Systematic analysis of JmjC gene family of *Brassica napus* and KDM5 subfamily involved in abiotic stress response

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Abstract

Background: Jumonji C (JmjC) proteins play an important role in plant development and stress response through the removal of lysine methylation from histones. *Brassica napus*, which originated from spontaneous hybridization between *Brassica rapa* and *Brassica oleracea*, is the most important oilseed crop after soybean, but evolutionary relationships and functions of JmjC proteins remain unclear. **Results:** 65 JmjC genes were identified from *B. napus* genome, 29 from *B. rapa*, and 23 from *B. oleracea*. These genes were grouped into seven clades according to conserved sequences, and their catalytic activities of demethylation were predicted. Group-KDM4/JHDM3 for H3K4/9/27/36, Group-KDM5A/B for H3K4, Group-JmjC domain-only A/B for H3K27/36, Group-KDM3/JHDM2 for H3K9, and Group-JMJD6 may be for arginine demethylases. *B. napus* inherited most of its JmjC genes from its parents. The average retention rate of *B. napus* JmjC gene from *B. rapa* (93.1%) and *B. oleracea* (82.6%) exceeded that of all homologous gene pairs (83.7%) across the whole *B. napus* genome. Thirteen new or duplicated JmjC genes have emerged in *B. napus*. Sequence similarity and domain organization analyses suggest that the functions of these genes might be diversified. Furthermore, KDM5 genes were examined under stress conditions due to H3K4 demethylation. Expression profiles indicated that the genes from *B. napus* are possibly involved in various stress responses. **Conclusion:** This study provides the first genome-wide characterization of JmjC genes in *Brassica* species. Its JmjC genes potentially have diverse functions, and its KDM5 genes might be involved in stress response. The results of this study facilitate the future functional characterization of the demethylation of JmjC family in *Brassica* crops.

Background

Epigenetics refers to heritable change for gene function that occurs without a change in DNA sequence and can dynamically regulate global gene expression through reversible chemical modifications on DNA and histones in eukaryotic chromatin [1]. Epigenetics regulation mainly includes acetylation, phosphorylation, histone methylation, DNA methylation, and small non-coding RNAs. Histone modification is an important epigenetics mechanism. Various post-translational covalent modifications, which primarily occur on histone (H3, H4, H2A, and H2B) lysines and arginines residues, form “histone code” to regulate various biological processes [2]. Histone methylation is usually catalyzed by three protein families of histone methyltransferases: protein arginine methyltransferase family, Su (var)3-9, Enhancer-of-zeste and Trithorax (SET) domain family, and telomeric silencing disruptor that is also known as DOT1-Like (Kmt4/DOT1L) [3]. Histone lysine methylation, playing many different roles in biological processes ranging from heterochromatin formation to transcription regulation, is dynamically regulated by histone lysine methyltransferases (KMTs) and histone lysine demethylases (KDMs), and can be distinguished depending on the position of lysine residue and the number of added methyl groups in lysine residues, which carry mono-, di-, or tri-methylated groups [4].

As sessile organisms, plants are more susceptible to environmental factor than animals, though the former has developed complex mechanisms that perceive environmental stimuli and respond and adapt accordingly to the changing external environment. Histone modifications play important roles in a wide

range of biological processes by affecting gene expression. For example, H3K4me3 and H3K36me3 are correlated to active gene expression, and H3K9me2 and H3K27me3 are associated to gene silencing [5]. The reversible epigenetic modifications dynamically regulate the plant's response to stress, including cold, freezing, saline, drought and submergence [6]. Abiotic stresses might induce various histone modifications to regulate the expression of genes with enhancing stress tolerance [2]. The active change in histone lysine methylation denotes a crucial role in plant adaptation to adverse environmental conditions. *Athaliana* genome-wide H3K4 methylation patterns (H3K4me1, H3K4me2 and H3K4me3) show dynamic responses to dehydration stress [7]. *Arabidopsis* ATX1, H3K4me3, is involved in dehydration response through ABA-dependent and -independent pathways [8]. Under drought stress, H3K4me3 enrichment is correlated with the activation of *Arabidopsis* drought stress-responsive genes, such as *RD29A* and *RD20* [9, 10]. H3K4me3 can be maintained at low levels after rehydration and which could function as an epigenetic mark of drought stress memory [10]. Under heat stress, H3K4 methylation accumulates to induce gene expression and can be sustained after heat stress to positively respond on a future stress incident [11]. High-dose oxidative stress increases several histone methylation marks, including H3K4me3, and low-dose oxidative stress increases the global levels of H3K4me3 and H3K27me3 [12]. Rice genome-wide H3K4me3 profiling showed that H3K4me3 modification is remarkably and positively correlated with the transcript level of drought stress-responsive genes [13]. Under submergence stress, the H3K4 methylation status of rice submergence-inducible *ADH1* and *PDC1* genes switches from dimethylation to trimethylation at the coding regions and is correlated with the increased expression of *ADH1* and *PDC1* [9].

