Postprint: Analysis of Epigenetic Diversity in Choy Sum Using F-MSAP

Authors: Shi Weidong

Date: 2022-05-21T20:03:12+00:00

Abstract

Hybridization of Chinese flowering cabbage can induce not only DNA sequence variations but also epigenetic changes independent of DNA sequence. To elucidate the formation mechanism of epigenetic diversity in Chinese flowering cabbage, this study employed F-MSAP to detect DNA methylation levels and pattern variations in 49 accessions of Chinese flowering cabbage. The results demonstrated that: (1) F-MSAP exhibited high detection efficiency, and Chinese flowering cabbage displayed high DNA methylation polymorphism, which could be enhanced by hybridization. (2) The epigenetic diversity of Chinese flowering cabbage was relatively low, with severe homogenization; most genetic variation originated from intraspecific sources. Selfing increased epigenetic divergence among inbred lines, while hybridization increased epigenetic divergence among hybrids. (3) The 49 accessions exhibited relatively high DNA methylation levels, predominantly in the full-methylation pattern. Selfing reduced DNA methylation levels, whereas hybridization elevated DNA methylation levels in inbred lines and hybrids through alterations in DNA methylation patterns. (4) The 49 accessions were classified into 5 groups, with consistent results between cluster analysis and principal component analysis. Hybrids tended to be categorized according to maternal genetic relationships. This research, through analysis of epigenetic diversity in Chinese flowering cabbage, enhanced the accuracy and efficiency of identification, providing both theoretical foundation and technical support for further hybrid breeding.

Full Text

Epigenetic Diversity of Chinese Flowering Cabbages Revealed by F-MSAP

SHI Weidong*
Vegetable Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, China

Abstract

Hybridization in Chinese flowering cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Le) may induce epigenetic changes independent of DNA sequence variation in addition to genetic changes. To elucidate the mechanism underlying epigenetic diversity formation in Chinese flowering cabbage, this study employed fluorescence-labeled methylation-sensitive amplified polymorphism (F-MSAP) to examine DNA methylation levels and patterns across 49 accessions. The results demonstrated: (1) F-MSAP exhibited high detection efficiency, revealing substantial DNA methylation polymorphism in Chinese flowering cabbage that could be enhanced through hybridization. (2) Epigenetic diversity was relatively low in Chinese flowering cabbage, with severe homogenization and most genetic variation originating within species. Selfing increased epigenetic divergence among inbred lines, while hybridization enhanced epigenetic differences in hybrids. (3) The 49 accessions exhibited high DNA methylation levels, predominantly in the full methylation pattern. Selfing reduced DNA methylation levels, whereas hybridization elevated methylation levels in inbred hybrids through alterations in DNA methylation patterns. (4) The 49 accessions clustered into five groups, with consistent results between cluster analysis and principal component analysis. Hybrids tended to be classified according to maternal genetic relationships. This study enhances the accuracy and efficiency of Chinese flowering cabbage identification through epigenetic diversity analysis and provides a theoretical foundation and technical support for hybrid breeding.

Keywords: Chinese flowering cabbage, selfing, hybridization, epigenetic diversity, F-MSAP, DNA methylation

---

Chinese flowering cabbage, a cruciferous vegetable originating from southern China, features high cropping indices, extensive cultivation areas, and numerous varieties. However, its germplasm resources are narrow, making germplasm innovation and identification crucial for variety breeding. Currently, genomic molecular markers are widely applied in genetic diversity analysis of Chinese flowering cabbage. ISSR, SCoT, and AFLP analyses have indicated low genetic diversity (Sun et al., 2010; Shi et al., 2011; Shi et al., 2015), while AFLP and SCoT analyses revealed that genetic variation primarily originates within species (Shi et al., 2011; Shi et al., 2015). Classification based on SRAP polymorphisms showed general consistency with phenotypic characteristics (Li et al., 2012), and SCoT marker classification aligned relatively well with maturity grouping (Shi et al., 2015). However, discrepancies between molecular marker clustering and phenotypic classification have also been observed (Sun et al., 2010; Li et al., 2012), suggesting that Chinese flowering cabbage undergoes epigenetic changes independent of DNA sequence variation that cannot be detected by genomic markers. Therefore, investigating epigenetic diversity in Chinese flowering cabbage is necessary to improve identification accuracy and efficiency.

