effectively. Unfortunately, highly discrepant results have often been obtained regarding the reliability or utility of the frequently used DNA molecular markers that reveal nucleotide sequence-encoded parental genetic differences (Lee et al., 2007; Qi et al., 2010). Therefore, looking for alternative molecular markers that are more intrinsically correlating with heterosis represents an active

*Corresponding author. E-mail: baoliu6677@yahoo.com.cn. Tel: 86-431-85099822. Fax: 86-431-85099822.

Abbreviations: PGD, Parental genetic distance; **MPH,** middle parent heterosis; **BPH**, better parent heterosis; NRE, number of rows per ear; **NKR,** number of kernels per row; **HKW,** one-hundred kernel weight; **KWE,** kernel weight per ear; **MSAP,** methylation-sensitive amplified polymorphism.

research area.

Epigenetic markers, which are not dependent on the nucleotide-sequence but on covalent modifications of DNA and/or chromatins, are being increasingly recognized as playing biologically functional roles in eukaryotic development, primarily via their heritable regulation on gene expression. DNA methylation is one of the most prominent epigenetic markers existing in a vast range of eukaryotes and is particularly abundant in higher plants, in which it plays diverse roles during normal growth and development, as well as in times of stress (Lukens and Zhan, 2007; Zilberman, 2008). Accumulating studies have documented that patterns of DNA methylation in maize F1 hybrids can be conspicuously remodeled relative to their inbred parents, which may function to regulate non-additive gene expression in the F1 hybrids (Zhang et al., 2007; Zhao et al., 2007), thus, suggesting a possible role in heterosis. Indeed, it was found recently that there exists a statistically positive correlation between particular patterns of DNA methylation and heterosis in grain-yield heterosis in maize (Qi et al., 2010). Nonetheless, it remains unknown which (if any) specific yield components are influenced by parental differences in DNA methylation, and in particular, what are the possible reasons that this relationship is sometimes undetectable or cryptic but at other times can be clearly discernible. Evidently, further investigations are needed to elucidate these issues.

The objective of this study was to explore if there exists a relationship between parental DNA methylation difference and several important agronomic traits related to grain-yield in maize. We report that there exist statistically significant correlations between particular DNA methylation patterns and heterotic manifestation of the agronomic traits, but the correlations can be in opposite directions, and hence, offsetting each other to render the cumulative effects of DNA methylationon grain-yield itself being cryptic.

MATERIALS AND METHODS

Maize inbred lines and field data collection for agronomic traits

Eleven maize inbred lines widely used in the Northeastern China Corn-Belt and 30 of their resultant F1 hybrids produced by half-diallel crossing were used in this study (Table 1). These lines were grown for agronomic performance at Jilin Agricultural University, Changchun, China, with three replications by the field experimental design, described earlier (Qi et al., 2010). The agronomic traits studied here included the following: number of rows per ear (NRE), number of kernels per row (NKR), one-hundred kernel weight (HKW), and kernel weight per ear (KWE). Each of the four traits was subjected to analysis of variance, mid-parent heterosis (MPH) and better parent heterosis (BPH) by the formula:

$$\text{MPH} = \frac{F_1 - MP}{MP} \times 100$$

$$\text{BPH} = \frac{F_1 - BP}{BP} \times 100$$

Where, F1is the value of a particular trait of given hybrid; MP = (P1 + P2)/2 in which P1 and P2 are the values of a particular trait of a given pair of inbred parents, and BP is the value of the better parent (Table 1).

Genomic DNA isolation and AFLP and MSAP analysis

Genomic DNA was extracted from expanded leaves at the 7- to 8-leaf stage for each of the 11 maize inbred lines by a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Kidwell and Osborn, 1992). The DNA was then purified by phenol extractions and quality and quantity checked by a spectrophotometer.

The standard amplified fragment length polymorphism (AFLP) procedure (Vos et al., 1995) with modifications for silver-staining (Wang et al., 2005) was used to assess the pairwise parental genetic distance (PGD) among the 11 maize inbred lines. Briefly, 300 ng of genomic DNA was double-digested with *Eco*RI and *Mse*I at 37°C for 6 h followed by ligation of the restriction fragments to the adaptors for 4 h. Pre-amplification was performed with nonselective primers in a total volume of 20 µl containing 2 µl of 5-fold dilutions of the ligation products. In the selective amplification, the templates were prepared by diluting 10 to 20 times from the pre-amplified products and a total of 18 combinations of selective primers were used (Table 2).

The methylation-sensitive amplified polymorphism (MSAP) procedure was essential as reported (Reyna-López et al., 1997) but with modifications for silver-staining (Dong et al., 2006). The MSAP marker is a version of modified AFLP, which uses *Eco*RI and either of a pair of isoschizomers, *Hpa*II and *Msp*I, which recognize the same sequence 5'-CCGG-3' but with differential sensitivity to DNA methylation at the two cytosine residues. *Hpa*II will not cut if either cytosine is fully (double-stranded) methylated, whereas *Msp*I will not cut if the external cytosine is fully- or hemi- (single-stranded) methylated. Therefore, difference in methylation states at either or both of the cytosines will lead to differential digestion by the two enzymes, and hence, difference in the polyacrylamide gel electrophoresis (PAGE) profiles. Thus, MSAP was used to investigate the pairwise parental epigenetic difference (PEGD) in DNA methylation among the 11 maize inbred lines. In total, one pair of pre-selective primers and 23 pairs of selective primers were used (Table 2). All restriction enzymes were purchased from New England Biolabs (Ipswich, MA). The AFLP and MSAP amplification products were fractionated by 5% PAGE. Only clear and reproducible bands that appeared in two independent polymerase-chain-reaction amplifications and gel-running (starting from the digestion/ligation step, that is, the first step of AFLP or MSAP) were scored.