Histone methylation is also a reversible process regulated by methylases and demethylases and might involve three distinct classes of demethylase. Petidylarginine deiminase 4 was identified as the first enzyme capable of antagonizing histone arginine methylation, but it cannot be strictly considered as a histone demethylase for its target citrulline [3]. KDMs mainly consist of LSD1/KDM1s (Lysine specific demethylase 1) and JmjC-domain enzymes, which both utilize oxidative mechanisms. LSD1, a flavin-dependent lysine specific demethylase member, was first reported as a histone lysine demethylase and transcriptional co-repressor specifically for histone H3 lysine 4 in humans [3]. *Arabidopsis* homologues of human LSD1 also exhibit similar demethylation and reduce H3K4 methylation in chromatin to promote floral transition [14].

JmjC, a highly conserved domain, was first reported by Takeuchi and colleagues in 1995 and was named as JmjC domain in 2000 [15, 16]. This domain carries eight β -sheets forming enzymatically-active pocket with three conserved and necessary amino-acid residues for binding with Fe (II) cofactor and two additional residues for binding with α -ketoglutarate (α KG) [17, 18]. *Arabidopsis* JmjC proteins are divided into five subfamilies: KDM4/JHDM3 (AtJMJ11-13), KDM5/JARID1 (AtJMJ14-19), JMJD6 (AtJMJ21/22), KDM3/JHDM2 (AtJMJ24-29) and JmjC domain-only (AtJMJ20 and AtJMJ30-32) [19]. The H3K4 methylases and demethylases dynamically balance the H3K4 methylation status among H3K4me1, H3K4me2 and H3K4me3, to maintain the optimum level of H3K4 methylation and adapt to external environment. KDM5 is a specific subfamily that specifically removes H3K4 methylation modifications. However, most reports on H3K4 demethylase functions were mainly focused on regulating plant

development. For example, AtJMJ14/PKDM7B, a histone H3K4 demethylase, represses floral integrators *Flowering Locus T (FT)*, *AP1*, *SOC1* and *LFY* during vegetative growth [20, 21]. AtJMJ15 regulates flowering time by demethylating H3K4me3 at *Flowering Locus C (FLC)* chromatin [22]. AtJMJ18 is dominantly expressed in companion cells exhibiting H3K4me3 and H3K4me2 demethylase activity of *FLC*. *atjnj18* mutation results in a weak late-flowering phenotype, and its overexpression induces early-flowering [23]. Moreover, the overexpression of *AtJMJ15* may regulate gene expression that enhances stress tolerance [24]. Although several functions of H3K4 methylation modifications in response to abiotic stresses have been reported, only a few were evaluated.

Allotetraploid species *Brassica napus* (oilseed rape, AACC, 2n=38) originated from interspecific spontaneous hybridization between *Brassica rapa*(AA, 2n = 20) and *Brassica oleracea*(CC, 2n=18) [21]. *Brassica* species might have diverged from a common ancestor with an *Arabidopsis* lineage from 14.5–20.4 million years ago [25]. The protein organization and function of JmjC domain in *Brassica* species and its relative relationship with model plant *Arabidopsis* remain uncharacterized. *B. napus* is currently the most important oilseed crop, preceded only by soybean. However, *B. napus* is vulnerable to abiotic stress that limits its growth and productivity and reduces its economic benefits. KDM5/JARID1 subfamily may regulate many abiotic stress responses genes through H3K4me3 and H3K4me2 downregulation but the roles of H3K4 demethylation in abiotic stress remain unknown.

Results

Chromosome maps of JmjC genes in *Brassica*

In this study, 21 *Arabidopsis* JmjC proteins were used as queries to Blastp in *Brassica* genomics (<http://brassicadb.org/brad/>). *B. napus* carried 65 JmjC genes, whereas its parents *B. rapa* and *B. oleracea* had 29 and 23, respectively (Additional file 1). 57 JmjC genes of *B. napus* were mapped on 19 chromosomes (AACC, 2n=38), and 8 genes were still on scaffolds, in which 5 were from C-genomics and 2 from A-genomics. In addition, 23 *B. oleracea* genes and 29 *B. rapa* genes located on C01-C09 and A01-A10 chromosomes, respectively.

The JmjC genes in A and C subgenomes of *B. napus* show nearly identical distributions to its ancestor genomes *B. rapa* (A-genome, 29) and *B. oleracea* (C-genome, 23) (Fig. 1). A02 and A07 chromosomes only exist in one member of *B. napus*, which is similar to its ancestor *B. rapa* genomes. A09 chromosome carries the highest number, seven genes. Four tandem JmjC genes pairs located on chromosomes A03, A09, and C03 in *B. rapa* (Fig. 1). The tandem duplicated genes *BnJMJ27;e* and *BnJMJ27;f* on A03 subgenome are derived from *BrJMJ27;a* and *BrJMJ27;b*, which belong to *AtJMJ27* orthology. Tandem duplicated gene pairs *BnJMJ27;d/BnJMJ27;b* and *BnJMJ17;a/BnJMJ17;c* might have resulted from the forming processes allotetraploidy of *B. napus*. However, *BnJMJ27;a* and *BnJMJ27;g* of C03 sub-genome are absent from the ancestor *B. oleracea* genomes, and the orthologous genes of these tandemly duplicated genes appear in the corresponding location of A03 subgenome, which indicate that *BnJMJ27;a* and *BnJMJ27;g* might have derived from the cross duplication of A03 subgenome.