Epigenetics refers to heritable modifications that do not alter DNA sequences.

DNA methylation has become the most important epigenetic marker in plants because it can be stably inherited during both meiosis and mitosis (Kakutani et al., 1999). DNA methylation can occur in all sequence contexts, including symmetric CG and CHG sequences and asymmetric CHH sequences (Chan et al., 2005). Methylation-sensitive amplification polymorphism (MSAP) is commonly used to detect methylation status at CG sequences. Due to its simplicity, reliability, and low cost, MSAP has been widely applied in DNA methylation analysis of cruciferous plants such as *Arabidopsis*, cabbage, kale, and rapeseed (Cervera et al., 2002; Lu et al., 2005; Salmon et al., 2008; Shi et al., 2012; Zhang et al., 2013). Fluorescence-labeled F-MSAP has also been applied to DNA methylation analysis in animals and plants including pepper, chicken, and oyster (Xu et al., 2005; Jiang et al., 2014; Xu et al., 2021).

This study aims to reveal the mechanism of epigenetic diversity formation in Chinese flowering cabbage by detecting DNA methylation level and pattern changes in 49 accessions, thereby providing a theoretical basis and technical support for improving identification accuracy and efficiency in hybrid breeding.

Materials and Methods

Plant Materials The 49 Chinese flowering cabbage accessions were collected and developed by the Vegetable Research Institute of Guangxi Academy of Agricultural Sciences, comprising 7 inbred parental lines (Nos. 1, 2, 3, 5, 6, 7, 14), 8 double-inbred hybrids (Nos. 21, 24, 26, 31, 34, 36, 47, 48), 17 single-inbred hybrids (Nos. 15, 18, 19, 20, 22, 23, 25, 29, 30, 32, 33, 35, 37, 39, 43, 44, 49), 7 commercial varieties (Nos. 4, 8, 9, 10, 11, 12, 13), and 10 commercial variety hybrids (Nos. 16, 17, 27, 28, 38, 40, 41, 42, 45, 46), totaling 14 varieties and 35 hybrids. Field experiments were conducted in 2014 at the Lijian Research Base of Guangxi Academy of Agricultural Sciences. Each accession was planted in a 5 m^2 plot, and at harvest, five plants were randomly selected for morphological index recording. Tender leaves were collected, pooled, and stored at -20°C.

Methods Genomic DNA Extraction: Total genomic DNA was extracted using the CTAB method.

F-MSAP Analysis: Taq DNA polymerase, buffer, dNTPs, and fluorescence-labeled primers were provided by Beijing Dingguo Changsheng Biotechnology Co., Ltd. The one-step restriction-ligation reaction system contained: DNA (50 ng $\cdot$ L^{-1}) 4 L, Adapter 1 L, EcoR I/Msp I or EcoR I/Hpa II 2 L, 10× Reaction buffer 2.5 L, 10 mmol $\cdot$ L^{-1} ATP 2.5 L, T4 Ligase 1 L, and H_2O 7 L. The same genomic DNA was digested with EcoR I/Msp I and EcoR I/Hpa II combinations, mixed, centrifuged briefly, incubated at 37°C for 5 h, 8°C for 4 h, and 4°C overnight.

Pre-amplification Reaction: The 25 L reaction system contained: DNA 2 L, pre-amplification primers 1 L, dNTPs 0.5 L, 10× PCR buffer 2.5 L, Taq enzyme 0.5 L, and ddH_2O 18.5 L. After brief centrifugation, the reaction

conditions were: 94°C for 2 min; 30 cycles of 94°C for 30 s, 56°C for 30 s; 72°C for 80 s, final extension at 72°C for 5 min, and hold at 4°C.

