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REVIEW

Epigenetic regulation of agronomical traits in Brassicaceae

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Abstract Epigenetic regulation, covalent modification of DNA and changes in histone proteins are closely linked to plant development and stress response through flexibly altering the chromatin structure to regulate gene expression. In this review, we will illustrate the importance of epigenetic influences by discussing three agriculturally important traits of Brassicaceae. (1) Vernalization, an acceleration of flowering by prolonged cold exposure regulated through epigenetic silencing of a central floral repressor, *FLOWERING LOCUS C*. This is associated with cold-dependent repressive histone mark accumulation, which confers competency of consequence vegetative-to-reproductive phase transition. (2) Hybrid vigor, in which an F_1 hybrid shows superior performance to the parental lines. Combination of distinct epigenomes with different DNA methylation states between parental lines is important for increase in growth rate in a hybrid progeny. This is independent of siRNA-directed DNA methylation but dependent on the chromatin remodeler DDM1. (3) Self-incompatibility, a reproductive mating system to prevent self-fertilization. This is controlled by the *S*-locus consisting of *SP11* and *SRK* which are responsible for self/non-self recognition. Because self-incompatibility in Brassicaceae is sporophytically controlled, there are dominance relationships between S haplotypes in the stigma and pollen. The dominance relationships in the pollen rely on de novo DNA methylation at the promoter region of a recessive allele, which is triggered by siRNA production from a flanking region of a dominant allele.

Keywords *Brassicaceae* · Epigenetics · Vernalization · Hybrid vigor · Self-incompatibility

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Introduction

Throughout the lifecycle, angiosperms need to carry out multicellular plant body development and adapt to changing environments. This means that the plant must possess gene sets required for these processes, and each tissue orchestrates the appropriate genes to be transcribed depending on the developmental stage and biotic/abiotic stimuli. Epigenetic regulation, covalent modifications of DNA or histone proteins, controls gene activation or repression by altering the physical properties of the target DNA region beyond the nucleotide sequence, which enables the plant to quickly respond to developmental and environmental cues. These modifications are inherited through multiple cell divisions, but can be removed as necessary to act as reversible switches creating spatially and temporally diverse epigenomes derived from an identical genome sequence. Furthermore, epigenetic silencing of transposable elements (TEs) must occur in whole plant to protect genome integrity. Thus, by integrating genetic and epigenetic information, plants undergo major developmental transitions and modulate environmental adaptability to successfully complete their lifecycle.

The Brassicaceae are attractive material for basic and applied research, as they include not only the model plant *Arabidopsis thaliana*, but also the *Brassica* genus

comprising economically valuable plants, such as oil seed and vegetable crops. Among six species that belong to the *Brassica* genus, three species are diploids (*Brassica rapa;* $n=10$ (AA genome), *B. nigra;* $n=8$ (BB), and *B. oleracea;* $n=9$ (CC)), and the others are allotetraploids, a doubled hybrid of each diploid genome [*B. juncea;* $n=18$ (AABB), *B. carinata;* $n=17$ (BBCC), and *B. napus;* $n=19$ (AACC)]. Additionally, there is a difference in the genome size between *A. thaliana* (130 Mb) and diploid Brassica crops (500–600 Mb) probably due to a whole genome triplication event that likely occurred just after the divergence between the ancestral *Brassica* genus and the Arabidopsis lineages. Recently, it has become clear that epigenetic regulation is closely associated with several agriculturally important traits observed in Brassicaceae. In this review, we will illustrate the importance of epigenetic modifications through three examples of traits in Arabidopsis and Brassica crops, vernalization, hybrid vigor and self-incompatibility.

Epigenetic regulation that influences gene expression level

The DNA molecule is compacted into the nuclear space through the formation of higher-order chromatin structure. DNA along with a histone octamer, which consists two copies each of four core proteins, H2A, H2B, H3 and H4, forms a basic chromatin unit termed as nucleosome. These histone proteins can be covalently modified at the N-terminal tails by acetylation, methylation, phosphorylation and ubiquitination. Post-translational modifications at a specific residue confer distinct physical properties of chromatin altering an accessibility of general transcription machinery including DNA or RNA polymerases to the DNA strand. For example, acetylation of lysine residues of histone H3 and histone H4 by histone acetyltransferases leads to a transcriptionally active state by loosening association between histone proteins and DNA. Conversely, deacetylation, the removal of an acetyl group by histone deacetylases, is associated with transcriptionally inactive chromatin. The case of histone methylation is more complicated, as it is associated with both transcriptional activation and repression depending on the residue and the position where methylation occurs. For example, methylation of histone H3 lysine 4 (H3K4) and histone H3 lysine 36 (H3K36) is associated with transcriptional activation, whereas dimethyl histone H3 lysine 9 (H3K9me2) and trimethyl histone H3 lysine 27 (H3K27me3) are repressive marks. These reactions are mediated by histone methyltransferases containing a conserved SET domain (Pien and Grossniklaus 2007). There are also histone demethylases to remove methyl groups from histones, which establishes chromatin state dynamics in order to respond to developmental programs and environmental signals.

As a component of a nucleosome unit, as well as the canonical histone proteins, multiple histone variants are encoded in the plant genome. Histone variants can be replaced with canonical histone proteins that will influence the physical properties of the nucleosome and nucleosome dynamics. Some variants of H2A and H3 are involved in various processes including transcription, DNA repair and chromatin remodeling, and have distinct distribution in the genome (Talbert et al. 2012). H3.1 is enriched in transcriptionally silent region, whereas H2A.Z and H3.3 are predominantly enriched in actively transcribed genes (Zilberman et al. 2008; Stroud et al. 2012). The deposition of histone variants into chromatin is mediated through various histone chaperones and chromatin remodeling complexes (Schönrock et al. 2006; Choi et al. 2007; Deal et al. 2007; March-Diaz et al. 2008; Nie at al. 2014).

DNA methylation, a covalent addition of the methyl group at the C-5 position of cytosine, is a major epigenetic modification that is widely associated with the expression levels of transcriptionally activated or silenced regions. Whereas in mammals, DNA methylation is predominantly in the symmetrical CG context; in plants, it occurs in three sequence contexts, CG, CHG and CHH (H: A/T/C). The Arabidopsis genome encodes four DNA methyltransferases with distinct biological functions and target specificities. For the maintenance of CG methylation, DNA METHYLTRANSFERASE 1 (MET1) methylates on the newly synthesized strand of the hemi-methylated strand during DNA replication (Finnegan et al. 1996; Ronemus et al. 1996; Kankel et al. 2003). For non-CG contexts, two plant-specific DNA methyltransferases, CHROMOMETHYLASE 2 (CMT2) and CMT3 are responsible for maintaining methylation at CHH and CHG sites, respectively. Both CMT2 and CMT3 are targeted by the H3K9me2 mark that is added by the histone methyltransferase KRYPTONITE (KYP) leading to DNA methylation nearby the methylated histone residues (Bartee et al. 2001; Lindroth et al. 2001; Jackson et al. 2002; Stroud et al. 2014). Methylated cytosine, in turn, can be a substrate for binding by KYP and triggers H3K9me2 creating a self-reinforcing loop between histone and DNA methylation. To establish de novo methylation, DOMAINS REARRANGED METHYLASE 1 and DOMAINS REARRANGED METHYLASE 2 (DRM2) function as methyltransferases, and DRM2 plays a central role in the RNA-directed DNA methylation (RdDM) pathway (Cao and Jacobsen 2002; Cao et al. 2003; Matzke and Mosher 2014). Cooperating with two plant-specific RNA polymerases and enzymes involved with 24-nt small interfering RNA (siRNA) production and its guide to nascent sequence, DRMs transfer methyl group onto unmethylated cytosine in all three contexts resulting in transcriptional gene silencing and promotion of heterochromatin formation (Matzke and Mosher 2014).

Furthermore, RdDM is also required to maintain CHH methylation without overlapping target sites with CMT2.

In addition to the enzymes that can directly transfer methyl groups, there are proteins whose catalytic activities can indirectly affect DNA methylation levels, such as DECREASED IN DNA METHYLATION1 (DDM1). The *ddm1* mutant shows overall reduction of DNA methylation at both CG and non-CG sites, especially in heterochromatic regions (Vongs et al. 1993; Lippman et al. 2004). *DDM1* encodes a chromatin-remodeling factor, SWI2/SNF2 (Jeddeloh et al. 1999). The first generation of the *ddm1* mutant shows relatively normal growth, but notable developmental defects have been observed after repeated self-pollination for several generations (Kakutani et al. 1996). However, how the DDM1 function leads to changes in the genome wide DNA methylation pattern is still largely unknown.

Genome-wide profiling of epigenetic modifications showed distinct epigenomes depending on the genomic features. DNA methylome analyses revealed that methylated DNA for both CG and non-CG contexts is highly enriched at silenced TEs, which are found primarily in the pericentromeric heterochromatin (Zhang et al. 2006; Zilberman et al. 2007). In the genic regions, CG and non-CG methylation at the promoter is associated with gene silencing, whereas CG methylation is also detected within the transcribed region of moderately expressed genes (Zhang et al. 2006; Zilberman et al. 2007). Several studies on genome-wide landscape of histone modification, most of which focuses on the methylation and acetylation of lysine residue of histone H3, showed that they are enriched in euchromatic regions. These histone modifications are associated with transcriptional states throughout development and stress responses, except H3K9me2 at heterochromatic regions that are required for constitutive TE silencing (He et al. 2011). In many cases, the larger genome size depends on the abundance of retrotransposons. As active TEs may cause genetic instability, epigenetic gene silencing is important to protect the genome integrity in plant species possessing a large number of TEs.

Epigenetic regulation of the vernalization response

In flowering plants, the timing of the vegetative-to-reproductive phase transition is one of the most important processes in a lifecycle. It relies on several seasonal cues such as daylength and temperature. For long-day (LD) plants of which Brassicaceae is one, premature flowering during the undesirable cold season severely decreases seed productivity. To avoid this, LD plants establish the requirement for prolonged cold exposure to be competent to initiate inflorescence meristem differentiation, termed as vernalization requirement. This can be explained by up- or downregulation of *FLOWERING LOCUS C* (*FLC*). FLC, a MADS-box protein, acts as a floral repressor in a dose-dependent manner by binding to regulatory elements of the floral inducer genes, *FT* and *SOC1*, to block their LD-dependent expression (Michael and Amasino 1999; Sheldon et al. 1999; Helliwell et al. 2006). Within a lifecycle, the *FLC* expression can first be detected during embryo development, and continues throughout the vegetative stage (Sheldon et al. 2008; Choi et al. 2009). On the plant being exposed to cold temperature, *FLC* transcripts gradually decrease proportional to the cold duration, and if the prolonged cold is long enough, the repressed state of *FLC* is maintained even after returning the plants to warm temperature. This sets a floral competency, in terms of derepression of *FT* and *SOC1*, which leads to flowering under the LD conditions (Helliwell et al. 2006). As plants can be vernalized at very young stage, e.g., even at the seed stage in *A. thaliana*, there is temporal separation between cold exposure and actual phase transition. This means that epigenetic modifications must occur to maintain stable silencing of *FLC* through multiple cell divisions, and specific covalent modifications on histone proteins are involved in this process. Vernalization every generation is required to flower except for perennial plants, thus cellular memories of vernalization experience should be reset at the end of the lifecycle. In this chapter, we will provide current understanding of epigenetic regulation in Arabidopsis vernalization focusing on the histone methylation occurring at the *FLC* locus and key players that directly or indirectly cause those modifications, and will discuss similarities and diversities in vernalization-associated epigenetic events between Arabidopsis and some Brassica crops.