Statistical treatments for the molecular data

The scored AFLP and MSAP bands were transformed into a binary character matrix, 1 for presence and 0 for absence of a band at a particular position in the AFLP or MSAP profiles, as detailed in Qi et al. (2010). Specifically, genetic distances (GD = 1 – GS) among the 11 maize inbred lines were calculated according to the Nei and Li (Nei and Li, 1979), similarity coefficient: GS = 2Nij/(Ni + Nj), where Nij is the number of bands common to lines *i* and *j*, and *Ni* and *Nj*, are the numbers of bands specific to lines *i* and *j*. The distance matrix was subject to cluster analysis by the unweighted pair group method with arithmetic (UPGMA), and the dendrogram was constructed using NTSYS-PC v. 2.2 g (Rohlf, 1987). Cluster analysis based on AFLP was performed using the Jaccard coefficients with

Table 1. Eleven maize inbred lines and their agronomic traits including number of rows per ear (NRE), number of kernels per row(NKR), one-hundred kernel weight (HKW), and kernel weight per ear (KWE). Variant analysis based on MPH and BPH of four agronomic traits of half-diallel crossing hybrids.

Maize line		Agronomic trait			
Symbol	Name	Number of row per ear (NRE)	Number of kernel per row (NKR)	100-kernel weight (HKW)	Ear kernel weight (EKW)
L1	Mo17	9.73±0.46	41.5±1.30	33.83±0.57	123.27±2.57
L2	Qi318	12.67±2.31	39.63±0.91	34.18±0.52	126.8±3.74
L3	364	11.56±0.38	39.00±1.73	25.69±0.52	106.71±5.00
L4	ZaC546	11.17±1.04	25.87±0.42	20.83±0.57	80.67±0.84
L5	He344	10.36±0.34	26.69±0.30	24.34±0.85	69.70±2.95
L6	Ji853	14.8±0.61	27.00±0.87	31.90±0.86	114.62±1.04
L7	444	13.33±0.58	26.58±0.14	37.07±0.52	129.85±0.05
L8	C8605-2	13.65±0.60	27.32±1.40	25.71±1.79	79.24±1.94
L9	7884	15.16±0.29	30.61±0.76	28.20±6.72	111.00±3.85
L10	B73	17.67±0.58	28.78±1.95	27.96±1.51	145.87±8.55
L11	Dan340	19.67±0.58	19.89±0.84	31.43±0.57	99.83±9.34

Maize line		Variant analysis based on MPH of four agronomic trait			
Symbol	Name	Number of row per ear (NRE)	Number of kernel per row (NKR)	100-kernel Weight (HKW)	Ear kernel weight (EKW)
L1×L6	Mo17×Ji853	0.2228	0.2944	0.2397	1.1167
L1×L7	Mo17×444	0.2428	0.2338	0.3485	0.9288
L1×L8	Mo17×C8605-2	0.2543	0.0172	0.3618	0.9324
L1×L9	Mo17×7884	0.1781	0.1696	0.2016	0.9016
L1×L10	Mo17×B73	0.0462	0.1905	0.2563	0.6357
L1×L11	Mo17×Dan340	0.1791	0.4693	0.2842	1.1142
L2×L6	Qi318×Ji853	0.0680	0.3037	0.1608	1.2343
L2×L7	Qi318×444	-0.0256	0.1981	0.0666	0.5444
L2×L8	Qi318×C8605-2	-0.0121	0.1700	0.1408	0.7921
L2×L9	Qi 318×7884	-0.0179	0.1010	0.2131	0.7385
L1×L10	Qi 318×B73	-0.0769	0.1489	0.2986	0.6138
L2×L11	Qi 318×Dan340	-0.0515	0.5311	0.3047	1.2883
L3×L6	364×Ji853	0.1130	0.3949	0.5140	1.3565
L3×L7	364×444	0.1518	0.3581	0.3730	0.9899
L3×L8	364×C8605-2	0.1107	0.2264	0.3949	1.3204
L3×L9	364×7884	0.0479	0.2116	0.4102	1.0688
L3×L10	364×B73	-0.0646	0.2167	0.3283	0.7931
L3×L11	364×Dan340	0.0463	0.5442	0.4630	1.4149
L4×L6	He344×Ji853	0.1297	0.6810	0.4692	1.2979
L4×L7	He344×444	0.1701	0.6371	0.4587	1.1539
L4×L8	He344×C8605-2	-0.0062	0.4139	0.6002	1.6078
L4×L9	He344×7884	0.0381	0.3458	0.4504	1.1093
L4×L10	He344×B73	-0.0751	0.4652	0.6539	0.6871
L4×L11	He344×Dan340	-0.0919	0.8300	0.5429	1.4233
L5×L6	ZaC546×Ji853	0.0866	0.5770	0.5056	1.3678
L5×L7	ZaC546×444	0.1257	0.5643	0.3092	1.2098
L5×L8	ZaC546×C8605-2	0.0829	0.4813	0.4909	1.7457
L5×L9	ZaC546×7884	0.1233	0.2857	0.4084	1.1909
L5×L10	ZaC546×B73	-0.0008	0.4772	0.5589	0.9220
L5×L11	ZaC546×Dan340	-0.0452	0.8650	0.5590	1.6521

Table 1. Continue.