Selective Amplification Reaction: Pre-amplification products were diluted 1:20 as templates for selective amplification. The 25 L reaction system contained: DNA 2 L, 10× PCR buffer 2.5 L, dNTP 0.5 L, EcoR I primer 1 L, Hpa II/Msp I primer 1 L, Taq enzyme 0.5 L, and ddH_2O 17.5 L. Reaction conditions were: 94°C for 30 s, 65°C for 30 s, 72°C for 80 s, with temperature decreasing by 0.7°C per cycle for 12 cycles; followed by 23 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 80 s; final extension at 72°C for 10 min, and hold at 4°C. Adapter and primer sequences are listed in Table 1.

Data Scoring and Statistical Analysis: Selective amplification products were separated by polyacrylamide gel electrophoresis and detected using an ABI377 sequencer. Data matrices were recorded as "0" for absence and "1" for presence of bands using GENESCAN software. Excel 2007 was used to calculate methylation polymorphism ratios and methylation types. DNA methylation patterns were classified into four types: Type I (1,1) from double digestion by Hpa II and Msp I, indicating unmethylated CCGG sites; Type II (1,0) from Hpa II digestion but not Msp I, indicating hemimethylation of the outer C in CCGG sites; Type III (0,1) from Msp I digestion but not Hpa II, indicating full methylation of the inner C in CCGG sites; and Type IV (0,0) from no digestion by either enzyme, indicating inhibited digestion of fully methylated CCGG sites or mutated sites.

POPGENE 1.32 software was used for epigenetic diversity analysis, calculating the percentage of polymorphic loci (P%), Nei' s gene diversity index (He), Shannon' s information index (I), Nei' s genetic distance (D), genetic similarity coefficient (GI), and gene flow (Nm). MEGA 4.0 software was used for cluster analysis using the UPGMA method. GenAlEx 6.41 software was used for principal component analysis and AMOVA analysis. Polymorphism information content (PIC) for primers was calculated using the formula PIC = 1 - Σfi^2, where fi represents the frequency of the ith genotype.

Results

DNA Methylation Polymorphism Analysis Using eight primer pairs that produced clear and polymorphic bands, F-MSAP amplification of 49 Chinese flowering cabbage accessions yielded a total of 1,728 bands, of which 1,479 were polymorphic, representing 86% polymorphism. The eight primer pairs produced 196, 186, 200, 173, 188, 200, 200, and 178 polymorphic bands respectively, averaging 190 bands per primer pair with 88% polymorphism. PIC values were 0.2304, 0.2012, 0.2478, 0.2373, 0.2447, 0.2418, 0.2340, and 0.2246, with a mean of 0.2327, all falling within the 0–0.5 range. Classification analysis by overall average, inbred lines, varieties, and hybrids revealed average polymorphism rates of 68.15%, 65.33%, 68.55%, 67.25%, 69.54%, and 70.09% for all 49 accessions, 7 inbred parents, 8 double-inbred hybrids, 17 single-inbred hybrids, 7 commer-

cial varieties, and 10 commercial variety hybrids, respectively. These results indicate high F-MSAP detection efficiency and substantial DNA methylation polymorphism in Chinese flowering cabbage, with commercial varieties showing higher polymorphism than inbred lines and their hybrids, and hybrids displaying higher polymorphism than parents, demonstrating that hybridization can enhance DNA methylation polymorphism.