Determinants affecting the basal level of *FLC* before vernalization

Multiple factors can affect the basal *FLC* expression before vernalization. *FRIGIDA* (*FRI*) is a major determinant of natural variation in flowering time by activating the basal expression level of *FLC* before cold exposure (Johanson et al. 2000). FRI interacts with FRIGIDA LIKE 1 (FRL1), SUPRESSOR OF FRIGIDA 4 (SUF4), FLC EXPRESSOR (FLX) and FRIGIDA ESSENTIAL 1 (FES1) to form a complex termed FRI-containing complex (FRI-C) (Choi et al. 2011). As well as increasing in the proportion of 5′-capped *FLC* mRNA, FRI-C enriches the COMPASS-like complex including trxG H3K4 methylase, such as ARABIDOPSIS TRITHORAX 1 (ATX1) and ATX-RELATED 7 (ATXR7) at the *FLC* chromatin that leads to H3K4 trimethylation and *FLC* upregulation (Pien et al. 2008; Geraldo et al. 2009; Jiang et al. 2009; Tamada et al. 2009; Berr et al. 2009). Furthermore, it has been demonstrated that an additional SET domain protein, EARLY FLOWERING IN SHORT DAYS (EFS) with dual substrate-specificity for H3K4 and H3K36, recruits FRI to the *FLC* locus, which leads to

further recruitment of trithorax group complex (Kim et al. 2005; Zhao et al. 2005; Xu et al. 2008; Ko et al. 2010). In contrast to the *FRI*-dependent pathway, an autonomous pathway, composing some independent repressive activities FCA, FPA, FY, FLD, FVE, LUMINIDEPENDENS (LD) and FLOWERING LATE KH DOMAIN (FLK), represses basal *FLC* expression. In this pathway, it is proposed that FCA, an RNA-binding protein, physically interacts with FY, a polyadenylation/3′ RNA processing factor, and affects spliced transcript accumulation at *FLC* locus (Macknight et al. 1997; Simpson et al. 2003; Liu et al. 2007).

FRI-C also recruits an SWR1-like chromatin remodeling complex that catalyzes the H2A/H2A.Z replacement to the *FLC* (Deal et al. 2007). Disruption of SWR1-like complex components resulted in decreased *FLC* expression and early flowering without cold treatment, indicating the requirement of H2A/H2A.Z replacement for basal *FLC* expression (Deal et al. 2007; Choi et al. 2007). On the other hand, removal of H2A.Z is not essential for *FLC* repression by vernalization because this variant still expressed abundant *FLC* even after cold treatment (Finnegan and Dennis 2007).

Molecular basis of epigenetic silencing of *FLC* during the course of vernalization

Initial events that occur during cold exposure are transient upregulation of COOLAIR, multiple antisense long non-coding RNAs (lncRNAs) and concomitant downregulation of *FLC* (Swiezewski et al. 2009). COOLAIR is transcribed from the region just downstream of the *FLC* polyadenylation site and alternatively spliced. There are two classes of COOLAIR transcript with distinct polyadenylation sites, namely proximally terminated shorter group (Class I) and distally terminated longer group (Class II) (Swiezewski et al. 2009). The quantitative abundance of Class I transcript is important for *FLC* repression, and it is associated with the functions of FCA, FPA and FY as RNA processing factors (Liu et al. 2007, 2010; Marquardt et al. 2014). The choice of this proximally polyadenylated transcript results in FLD-dependent H3K4 demethylation at *FLC* by unknown mechanism (Liu et al. 2007, 2010). While several reports have shown the importance of COOLAIR in the early process of *FLC* repression, its role in the establishment of epigenetic silencing of *FLC* is still controversial (Sheldon et al. 2002; Helliwell et al. 2011).

When the plant is returned to warm conditions, *FLC* repression is stably maintained. Characterization of several flowering mutant lines revealed that Polycomb Repressive Complex 2 (PRC2) including VERNALIZATION 2 (VRN2) contributed to this silencing by increasing H3K27me3 level at the *FLC* locus (Gendall et al. 2001; Bastow et al. 2004; Sung et al. 2006a). Indeed, dynamic changes is seen in the H3K27me3 distribution of the *FLC* gene. H3K27me3 peaks around the nucleation site within the 5′ region of the *FLC* intron 1 during the cold, and spreads across the gene after returning to warm conditions (Sung and Amasino 2004; Angel et al. 2011; Yang et al. 2014). The vernalization-associated PRC2 complex prelocalizes across the *FLC* without cold exposure, but interacts with plant homeodomain (PHD) proteins, VERNALIZATION INSENSITIVE 3 (VIN3), VRN5 and VEL1 to form a PHD-PRC2 complex under cold temperature (Sung and Amasino 2004; Sung et al. 2006b; Wood et al. 2006; De Lucia et al. 2008). This cold-dependent complex is required for further spreading of H3K27me3 after returning to warm temperature.

Following reduction of COOLAIR, another cold-inducible lncRNA, COLDAIR is transiently transcribed from the *FLC* intron 1 in the sense direction (Heo and Sung 2011). Along with in vivo interaction of COLDAIR with PRC2 component, similarity of phenotypes between COLDAIR knockdown line and PHD-PRC2 component mutant lines indicates that COLDAIR may take part in epigenetic silencing by recruiting PRC2 to *FLC* (Gendall et al. 2001; Sung and Amasino 2004; Sung et al. 2006b; Mylne et al. 2006; Greb et al. 2007; Heo and Sung 2011). Recently, a third cold-inducible lncRNA has been identified proximal to the *FLC* promoter, and is termed COLDWRAP (Kim and Sung 2017). In contrast to two other lncRNAs, COLDWRAP transcript remains expressed even after returning to warm condition (Kim and Sung 2017). It is proposed that intragenic chromatin loop formation by COLDWRAP is also associated with the maintenance of *FLC* repression through assisting PHD-PRC2 in spreading across the gene.

The epigenetic memories written during vernalization response must be erased at the end of the lifecycle to confer a vernalization requirement to the next generation. Indeed, *FLC* repressed by vernalization is reactivated during the development of reproductive tissue and in early embryogenesis (Sheldon et al. 2008; Choi et al. 2009). EARLY FLOWERING 6 (ELF6), a histone lysine demethylase, has been characterized to remove methyl groups from *FLC* chromatin in reproductive tissue (Crevillén et al. 2014). In a hypomorphic *elf6* mutant line, *FLC* expression could not fully recover and H3K27me3 accumulated at higher level in the next generation as compared with WT, indicating that the presence or absence of H3K27me3 mark is important for resetting of vernalization experience (Crevillén et al. 2014).

Conservation of basic molecular events underlying vernalization in Brassicaceae

In *B. rapa, B. oleracea* and *B. napus*, multiple *FLC* paralogues, with seven exons and a large intron 1 similar to Arabidopsis *FLC*, are encoded, and their functionalities as floral repressors have been shown in part by transgenic experiments (Tadege et al. 2001; Kim et al. 2007; Zou et al.

2012; Shea et al. 2017). In *B. rapa*, four *FLC* orthologues (termed *BrFLC1-3, 5*) are encoded, and QTL analyses and sequence comparative analyses indicated that every *FLC* orthologue could affect flowering time variation among cultivars (Schrantz et al. 2002; Kakizaki et al. 2011; Wu et al. 2012; Kitamoto et al. 2014; Shea et al. 2017). In *B. oleracea*, among four *FLC* orthologues (termed *BoFLC1-3, 5; BoFLC2* is also termed *BoFLC4*), *BoFLC2* has been proposed to contribute to determine variation in flowering-time trait thus far (Schrantz et al. 2002; Lin et al. 2005; Okazaki et al. 2007; Ridge et al. 2015; Irwin et al. 2016).

In Chinese cabbage (*B. rapa* var. *pekinensis*), the expression of *BrFLC* paralogues were repressed during cold exposure and stably maintained after returning to warm conditions (Kawanabe et al. 2016a). Active marks, trimethyl histone H3 lysine 4 (H3K4me3) and trimethyl histone H3 lysine 36 (H3K36me3) accumulated at *BrFLCs* under ambient temperature and were reduced by cold treatment and, conversely, H3K27me3 increased after returning to warm conditions, indicating that the roles of epigenetic histone modifications in the vernalization response might be conserved between Arabidopsis and Brassica crops (Kawanabe et al. 2016a). Li et al. (2016) have identified natural antisense transcripts (NATs) derived from the terminator region of *BrFLC2* and grouped them into two classes according to whether they are proximally (Class I) or distally (Class II) terminated. It seemed that upregulation of NATs from Class II is associated with flowering acceleration and *BrFLC2* repression, rather than the proximally terminated transcript (Class I) repressing *FLC* like in Arabidopsis (Swiezewski et al. 2009; Liu et al. 2007, 2010; Li et al. 2016). As is the case in Arabidopsis, in both Chinese cabbage and Cauliflower (*B. oleracea* var. *botrytis*), *VIN3* orthologues were transiently upregulated during cold exposure, indicating that PHD-PRC2-mediated epigenetic silencing might be conserved (Ridge et al. 2015; Kawanabe et al. 2016a). However, the intron 1 of *FLC* and its paralogues appear highly diverse in its size and nucleotide sequence between Arabidopsis and Brassica crops, and COLDAIR-like lncRNA has not been discovered in Brassica crops thus far (Zou et al. 2012; Shea et al. 2017). This suggests that Brassica crops do not have a lncRNA-mediated pathway to stably silence *FLC* paralogues. Interestingly, two tandem *cis*-elements found near the *FLC* nucleation site, RY-1 and RY-2 which are bound by the transcriptional repressors VAL1 and VAL2, are conserved in *B. rapa, B. oleracea* and *B. napus* (Qüesta et al. 2016; Yuan et al. 2016). These repressors may be associated with H3K27me3 accumulation at *FLC* and epigenetic silencing by recruiting other negative regulator protein complex (Qüesta et al. 2016; Yuan et al. 2016). The findings above suggested that there are some similarities to *A. thaliana*, but different mechanisms may be controlling the expression profiles of multiple *FLC* paralogues during the vernalization response in Brassica crops. Further exploration of novel or conserved factors, such as more lncRNAs and components of PRC2-PHD-dependent or -independent pathway, will provide new insights into conserved molecular basis and diversities of vernalization response in Brassicaceae.