BnJMJ31;a of C03 subgenome, *BnJMJ18;c* of C08 subgenome, *BnJMJ17;b*, and *BnJMJ29;c* of C09 subgenome may have similar origins.

Phylogenetic analysis of JmjC proteins in *B. napus*

A phylogenetic tree was constructed from 21 JmjC proteins from *Arabidopsis*, 19 from *O. sativa*, 29 from *B. rapa*, 23 from *B. oleracea*, and 65 from *B. napus* to examine the evolutionary relationships of JmjC proteins. The proteins were renamed using lysine demethylases nomenclature by Chromdb database (<http://www.chromdb.org/>). The JmjC proteins of *Brassica* were divided into seven clades, except BoJMJ19;c and BoJMJ19;d: KDM4/JHDM3, KDM5, JmjC domain only A/B, JMJD6 and KDM3/JHDM2 groups. This classification pattern was similar to the one previously reported for JmjC-domain proteins in the green lineage [26]. JmjC, Jumonji N (JmjN) and zinc-finger (ZnF) motifs were the special motifs for KDM4/JHDM3; JmjC, JmjN, F/Y-rich N terminus (FYRN) and F/Y-rich C terminus (FYRC) for KDM5A; JmjC, JmjN and plant homeodomain (PHD) for KDM5B; JmjC and F-box for JMJD6; JmjC and RING (really interesting new gene) for KDM3/JHDM2; and JmjC domain for JmjC domain only A/B (Figs. 3-7). JmjN domain specifically exists in all proteins of KDM4/JHDM3, KDM5A and KDM5B group, except for BrJMJ14;a, BnJMJ16;b, BrJMJ17 and BnJMJ17;c. *B. rapa*, *Arabidopsis* and *O. sativa* possess similar amounts of JmjC proteins in KDM5B, JmjC domain-only A, JMJD6 and JmjC domain-only B group. However, *B. oleracea* does not have JmjC protein of KDM5B and JmjC domain-only A. *B. napus* has 63 JmjC proteins, which is more than the sum of those for *B. oleracea* and *B. rapa* (Fig. 2; Additional file 1). The gene pairs imply the closest relatives within the phylogenetic tree. JmjC phylogenetic tree identified 39 sister pairs consisting of 22 An-Ar and 17 Cn-Co (Fig. 2). Moreover, most of the sister pairs are also paralogous gene pairs between the An- and Cn- subgenomes. JmjC genes show partial evolution but are still conserved on the whole genome of *Brassica*.

Group KDM4/JHDM3

Group-KDM4/JHDM3 contains nine JmjC proteins from *B. napus*, four from *B. rapa*, five from *B. oleracea*, five from *O. sativa* and three from *Arabidopsis* (Fig. 3). Group-KDM4/JHDM3 can be divided into two subgroups according to phylogenetic relationship, domain characteristic and gene structure: subgroup-I with eight *Brassica* members and two *Arabidopsis* homologous genes, *AtJMJ11* and *AtJMJ12*.

The domain organization of subgroup-I members show highly-conserved and shared JmjC, JmjN and ZnF domain. Subgroup-II contains 10 *Brassica* members and *Arabidopsis* homologous gene *AtJMJ13* shared JmjC, and JmjN (Figs. 3A and 3B). KDM4 subfamily shares the JmjN and JmjC motifs. JmjN domain is the second highly-conserved domain that is close to the N terminus and shorter than JmjC domain [16]. The four tandem array ZnF domain of RELATIVE OF EARLY FLOWERING 6 (REF6)/*AtJMJ12* targets motif CTCTGYTY, and the ZnF domain only exists in subgroup-I [27]. REF6 also tends to bind to hypo-methylated CTCTGYTY motifs in vivo [28]. Subgroup-I generally harbors 7–8 exons, but subgroup-II keeps highly similar gene structures with 10 exons (Fig. 3C).

JmjC proteins have been discovered as Fe(II)- and α KG-dependent histone demethylases [17, 18]. The JmjN and JmjC domains, two non-adjacent domains, interact with each other through two β -sheets and form a single functional unit to ensure the stability and appropriate transcription activity of Gis1 and maintain the overall protein levels and function of Jhd2 H3K4-specific demethylase in budding yeast [29, 30]. KDM4/JHDM3 has conserved Fe(II) binding site (His and Glutresidues) and α KG binding site (Phe and Lysresidues) (Fig. 3D).