Epigenetic Diversity Analysis Genetic diversity analysis revealed that the 49 Chinese flowering cabbage accessions had average observed allele number (Na), effective allele number (Ne), Shannon' s diversity index (I), and expected heterozygosity (He) of 1.7020, 1.2010, 0.1427, and 0.2410, respectively. Corresponding values were 1.6680, 1.1900, 0.1354, and 0.2289 for the 7 inbred parents; 1.6829, 1.1889, 0.1354, and 0.2301 for the 8 double-inbred hybrids; 1.7075, 1.2120, 0.1487, and 0.2491 for the 17 single-inbred hybrids; 1.7295, 1.2020, 0.1435, and 0.2434 for the 7 commercial varieties; and 1.7121, 1.2000, 0.1434, and 0.2432 for the 10 commercial variety hybrids. These results indicate relatively low epigenetic diversity in Chinese flowering cabbage, with inbred hybrids showing greater diversity than their parents, and single-inbred hybrids displaying greater diversity than double-inbred hybrids, confirming that hybridization can increase epigenetic differences in inbred hybrids.

Epigenetic distances were 0.0094 for all 49 accessions, 0.0095 for the 7 inbred parents, 0.0094 for the 8 double-inbred hybrids, 0.0094 for the 17 single-inbred hybrids, 0.0086 for the 7 commercial varieties, and 0.0096 for the 10 commercial variety hybrids. These results show that epigenetic distances among inbred parents were greater than those among inbred hybrids and commercial varieties, with single- and double-inbred hybrids showing similar distances but both smaller than commercial varieties, and commercial variety hybrids showing greater distances than commercial varieties. This indicates that selfing increases epigenetic distances among inbred parents, while hybridization increases epigenetic distances in commercial variety hybrids. AMOVA analysis revealed that epigenetic variation primarily originated within species (96%) rather than between species (4%) ($P = 0.036$). Gene flow (Nm) was 8.6815, greater than 1, indicating severe homogenization in Chinese flowering cabbage with suppressed genetic differentiation, where most genetic variation occurs within species and only a small portion exists between species.

DNA Methylation Analysis As shown in Table 2, DNA methylation level analysis indicated methylation rates of 68.14%, 65.26%, 68.99%, 67.39%, 69.86%, and 69.29% for all 49 accessions, 7 inbred parents, 8 double-inbred hybrids, 17 single-inbred hybrids, 7 commercial varieties, and 10 commercial variety hybrids, respectively. DNA methylation pattern analysis revealed unmethylation rates of 31.86%, 34.74%, 31.01%, 32.61%, 30.14%, and 30.71%; hemimethylation rates of 33.18%, 32.54%, 37.80%, 31.09%, 33.01%, and 33.67%; and full methylation rates of 34.96%, 32.72%, 31.20%, 36.30%, 36.85%, and 35.62%, respectively. These results demonstrate that the 49 Chinese flowering cabbage accessions

had high DNA methylation levels, with full methylation predominating over unmethylation and hemimethylation. Inbred hybrids showed higher DNA methylation levels than their parents, with the 7 inbred lines exhibiting decreased methylation due to elevated unmethylation levels, the 8 double-inbred hybrids showing increased demethylation, and the 17 single-inbred hybrids displaying increased methylation due to elevated full methylation levels. Commercial varieties and their hybrids showed minimal changes in DNA methylation levels and patterns, indicating that selfing can reduce DNA methylation levels while hybridization elevates methylation levels in inbred hybrids through changes in DNA methylation patterns.