Epigenetic regulation of hybrid vigor

Heterosis or hybrid vigor describes the phenomenon where hybrids exhibit superior performance relative to their parental inbred lines in many traits, such as biomass, yield, fertility, and abiotic and biotic stress resistance (Lippmann and Zamir 2007). Heterosis has been used in the breeding of crop and vegetable cultivars through F_1 hybrid seed production where F_1 hybrid cultivars have increased production. However, the underlying biological mechanisms are not well understood. Recently developed high-throughput molecular analyses such as transcriptomes, proteomes, metabolomes, epigenomes (including DNA methylome, small RNAomes, and genome wide distribution of histone modifications) allow us to clarify the molecular mechanism of heterosis (Hochholdinger and Hoecker 2007; Birchler et al. 2010; Baranwal et al. 2012; Chen 2013; Groszmann et al. 2013; Schnable and Springer 2013). In this chapter, we introduce recent research in heterosis, focusing on epigenetic regulation in Brassicaceae.

Historical models of heterosis

Several genetic hypotheses have been presented to explain the development of heterosis. The first hypothesis is the dominance model; superior performance of hybrids results in the suppression/complementation of deleterious recessive alleles from one parent by dominant alleles from the other (Davenport 1908; Bruce 1910; Crow 1998). The second hypothesis is the overdominance model; heterozygosity at individual key loci leads to superior performance compared with either homozygote (East 1936; Crow 1998). A single heterozygous gene, *SINGLE FLOWER TRUSS*, contributes to fruit yield heterosis in tomato, demonstrating the first example of a single overdominant gene in plants (Krieger et al. 2010). The third hypothesis is the epistasis model; interactions between two or more non-allelic genes derived from the parental lines generate superior performance (Richey 1942; Powers 1944; Williams 1959). In 2000s, a genetic approach using QTL (quantitative trait locus) analysis was performed in multiple species and revealed that a large number of genes contribute to heterotic phenotypes by dominance, overdominance, or epistatic effect (Frascaroli et al. 2007; Lippman and Zamir 2007; Radoev et al. 2008; Meyer et al. 2010; Schnable and Springer 2013).

Heterosis phenotypes in Brassicaceae

In *A. thaliana*, hybrids of combinations of accessions show strong heterosis, especially in vegetative biomass (Barth et al. 2003; Meyer et al. 2004; Groszmann et al. 2014). A heterosis phenotype is seen in early development with hybrids having increased cotyledon size only a few days after sowing (Fujimoto et al. 2012; Meyer et al. 2012; Groszmann et al. 2014). Heterosis in vegetative biomass is largely dependent on a larger leaf size but not on increased leaf number (speed of development) (Meyer et al. 2004, 2012; Fujimoto et al. 2012; Groszmann et al. 2014). The larger leaf area is associated with increased cell size and number of the photosynthetic palisade mesophyll cells. In Chinese cabbage (*B. rapa* var. *pekinensis*), the commercial F_1 hybrid cultivar, 'W39', also showed increased cotyledon area at a few days after sowing compared with parental lines. The F_1 hybrid, 'W39', combines the parental properties, larger cell size of paternal line and increased cell number in maternal line (Saeki et al. 2016). The combination of cell proliferation (increased cell number) and post-mitotic cell expansion (increased cell size) regulates the leaf area (Hisanaga et al. 2015). Difference in cell number or size does not result in a difference in the cotyledon/leaf size between parental lines, but the increased cell number and size in the F_1 hybrid result in an increased cotyledon/leaf size in *A. thaliana* and *B. rapa*, suggesting that heterotic hybrids have different mechanism of increasing capacity of increased cell size and numbers.

In *A. thaliana*, cell size and chloroplast numbers correlate both in the heterotic hybrid and its parental lines (Fujimoto et al. 2012), suggesting that increased cell numbers and size in hybrids are coordinated with increased chloroplast numbers. Indeed, chlorophyll content per fresh weight and the rate of photosynthesis per unit area are not changed in hybrids (Fujimoto et al. 2012). Heterotic hybrids in rice, wheat, and sorghum also did not show an increased rate of photosynthesis per unit area compared with parental lines (Yang et al. 2007; Zhang et al. 2007; Tazoe et al. 2016). Transcriptome analysis showed upregulation of chloroplast-targeted genes in F_1 hybrids at a few days in cotyledons, in both *A. thaliana* and *B. rapa*, which might coordinate with increased chloroplast numbers or chlorophyll contents following increased cell size or numbers. A chlorophyll biogenesis inhibitor, norflurazon, treatment on cotyledon stages eliminates the heterosis phenotype (Fujimoto et al. 2012; Saeki et al. 2016), suggesting that photosynthesis and chlorophyll biogenesis are important for increased leaf size in hybrids even at stages after the cotyledon stage in *A. thaliana* and *B. rapa*.

Changes in expression, siRNAs, and DNA methylation

Interactions between the two different parental genomes lead to the alteration of transcription, small RNA levels, and DNA methylation patterns in F_1, which may be involved in the heterosis phenotype (Birchler et al. 2010; Greaves et al. 2015). Comparison of global transcript profiling between heterotic hybrids and their parents has been performed in many plant species. These studies have revealed additive (gene expression being equal to the average of the parental gene expression level) and non-additive (gene expression being different to the average of the parental gene expression level) gene expression pattern in heterotic hybrids. In many cases, the majority of genes showed additive gene expression and a small proportion of genes showed non-additive gene expression (Swanson-Wagner et al. 2006:; Wei et al. 2009; Fujimoto et al. 2012; Meyer et al. 2012; Saeki et al. 2016). In addition, the non-additive gene expression profile is drastically changed through developmental stages even when they differ by only a few days (Fujimoto et al. 2012; Meyer et al. 2012).

Global small RNA expression has been compared between heterotic hybrids and their parents in *A. thaliana*, rice, and maize, and the differences in small RNA levels between them have been observed (He et al. 2010; Groszmann et al. 2011; Barber et al. 2012; Li et al. 2012; Shen et al. 2012). In the heterotic maize hybrid between B73 and Mo17, siRNA clusters were additive in the shoot apex, while siRNA clusters in the ear showed larger deviations, especially falling below midparent levels (Barber et al. 2012). 24-nt siRNAs tended to be downregulated in hybrids compared with their parental lines in rice, maize, and *A. thaliana*, thus global or local reduction in 24-nt siRNAs in hybrids may be a universal phenomenon (He et al. 2010; Groszmann et al. 2011; Barber et al. 2012; Li et al. 2012; Shen et al. 2012). There is a hypothesis that changes in siRNA expression levels in hybrids contribute to non-additive gene expression in hybrids or heterosis. However, maize hybrids having homozygous *mediator of paramutation 1* (*mop1*) mutation, the ortholog of RDR2, or *A. thaliana* hybrids having homozygous mutation in genes involved in 24-nt siRNAs biogenesis do not affect the heterosis phenotype, suggesting that changes in siRNA expression in heterotic hybrids are independent from the heterosis phenotype (Barber et al. 2012; Kawanabe et al. 2016b; Zhang et al. 2016a).

Genetic distance between parental lines might be a good predictor of the level of heterosis, though the relationship between genetic distance and heterosis is controversial (Barth et al. 2003; Geleta et al. 2004; Meyer et al. 2004; Dreisigacker et al. 2005; Yu et al. 2005; Flint-Garcia et al. 2009; Kawamura et al. 2016). In *A. thaliana* and *B. rapa*, there is no correlation between genetic distance and heterosis (Barth et al. 2003; Meyer et al. 2004; Kawamura et al.

2016), suggesting that the different epigenomes of the two parental lines might be involved in heterosis phenotypes as well as the genetic interactions at the specific loci (Greaves et al. 2012a, 2015). DNA methylation has a potential to generate the F_1 specific epigenome because non-additive DNA methylation states caused by trans-chromosomal methylation (TCM) (an increase in methylation at a locus with a previously low methylation allele gaining methylation to resemble the more heavily methylated allele) and trans-chromosomal demethylation (TCdM) (loss of methylation at a genomic segment) in the F_1 has been observed in heterotic *A. thaliana* (Greaves et al. 2012a, b; Shen et al. 2012). TCM and TCdM events in hybrids are largely dependent on the 24-nt siRNAs, but abolition of the TCM and TCdM by the *pol iv* or *pol v* mutations (genes critical for 24-nt siRNA biogenesis) does not affect the heterosis phenotype (Zhang et al. 2016a). More than 10,000 regions of non-additively inherited DNA methylation in epihybrids occur between *met1* and wild type, though these F_1 plants do not show superior performance (Rigal et al. 2016). There is still a possibility that RdDM-independent TCM and TCdM are involved in heterosis and further study will be required to clarify this possibility.

The chromatin remodeler DDM1 is a key gene for promotion of heterosis

Populations of epigenetic recombinant inbred lines (epiRILs) between parents, which differed only in epigenetic marks (hybrids between *met1* and WT or between *ddm1* and WT), have been established in *A. thaliana*, and these populations have a variation of phenotypes including biomass (Johannes et al. 2009; Reinders et al. 2009). Several hybrids between WT and specific epiRIL lines derived from the hybrids between *met1* and WT or between *ddm1* and WT showed enhanced vegetative growth, suggesting that epigenetic diversity and epigenetic regulation of transcription play a role in heterosis (Dapp et al. 2015; Lauss et al. 2016). MutS HOMOLOG1 (MSH1) encodes a protein dually targeted to mitochondria and plastids and is involved in organelle genome stability (Abdelnoor et al. 2003; Xu et al. 2011). Disruption of MSH1 causes change of DNA methylation and enhanced vigor was observed in F_4 generations derived from the hybrids between WT and *msh1*, suggesting that epigenetic reprogramming can result in enhanced growth (Virdi et al. 2015).