Maize line		Variant analysis based on BPH of four agronomic trait			
Symbol	Name	Number of row per ear (NRE)	Number of Kernel per row (NKR)	100- Kernel weight (HKW)	Ear kernel weight (EKW)
L1×L6	Mo17×Ji853	0.0135	0.0683	0.2043	1.0425
L1×L7	Mo17×444	0.0750	0.0120	0.2895	0.8799
L1×L8	Mo17×C8605-2	0.0742	-0.1566	0.1983	0.5873
L1×L9	Mo17×7884	-0.0328	0.0161	0.1017	0.8071
L1×L10	Mo17×B73	-0.1887	0.0080	0.1473	0.5090
L1×L11	Mo17×Dan340	-0.1186	0.0867	0.2386	0.9132
L2×L6	Qi318×Ji853	-0.0090	0.0959	0.1219	1.1270
L2×L7	Qi318×444	-0.0500	0.0008	0.0250	0.5262
L2×L8	Qi318×C8605-2	-0.0479	-0.0118	-0.0006	0.4560
L2×L9	Qi 318×7884	-0.0988	-0.0244	0.1070	0.6303
L×L10	Qi 318×B73	-0.2075	-0.0084	0.1803	0.5083
L2×L11	Qi 318×Dan340	-0.2203	0.1497	0.2520	1.0450
L3×L6	364×Ji853	-0.0090	0.1803	0.3666	1.2753
L3×L7	364×444	0.0750	0.1419	0.1621	0.8126
L3×L8	364×C8605-2	0.0254	0.0427	0.3943	1.0217
L3×L9	364×7884	-0.0768	0.0812	0.3473	1.0288
L3×L10	364×B73	-0.2264	0.0573	0.2744	0.5524
L3×L11	364×Dan340	-0.1695	0.1658	0.3294	1.3370
L4×L6	He344×Ji853	-0.0090	0.6457	0.2143	0.9575
L4×L7	He344×444	0.0750	0.6150	0.1391	0.7460
L4×L8	He344×C8605-2	-0.0967	0.3764	0.4483	1.5847
L4×L9	He344×7884	-0.0988	0.2416	0.2607	0.8210
L4×L10	He344×B73	-0.2453	0.3911	0.4430	0.3101
L4×L11	He344×Dan340	-0.2881	0.6186	0.2827	1.1908
L5×L6	ZaC546×Ji853	-0.0766	0.5679	0.3273	0.9038
L5×L7	ZaC546×444	0.0000	0.5612	0.0844	0.6980
L5×L8	ZaC546×C8605-2	-0.0479	0.4642	0.4512	1.5804
L5×L9	ZaC546×7884	-0.0548	0.2034	0.3119	0.7832
L5×L10	ZaC546×B73	-0.2075	0.4236	0.4580	0.4202
L5×L11	ZaC546×Dan340	-0.2712	0.6274	0.3832	1.2519

the same program. Three kinds of cytosine methylation levels -CG, CHG, and total (CG+CHG) -for each of the 11 inbred lines were tabulated (angle matrices). Correlation coefficients of the AFLP-based GD and each of the three kinds of methylation (CG, CHG, and total) based PEGD with MPH and BPH (note that data of MPH and BPH were not symmetrical matrices) in GY and other traits were separately calculated by Mantel's test (Mantel, 1967), and the statistical significance was determined based on 999 random permutations.

RESULTS

Parental genetic distances (PGDs) among the 11 maize inbred lines revealed by genetic marker AFLP

By 18 combinations of selective AFLP primers, a total of 1205 clear and reproducible (between two technical replications, as earlier discussed) bands were scored across the 11 maize inbred lines. We computed parental genetic distances (PGDs) involving the 11 inbred lines based on the AFLP markers. The distance values ranged from 0.25 (Line 1 vs. Line 4) to 0.40 (Line 3 vs. line 6) (Table 3), with an average of 0.345 across the 30 parental pairs used to produce the F1 hybrids. A dendrogram built on the PGDs divided the 11 maize inbred lines into several distinct groups (Figure 1).

DNA methylation-level difference among the 11 maize inbred lines revealed by the epigenetic marker MSAP

By using a total of 23 pairs of selective primers, we scored 1197 clear and reproducible MSAP bands across all 11 maize inbred lines, of which, 997 are polymorphic in

Table 2. List of adaptors and primers used in the AFLP and MSAP analysis.

Adaptor	Sequence
Mse I-adaptor I	5'-GACGATGAGTCCTGAG-3'
Mse I-adaptor II	5'-TACTCAGGACTCAT-3'
EcoR I-adaptor I	5'-CTCGTAGACTGCGTACC-3'
EcoR I-adaptor II	5'-AATTGGTACGCAGTC-3'
H/M-adaptor I	5'-GATCATGAGTCCTGCT-3'
H/M –adaptor II	5'-CGAGCAGGACTCATGA-3'
Pre-selective primer	Sequence
*Eco*RI+A	5'-GACTGCGTACCAATTCA-3'
*Mse*I+C	5'-GATGAGTCCTGAGTAAC-3'
H/M +O	5'-ATCATGAGTCCTGCTCGG-3'
*Eco*RI+3 Primer	Sequence
a. *E*-AAC	5'-GACTGCGTACCAATTCAAC-3'
b. *E*-AAG	5'-GACTGCGTACCAATTCAAG-3'
c. *E*-ACA	5'-GACTGCGTACCAATTCACA-3'
d. *E*-ACT	5'-GACTGCGTACCAATTCACT-3'
e. *E*-ACC	5'-GACTGCGTACCAATTCACC-3'
f. *E*-ACG	5'-GACTGCGTACCAATTCACG-3'
g. *E*-AGC	5'-GACTGCGTACCAATTCAGC-3'
h. *E*-AGG	5'GACTGCGTACCAATTCAGG-3'
i. *E*-AGA	5'-GACTGCGTACCAATTCAGA-3'
j. *E*-ATC	5'-GACTGCGTACCAATTCATC-3'
MseI+3 Primer	Sequence
1. M-CAA	5'-GATGAGTCCTGAGTAACAA-3'
2. M-CAC	5'-GATGAGTCCTGAGTAACAC-3'
3. M-CAG	5'-GATGAGTCCTGAGTAACAG-3'
4. M-CAT	5'-GATGAGTCCTGAGTAACAT-3'
5. M-CTA	5'-GATGAGTCCTGAGTAACTA-3'
6. M-CTC	5'-GATGAGTCCTGAGTAACTC-3'
7. M-CTG	5'-GATGAGTCCTGAGTAACTG-3'
8. M-CTT	5'-GATGAGTCCTGAGTAACTT-3'
9. M-CCA	5'-GATGAGTCCTGAGTAACCA-3'
H/M+3 Primer	Sequence
1. H/M-TCT	5'-ATCATGAGTCCTGCTCGGTCT-3'
2. H/M-TCG	5'-ATCATGAGTCCTGCTCGGTCG-3'
3. H/M-TCC	5'-ATCATGAGTCCTGCTCGGTCC-3'
4. H/M-TTC	5'-ATCATGAGTCCTGCTCGGTTC-3'
5. H/M-TTG	5'-ATCATGAGTCCTGCTCGGTTG-3'
6. H/M-TTA	5'-ATCATGAGTCCTGCTCGGTTA-3'
7. H/M-TGA	5'-ATCATGAGTCCTGCTCGGTGA-3'
8. H/M-TGT	5'-ATCATGAGTCCTGCTCGGTGT-3'
9. H/M-TGC	5'-ATCATGAGTCCTGCTCGGTGC-3'
10. H/M-TAC	5'-ATCATGAGTCCTGCTCGGTAC-3'