Group-KDM5A/B

KDM5/JARID1 further be divided into two groups: KDM5A and KDM5B (Fig. 2; Additional file 2). Group-KDM5A contains 18 JmjC proteins from *B. napus*, 2 from *O. sativa*, 10 from *B. rapa*, 7 from *B. oleracea* and 5 from *Arabidopsis* (Fig. 4A). Group-KDM5B only has 4 JmjC proteins from *Brassica* and 1 from *Arabidopsis* homologous gene *AtJMJ17*. *B. oleracea* does not have KDM5B JmjC proteins, and *B. napus* carries 3 members.

Group-KDM5A is distinguished by JmjC, JmjN, FYRN and FYRC motifs (Fig. 4B). FYRN and FYRC are present in many chromatin-associated proteins associated with histone H3K4 methyltransferases [31]. The FYRN and FYRC domains of JMJ14 interact with NAC050 and NAC052 transcription factors to facilitate H3K4 demethylase recruitment [32]. Group-KDM5A group can be further divided into three subgroups: subgroup-I with 18 *Brassica* members and 3 *Arabidopsis* homologous genes, *AtJMJ14*, *AtJMJ15* and *AtJMJ18*. These members show highly-conserved domain organization sharing JmjC, JmjN, FYRN, and FYRC domains, except *BrJMJ14;a*. The phylogenetic tree showed that *B. oleracea* does not have *AtJMJ18* homologues. Moreover, *BrJMJ18;b* is clustered with *BnJMJ18;a* and *BnJMJ18;b*, as well as *BrJMJ18;a* with *BnJMJ18;c* and *BnJMJ18;d* (Fig. 4A). However, *B. napus* does not have a gene clustered with *BrJMJ14;b*, *BoJMJ15;b*, and *BrJMJ15;b*. Subgroup-II has seven *Brassica* members and *Arabidopsis* homologous gene *AtJMJ16* sharing JmjC, FYRN, JmjN and FYRC domains, except *BnJMJ16;e*, which display highly-conserved domain organization, in addition to *BrJMJ16;a* with additional helicase superfamily C-terminal and DEAD-like helicases superfamily domains. Subgroup-III has 10 *Brassica* members and *Arabidopsis* homologous genes *AtJMJ19* and share JmjC and JmjN domains, besides *BnJMJ19;a* and *BnJMJ19;b* with an additional transmembrane domain. This finding suggested that subgroup-III may have a relatively stable inheritance during the evolutionary process of allotetraploidy.

Group-KDM5B differs from group-KDM5A group in domain organization, which has BRIGHT and PHD but lacking FYRN and FYRC (Fig. 4F). BRIGHT is associated with H3K4 demethylase by DNA binding motif (CCGCCC) to regulate transcription [33]. PHD mainly exerts epigenetic effectors capable of recognizing or “reading” post-translational histone modifications and unmodified histone tails [34]. The original PHD role in gene transcription is acted as a reader of H3K4me3 in 2006 [35]. Many sophisticated functions of PHD were also determined, including H3K9me3 recognition and binding to the N-terminus of H3, indicating its key roles in regulating transcription and chromatin structure [36]. All members of group-KDM5B group have BRIGHT or PHD domains (Fig. 4F), indicating their involvement in demethylation

using JmjC domain associated with BRIGHT and PHD domains. The tandem duplication genes of *BnJMJ17;a* and *BnJMJ17;c* (An 09) are derived from the ancestor gene *BrJMJ17* (Ar 09), but *BnJMJ17;c* is clustered with *BnJMJ17;b* (Cn 09) (Fig. 4E; Additional file 3).

Group-KDM5A/B shows a wide range intron/exon number (5-36), but sister gene pairs are relatively conserved in gene structure (Fig. 4). In group-KDM5A, subgroups-I/II are highly conserved in Fe(II) and α KG binding sites, except for *BrJMJ16;b* in which Phe is replaced by Met in α KG binding site, and His is replaced by Arg Fe(II) binding site. In subgroup-III, Phe is replaced by Gln in α KG binding site, and *BoJMJ19;c/d* is variable in other Fe(II) and α KG binding sites (Fig. 4D). In group-KDM5B, *BnJMJ17a* gene structure is similar to its parent *BrJMJ17* (Fig. 4G). Group-KDM5B is highly conserved in Fe(II) and α KG binding sites, similar to KDM4/JHDM3 group (Fig. 4H).

Group-JmjC domain-only A/B

Group-JmjC domain-only A/B and JMJD6 are distributed in different branches of a large clade. Group-JmjC domain-only A is close to group-JMJD6 but far from group-JmjC domain-only B. Group-JmjC domain-only A and B have same domain organization and only exist in JmjC domain (Fig. 2).

Group-JmjC domain-only A possesses the least number of JmjC proteins among the groups (Figs. 2 and 5A) and contains three *Brassica* members and one *Arabidopsis* homologous gene *AtJMJ20*. *B. oleracea* is lack of Group-JmjC domain-only A JmjC proteins (Fig. 5). *BnJMJ20;b* shares coincident gene structures, domain organizations and chromosomal map with *BrJMJ20* (Figs. 1, 5B and 5C) indicating that the former may have originated from the latter. Chromosomal map, CDS cover and protein ID reveal that *BnJMJ20;a* might be the duplicate of *BnJMJ20;b* (Additional file 2).