Recently two groups showed that DDM1 is a major regulator of heterosis using genetic tests (Zhang et al. 2016b; Kawanabe et al. 2016b). Hybrids between homozygous mutants in some genes involved in epigenetic regulation in the C24 and Col background were developed. The F_1 having homozygous mutations in *rdr2, dms3, drd1, rdm1, nrpd1, nrpe1, ago4, ago6*, and *rdm3* showed the same level of heterosis as the wild type F_1, while the F_1 with homozygous mutations in *ddm1* (termed *ddm1* hybrids hereafter) reduced the vegetative heterosis (Zhang et al. 2016b; Kawanabe et al. 2016b). In the hybrid between a heterozygous *ddm1-9* mutation in C24 and a *ddm1-1* homozygous mutant in Col, plants having a homozygous *ddm1* mutation were smaller than those having heterozygous *ddm1* mutation. However, some plants having a *ddm1-1* homozygous mutation showed heterosis as great as the plants having a heterozygous *ddm1* mutation, and some plants having heterozygous *ddm1* mutation reduced heterosis like the *ddm1* mutant hybrid plants (Kawanabe et al. 2016b). Both cases had an identical genetic background except for the *ddm1* mutation. These effects may result from the previous methylation state of the genome in the *ddm1* parent, and the gene or segments important for heterosis coming from the *ddm1* parent might already have an altered level of DNA methylation. By transcriptome analysis, *ddm1* hybrids showed non-additive expression of genes involved in salicylic acid metabolism without any association with DNA methylation (Zhang et al. 2016b). SA concentrations in Col, C24, and wild type hybrids are higher than those in *ddm1* (Col), *ddm1* (C24), and *ddm1* hybrids, respectively. Regardless of whether DDM1 is functional or not, the SA concentrations in C24 are much higher than in Col and the F_1, leading to concentrations in F_1 lower than MPV (mid parent value) (Groszmann et al. 2015; Zhang et al. 2016b). The authors suggested that low endogenous SA concentrations stimulate growth but when the level is beyond a threshold, SA inhibits growth. The endogenous SA concentration in wild-type hybrids is best for heterosis, while SA concentrations in *ddm1* hybrids exceed the appropriate range for showing heterosis (Zhang et al. 2016b). However, the difference of SA concentration between F_1 and MPV is largely dependent on the high level of SA concentration in C24, and the difference of SA concentrations between wild-type Col and C24 x Col hybrids or between L*er* and C24 × L*er* hybrids is small (Groszmann et al. 2015; Zhang et al. 2016b). Further study will be required to confirm this hypothesis.

There are epigenetic changes in heterotic hybrids. 24-nt siRNAs are changed and affect DNA methylation but these do not appear to be associated with the generation of heterosis. Alterations in DNA methylation in the chromatin remodeler DDM1 affect the level of heterosis, but the mechanism is unclear. Further study will be required to understand how DDM1 regulate heterosis.

Epigenetic regulation of self-incompatibility

Self-incompatibility is a classical area in plants involved in the mechanism to prevent self-fertilization (Bateman 1955). This mating system is controlled by the interaction between *S*-locus protein 11/*S* locus cysteine-rich protein (SP11/

SCR) on the pollen grain and *S*-receptor kinase (SRK) in the stigma (Schopfer et al. 1999; Takasaki et al. 2000; Takayama et al. 2000). These two proteins are encoded on the single *S*-locus and inherited together from a parent, so they are defined as the '*S*-haplotype'. In Brassicaceae, self-recognition is controlled by multiple *S*-haplotypes (*S-1, S-2*, …, and *S-n*). There is considerable polymorphism for the two genes in the *S*-haplotypes, and the interaction between SP11 and SRK occurs only when they are produced from the same *S*-haplotypes. As a result, this interaction inhibits the germination of pollen carrying the same *S*-haplotype of SP11 (Kachroo et al. 2001; Takayama et al. 2001). Although self/nonself recognition is an interaction between haploid (pollen) and diploid (pistil), pollen changes its behavior by the dominance relationship between the *S*-haplotype of the parent. This phenomenon is due to the fact that *SP11* is expressed in the sporophytic anther tapetum cells, which surround the microspores (Shiba et al. 2002). A dominance relationship between the *S*-haplotype has been reported in some self-incompatible plants such as *Arabidopsis halleri, Arabidopsis lyrata, B. oleracea, B. rapa, Ipomoea trifida*, and *Senecio squalidus* (Brennan et al. 2011; Hatakeyama et al. 1998; Kowyama et al. 1994; Kusaba et al. 2001; Llaurens et al. 2008; Thompson and Taylor 1966). Recently, it has become clear that epigenetic regulation is involved in this phenomenon. In this chapter, we introduce the latest findings on the epigenetic control of dominance relationship among the *S*-haplotypes in self-incompatibility.

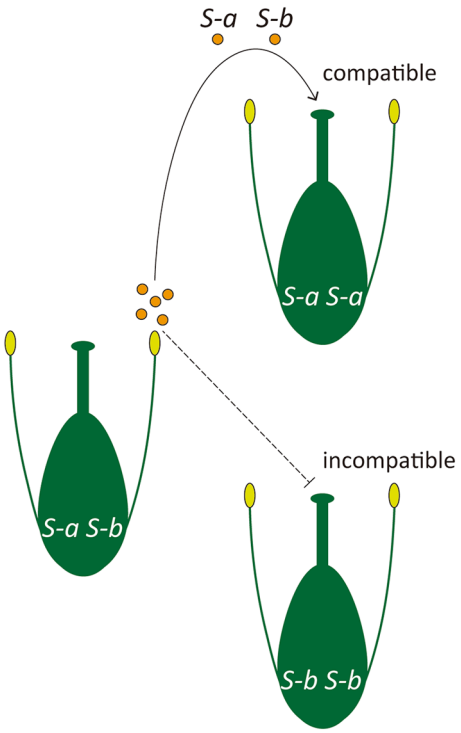

Fig. 1 Interaction of the *S*-haplotypes in pollen. Pollen grains from the *S*-heterozygous plant (*S-a* and *S-b*) are compatible for *S-a* homozygous plant (solid line), but incompatible for *S-b* plant (dashed line). In this case, *S-b* haplotype is dominant over the *S-a* haplotype in pollen

Dominance relationship in pollen *S* gene

Self-recognition occurs when pollen and pistil have the same *S*-haplotype. The pollen recognition phenotype is determined by one of the two alleles of dominance-recessive interactions between *S* alleles of *SP11*. If the dominance relationship between the two haplotypes is equal (co-dominant), the pollen shows both phenotypes. In Brassicaceae, dozens of *S*-haplotypes have been identified and classified into two classes, class-I and class-II, based on their nucleotide sequence. The class-I *S*-haplotypes are dominant over class-II in the pollen of heterozygotes of class-I and class-II *S*-haplotypes (Nasrallah et al. 1991). Therefore, pollen derived from heterozygous plants of class-I and class-II *S*-haplotype shows phenotype of class-I regardless of the pollen genotype (Fig. 1). This phenomenon is due to the reduction of *SP11* mRNA from the class-II *S*-haplotype in the class-I/class-II *S*-heterozygote in the tapetum cells (Kusaba et al. 2002; Shiba et al. 2002).

The dominance-recessive interactions are observed within the same classes of *S*-haplotypes in *B. rapa* (Hatakeyama et al. 1998). Interestingly, class-II *S*-haplotypes exhibit a complicated dominance hierarchy, such as *BrS-44* > *BrS-60* > *BrS-40* > *BrS-29* (Hatakeyama et al. 1998; Kakizaki et al. 2003). Thus, *BrS-44* is most dominant and *BrS-29* is most recessive. Likewise, dominance hierarchy of the five *S*-haplotypes (*AhS-20* > *AhS-12* > *AhS-04* > *AhS-03* > *AhS-01*) is also observed in *A. halleli* (Llaurens et al. 2008). Therefore, the *S*-haplotypes in the middle of the hierarchy (e.g., *BrS-40* and *AhS-12*) act dominantly or recessively dependent on the other partner *S*-haplotype in the heterozygote.

How do dominance relationships arise?

Because selfed progeny derived from the *S*-heterozygote shows self-incompatibility, repression of *SP11* in the recessive haplotype of *S*-heterozygote is released in the next generation (Shiba et al. 2006). This result suggests that suppression of *SP11* is epigenetically controlled. To elucidate the molecular mechanism of monoallelic expression of *SP11* in a heterozygote, methylation states of genomic DNA for several tissues were examined (Kusaba et al. 2002; Shiba et al. 2006). As a result, monoallelic expression in the anther tapetum was suppressed by DNA methylation of a 300 bp region in the promoter region of the recessive allele but there was no DNA methylation in the promoter region of the dominant allele or in the promoter region of recessive alleles of other

tissues (Shiba et al. 2006). In early stage of pollen development, such as the uni-nucleate stage, there is no DNA methylation even in the recessive allele but methylation increases as the anther develop. These observations suggest that de novo DNA methylation of a recessive allele occurs in the early stages of anther development just before the initiation of *SP11* transcription. Monoallelic DNA methylation in recessive alleles can explain the dominance relationship of all combinations consistently with phenotypic expression and *SP11* expression, which is considered to be the cause of the dominance-recessive mechanism.

Dominance modifier

The next question is how de novo DNA methylation is controlled. Fujimoto et al. (2006) showed that a class-I haplotype that has a defect in the *SP11* promoter can also suppress the class-II *SP11* expression. This result indicates that the expression of *SP11* is not necessary for the suppression of recessive haplotype and other element(s) are involved in the dominance relationship. Ninety years ago, there was intense discussion among statisticians on the existence of genetic elements controlling the dominance-recessive relation (Billiard and Castric 2011). This element was named 'dominance modifier' but until recently, its entity remained unclear.

It has been known that small RNAs are involved in regulation of gene expression (Carthew and Sontheimer 2009; Voinnet 2009). One of the small RNAs, 24-nt siRNA, regulates de novo DNA methylation of a homologous region by the RdDM pathway (Daxinger et al. 2009). Tarutani et al. (2010) identified a 24-nt siRNA (named *Smi*) transcribed from the dominant allele (class-I) that directs DNA methylation of the promoter region of recessive *SP11* alleles (class-II) in *B. rapa* (Fig. 2a). The precursor of *Smi, SP11 Methylation inducer* (*SMI*), flanks the *SP11* and is expressed in the tapetum before de novo DNA methylation is initiated (Tarutani et al. 2010). This study provides the first evidence for 24-nt siRNA may act as a 'dominance modifier' thought the allele-specific DNA methylation. Interestingly, *SMI* also exists on the class-II alleles, but a single nucleotide substitution in *Smi* causes the recessive *SMI* to be non-functional. Therefore, the more complex dominance hierarchy in the class-II haplotypes in *B. rapa* or *A. halleli* cannot be explained by the function of *Smi* alone (Kakizaki et al. 2003; Llaurens et al. 2008).

Recently, two independent research teams have proposed different models of complicated dominance hierarchy using different plant species. Durand et al. (2014) proposed a 'multiple dominance modifier model'. They identified that the most dominant haplotype has multiple small RNA candidates in *A. halleli* by comprehensive genomic and transcriptome analysis (Durand et al. 2014). The number of small RNA candidates was associated with the dominance hierarchy, and the most recessive haplotype has the least small RNA candidates and many target sites. In this model, an individual small RNA candidate from a dominant haplotype was predicted to target more *SCR* alleles (Fig. 2b). Several siRNA candidates were predicted to bind to introns. Although this result suggests the involvement of gene-body-methylation or post-transcriptional gene silencing, there is no report that these regulations are involved in suppressing the *SCR* expression. Yasuda et al. (2016) proposed a different model behind the dominance hierarchy among four class-II *S*-haplotype of *B. rapa* (Fig. 2c). They identified a single polymorphic 24-nt small RNA, named *SP11 methylation inducer 2* (*Smi2*), transcribed from downstream of *SRK* in all of class-II *S*-haplotypes (Yasuda et al. 2016). Target sites of *Smi2* were found in all the promoters of class-II *SP11* but not in class-I *SP11* promoters. This implies that *Smi2* controls dominance hierarchy among class-II haplotypes. They named this model 'Polymorphic dominance modifier model' because the allelic *Smi2* and their targets control dominance hierarchy depending on the similarity of nucleotides. For example, *Smi2-44*, which is derived from the most dominant haplotype, shows high similarity to the promoter of other *SP11* promoter and induces DNA methylation. In contrast, *Smi2-40*, which is derived from the third dominant haplotype, shows similarity to the most recessive haplotype of *SP11-29* promoter.