*Eco*RI+*Mse*I primer combinations

*Eco*RI+3	g	e	c	f	j	j	f	f	a	h	e	a	a	f	c	c	f	j	f
*Mse*I+3	1	6	1	7	5	2	8	5	1	1	7	7	2	1	7	2	2	7	6

*Eco*RI+*Hpa*II/*Msp*I primer combinations

E+3	e	i	h	c	b	b	b	b	i	j	c	j	b	b	b	c	c	
H/M+3	9	7	8	3	6	4	5	7	8	8	4	7	2	1	3	7	8	
E+3	h	h	f	f	h	g	h	h	j	j	j	g	i	g	j	e	f	
H/M+3	7	1	6	9	5	5	4	6	6	5	1	6	4	3	3	1	1	

Table 3. The parental genetic difference (PGD) values of the 11 maize inbred lines based on AFLP marker[&].

Parental inbred line	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}	L_{11}
L_1	0										
L_2	0.3591	0									
L_3	0.3487	0.3068	0								
L_4	0.2454	0.3509	0.3043	0							
L_5	0.2392	0.3587	0.3288	0.2618	0						
L_6	0.3777	0.3855	0.3969	0.3727	0.3476	0					
L_7	0.3136	0.3820	0.3776	0.3571	0.3060	0.3216	0				
L_8	0.3229	0.3234	0.2652	0.3275	0.3054	0.3780	0.3716	0			
L_9	0.3410	0.3736	0.3314	0.3424	0.3533	0.3925	0.3608	0.3475	0		
L_{10}	0.3347	0.3584	0.3280	0.3652	0.3373	0.3907	0.3743	0.2904	0.2996	0	
L_{11}	0.3327	0.3898	0.3327	0.3531	0.3288	0.3807	0.3104	0.3164	0.3086	0.3541	0

The PGD values were calculated by the Jaccard coefficients (1908).

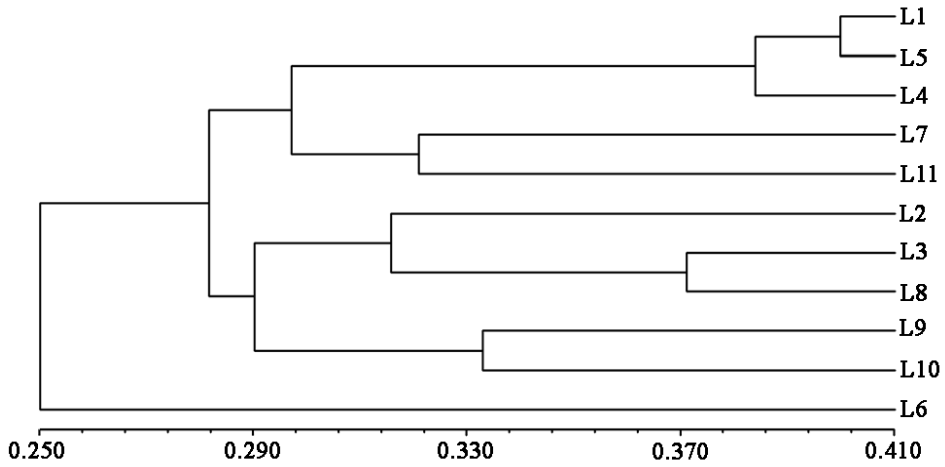

Figure 1. Dendrogram of the 11 maize inbred lines built on the AFLP-based parental genetic differences (PGDs).

either but not both of the two enzyme digestions, *Eco*RI+ *Hpa*II and *Eco*RI +*Msp*I. The number of CG and CHG methylated bands at the 5'-CCGG-3' sites were tabulated based on the criterion specified in Qi et al. (2010). The relative methylation levels of three types, CG, CHG and total (CG+ CHG), were calculated for each of the 11 inbred lines, and presented in percentage (Figure 2). It was found that the CG methylation levels of these 11 inbred lines ranged from 19.69 to 21.81% (average 20.25%), the CHG methylation levels ranged from 9.47 to 13.74% (average 11.90%), and total methylation levels ranged from 29.68 to 34.66% (average 32.15%) (Figure 2).

Based on the difference in each of the three types of relative methylation levels (CG, CHG and total) (Table 4), the corresponding dendrograms were constructed, which are similar to each other (Figure 3), thus, indicating intrinsic correlations among the three types of relative methylation levels, as indeed verified by a correlation analysis (Table).

Correlation of the AFLP-based parental epigenetic difference (PEGD) with the agronomic traits in MPH and BPH

We calculated for possible correlating relationships between each of the four scored agronomic traits: number of rows per ear (NRE), number of kernels per row (NKR), one-hundred kernel weight (HKW) and kernel weight per ear (KWE), which were known to contribute significantly to grain-yield, with the AFLP-based parental genetic difference (PGD). Data showed that no correlation was significant at the statistical level (Table).