JmjC domain-only B contains 17 JmjC proteins: 6 from *B. napus*, 2 from *B. oleracea*, 1 from *B. rapa*, 1 from *O. sativa* and 3 from *Arabidopsis*. Group-JmjC domain-only B can be further divided into three subgroups. Subgroup-I contains four *Brassica* members and *Arabidopsis* homologous gene *AtJMJ30* (Fig. 5E). Subgroup-II contains three *Brassica* members and *Arabidopsis* homologous gene *AtJMJ31*. Subgroup-III contains four *Brassica* members and *Arabidopsis* homologous gene *AtJMJ32* (Fig. 5E). Subgroups-I and III show high conservation during the forming process of allotetraploid. *B. napus* perfect inherited JmjC genes from its parents *B. oleracea* and *B. rapa*: *BnJMJ30;b* originating from *BoJMJ30;a* and *BnJMJ30;a* from *BrJMJ30;a* within subgroup-I; *BnJMJ32;b* originating from *BoJMJ32;a* and *BnJMJ32;a* from *BrJMJ32* in subgroup-III. *B. oleracea* lacks JmjC proteins in subgroup-II. *BnJMJ31;a* exhibits notable similarity with *BnJMJ31;b* in terms of domain component and gene structure, indicating that *BnJMJ31;a* may have originated from the inserted duplicate of *BnJMJ31;b* belonged to paralogues gene (Figs. 5F and 5G).

Group-JmjC domain-only A has stable exon distribution harboring approximately 7–9 exons. Sequence alignment and logos analysis of JmjC domain reveal that JmjC domain-only A group is highly conserved in Fe(II) and α KG binding sites. However, compared with that in the KDM4/JHDM3 group, Phe is replaced by Thr in Fe(II) binding site (Fig. 5D). In group-JmjC domain-only B, subgroup-I genes contains 6 exons,

subgroup-III harbors 4 exons, and subgroup-II has many exons (Fig. 5G). As compared with that in the KDM4/JHDM3 group, the Phe residue is replaced by Ser within AtJMJ31 orthology (Fig. 5H).

Group-JMJD6

The phylogenetic tree showed that the JMJD6 group is close to JmjC domain-only A group and includes five JmjC proteins from *B. napus*, three from *B. oleracea*, three from *Arabidopsis*, two from *B. rapa* and two from *O. sativa*. Each JmjC gene of *B. napus* is clustered with a corresponding homologous gene from *B. oleracea* or *B. rapa* (Figs. 1 and 6A).

On the basis of phylogenetic tree analysis and schematic diagrams, group-JMJD6 can further be divided into two subgroups (Fig. 6). Subgroup-I contains six *Brassica* members and *Arabidopsis* homologous gene *AtJMJ21* having only JmjC domain, besides BoJMJ21;b protein with an additional F-box domain (Figs. 6A and 6B). Subgroup-II contains four *Brassica* members and *AtJMJ22* sharing JmjC and F-box domains, except *AtJMJ22* missing F-box domain. However, their gene structure shows high conservation (Fig. 6). F-box domain recognizes a wide array of substrates and regulates many important biological processes by degrading cellular proteins in plants [37].

Subgroup-I generally harbors 15–16 exons, except BoJMJ21;a (4 exon) and BnJMJ21;a (9 exon). Subgroup-II keeps highly similar gene structures with 2–3 exons (Fig. 6C). Compared with that in KDM4/JHDM3 group, Phe is replaced by Ala within AtJMJ21 orthology and by Ser within AtJMJ22 orthology in JMJD6 (Fig. 6D).

Group-KDM3/JHDM2

The KDM3 & JHDM2 group is the largest group with 48 JmjC proteins: 6 from *Arabidopsis* (JMJ24-29), 22 from *B. napus*, 8 from *B. rapa*, 5 from *O. sativa*, and 6 from *B. oleracea* (Figs. 2 and 7A). Group-KDM3 & JHDM2 can be divided into four subgroups: subgroup-I containing 14 *Brassica* members and 3 *Arabidopsis* homologous genes, *AtJMJ25*, *AtJMJ26* and *AtJMJ29*. These proteins have AT-hook motif, RING and DM domains, except JmjC domain. Subgroups-II/III/IV contain *AtJMJ27*, *AtJMJ24* and *AtJMJ28* and their homologue genes, respectively. Subgroups-III/IV show highly-conserved and shared JmjC and RING domains, except BoJMJ24;a (Fig. 7B). Moreover, their gene structure also shows corresponding conservation (Fig. 7C).

In group-KDM3/JHDM2 (Fig. 7B), half of the member harbors RING domain as the second primary domain. Cys-X₂-Cys-X₍₉₋₃₉₎-Cys-X₍₁₋₃₎-His-X₍₂₋₃₎-Cys-X₂-Cys-X₍₄₋₄₈₎-Cys-X₂-Cys is the canonical RING [38]. The RING domain of many proteins mainly binds to ubiquitin-conjugating enzymes and mediates the direct transfer of ubiquitin to substrate [38]. The AT-hook is a small DNA-binding motif with a preference for A/T rich regions found in various proteins, such as the high mobility group proteins [39].