The past decade of research in the field of self-incompatibility in Brassicaceae revealed that epigenetic mechanism control monoallelic gene expression. Particularly, more complex dominance relationships, such as dominance hierarchy, were determined by polymorphism between a 24-nt siRNA and its target. Although this epigenetic regulation fits the Brassicaceae, other self-incompatible species which retain dominance hierarchy in the Asteraceae and Convolvulaceae remain unclear. Further analysis will be required to ascertain the commonality of the systems found in Brassicaceae.

Conclusion and perspectives

In plants, throughout the life cycle and beyond generation, various epigenetic modifications occur. They bring spatio-temporal dynamics in gene expression associated with important aspects such as plant body development and response to internal or external signals. Interaction between individuals with distinct epiallele or epigenome background can also affect various aspects including vegetative growth rate and reproduction. A series of genetic studies and genome wide profiling of epigenetic states, such as DNA methylation, small RNA production and histone modifications, have identified a broad range of molecules required for these modifications, and revealed that they play pivotal roles

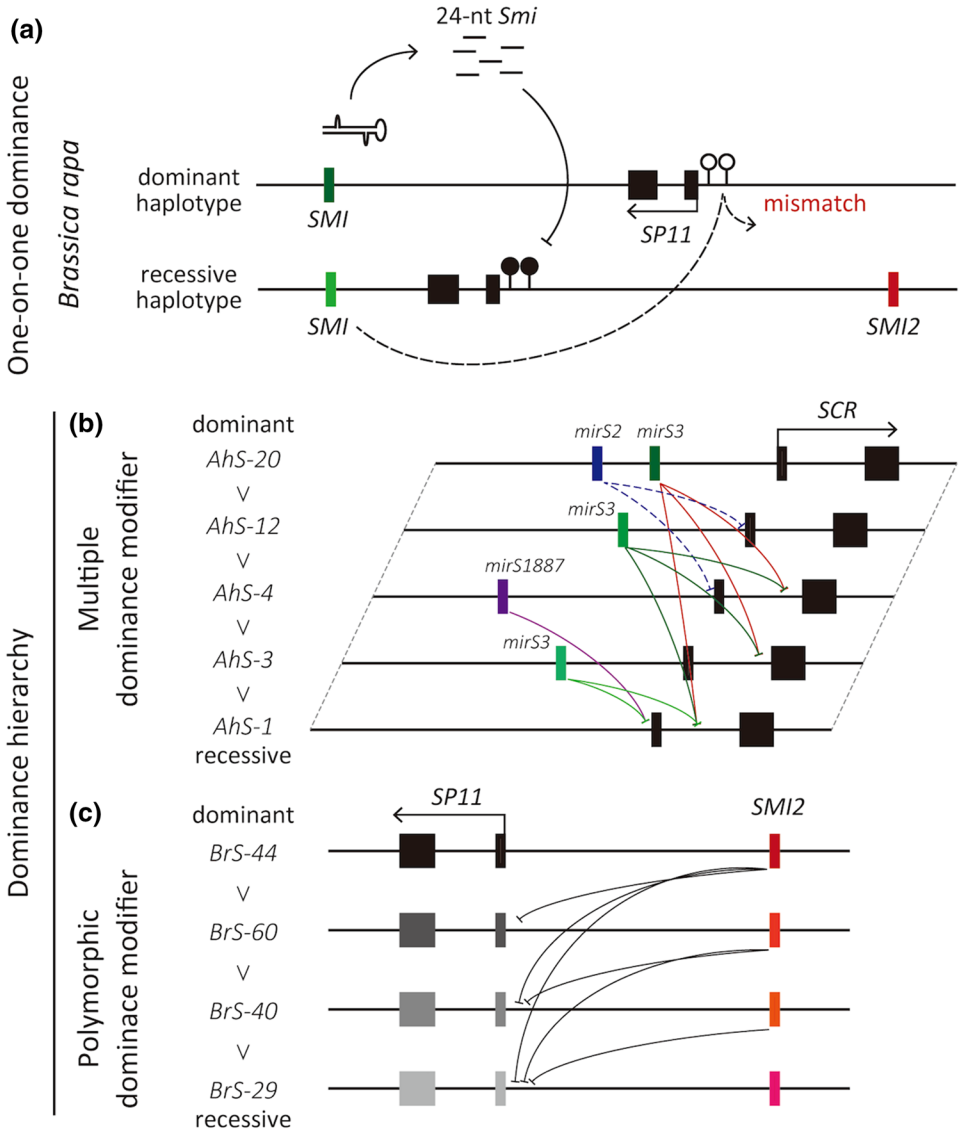

Fig. 2 Mode of action of the dominance relationships via trans-acting small RNA in Brassicaceae. **a** Canonical model for dominance relationship. In *S*-heterozygote having class-I (dominant) and class-II (recessive) haplotypes, expression of *SP11* from recessive allele is repressed by DNA methylation triggered by a 24-nt small RNA "*Smi*". *Smi* fails to repress the dominant *SP11* expression due to no homologous region in dominant *SP11* promoter. *Smi* derived from recessive haplotype cannot trigger DNA methylation. Black boxes indicates the exons of *SP11*. Open and solid circles indicate that the unmethylated and methylated status of *SP11* promoters, respectively. **b** A mode of "Multiple dominance modifier" model in *A. halleli*. Dominant *S*-haplotype have a larger set of small RNA "*mirS*" precursor genes. **c** The "Polymorphic dominance modifier" model in *B. rapa*. The single *SMI2* gene regulate dominance hierarchy via a homology-dependent manner. In all dominant-recessive interactions, *Smi2* variants derived from dominant *SMI2* region exhibited high similarity to the recessive *SP11* promoters

in complex multiple epigenetic layers as introduced in this review. However, there are still a number of questions that await further experimentation as exemplified below.

For vernalization, how different accessions respond differently to varied cold duration is one of the fundamental questions, which is likely associated with sequence variation in the *FLC* intron 1 (Coustham et al. 2012). Moreover, it is proposed that the cold signal is perceived in a digital fashion, namely every cell is either of one of a bistable state in *FLC* expression, ON or OFF. For hybrid vigor, it is emerging that specific interaction of parents with distinct epigenome background and non-additive gene expression in hybrid determines the level of hybrid vigor through DDM1 function. It is still largely unknown how DDM1 consequently renders hybrid vigor. Additionally, whether the function of DDM1 as a chromatin remodeler or genome-wide DNA methylation is important for heterosis phenotype is still to be determined. Understanding how the increase of cell number is accelerated and how its variation depends on sequence diversity is another challenge in hybrid vigor. For self-incompatibility, in general, the canonical RdDM pathway silences TEs in all tissues, whereas de novo methylation associated with self/non-self recognition is stage and tissue dependent. This is the first example of the RdDM pathway not functioning ubiquitously. Identifying the determinant(s) of the first step to confer the stage and tissue-specificity is an interesting theme in the future. Further identification of key factors would extend our understanding and allow us to elucidate fundamental principles underlying epigenetic regulation important for the plant lifecycle. It is also worthwhile to find potential benefits underlying epigenetic basic research to develop strategies for applying them to agricultural science in the Brassicaceae.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest relevant to this article.

References

Abdelnoor RV, Yule R, Elo A, Christensen AC, Meyer-Gauen G, Mackenzie SA (2003) Substoichiometric shifting in the plant mitochondrial genome is influenced by a gene homologous to MutS. Proc Natl Acad Sci USA 100:5968–5973

Angel A, Song J, Dean C, Howard M (2011) A Polycomb-based switch underlying quantitative epigenetic memory. Nature 476:105–108

Baranwal VK, Mikkilineni V, Zehr UB, Tyagi AK, Kapoor S (2012) Heterosis: emerging ideas about hybrid vigour. J Exp Bot 63:6309–6314

Barber WT, Zhang W, Win H, Varala KK, Dorweiler JE, Hudson ME, Moose SP (2012) Repeat associated small RNAs vary among parents and following hybridization in maize. Proc Natl Acad Sci USA 109:10444–10449

Bartee L, Malagnac F, Bender J (2001) *Arabidopsis cmt3* chromomethylase mutations block non-CG methylation and silencing of an endogenous gene. Genes Dev 15:1753–1758

Barth S, Busimi AK, Friedrich Utz H, Melchinger AE (2003) Heterosis for biomass yield and related traits in five hybrids of *Arabidopsis thaliana* L. Heynh. Heredity 91:36–42

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C (2004) Vernalization requires epigenetic silencing of *FLC* by histone methylation. Nature 427:164–167

Bateman AJ (1955) Self-incompatibility systems in angiosperms. III Cruciferae Heredity 9:52–68

Berr A, Xu L, Gao J, Cognat V, Steinmetz A, Dong A, Shen WH (2009) *SET DOMAIN GROUP25* Encodes a Histone Methyltransferase and Is Involved in *FLOWERING LOCUS C* Activation and Repression of Flowering. Plant Physiol 151:1476–1485

Billiard S, Castric V (2011) Evidence for Fisher's dominance theory: how many 'special cases'? Trends Genet 27:441–445

Birchler JA, Yao H, Chudalayandi S, Vaiman D, Veitia RA (2010) Heterosis. Plant Cell 22:2105–2112

Brennan AC, Tabah DA, Harris SA, Hiscock SJ (2011) Sporophytic self-incompatibility in *Senecio squalidus* (Asteraceae): *S* allele dominance interactions and modifiers of cross-compatibility and selfing rates. Heredity 106:113–123

Bruce AB (1910) The Mendelian theory of heredity and the augmentation of vigor. Science 32:627–628

Cao X, Jacobsen SE (2002) Role of the *Arabidopsis DRM* methyltransferases in de novo DNA methylation and gene silencing. Curr Biol 12:1138–1144

Cao X, Aufsatz W, Zilbermen D, Mette MF, Huang MS, Matzke M, Jacobsen SE (2003) Role of the *DRM* and *CMT3* methyltransferases in RNA-directed DNA methylation. Curr Biol 13:2212–2217

Carthew RW, Sontheimer EJ (2009) Origins and Mechanisms of miRNAs and siRNAs. Cell 136:642–655

Chen ZJ (2013) Genomic and epigenetic insights into the molecular bases of heterosis. Nat Rev Genet 14:471–482

Choi K, Park C, Lee J, Oh M, Noh B, Lee I (2007) *Arabidopsis* homologs of components of the SWR1 complex regulate flowering and plant development. Development 134:1931–1941