Correlation of the DNA methylation level-based parental epigenetic difference (PEGD) with the agronomic traits in MPH and BPH

We calculated for possible correlating relationships between each of the same four scored agronomic traits:

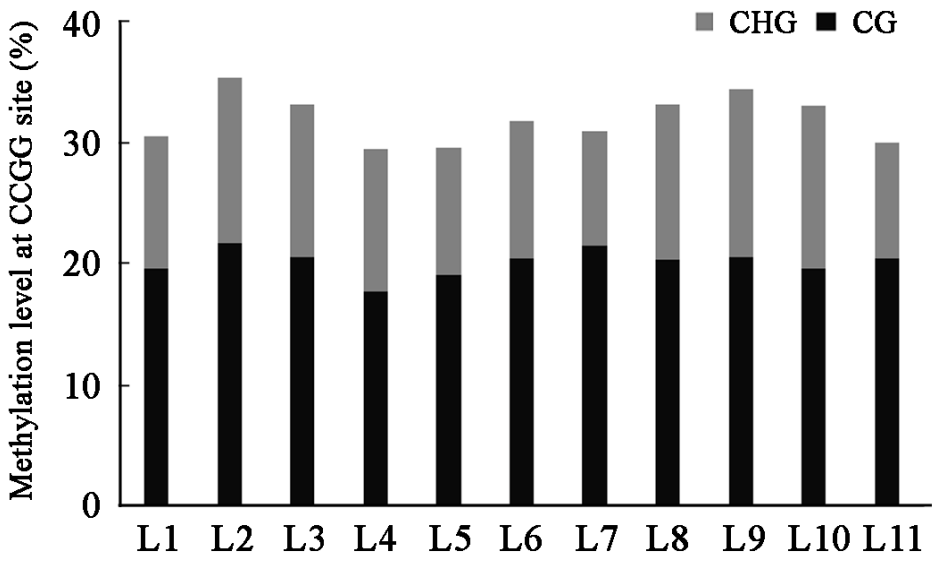

Figure 2. Relative methylation levels (%) of three types, CG, CHG and total (CG+ CHG) of the 11 inbred lines revealed by the MSAP marker.

number of rows per ear (NRE), number of kernels per row (NKR), one-hundred kernel weight (HKW) and kernel weight per ear (KWE), with the DNA methylation level-based parental epigenetic differences (PEGD). These data showed that the parental CHG methylation levels showed significant negative and positive correlations with two of the agronomic traits, namely, NRE and NKR, respectively. Specifically, (1) CHG methylation level differences showed significant negative correlations with both the middle parents heterosis (MPH) and better parent heterosis (BPH) for NRE ($r = -0.477$ and -0.493, respectively, $p < 0.01$) (Figure 4A and B; Table); (2) CHG methylation level differences showed significant positive correlation with MPH of NKR ($r = 0.385$, $p < 0.05$; Table) (Figure 4C), but insignificant correlation with BPH of NKR ($r = 0.247$, $p > 0.05$; Table) (Figure 4D). All the rest correlations are statistically insignificant (Table 5).

DISCUSSION

Although, various nucleotide sequence-based DNA molecular markers have been used to categorize inbred germplasm into different “heterotic groups” in maize and other crops for the purpose of heterosis prediction, inconsistent and sometimes discrepant results have been obtained (Lee et al., 2007; Hochholdinger and Hoecker, 2007; Zhao et al., 2006). This has fostered the proposal that more bio-logically meaningful markers need to be exploited for this purpose. Therefore, it has been suggested that parental differences in gene expression levels from a genomewide perspective (transcriptome) will likely produce more reliable markers for the prediction of heterosis, and indeed, some promising results were obtained (Stupar et al., 2008; Swanson-Wagner et al., 2009; Frisch et al., 2010; Thiemann et al., 2010). Nonetheless, trans-criptome- based molecular makers are expensive to develop and, at the current stage, unrealistic to be readily used by breeders. Therefore, developing alternative and more robust markers is urgently needed (Qi et al., 2010).

Accumulated recent evidence has established that genetic difference at the primary nucleotide sequence level is not the only determinant of organismal phenol-types; instead, epigenetic differences dependent on heritable covalent modification of DNA or chromatin also plays important roles in regulating heritable but potentially reversible changes in gene expression, and hence, phenotypic novelty (Lukens and Zhan, 2007; Kimatu and Liu, 2010; King et al., 2010; Zhong et al., 2009).

The combination of two differentiated parental genomes into a common nucleus with only one parent’s cytoplasm by hybridization (that is, F1 hybrids) conceivably may cause a cascade of epigenetic perturbations resulting in remodeled epigenetic landscape that cause alterations of gene expression (Chen et al., 2006; Liu and Wendel, 2002). Indeed, several studies have shown that even intraspecific hybridization may cause large-scale alterations in DNA methylation pattern and level, and novel patterns of gene expression, for example, non-additive expression (Tani et al., 2005; Zhang et al., 2007; Zhao et al., 2007, 2008), implicating its potential roles in heterosis. Recent studies in several crop plants including potato, cotton, *Brassica*, rice and maize showed that cytosine methylation might be directly or indirectly related to heterosis, though, the results can be variable in specific cases (Qi et al., 2010; Zhang et al., 2007; Zhao et al., 2007, 2008; Nakamura and Hosaka, 2010).

Table 4. The parental epigenetic difference (PEGD) values of the 11 maize inbred lines based on CG, CHG and total (CG+CHG) methylation levels.

PEGD based on CG methylation level	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}	L_{11}
L_1	0										
L_2	0.8069	0									
L_3	0.7297	0.7064	0								
L_4	0.6864	0.7991	0.7536	0							
L_5	0.4779	0.8019	0.7216	0.6176	0						
L_6	0.7865	0.7824	0.7644	0.7861	0.7348	0					
L_7	0.7248	0.8150	0.7581	0.7684	0.7152	0.6914	0				
L_8	0.7104	0.7422	0.6287	0.7122	0.7231	0.7164	0.7660	0			
L_9	0.7700	0.7479	0.7072	0.8087	0.7840	0.7418	0.7778	0.7424	0		
L_{10}	0.7839	0.7380	0.5920	0.7671	0.7799	0.7308	0.7919	0.6214	0.7217	0	
L_{11}	0.7468	0.7931	0.7151	0.7979	0.7212	0.6905	0.7320	0.6885	0.7437	0.7120	0
PEGD based on CHG methylation levels											
L_1	0										
L_2	0.6163	0									
L_3	0.6024	0.5506	0								
L_4	0.4923	0.6296	0.5882	0							
L_5	0.3969	0.6514	0.5802	0.4603	0						
L_6	0.5260	0.6324	0.6124	0.6118	0.5669	0					
L_7	0.5404	0.6263	0.6141	0.5592	0.5617	0.4940	0				
L_8	0.5283	0.6243	0.5730	0.5844	0.5939	0.5439	0.5879	0			
L_9	0.5723	0.5543	0.5556	0.6118	0.6025	0.5765	0.5795	0.5771	0		
L_{10}	0.6494	0.5892	0.5174	0.6564	0.6532	0.6559	0.6277	0.5956	0.5465	0	
L_{11}	0.6118	0.6211	0.6011	0.6335	0.6395	0.5322	0.5611	0.5341	0.5657	0.6075	0
PEGD based on total methylation levels											
L_1	0										
L_2	0.7019	0									
L_3	0.6385	0.6047	0								
L_4	0.5685	0.6976	0.6620	0							
L_5	0.4144	0.7037	0.6145	0.5358	0						
L_6	0.6327	0.6667	0.6524	0.6812	0.6201	0					
L_7	0.5880	0.6825	0.6348	0.6215	0.5890	0.5652	0				
L_8	0.6053	0.6459	0.5531	0.6346	0.6257	0.5917	0.6379	0			
L_9	0.6524	0.6384	0.6094	0.6962	0.6776	0.6472	0.6371	0.6266	0		
L_{10}	0.6860	0.6352	0.5341	0.6639	0.6775	0.6545	0.6631	0.5612	0.6122	0	
L_{11}	0.6563	0.6762	0.6173	0.6805	0.6391	0.5684	0.5932	0.5731	0.6311	0.6164	0