Sequence alignment and logos analysis of the JmjC domain reveal that subgroups-I and II are highly conserved in Fe(II) binding sites (His, Asp and Cys) and αKG binding sites (Thr and Lys), except

BoJMJ29;a. Moreover, both sites of subgroup-IV are different: the His and Asp residues of Fe(II) binding sites are replaced by Gly and Glu residues, and the Thr of α KG binding sites is replaced by Lys residue (Fig. 7D). However, subgroup-III does not present conservation.

KDM5 expression in abiotic stress

Arabidopsis KDM5 genes play central roles in stress-responsive gene expression and gene priming by H3K4me3 demethylation [40]. The expression of genes related to the response for drought, high temperature and saline stresses was determined to characterize the corresponding function of KDM5 group homologues in *B. napus* abiotic stress response.

The expression profiles of JmjC genes in *B. napus* leaves at three different stress conditions were detected by real-time PCR (Fig. 10). *BnJMJ16;a*, *BnJMJ17;b/c* and *BnJMJ18;a* showed remarkably elevated expression under salt, drought and high temperature. However, *BnJMJ19;a/c* did not show significant expression changes and thus might not be involved in the stress response or are less important. The vast majority of JmjC genes showed remarkably elevated expression under drought treatment, except for *BnJMJ14;a* and *BnJMJ19;a/c/e*. Most of the genes had higher expression under drought 5 or/and 10 day than under drought 15, except for *BnJMJ17;c* and *BnJMJ19;b* (Fig. 10A). However, only 6 (*BnJMJ16;a*, *BnJMJ17*, *BnJMJ18;a* and *BnJMJ19;e*) out of the 20 JmjC genes showed elevated expression under high temperature treatment. *BnJMJ16;a*, *BnJMJ17a/b* and *BnJMJ18;a* expression was induced under 12 hours of high temperature treatment, but *BnJMJ17;c* and *BnJMJ19;e* were not substantially expressed until 36 hours (Fig. 10B). Moreover, nearly half of the JmjC genes (*BnJMJ14*, *BnJMJ15;a*, *BnJMJ16;a*, *BnJMJ17;b/c* and *BnJMJ18;a/d*) showed remarkable expression under 100 mM NaCl treatment, besides *BnJMJ15;c* that was strongly induced by 200 mM NaCl stress (Fig. 10C).

Discussion

JmjC genes evolution of *B. napus*

Brassica species may diverge from a common ancestor with the *Arabidopsis* lineage from 14.5–20.4 million years ago [25]. *Arabidopsis* JmjC genes only possess one orthologues gene in the *B. rapa* and *B. oleracea* genomes, except for the six and four *Arabidopsis* JmjC genes with two orthologues genes in *B. rapa* and *B. oleracea* genomes, respectively. In addition, most of the genes originated from KDM5A subfamily. Only one *Arabidopsis* JmjC genes had three orthologues genes in *B. rapa* and *B. oleracea* genomes. Six *Arabidopsis* JmjC genes lack orthologues in *B. oleracea* (Fig. 1; Additional file 2).

B. napus (AACC, $2n = 38$) is an amphidiploid species derived from interspecific hybridization between two diploid progenitors, *B. rapa* (AA, $2n = 20$) and *B. oleracea* (CC, $2n = 18$) [41]. Nuclear genomes have remained essentially unaltered since amphidiploid species formation [42]. Similarly, the JmjC protein family appears to be extremely conserved during *B. napus* formation. Compared with the progenitor genomes of *B. rapa* and *B. oleracea*, 27 (93.1%) JmjC orthologous genes pairs between An subgenome

and 19 (82.6%) between Cn subgenome in *B. napus* were conserved (Fig. 2). The average retention rates from ancestor exceed the rate of all homologous gene pairs (83.7%) across the whole *B. napus* genome [43]. Each member of *B. rapa* and *B. oleracea* can be paired to at least one homologue of *B. napus*, except for five members of KDM5A subfamily: *BrJMJ14;b*, *BrJMJ15;b*, *BoJMJ15;b*, *BoJMJ19;c* and *BoJMJ19;d*. This finding indicated their absence during allotetraploid formation (Fig. 4). Gene duplication expands genome content and changes gene function to ensure the optimal adaptability and evolution of plants [44]. The 65 JmjC proteins from *B. napus* were more than the total number of proteins for *B. rapa* (29) and *B. oleracea* (23) (Addition file 1). According to the systematic analysis of JmjC proteins (Figs. 2-7), *BrJMJ13;b*, *BrJMJ14;b*, *BoJMJ15;b*, *BoJMJ19;c/d* and *BoJMJ24;a* might have been lost, and 13 new or duplicated JmjC gene have emerged in *B. napus*. Gene duplication events were confirmed by Yang et al. [45] and Sun et al. [46]. *BnJMJ16;e/BnJMJ16;d*, *BnJMJ18;a/BnJMJ18;b*, *BnJMJ18;d/BnJMJ18;c*, *BnJMJ31;a/BnJMJ31;b*, *BnJMJ29;b/BnJMJ29;d* and *BnJMJ17;a/BnJMJ17;b* duplicated genes pairs may have been derived from the existing JmjC gene from *B. rapa* and *BnJMJ28;a/BnJMJ28;b* and *BnJMJ29;a/BnJMJ29;c* pairs from *B. oleracea* (Addition file 1). These genes pairs were duplicated through segmental duplication (Addition file2). The parent of *BnJMJ17;c* was not found using the method by Yang et al. [45] and Sun et al. [46], but the JmjC domain sequence was consistent with the *BnJMJ17;b*.