Choi J, Hyun Y, Kang MJ, Yun H, Yun JY, Lister C, Dean C, Amasino RM, Noh B, Noh YS, Choi Y (2009) Resetting and regulation of *FLOWERING LOCUS C* expression during Arabidopsis reproductive development. Plant J 57:918–931

Choi K, Kim J, Hwang HJ, Kim S, Park C, Kim SY, Lee I (2011) The FRIGIDA complex activates transcription of *FLC*, a strong flowering repressor in *Arabidopsis*, by recruiting chromatin modification factors. Plant Cell 23:289–303

Coustham V, Li P, Strange A, Lister C, Song J, Dean C (2012) Quantitative modulation of Polycomb silencing underlies natural variation in vernalization. Science 337:584–587

Crevillén P, Yang H, Cui X, Greeff C, Trick M, Qiu Q, Cao X, Dean C (2014) Epigenetic reprogramming that prevents transgenerational inheritance of the vernalized state. Nature 515:587–590

Crow JF (1998) 90 years ago: the beginning of hybrid maize. Genetics 148:923–928

Dapp M, Reinders J, Bédiée A, Balsera C, Bucher E, Theiler G, Granier C, Paszkowski J (2015) Heterosis and inbreeding depression of epigenetic *Arabidopsis* hybrids. Nat Plants 1:15092

Davenport CB (1908) Degeneration, albinism and inbreeding. Science 28:454–455

Daxinger L, Kanno T, Bucher E, van der Winden J, Naumann U, Matzke AJM, Matzke M (2009) A stepwise pathway for biogenesis of 24-nt secondary siRNAs and spreading of DNA methylation. EMBO J 28:48–57

De Lucia F, Crevillén P, Jones AM, Greb T, Dean C (2008) A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of *FLC* during vernalization. Proc Natl Acad Sci USA 105:16831–16836

Deal RB, Topp CN, McKinney EC, Meagher RB (2007) Repression of flowering in *Arabidopsis* requires activation of *FLOWERING LOCUS C* expression by the histone variant H2A.Z. Plant Cell 19:74–83

Dreisigacker S, Melchinger AE, Zhang P, Ammar K, Flachenecker C, Hoisington D, Warburton ML (2005) Hybrid performance and heterosis in spring bread wheat, and their relations to SSR-based genetic distances and coefficients of parentage. Euphytica 144:51–59

Durand E, Meheust R, Soucaze M, Goubet PM, Gallina S, Poux C, Fobis-Loisy I, Guillon E, Gaude T, Sarazin A, Figeac M, Prat E, Marande W, Berges H, Vekemans X, Billiard S, Castric V (2014) Dominance hierarchy arising from the evolution of a complex small RNA regulatory network. Science 346:1200–1205

East EM (1936) Heterosis Genetics 21:375–397

Finnegan EJ, Dennis ES (2007) Vernalization-induced trimethylation of histone H3 lysine 27 at *FLC* is not maintained in mitotically quiescent cells. Curr Biol 17:1978–1983

Finnegan EJ, Peacock WJ, Dennis ES (1996) Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. Proc Natl Acad Sci USA 93:8449–8454

Flint-Garcia SA, Buckler ES, Tiffin P, Ersoz E, Springer NM (2009) Heterosis is prevalent for multiple traits in diverse maize germplasm. PLoS One 4:e7433

Frascaroli E, Canè MA, Landi P, Pea G, Gianfranceschi L, Villa M, Morgante M, Pè ME (2007) Classical genetic and quantitative trait loci analyses of heterosis in a maize hybrid between two elite inbred lines. Genetics 176:625–644

Fujimoto R, Sugimura T, Fukai E, Nishio T (2006) Suppression of gene expression of a recessive *SP11*/*SCR* allele by an untranscribed

SP11/SCR allele in *Brassica* self-incompatibility. Plant Mol Biol 61:577–587

Fujimoto R, Taylor JM, Shirasawa S, Peacock WJ, Dennis ES (2012) Heterosis of Arabidopsis hybrids between C24 and Col is associated with increased photosynthesis capacity. Proc Natl Acad Sci USA 109:7109–7114

Geleta LF, Labuschagne MT, Viljoen CD (2004) Relationship between heterosis and genetic distance based on morphological traits and AFLP markers in pepper. Plant Breed 123:467–473

Gendall AR, Levy YY, Wilson A, Dean C (2001) The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. Cell 107:525–535

Geraldo N, Bäurle I, Kidou S, Hu X, Dean C (2009) FRIGIDA delays flowering in Arabidopsis via a cotranscriptional mechanism involving direct interaction with the nuclear cap-binding complex. Plant Physiol 150:1611–1618

Greaves IK, Groszmann M, Ying H, Taylor JM, Peacock WJ, Dennis ES (2012a) Trans chromosomal methylation in Arabidopsis hybrids. Proc Natl Acad Sci USA 109:3570–3575

Greaves IK, Groszmann M, Dennis ES, Peacock WJ (2012b) Transchromosomal methylation. Epigenetics 7:800–805

Greaves IK, Gonzalez-Bayon R, Wang L, Zhu A, Liu PC, Groszmann M, Peacock WJ, Dennis ES (2015) Epigenetic changes in hybrids. Plant Physiol 168:197–205

Greb T, Mylne JS, Crevillén P, Geraldo N, An H, Gendall AR, Dean C (2007) The PHD finger protein VRN5 functions in the epigenetic silencing of *Arabidopsis FLC*. Curr Biol 17:73–78

Groszmann M, Greaves IK, Albertyn ZI, Scofield GN, Peacock WJ, Dennis ES (2011) Changes in 24-nt siRNA levels in Arabidopsis hybrids suggest an epigenetic contribution to hybrid vigor. Proc Natl Acad Sci USA 108:2617–2622

Groszmann M, Greaves IK, Fujimoto R, Peacock WJ, Dennis ES (2013) The role of epigenetics in hybrid vigour. Trends Genet 29:684–690

Groszmann M, Gonzalez-Bayon R, Greaves IK, Wang L, Huen AK, Peacock WJ, Dennis ES (2014) Intraspecific Arabidopsis hybrids show different patterns of heterosis despite the close relatedness of the parental genomes. Plant Physiol 166:265–280

Groszmann M, Gonzalez-Bayon R, Lyons RL, Greaves IK, Kazan K, Peacock WJ, Dennis ES (2015) Hormone-regulated defense and stress response networks contribute to heterosis in *Arabidopsis* F1 hybrids. Proc Natl Acad Sci USA 112:E6397-6406

Hatakeyama K, Watanabe M, Takasaki T, Ojima K, Hinata K (1998) Dominance relationships between *S*-alleles in self-incompatible *Brassica campestris* L. Heredity 80:241–247

He G, Zhu X, Elling AA, Chen L, Wang X, Guo L, Liang M, He H, Zhang H, Chen F, Qi Y, Chen R, Deng XW (2010) Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids. Plant Cell 22:17–33

He G, Zhu X, Elling AA, Deng XW (2011) The epigenome and plant development. Annu Rev Plant Biol 62:411–435

Helliwell CA, Wood CC, Robertson M, Peacock WJ, Dennis ES (2006) The Arabidopsis FLC protein interacts directly in vivo with *SOC1* and *FT* chromatin and is part of a high-molecular-weight protein complex. Plant J 46:183–192

Helliwell CA, Robertson M, Finnegan EJ, Buzas DM, Dennis ES (2011) Vernalization-repression of Arabidopsis *FLC* requires promoter sequences but not antisense transcripts. PLoS One 6:e21513

Heo JB, Sung S (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science 331:76–79

Hisanaga T, Kawade K, Tsukaya H (2015) Compensation: a key to clarifying the organ-level regulation of lateral organ size in plants. J Exp Bot 66:1055–1063

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

Irwin JA, Soumpourou E, Lister C, Ligthart JD, Kennedy S, Dean C (2016) Nucleotide polymorphism affecting *FLC* expression underpins heading date variation in horticultural brassicas. Plant J 87:597–605

Jackson JP, Lindroth AM, Cao X, Jacobsen SE (2002) Control of CpNpG DNA methylation by the KRYPTONOTE histone H3 methyltransferase. Nature 416:556–560

Jeddeloh JA, Stokes TL, Richards EJ (1999) Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat Genet 22:94–97

Jiang D, Gu X, He Y (2009) Establishment of the winter-annual growth habit via *FRIGIDA*-mediated histone methylation at *FLOWERING LOCUS C* in *Arabidopsis*. Plant Cell 21:1733–1746

Johannes F, Porcher E, Teixeira FK, Saliba-Colombani V, Simon M, Agier N, Bulski A, Albuisson J, Heredia F, Audigier P, Bouchez D, Dillmann C, Guerche P, Hospital F, Colot V (2009) Assessing the impact of transgenerational epigenetic variation on complex traits. PLoS Genet 5:e1000530

Johanson U, West J, Lister C, Michaels S, Amasino R, Dean C (2000) Molecular analysis of *FRIGIDA*, a major determinant of natural variation in *Arabidopsis* flowering time. Science 290:344–347

Kachroo A, Schopfer CR, Nasrallah ME, Nasrallah JB (2001) Allele-specific receptor-ligand interactions in *Brassica* self-incompatibility. Science 293:1824–1826

Kakizaki T, Takada Y, Ito A, Suzuki G, Shiba H, Takayama S, Isogai A, Watanabe M (2003) Linear dominance relationship among four class-II *S* haplotypes in pollen is determined by the expression of *SP11* in *Brassica* self-incompatibility. Plant Cell Physiol 44:70–75

Kakizaki T, Kato T, Fukino N, Ishida M, Hatakeyama K, Matsumoto S (2011) Identification of quantitative trait loci controlling late bolting in Chinese cabbage (*Brassica rapa* L.) parental line Nou 6 gou. Breed Sci 61:151–159

Kakutani T, Jeddeloh JA, Flowers SK, Munakata K, Richards EJ (1996) Developmental abnormalities and epimutations associated with DNA hypomethylation mutants. Proc Natl Acad Sci USA 22:12406–12411

Kankel MW, Ramsey DE, Stokes TL, Flowers SK, Haag JR, Jeddeloh JA, Riddle NC, Verbsky ML, Richards EJ (2003) Arabidopsis MET1 cytosine methyltransferase mutants. Genetics 163:1109–1122

Kawamura K, Kawanabe T, Shimizu M, Nagano AJ, Saeki N, Okazaki K, Kaji M, Dennis ES, Osabe K, Fujimoto R (2016) Genetic distance of inbred lines of Chinese cabbage and its relationship to heterosis. Plant Gene 5:1–7

Kawanabe T, Osabe K, Itabashi E, Okazaki K, Dennis ES, Fujimoto R (2016a) Development of primer sets that can verify the enrichment of histone modifications, and their application to examining vernalization-mediated chromatin changes in *Brassica rapa* L. Genes Genet Syst 91:1–10