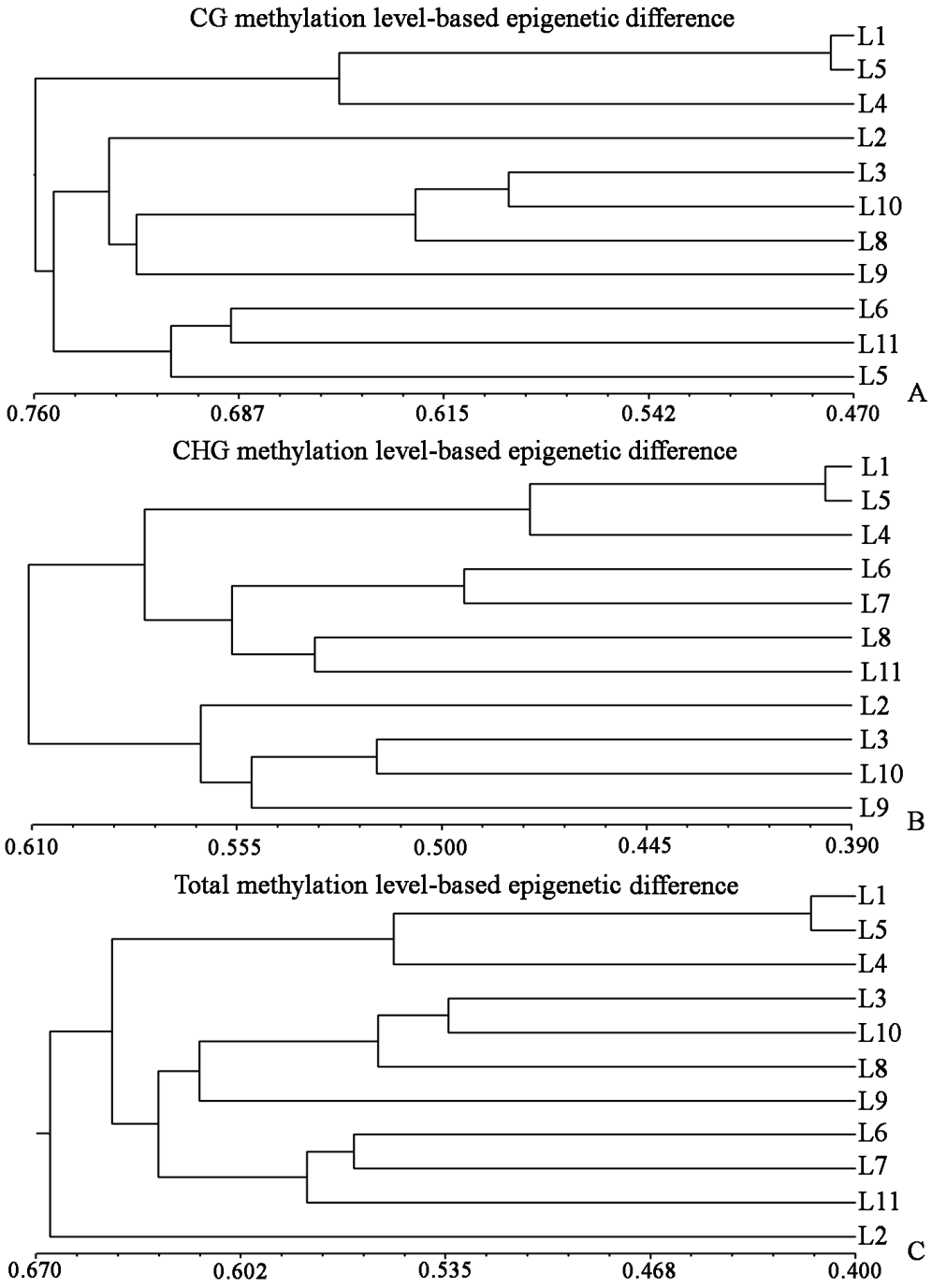

Figure 3. Dendrograms of the 11 maize inbred lines built on the relative DNA methylation levels (%) of three types; A) CG; B) CHG and; C) total (CG+ CHG), revealed by the MSAP marker.

Table 5. Correlations among the three types of relative DNA methylation levels, CG, CHG and total (CG + CHG), for the 11 maize inbred lines.

Parameter	CG methylation level	CHG methylation level	Total methylation level
CG methylation level	1	-	-
CHG methylation level	0.546**	1	
Total methylation level	0.916**	0.742**	1

** Correlation is significant at the 0.01 statistic level (2-tailed).

Table 6. Correlation of the AFLP/MSAP-based parental genetic/epigenetic difference (GD/EPGD) with MPH and BPH in four agronomic traits.