Conservation and function of JmjC proteins of *B. napus*

Histone modification regulates plant development events by epigenetically silencing or activating target gene expression. Histone methylation is an important method to control plant development and stress response. In general, H3K4me2/me3 and H3K36 correlates with transcriptional activation, and H3K9me2 and H3K27me3 correlates with gene silencing [5]. Histone modification status is regulated by KMTs and KDMs. KDMs can balance histone methylation through the removal of methylated residue of histone lysine or arginine. Histone demethylation, catalyzed by JmjC proteins, mainly occurs at the Lys residues of histone H3 (K4, K9, K27, and K36), which is involved in plant developmental stages [47], defense against [48,49], proteasomal degradation [50] and circadian clock [51]. In addition, JmjC protein regulates cell cycle progression by regulating H4K20 methylation [52]. Recent studies showed that arginine demethylation can also be catalyzed using a subset of JmjC KDMs [53].

JmjC domain proteins have been claded into seven groups [26]. KDM4/JHDM3 was involved in multi-demethylation, e.g., *AtJMJ11* contributes to the control of flowering time and other developmental processes by H3K27me3, H3K9me3 and H3K4me3 demethylation [54-57]. *AtJMJ12/REF6* is involved in H3K4me2/3, H3K27me2/3 and H3K36me2/3 demethylation [27, 47, 58-61]. *OsJM12;a/JMJ705* is involved in biotic stress-response through the H3K27me2/3 demethylation of defense-related genes and increases basal and induced expression during pathogenic infection [62]. KDM5A might be involved in H3K4 demethylase activity, e.g., *AtJMJ14* for histone H3K4 demethylation of *FT* [20, 21], *AtJMJ15* for histone H3K4me3 demethylation of *FLC* [22] and H3K4me2/3 demethylation for stress response genes [24] and *AtJMJ18* for H3K4me2/3 demethylation for *FLC* [23]. *JMJ703/OsJM16;a* is also a histone H3K4 demethylase involved in transposon regulation and stem elongation [63, 64]. *JMJ704/OsJM14;a* exhibits

H3K4me2/3 demethylase activity and positively regulates rice defense response against *Xanthomonas oryzae* pv. *oryzae* [65]. The BRIGHT and PHD domains of KDM5B suggest its association with H3K4 demethylase [33-35]. JmjC domain-only A AtJMJ20 has a crucial role in removing histone arginine methylases at *Gibberellin 3 β -hydroxylase 1* (*GA3ox1*) and *GA3ox2* and can positively regulate seed germination through demethylation increasing the gibberellic acid levels [66]. JmjC domain-only B AtJMJ30 mediates plant responses to temperature and light to determine the timing of reproduction through H3K36me2 demethylation of *FT* [67] or H3K27me3 demethylation of *FLC* [68]. JMJD6 AtJMJ22 acts as histone arginine demethylases and positively regulates seed germination with AtJMJ20 [66]. KDM3/JHDM2 IBM1/AtJMJ25 can negatively regulate DNA methylation, which prevents the ectopic accumulation of DNA methylation through the removal of heterochromatic H3K9 methylation mark [69]. AtJMJ27 modulates defense against pathogens and flowering time as H3K9 histone demethylase [48].

65 JmjC proteins of *B. napus* were clustered into seven groups based on phylogenetic and domain organization (Figs. 2-7; Supplement file 1), which displayed various histone demethylases activity. JmjC proteins of *B. napus* were an evolutionarily-conserved family during allotetraploid formation (Figs. 1 and 2). According to gene structure, domain origination, phylogenetic tree, and relationship with known functional proteins from model plant *Arabidopsis*, the substrate specifics of BnJmjC proteins can be inferred [26]. Group-KDM4/JHDM3 for demethylation of H3K4me2/3, H3K9me3, H3K27m2/3 and H3K36me2/3; group-KDM5A for demethylation of H3K4me2/3; group-KDM5B for demethylation of H3K4; group-JmjC domain-only A and group-JMJD6 for demethylation of arginine; group-JmjC domain-only B for demethylation of H3K36me2 and H3K27me3; group-KDM3/JHDM2, indirectly correlate with demethylation of H3K9.