Kawanabe T, Ishikura S, Miyaji N, Sasaki T, Wu LM, Itabashi E, Takada S, Shimizu M, Takasaki-Yasuda T, Osabe K, Peacock WJ, Dennis ES, Fujimoto R (2016b) Role of DNA methylation in hybrid vigor in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 113:E6704–E6711

Kim DH, Sung S (2017) Vernalization-triggered intragenic chromatin loop formation by long noncoding RNAs. Dev Cell 40:302–312

Kim SY, He Y, Jacob Y, Noh YS, Michaels S, Amasino R (2005) Establishment of the vernalization-responsive, winter-annual habit in *Arabidopsis* requires a putative histone H3 methyl transferase. Plant Cell 17:3301–3310

Kim SY, Park BS, Kwon SJ, Kim J, Lim MH, Park YD, Kim DY, Suh SC, Jin YM, Ahn JH, Lee YH (2007) Delayed flowering time in Arabidopsis and *Brassica rapa* by the overexpression of *FLOWERING LOCUS C* (*FLC*) homologs isolated from

Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Plant Cell Rep 26:327–336

Kitamoto N, Yui S, Nishikawa K, Takahata Y, Yokoi S (2014) A naturally occurring long insertion in the first intron in the *Brassica rapa FLC2* gene causes delay bolting. Euphytica 196:213–223

Ko JH, Mitina I, Tamada Y, Hyun Y, Choi Y, Amasino RM, Noh B, Noh YS (2010) Growth habit determination by the balance of histone methylation activities in *Arabidopsis*. EMBO J 29:3208–3215

Kowyama Y, Takahasi H, Muraoka K, Tani T, Hara K, Shiotani I (1994) Number, frequency and dominance relationships of *S*-alleles in diploid *Ipomoea trifida*. Heredity 73:275–283

Krieger U, Lippman ZB, Zamir D (2010) The flowering gene *SINGLE FLOWER TRUSS* drives heterosis for yield in tomato. Nat Genet 42:459–463

Kusaba M, Dwyer K, Hendershot J, Vrebalov J, Nasrallah JB, Nasrallah ME (2001) Self-incompatibility in the genus *Arabidopsis*: characterization of the *S* locus in the outcrossing *A. lyrata* and its autogamous relative *A. thaliana*. Plant Cell 13:627–643

Kusaba M, Tung CW, Nasrallah ME, Nasrallah JB (2002) Monoallelic expression and dominance interactions in anthers of self-incompatible *Arabidopsis lyrata*. Plant Physiol 128:17–20

Lauss K, Wardenaar R, van Hulten MHA, Guryev V, Keurentjes JJB, Stam M, Johannes F (2016) Epigenetic divergence is sufficient to trigger heterosis in Arabidopsis thaliana. bioRxiv

Li Y, Varala K, Moose SP, Hudson ME (2012) The inheritance pattern of 24 nt siRNA clusters in Arabidopsis hybrids is influenced by proximity to transposable elements. PLoS One 7:e47043

Li X, Zhang S, Bai J, He Y (2016) Tuning growth cycles of *Brassica* crops via natural antisense transcripts of *BrFLC*. Plant Biotechnol J 14:905–914

Lin SI, Wang JG, Poon SY, Su CL, Wang SS, Chiou TJ (2005) Differential regulation of *FLOWERING LOCUS C* expression by vernalization in cabbage and Arabidopsis. Plant Physiol 137:1037–1048

Lindroth AM, Cao X, Jackson JP, Zilberman D, McCallum CM, Henikoff S, Jacobsen SE (2001) Requirement of *CHROMOMETHYLASE3* for maintenance of CpXpG methylation. Science 292:2077–2080

Lippman ZB, Zamir D (2007) Heterosis: revisiting the magic. Trends Genet 23:60–66

Lippmen Z, Gendrel AV, Black M, Vaughn MW, Dedhia N, McCombie WR, Lavine K, Mittal V, May B, Kasschau KD, Carrington JC, Doerge RW, Colot V, Martienssen R (2004) Role of transposable elements in heterochromatin and epigenetic control. Nature 430:471–476

Liu F, Quesada V, Crevillén P, Bäurle I, Swiezewski S, Dean C (2007) The *Arabidopsis* RNA-binding protein FCA requires a lysine-specific demethylase 1 homolog to downregulate *FLC*. Mol Cell 28:398–407

Liu F, Marquardt S, Lister C, Swiezewski S, Dean C (2010) Targeted 3′ processing of antisense transcripts triggers *Arabidopsis FLC* chromatin silencing. Science 327:94–97

Llaurens V, Billiard S, Leducq JB, Castric V, Klein EK, Vekemans X (2008) Does frequency-dependent selection with complex dominance interactions accurately predict allelic frequencies at the self-incompatibility locus in *Arabidopsis halleri*? Evol Int J org Evol 62:2545–2557

Macknight R, Bancroft I, Page T, Lister C, Schmidt R, Love K, Westphal L, Murphy G, Sherson S, Cobbett C, Dean C (1997) FCA, a gene controlling flowering time in Arabidopsis, encodes a protein containing RNA-binding domains. Cell 89:737–745

March-Diaz R, Garcia-Dominguez M, Lozano-Juste J, Leon J, Florencio FJ, Reyes JC (2008) Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in Arabidopsis. Plant J 53:475–487

Marquardt S, Raitskin O, Wu Z, Liu F, Sun Q, Dean C (2014) Functional consequences of splicing of the antisense transcript *COOLAIR* on *FLC* transcription. Mol Cell 54:156–165

Matzke MA, Mosher RA (2014) RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. Nat Rev Genet 15:394–408

Meyer RC, Törjék O, Becher M, Altmann T (2004) Heterosis of biomass production in Arabidopsis. Establishment during early development. Plant Physiol 134:1813–1823

Meyer RC, Kusterer B, Lisec J, Steinfath M, Becher M, Scharr H, Melchinger AE, Selbig J, Schurr U, Willmitzer L, Altmann T (2010) QTL analysis of early stage heterosis for biomass in Arabidopsis. Theor Appl Genet 120:227–237

Meyer RC, Witucka-Wall H, Becher M, Blacha A, Boudichevskaia A, Dörmann P, Fiehn O, Friedel S, von Korff M, Lisec J, Melzer M, Repsilber D, Schmidt R, Scholz M, Selbig J, Willmitzer L, Altmann T (2012) Heterosis manifestation during early Arabidopsis seedling development is characterized by intermediate gene expression and enhanced metabolic activity in the hybrids. Plant J 71:669–683

Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11:949–956

Mylne JS, Barrett L, Tessadori F, Mesnage S, Johnson L, Bernatavichute YV, Jacobsen SE, Fransz P, Dean C (2006) LHP1, the *Arabidopsis* homologue of HETEROCHROMATIN PROTEIN1, is required for epigenetic silencing of *FLC*. Proc Natl Acad Sci USA 103:5012–5017

Nasrallah JB, Nishio T, Nasrallah ME (1991) The Self-Incompatibility genes of Brassica—expression and use in genetic ablation of floral tissues. Annu Rev Plant Phys 42:393–422

Nie X, Wang H, Li J, Holec S, Berger F (2014) The HIRA complex that deposits the histone H3.3 is conserved in Arabidopsis and facilitates transcriptional dynamics. Biol Open 3:794–802

Okazaki K, Sakamoto K, Kikuchi R, Saito A, Togashi E, Kuginuki Y, Matsumoto S, Hirai M (2007) Mapping and characterization of *FLC* homologs and QTL analysis of flowering time in *Brassica oleracea*. Theor Appl Genet 114:595–608

Pien S, Grossniklaus U (2007) *Polycomb* group and *trithorax* group proteins in *Arabidopsis*. Biochim Biophys Acta 1769:375–382

Pien S, Fleury D, Mylne JS, Crevien P, Inze D, Avramova Z, Dean C, Grossniklaus U (2008) ARABIDOPSIS TRITHORAX1 dynamically regulates *FLOWERING LOCUS C* activation via histone 3 lysine 4 trimethylation. Plant Cell 20:580–588

Powers L (1944) An expansion of Jones's theory for the explanation of heterosis. Am Nat 178:275–280

Qüesta JI, Song J, Geraldo N, An H, Dean C (2016) *Arabidopsis* transcriptional repressor VAL1 triggers Polycomb silencing at *FLC* during vernalization. Science 353:485–488

Radoev M, Becker HC, Ecke W (2008) Genetic analysis of heterosis for yield and yield components in rapeseed (*Brassica napus* L.) by quantitative trait locus mapping. Genetics 179:1547–1558

Reinders J, Wulff BB, Mirouze M, Marí-Ordóñez A, Dapp M, Rozhon W, Bucher E, Theiler G, Paszkowski J (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. Genes Dev 23:939–950

Richey FD (1942) Mock-dominance and hybrid vigor. Science 96: 280–281

Ridge S, Brown PH, Hecht V, Driessen RG, Weller JL (2015) The role of *BoFLC2* in cauliflower (*Brassica oleracea* var. *botrytis* L.) reproductive development. J Exp Bot 66:125–135

Rigal M, Becker C, Pélissier T, Pogorelcnik R, Devos J, Ikeda Y, Weigel D, Mathieu O (2016) Epigenome confrontation triggers immediate reprogramming of DNA methylation and transposon silencing in *Arabidopsis thaliana* F_1 epihybrids. Proc Natl Acad Sci USA 113:E2083–E2092

Ronemus MJ, Galbiati M, Ticknor C, Chen J, Dellaporta SL (1996) Demethylation-induced developmental pleiotropy in *Arabidopsis*. Science 237:654–657

Saeki N, Kawanabe T, Ying H, Shimizu M, Kojima M, Abe H, Okazaki K, Kaji M, Taylor JM, Sakakibara H, Peacock WJ, Dennis ES, Fujimoto R (2016) Molecular and cellular characteristics of hybrid vigour in a commercial hybrid of Chinese cabbage. BMC Plant Biol 16:45

Schnable PS, Springer NM (2013) Progress toward understanding heterosis in crop plants. Annu Rev Plant Biol 64:71–88

Schönrock N, Exner V, Probst A, Gruissem W, Henning L (2006) Functional genomic analysis of CAF-1 mutants in *Arabidopsis thaliana*. J Biol Chem 281:9560–9568

Schopfer CR, Nasrallah ME, Nasrallah JB (1999) The male determinant of self-incompatibility in *Brassica*. Science 286:1697–1700

Schranz ME, Quijada P, Sung SB, Lukens L, Amasino R, Osborn TC (2002) Characterization and effects of the replicated flowering time gene *FLC* in *Brassica rapa*. Genetics 162:1457–1468

Shea DJ, Itabashi E, Takada S, Fukai E, Kakizaki T, Fujimoto R, Okazaki K (2017) The role of *FLOWERING LOCUS C* in vernalization of *Brassica*: the importance of vernalization research in the face of climate change. Crop Pasture Sci. doi:10.1071/CP16468

Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES (1999) The *FLF* MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and methylation. Plant Cell 11:445–458