Cluster Analysis As shown in Figure 1, UPGMA cluster analysis divided the 49 Chinese flowering cabbage accessions into five groups at a Nei' s genetic distance of approximately 0.42. Group I contained 7 accessions (Nos. 1, 16, 18, 19, 20, 21, and 22), with No. 1 being the maternal parent of Nos. 19, 20, 21, and 22, and 'Teqing 60 Days' being the maternal parent of Nos. 16 and 18. Group II contained 2 accessions (Nos. 38 and 44), with 'Lübao 701' as the maternal parent of No. 38 and paternal parent of No. 44. Group III contained 14 accessions (Nos. 2, 5, 6, 8, 9, 10, 11, 15, 23, 35, 36, 39, 46, and 47), with No. 2 as the maternal parent of No. 23, No. 6 as the maternal parent of Nos. 35 and 36, and 'Lübao 701' as the paternal parent of Nos. 15 and 47. Group IV contained 11 accessions (Nos. 12, 14, 17, 24, 37, 40, 41, 42, 43, 45, and 48), with 'Lübao 701' as the maternal parent of Nos. 41, 42, 43, and 45, and as the paternal parent of Nos. 17, 40, and 37, while No. 14 was the maternal parent of No. 48. Group V contained 15 accessions (Nos. 3, 4, 7, 13, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, and 49), with No. 3 as the maternal parent of Nos. 25 and 26, No. 4 as the maternal parent of No. 27, and No. 5 as the maternal parent of Nos. 29, 30, 31, 32, and 33. The 14 varieties were distributed across different groups, while the 35 hybrids tended to cluster according to maternal genetic relationships, indicating that hybrids inherit DNA methylation states primarily from the maternal parent and that maternal DNA methylation significantly influences hybrid epigenetic diversity.

Principal Component Analysis Principal component analysis using the PCA module in GenAlEx 6.41 software clearly separated the 49 accessions into five groups (I, II, III, IV, and V) (Figure 2). The distribution of these five groups was largely consistent with the five clusters from UPGMA analysis, demonstrating high detection efficiency and accuracy of F-MSAP. The first and second principal coordinates contributed 19.44% and 11.81% respectively, explaining 31.25% of the epigenetic variation.

Discussion

DNA Methylation Polymorphism Analysis Conventional MSAP detects DNA methylation polymorphism using polyacrylamide gel electrophoresis, whereas F-MSAP employs fluorescence-labeled primers combined with capillary

electrophoresis, substantially improving detection efficiency and sensitivity. Studies using F-MSAP in chicken F_1 generations revealed that approximately 95% of methylation polymorphism patterns were inherited from parents, with only about 5% representing novel methylation sites, while detection efficiency in oysters was more than double that of MSAP, with polymorphism detection rate increasing by 9% (Jiang et al., 2014). A limitation of F-MSAP is its restriction to CCGG sites in the genome, unable to detect CHG sequences or asymmetric CHH sequences, potentially underestimating actual methylation levels (McClelland et al., 1994; Salmon et al., 2008). Additionally, as MSAP is based on AFLP technology, the size range of polymorphic fragments detected is limited. Compared with cruciferous relatives, the 49 Chinese flowering cabbage accessions in this study exhibited higher DNA methylation polymorphism than *Arabidopsis* (24–34%), Chinese cabbage seedling shoot tips (30.42%), cabbage (53.3–60.7%), rapeseed seeds (15.7%), kale (47%), and *Brassica napus* introgression lines (33.4–39.8%) (Cervera et al., 2002; Li et al., 2002; Salmon et al., 2008; Lu et al., 2005; Shi et al., 2012; Zhang et al., 2013), and also higher than genomic polymorphism detected by ISSR (56.31%), SRAP (40.2%), and SCoT (36%) in Chinese flowering cabbage (Sun et al., 2010; Li et al., 2012; Shi et al., 2015). These results demonstrate that F-MSAP is an effective method for detecting DNA methylation in Chinese flowering cabbage, substantially improving detection efficiency and sensitivity.

Epigenetic Diversity Analysis Shannon' s diversity index (I) is an indicator for evaluating intra- and inter-specific genetic diversity levels, with higher I values indicating greater diversity. The epigenetic Shannon' s diversity index for the 49 Chinese flowering cabbage accessions (0.1427) was lower than genomic Shannon' s diversity indices from ISSR (0.229), AFLP (0.472), and SCoT (0.217) (Sun et al., 2010; Shi et al., 2011; Shi et al., 2015). The epigenetic distance (0.0094) was also smaller than genetic distances from ISSR (0.029–0.344), AFLP (0.112), and SCoT (0.428) (Sun et al., 2010; Shi et al., 2011; Shi et al., 2015). These findings suggest that epigenetic diversity in Chinese flowering cabbage is low and lower than genomic genetic diversity, consistent with results showing low epigenetic diversity in kale (Shi et al., 2012), but contrasting with findings in watermelon, rice, and pepper where genomic methylation diversity exceeded genetic diversity (Nimmakayala et al., 2011; Peng et al., 2014; Xu et al., 2021). These differences may relate to different comparison methods or substantial variations in the relationship between epigenetic and genomic diversity across species. This study also found that epigenetic distances among inbred parents were greater than those among commercial varieties, indicating that selfing increases epigenetic diversity levels and enriches genetic backgrounds, while commercial varieties exhibit the lowest epigenetic diversity, which can be increased through hybridization.