MPH	Agronomic trait			
	NRE	NRK	HKW	EKW
AFLP-based PGD	-0.147	0.076	-0.152	-0.194
Total methylation level-based PEGD	-0.224	0.208	-0.004	-00.073
CG methylation level-based PEGD	0.001	0.187	-0.111	-0.119
CHG methylation level-based PEGD	-0.477**	0.385*	0.192	0.033
BPH				
AFLP-based PGD	-0.105	0.002	-0.196	-0.059
Total methylation level-based PEGD	-0.272	0.148	-0.197	-0.082
CG methylation level-based PEGD	-0.043	0.156	-0.292	-0.080
CHG methylation level-based PEGD	-0.493**	0.247	0.152	-0.101

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level.

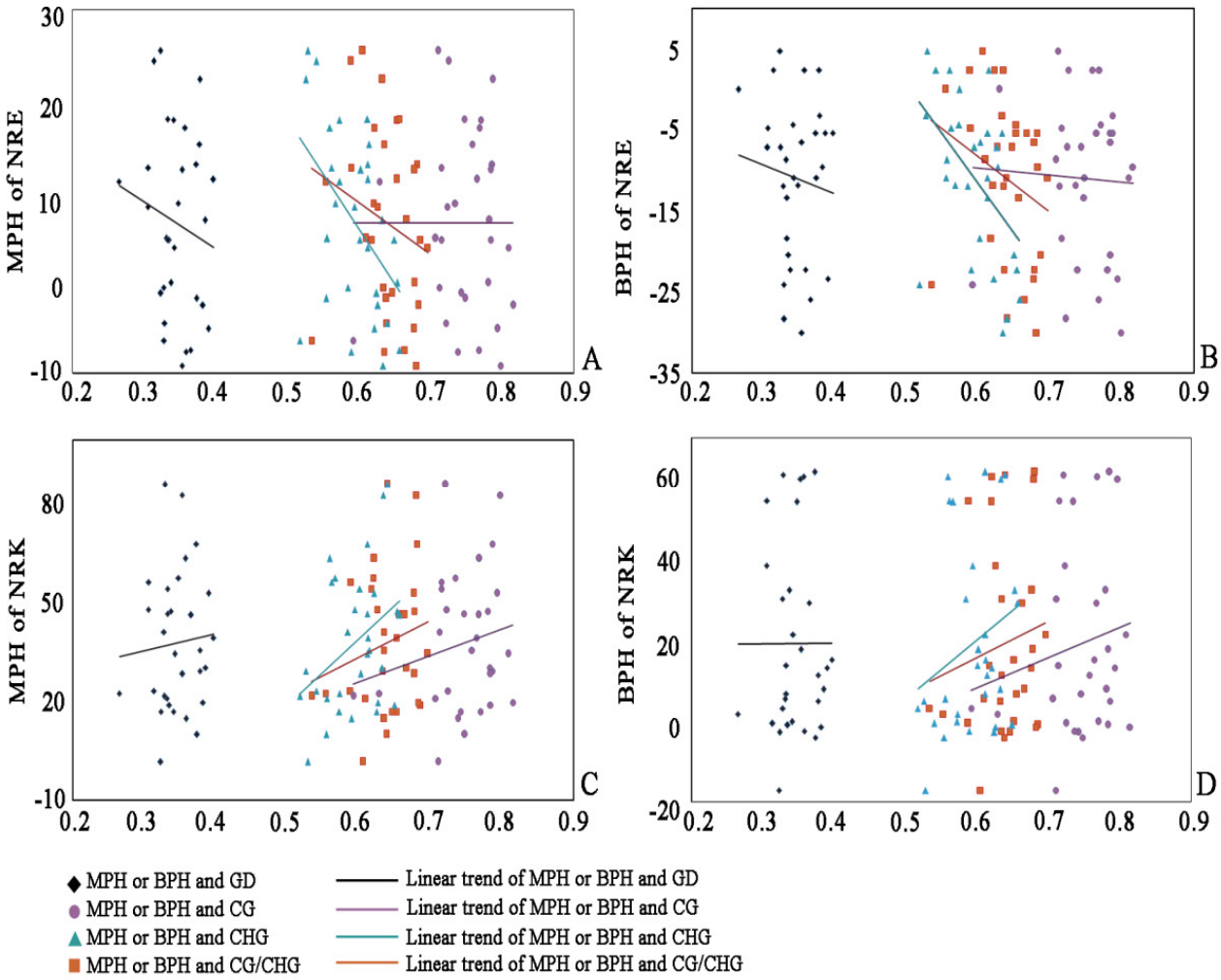

Figure 4. Correlations respectively between the AFLP-based parental genetic differences (PGDs) and middle parent heterosis (MPH) or better parent heterosis (BPH) for two agronomic traits, NRE and NKR, and between the MSAP-based various DNA methylation-level-based parental epigenetic differences (EPGDs) and MPH or BPH for the same two agronomic traits, NRE and NKR. The various correlations are denoted by different symbols. The statistical significance of the various coefficients are given in Table.

It has been documented in a recent study in maize that a specific type of parental DNA methylation difference, that is, CHG methylation difference, is positively correlated with heterosis in grain yield (Qi et al., 2010). Because grain-yield is a complex trait that is determined by many other traits in hierarchy, termed, yield-component traits, it is meaningful to further explore the degree to which these traits that are influenced by differences in DNA methylation. To address this issue in maize, we conducted the present investigation. We found that among the four agronomic traits related to grain-yield we investigated the number of rows per ear (NRE), the number of kernels per row (NKR), 100-kernel weight (HKW) and kernel weight per ear (KWE). NRE and NKR showed respectively, a positive and negative correlation with CHG methylation levels at the 5'-CCGG-3' sites randomly sampled across the maize genome, whereas, the other two traits showed no statistically meaningful correlation with any of the DNA methylation levels. Our results suggest that parental epigenetic difference in particular types of DNA methylation-level difference, may play a significant role in the manifestation of heterosis of specific grain-yield-component traits in maize, but the effects can be in opposite directions, and hence, may offset each other and cumulate in cryptic effects on grain-yield itself. Further knowledge of plant epigenetic modifications may enable manipulation towards modification of only genomic regions of interest, and enable their use as more reliable predictors for heterosis.