Sheldon CC, Conn AB, Dennis ES, Peacock WJ (2002) Different regulatory regions are required for the vernalization-induced repression of *FLOWERING LOCUS C* and for the epigenetic maintenance of repression. Plant Cell 14:2527–2537

Sheldon CC, Hills MJ, Lister C, Dean C, Dennis ES, Peacock WJ (2008) Resetting of *FLOWERING LOCUS C* expression after epigenetic repression by vernalization. Proc Natl Acad Sci USA 105:2214–2219

Shen H, He H, Li J, Chen W, Wang X, Guo L, Peng Z, He G, Zhong S, Qi Y, Terzaghi W, Deng XW (2012) Genome-wide analysis of DNA methylation and gene expression changes in two *Arabidopsis* ecotypes and their reciprocal hybrids. Plant Cell 24:875–892

Shiba H, Iwano M, Entani T, Ishimoto K, Shimosato H, Che FS, Satta Y, Ito A, Takada Y, Watanabe M, Isogai A, Takayama S (2002) The dominance of alleles controlling self-incompatibility in *Brassica* pollen is regulated at the RNA level. Plant Cell 14:491–504

Shiba H, Kakizaki T, Iwano M, Tarutani Y, Watanabe M, Isogai A, Takayama S (2006) Dominance relationships between self-incompatibility alleles controlled by DNA methylation. Nat Genet 38:297–299

Simpson GG, Dijkwel PP, Quesada V, Henderson I, Dean C (2003) FY is an RNA 3′ end-processing factor that interacts with FCA to control the Arabidopsis floral transition. Cell 113:777–787

Stroud H, Otero S, Desvoyes B, Ramírez-Parra E, Jacobsen SE (2012) Genome-wide analysis of histone H3.1 and H3.3 variants in Arabidopsis thaliana. Proc Natl Acad Sci USA 109:5370–5375

Stroud H, Do T, Du J, Zhong X, Feng S, Johnson L, Patel DJ, Jacobsen SE (2014) Non-CG methylation pattern shape the epigenetic landscape in Arabidopsis. Nat Struct Mol Biol 21:64–72

Sung S, Amasino RM (2004) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. Nature 427:159–164

Sung S, He Y, Eshoo TW, Tamada Y, Johnson L, Nakahigashi K, Goto K, Jacobsen SE, Amasino RM (2006a) Epigenetic maintenance of the vernalized state in *Arabidopsis thaliana* requires LIKE HETEROCHROMATIN PROTEIN 1. Nat Genet 38:706–710

Sung S, Schmitz RJ, Amasino RM (2006b) A PHD finger protein involved in both the vernalization and photoperiod pathways in *Arabidopsis*. Genes Dev 20:3244–3248

Swanson-Wagner RA, Jia Y, DeCook R, Borsuk LA, Nettleton D, Schnable PS (2006) All possible modes of gene action are observed in a global comparison of gene expression in a maize F_1 hybrid and its inbred parents. Proc Natl Acad Sci USA 103:6805–6810

Swiezewski S, Liu FQ, Magusin A, Dean C (2009) Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. Nature 462:799–802

Tadege M, Sheldon CC, Helliwell CA, Stoutjesdijk P, Dennis ES, Peacock WJ (2001) Control of flowering time by *FLC* orthologues in *Brassica napus*. Plant J 28:545–553

Takasaki T, Hatakeyama K, Suzuki G, Watanabe M, Isogai A, Hinata K (2000) The *S* receptor kinase determines self-incompatibility in *Brassica* stigma. Nature 403:913–916

Takayama S, Shiba H, Iwano M, Shimosato H, Che FS, Kai N, Watanabe M, Suzuki G, Hinata K, Isogai A (2000) The pollen determinant of self-incompatibility in *Brassica campestris*. Proc Natl Acad Sci USA 97:1920–1925

Takayama S, Shimosato H, Shiba H, Funato M, Che FS, Watanabe M, Iwano M, Isogai A (2001) Direct ligand-receptor complex interaction controls *Brassica* self-incompatibility. Nature 413:534–538

Talbert PB, Ahmad K, Almouzni G et al (2012) A unified phylogeny-based nomenclature for histone variants. Epigenetics Chromatin Epigenetics Chromatin 5:7

Tamada Y, Yun JY, Woo SC, Amasino RM (2009) *ARABIDOPSIS TRITHORAX-RELATED7* is required for methylation of lysine 4 of histone H3 and for transcriptional activation of *FLOWERING LOCUS C*. Plant Cell 21:3257–3269

Tarutani Y, Shiba H, Iwano M, Kakizaki T, Suzuki G, Watanabe M, Isogai A, Takayama S (2010) Trans-acting small RNA determines dominance relationships in *Brassica* self-incompatibility. Nature 466:983–986

Tazoe Y, Sazuka T, Yamaguchi M, Saito C, Ikeuchi M, Kanno K, Kojima S, Hirano K, Kitano H, Kasuga S, Endo T, Fukuda H, Makino A (2016) Growth properties and biomass production in the hybrid C4 crop *Sorghum bicolor*. Plant Cell Physiol 57:944–952

Thompson KF, Taylor JP (1966) Non-linear dominance relationships between *S* alleles. Heredity 21:345–362

Virdi KS, Laurie JD, Xu YZ, Yu J, Shao MR, Sanchez R, Kundariya H, Wang D, Riethoven JJ, Wamboldt Y, Arrieta-Montiel MP, Shedge V, Mackenzie SA (2015) *Arabidopsis* MSH1 mutation alters the epigenome and produces heritable changes in plant growth. Nat Commun 6:6386

Voinnet O (2009) Origin, biogenesis, and activity of plant MicroRNAs. Cell 136:669–687

Vongs A, Kakutani T, Martienssen RA, Richards EJ (1993) *Arabidopsis thaliana* DNA methylation mutants. Science 260:1926–1928

Wei G, Tao Y, Liu G, Chen C, Luo R, Xia H, Gan Q, Zeng H, Lu Z, Han Y, Li X, Song G, Zhai H, Peng Y, Li D, Xu H, Wei X, Cao M, Deng H, Xin Y, Fu X, Yuan L, Yu J, Zhu Z, Zhu L (2009) A transcriptomic analysis of superhybrid rice *LYP9* and its parents. Proc Natl Acad Sci USA 106:7695–7701

Williams W (1959) Heterosis and the genetics of complex characters. Nature 184:527–530

Wood CC, Robertson M, Tanner G, Peacock WJ, Dennis ES, Helliwell CA (2006) The *Arabidopsis thaliana* vernalization response requires a polycomb-like protein complex that also includes VERNALIZATION INSENSITIVE 3. Proc Natl Acad Sci USA 103:14631–14636

Wu J, Wei K, Cheng F, Li S, Wang Q, Zhao J, Bonnema G, Wang X (2012) A naturally occurring InDel variation in *BraA.FLC.b* (*BrFLC2*) associated with flowering time variation in *Brassica rapa*. BMC Plant Biol 12:151

Xu L, Zhao Z, Dong A, Soubigou-Taconnat L, Renou JP, Steinmetz A, Shen WH (2008) Di- and tri- but not monomethylation on histone H3 lysine 36 Marks active transcription of genes involved in flowering time regulation and other processes in *Arabidopsis thaliana*. Mol Cell Biol 28:1348–1360

Xu YZ, Arrieta-Montiel MP, Virdi KS, de Paula WB, Widhalm JR, Basset GJ, Davila JI, Elthon TE, Elowsky CG, Sato SJ, Clemente TE, Mackenzie SA (2011) MutS HOMOLOG1 is a nucleoid protein that alters mitochondrial and plastid properties and plant response to high light. Plant Cell 23:3428–3441

Yang X, Chen X, Ge Q, Li B, Tong Y, Li Z, Kuang T, Lu C (2007) Characterization of photosynthesis of flag leaves in a wheat hybrid and its parents grown under field conditions. J Plant Physiol 164:318–326

Yang H, Howard M, Dean C (2014) Antagonistic roles for H3K36me3 and H3K27me3 in the cold-induced epigenetic switch at *Arabidopsis FLC*. Curr Biol 24:1793–1797

Yasuda S, Wada Y, Kakizaki T, Tarutani Y, Miura-Uno E, Murase K, Fujii S, Hioki T, Shimoda T, Takada Y, Shiba H, Takasaki-Yasuda T, Suzuki G, Watanabe M, Takayama S (2016) A complex dominance hierarchy is controlled by polymorphism of small RNAs and their targets. Nat Plants 3:16206

Yu CY, Hu SW, Zhao HX, Guo AG, Sun GL (2005) Genetic distances revealed by morphological characters, isozymes, proteins and RAPD markers and their relationships with hybrid performance in oilseed rape (*Brassica napus* L.). Theor Appl Genet 110:511–518

Yuan W, Lou X, Li Z, Yang W, Wang Y, Liu R, Du J, He Y (2016) A *cis* cold memory element and a *trans* epigenome reader mediate Polycomb silencing of *FLC* by vernalization in *Arabidopsis*. Nat Genet 48:1527–1534

Zhang X, Yazaki J, Sundaresan A, Cokus S, Chan SW, Chen H, Henderson IR, Shinn P, Pellegrini M, Jacobsen SE, Ecker JR (2006) Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. Cell 126:1189–1201

Zhang CJ, Chu HJ, Chen GX, Shi DW, Zuo M, Wang J, Lu CG, Wang P, Chen L (2007) Photosynthetic and biochemical activities in flag leaves of a newly developed super high-yield hybrid rice (*Oryza sativa*) and its parents during the reproductive stage. J Plant Res 120:209–217

Zhang Q, Wang D, Lang Z, He L, Yang L, Zeng L, Li Y, Zhao C, Huang H, Zhang H, Zhang H, Zhu JK (2016a) Methylation interactions in *Arabidopsis* hybrids require RNA-directed DNA methylation and are influenced by genetic variation. Proc Natl Acad Sci USA 113:E4248-4256

Zhang Q, Li Y, Xu T, Srivastava AK, Wang D, Zeng L, Yang L, He L, Zhang H, Zheng Z, Yang DL, Zhao C, Dong J, Gong Z, Liu R, Zhu JK (2016b) The chromatin remodeler DDM1 promotes hybrid vigor by regulating salicylic acid metabolism. Cell Discov 2:16027

Zhao Z, Yu Y, Meyer D, Wu C, Shen WH (2005) Prevention of early flowering by expression of *FLOWERING LOCUS C* requires methylation of histone H3 K36. Nat Cell Biol 7:1256–1260

Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S (2007) Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. Nat Genet 39:61–69

Zilberman D, Coleman-Derr D, Ballinger T, Henikoff S (2008) Histone H2A.Z and DNA methylation are mutually antagonistic chromatin marks. Nature 456:125–129

Zou X, Suppanz I, Raman H, Hou J, Wang J, Long Y, Jung C, Meng J (2012) Comparative analysis of *FLC* homologues in Brassicaceae provides insight into their role in the evolution of oilseed rape. PLoS One 7:e45751