DNA Methylation Analysis Research has shown that 35–43% of CCGG sites are DNA methylation-sensitive positions in different *Arabidopsis* ecotypes,

while being highly conserved within the same ecotype (Cervera et al., 2002). Cabbage varieties or lines possess more methylated fragments (Salmon et al., 2008), and *Brassica napus* introgression lines exhibit high-frequency hypermethylation (Zhang et al., 2013). In this study, unmethylation, hemimethylation, and full methylation each accounted for more than one-third of patterns in inbred parents, inbred hybrids, commercial varieties, and commercial variety hybrids, but full methylation was higher in commercial varieties. This indicates that Chinese flowering cabbage predominantly exhibits methylation patterns, similar to methylation changes observed in *Arabidopsis* and cabbage, though whether this represents a universal phenomenon in cruciferous plants requires further investigation.

The reduced DNA methylation level in the 7 inbred lines in this study aligns with findings of decreased DNA methylation levels in Chinese cabbage inbred lines (Liu et al., 2018), providing evidence from the methylome perspective that Chinese flowering cabbage is closely related to Chinese cabbage and thus exhibits similar epigenetic changes. Studies have shown that methylation pattern changes in hybrid *Arabidopsis* frequently occur in differentially methylated regions between parents (Greaves et al., 2012), and methylation differences between parents may be the primary cause of methylation differences between parents and hybrids (Shen et al., 2012). This study found that both single- and double-inbred hybrids exhibited higher methylation levels than their parents, consistent with increased methylation levels in *Arabidopsis* C24/Landsberg F_1 hybrids (Greaves et al., 2012). However, the two hybrid types showed different DNA methylation pattern changes, likely due to DNA methylation differences between parental inbred lines and commercial varieties. As Chinese flowering cabbage inbred parents become increasingly homozygous through continuous selfing, genomic heterozygosity decreases, manifested by reduced DNA methylation levels and increased unmethylation levels. In contrast, commercial varieties, as open-pollinated seeds, maintain higher genomic heterozygosity, reflected by higher full methylation levels. Therefore, the high full methylation level in single-inbred hybrids may be attributed to commercial varieties, similar to findings in *Arabidopsis* reciprocal F_1 hybrids where the methylation level of one parental allele changed to that of the other parent (Greaves et al., 2012). The high demethylation level in double-inbred hybrids resembles the significantly higher hemimethylation levels in wheat-rye distant hybrid progeny compared with both parents (Zhu et al., 2018). The function and mechanism of active DNA demethylation remain controversial in biology, though active demethylation is important for pruning genome-wide methylation patterns, and dynamic regulation of methylation and demethylation is crucial for maintaining plasticity of the plant epigenome (Zhu et al., 2007). The DNA methylation changes resulting from parental differences in this study indicate that hybridization alters the methylome of Chinese flowering cabbage hybrids, reflecting the complexity of mechanisms underlying epigenetic diversity formation.