REFERENCES

Birchler JA, Auger DL, Riddle NC (2003). In search of the molecular basis of heterosis. Plant Cell. 15(10): 2236-2239.

Chen Y, Long LK, Lin X, Guo WL, Liu B (2006). Isolation and characterization of a set of disease resistance-gene analogs (RGAs) from wild rice, Zizania latifolia Griseb. I. Introgression, copy number lability, sequence change, and DNA methylation alteration in several rice-Zizania introgression lines. Genome, 49: 150-158.

Dong ZY, Wang YM, Zhang ZJ, Shen Y, Lin XY, Ou XF, Han FP, Liu B (2006). Extent and pattern of DNA methylation alteration in rice lines derived from introgressive hybridization of rice and Zizania latifolia Griseb. Theor. Appl. Genet. 113: 196-205.

Frisch M, Thiemann A, Fu J, Schrag TA, Scholten S, Melchinger AE (2010). Transcriptome-based distance measures for grouping of germplasm and prediction of hybrid performance in maize. Theor. Appl. Genet. 120: 441-450.

Hochholdinger F, Hoecker N (2007). Towards the molecular basis of heterosis. Trends Plant Sci. 12: 427-432.

Kidwell KK, Osborn TC (1992). Simple plant DNA isolation procedures. Plant genomes: methods for genetic and physical mapping. pp. 1-13.

Kimatu JN, Liu B (2010). Epigenetic polymorphisms could contribute to the genomic conflicts and gene flow barriers resulting to plant hybrid necrosis. Afr. J. Biotechnol. 9(48): 8125-8133.

King GJ, Amoah S, Kurup S (2010). Exploring and exploiting epigenetic variation in crops. Genome, 53(11): 856-868.

Lee EA, Ash MJ, Good B (2007). Re-examining the relationship between degree of relatedness, genetic effects, and heterosis in Maize. Crop Sci. 47: 629-635.

Liu B, Wendel JF (2002). Non-mendelian phenomena in allopolyploid genome evolution. Curr. Genomics, 3: 489-505.

Liu W, Tollenaar M (2009). Physiological mechanisms underlying heterosis for shade tolerance in Maize. Crop Sci. 49: 1817-1826.

Lukens LN, Zhan S (2007). The plant genome's methylation status and response to stress: implications for plant improvement. Curr. Opin. Plant Biol. 10: 317-322.

Mantel N (1967). The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209-220.

Nakamura S, Hosaka K (2010). DNA methylation in diploid inbred lines of potatoes and its possible role in the regulation of heterosis. Theor. Appl. Genet. 120(2): 205-214.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. 76: 5269-5273.

Qi X, Li ZH, Jiang LL, Yu XM, Ngezahayo F, Liu B (2010). Grain-yield heterosis in Zea mays L. shows positive correlation with parental difference in CHG methylation. Crop Sci. 50(10): 2338-2346.

Reyna-Lopez GE, Simpson J, Ruiz-Herrera J (1997). Differences in DNA methylation patterns are detectable during the dimorphic transition of fungi by amplification of restriction polymorphisms. Mol. Gen. Genet. 253: 703-710.

Rohlf FJ (1987). NTSYS-pc: Microcomputer programs for numerical taxonomy and multivariate analysis. Am. Stat. 41: p. 330.

Soengas P, Ordaìs B, Malvar RA, Revilla P, Ordaìs A (2003).Heterotic patterns among flint maize populations. Crop Sci. 43: 844-849.

Stupar RM, Gardiner JM, Oldre AG, Haun WJ, Chandler VL, Springer NM (2008). Gene expression analyses in maize inbreds and hybrids with varying levels of heterosis. BMC Plant Biol. 8: p. 33.

Swanson-Wagner RA, De Cook R, Jia Y, Bancroft T, Ji T, Zhao X, Nettleton, D, Schnable PS (2009). Paternaldominance of trans-eQTL influences gene expression patterns in maize hybrids. Science, 326: 1118-1120.

Tani E, Polidoros AN, Nianiou-Obeidat I, Tsaftaris AS (2005). DNA methylation patterns are differently affected by planting density in maize inbreds and their hybrids. Maydica, 50: 19-23.

Thiemann A, Fu J, Schrag TA, Melchinger AE, Frisch M, Scholten S (2010). Correlation between parental transcriptome and field data for the characterization of heterosis in *Zea mays* L. Theor. Appl. Genet. 120: 401-413.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Zabeau M (1995). AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

Wang YM, Dong ZY, Zhang ZJ, Lin XY, Shen Y, Zhou D, Liu B (2005). Extensive de novo genomic variation in rice induced by introgression from wild rice (*Zizania latifolia* Griseb.). Genetics, 170: 1945-1956.

Zhang MS, Wang HY, Zhao N, Lin XY, Pang JS, Xu KZ, Liu LX, Liu B (2007). Endosperm-specific hypomethylation, and meiotic inheritance and variation of DNA methylation level and pattern in sorghum (Sorghum bicolor L.) inter-strain hybrids. Theor. Appl. Genet. 115: 195-207.

Zhao X, Jia E, Yang W, Dong Y, Liu B (2006). DNA methylation polymorphism in a set of elite maize inbred lines revealed by methylation-sensitive ISSR analysis. Cereal Res. Commun. 34: 879-886.

Zhao XX, Chai Y, Liu B (2007). Epigenetic inheritance and variation of DNA methylation level and pattern in maize intra-specific hybrids. Plant Sci. 172: 930-938.

Zhao Y, Yu S, Xing C, Fan S, Song M (2008). Analysis of DNA methylation in cotton hybrids and their parents. Mol. Biol. 42: 169-178.

Zhong X, Wang Y, Liu X, Gong L, Ma Y, Qi B, Dong Y, Liu B (2009). DNA methylation polymorphism in annual wild soybean (Glycine soja Sieb. et Zucc.) and cultivated soybean (*G. max* L. Merr.) Can. J. Plant Sci. 8: 851-863.

Zilberman D (2008). The evolving functions of DNA methylation. Curr. Opin. Plant Biol. 11: 554-559.