Effects of Hybridization on Epigenetic Diversity Chinese flowering cabbage breeding frequently employs hybridization among early-, medium-, and late-maturing varieties, using phenotypic and molecular markers to detect genetic relationships. However, identification results from these approaches are not always consistent, possibly due to complex genetic backgrounds of variety resources, insufficient optimization or unsuitability of genomic molecular markers, and inability to detect epigenetic changes. The clustering results for the 49 accessions in this study were clear and reliable, with 14 varieties showing substantial epigenetic variation. The 7 inbred parents were distributed across different groups, while the 7 commercial varieties clustered in Groups III, IV, and V, consistent with the finding that selfing increases epigenetic distances, indicating that selfing enhances epigenetic differences among inbred parents. Studies have shown that reciprocal *Arabidopsis* hybrids possess identical genetic composition, yet maternal and paternal genomes may not equally influence DNA methylation changes in hybrids, and methylation remodeling in hybrids may contribute to heterosis (Shen et al., 2012). In this study, the 35 inbred and commercial variety hybrids were distributed across different groups and tended to cluster according to maternal genetic relationships, consistent with findings that methylation states in hybrid rice are more similar to the maternal parent (Peng et al., 2014), but contrasting with results showing that methylation polymorphism in different *Arabidopsis* genotypes is not correlated with genetic relationships and that closely related *Arabidopsis* accessions do not cluster together (Cervera et al., 2002), as well as findings that gene methylation is not correlated with genetic relationship-based clustering in *Arabidopsis* (Matthew et al., 2007). Compared with AFLP and SCoT analyses in Chinese flowering cabbage, this study further improved identification accuracy (Shi et al., 2011; Shi et al., 2015), with consistent results from principal component analysis. This novel finding has not been previously reported and carries extremely important guiding significance for Chinese flowering cabbage germplasm research and breeding. Given that maternal genetic relationships may substantially influence epigenetic diversity in Chinese flowering cabbage hybrids, maternal parent selection should be emphasized when utilizing different parental combinations for hybrid production, and classification of hybrid progeny based on maternal genetic relationships may achieve greater efficiency.

In summary, detection of DNA methylation changes in Chinese flowering cabbage through F-MSAP has revealed the mechanism of epigenetic diversity formation, enabled analysis and prediction of epigenetic differences in hybrid progeny, improved identification accuracy and efficiency, and provided a theoretical foundation and technical support for hybrid breeding.

References

CERVERA MT, RUIZ-GARCÍA L, MARTÍNEZ-ZAPATER J, et al., 2002. Analysis of DNA methylation in *Arabidopsis thaliana* based on methylation-sensitive AFLP markers[J]. *Mol Genet Genom*, 268: 543-552.

CHAN WL, HENDERSON IR, JACOBSEN SE, et al., 2005. Gardening the genome: DNA methylation in *Arabidopsis thaliana*[J]. *Not Rev Genet*, 6(5): 351-360.

GREAVES IK, GROSZMANN M, HUA Y, et al., 2012. Trans Chromosomal Methylation in *Arabidopsis* hybrids[J]. *Proc Natl Acad Sci USA*, 109(9): 3570-3575.

JIANG Q, YU H, KONG LF, et al., 2014. Analysis of DNA methylation in different tissues of the pacific oyster (*Crassostrea gigas*) with the fluorescence-labeled methylation-sensitive amplified polymorphism(F-MSAP)[J]. *Chin Fish Sci*, 21(4): 676-683.

KAKUTANI T, MUNAKATA K, RICHARDS EJ, et al., 1999. Meiotically and mitotically stable inheritance of DNA hypomethylation induced by ddm1 mutation of *Arabidopsis thaliana*[J]. *Genetics* 151(2): 831-838.

LI GH, CHEN HC, ZHANG Y, et al., 2012. Genetic diversity of *Brassica parachinensis* germplasm revealed by SRAP analysis[J]. *Chin Agric Sci Bull*, 28(4): 110-114.

LI ML, WANG QM, ZHU ZJ, et al., 2002. Studies on the changes of DNA methylation level, GA content and protein in non-heading Chinese cabbage during vernalization[J]. *Acta Hortic Sin*, (4): 353-357.

LIU Y, XU C, TANG XB, et al., 2018. Genomic methylation and transcriptomic profiling provides insights into heading depression in inbred *Brassica rapa* L. ssp. *pekinensis*[J]. *Gene*, 665:119-126.

LU GY, WU XM, CHEN BY, et al., 2005. Analysis on genomic DNA methylation modification of seed germination *Brassica* by methylation-sensitive amplification polymorphism[J]. *Chin Sci Bull*, 50(24): 2750-2756.

MCCLELLAND M, NELSON M, RASCHKE E, et al., 1994. Effect of site-specific modification on restriction endonucleases and DNA modification methyltransferases[J]. *Nucl Acids Res*, 22(17): 3640-3659.

NIMMAKAYALA P, VAJJA G, GIST RA, et al., 2011. Effect of DNA methylation on molecular diversity of watermelon heirlooms and stability of methylation specific polymorphisms across the genealogies[J]. *Euphytica*, 177(1): 79-89.

PENG H, JIANG GH, ZHANG J, et al., 2013. DNA methylation polymorphism and stability in Chinese indica hybrid rice[J]. *Sci Sin Vitae*, 44(1): 1097-1106.

SALMON A, CLOTAULT J, JENCZEWSKI E, et al., 2008. *Brassica oleracea* displays a high level of DNA methylation polymorphism[J]. *Plant Sci*, 174(1): 61-70.

SHEN HS, HE H, LI JG, et al., 2012. Genome-wide analysis of DNA methylation and gene changes in two *Arabidopsis* ecotypes and their reciprocal hybrids[J]. *Plant Cell*, 24(3): 875-892.

SHI WD, HUANG RK, CHEN ZD, et al., 2012. The epigenetic genetic diversity of 18 Chinese kales analyzed by msap when initiated flowering[J]. *Genom Appl Biol*, 31(5): 505-512.

SHI WD, HUANG RK, ZHOU SM, et al., 2011. Genetic diversity of 30 Cai-xins (*Brassica rapa* var. *parachinensis*) evaluated based on AFLP molecular data[J]. *Mol Plant Breed*, 2(7): 22-29.

SHI WD, JU XX, ZHANG L, et al., 2015. Analysis on genetic diversity of chinese flowering cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Le) germplasms based on SCoT markers[J]. *J S Agric*, 46(8): 1350-1355.

SUN XM, QIAO AM, SUN M, et al., 2010. ISSR analysis of genetic diversity of 27 flowering Chinese cabbage[J]. *J SW Norm Univ (Nat Sci Ed)*, 35(1): 119-123.

MATTHEW WV, MILOS T, ZACHARY L, et al., 2007. Epigenetic natural variation in *Arabidopsis thaliana*[J]. *Plos Biol*, 5(7): 1617-1629.

XU Q, SUN DX, ZHANG Y, et al., 2005. F-MSAP: A practical system to detect methylation in chicken genome[J]. *Chin Sci Bull*, 50(17): 2039-2044.

XU XW, XIA XB, WU ZM, et al., 2021. Surveying DNA epigenetic diversity in the foreign hot pepper germplasms[J]. *Hubei Agric Sci*, 60(6): 76-80.

ZHU CY, LI T, GAO YU, et al., 2018. DNA methylation-sensitive amplification polymorphism analysis of distant hybrid progeny between wheat and rye[J]. *Mol Plant Breed*,16(3): 859-864.

ZHU JH, KAPOOR A, SRIDHAR VV, et al., 2007. The DNA glycosylase/lyase ROS1 functions in pruning DNA methylation patterns in *Arabidopsis*[J]. *Curr Biol*, 17 (26): 54-59.

ZHANG XL, GE XH, SHAO YJ, et al., 2013. Genomic change, retrotransposon mobilization and extensive cytosine methylation alteration in *Brassica napus* introgressions from two intertribal hybridizations[J]. *PLoS ONE*, 8(2): 1-10.

Note: Figure translations are in progress. See original paper for figures.