KDM5 response to abiotic stress

Environmental conditions dramatically affect plant growth and crop production. Epigenetic mechanisms have also been involved in the regulation of stress response genes [70, 71]. Santos et al. reviewed rice environmental epigenetic factors. Chromatin modifier enzymes may be transcriptionally regulated by abiotic stresses. The rice H3K4me3 levels of *ADH1* and *PDC1* respond to submergence stress. H3K4me3 is particularly correlated to gene expression regulation in dehydration stress [72]. *Arabidopsis* H3K4me3 of *AHG3*, catalyzed by ATX4 and ATX5, plays an essential role in drought stress response [73]. *Arabidopsis* H3K4 hypermethylation is associated with transcriptional activation and maintenance in heat stress response [74].

KDMs balance histone methylation level. KDM5 may be involved in H3K4 demethylase activity, for example, AtJMJ14 for histone H3K4 demethylation of *FT* [20, 21], AtJMJ15 for histone H3K4me3 demethylation of *FLC* [22] and H3K4me2/3 demethylation for stress response genes [24] and AtJMJ18 for H3K4me2/3 demethylation of *FLC* [23]. Rice JMJ703/OsJM16;a is also a histone H3K4 demethylase involved in transposon activity regulation and stem elongation [63, 64]. JMJ704/OsJM14;a exhibits H3K4me2/3 demethylase activity, which positively regulates defense response of rice against *Xanthomonas oryzae* pv. *oryzae* [65]. The phylogenetic analysis demonstrates that BnKDM5A members

have conserved structural composition, and is almost consistent with *Arabidopsis* homologous genes indicating that *B. napus* members may also possess conserved function with H3K4 demethylation. Over-expression of *Arabidopsis* AtJMJ15 enhanced salt tolerance [24]. Group-KDM5/JARID1 may have H3K4 demethylase activity (Figs. 2 and 4). The much similar gene structures and domain organizations of JmjC genes between *Arabidopsis* and *B. napus* suggest their conserved biological functions (Fig. 4).

The expression patterns of BnKDM5 show its involvement in drought, high temperature and salt stress response (Fig. 8). KDM5B subgroup have three copies of *AtJMJ17* (*BnJMJ17;a/b/c*). The similar gene structures and domain organizations of JmjC genes between *Arabidopsis* and *B. napus* suggest their conserved biological functions (Fig. 4). Under drought, high temperature or salt stress, all members of BnKDM5B exhibited remarkable elevated expression, except for *BnJMJ17;a* under salt stress, suggesting that these homologous genes have similar responses to similar stress stimuli. Moreover, some members of BnKDM5B were relatively conserved between their homologous genes in certain stress. For instance, the homologous gene of *AtJMJ15* (*BnJMJ15;a/b/c*), *AtJMJ16* (*BnJMJ16;a/b/c/d*), and *AtJMJ18* (*BnJMJ18;a/b/c/d*) showed similar stress response to drought stress with remarkable increased expression. However, their transcriptional responses to other stress stimuli may be different, even among homologous genes. For example, *BnJMJ18;a* shows remarkably elevated expression under high temperature without the homologous genes *BnJMJ18;b/c/d* that indicates some of the homologous gene functions may differ. The expression profiling results present that BnKDM5A genes are relatively conserved and show remarkable response to identified stress. Moreover, most homologous genes of BnKDM4B genes demonstrated similar response to drought stress, but functions of some homologous genes may have differed to adapt to various growth environments, which indicated that functions of BnKDM5 members is conserved and evolutionary during allotetraploid formation.

Methods

Identification of JmjC proteins and chromosomal map construction

AtJMJ11-22 and AtJMJ24-32 JmjC protein sequences of *Arabidopsis thaliana* was obtained from official website of TAIR (<http://www.arabidopsis.org/>) and used as queries to BLASTp JmjC proteins of *B. rapa*, *B. oleracea* and *B. napus* in *Brassica* database (<http://brassicadb.org/brad/blastPage.php>). *O. sativa* were retrieved from Phytozome database (Version 12). The JmjC domain of candidate proteins was confirmed using SMART and NCBI, and proteins without JmjC domain were excluded. The JmjC gene loci information of 3 *Brassica* species were used to generate chromosome maps with the Mapchart 2.2 program [75].

Domain organization and phylogenetic analysis

The gene structures were visualized using the Gene Structure Display Server (<http://gsds.cbi.pku.edu.cn/>). The site information of the domain organization was used to construct a protein organization sketch map using DOG program [76].

Multiple sequence alignment of JmjC proteins ClustalW [77] and its resulting file were subjected to phylogenetic analysis using MEGA7.0 program [78]. A tree was constructed based on the full-length protein sequences using neighbor-joining method with pairwise deletion and p-distance model, and a Bootstrap test of 1000 replicates for internal branch reliability. CDS sequences of JmjC were aligned with ClustalW (<http://www.genome.jp/tools/clustalw/>), and the resulting files were used to create Logo maps (<http://weblogo.berkeley.edu/logo.cgi>).

Duplicated JmjCgenes in *B. napus*

Duplicated genes were defined according to Yang et al [45] and Sun et al [46]. The full-length CDS sequence coverage and amino acid identities were determined using Blastn/Blastp at the NCBI website. The number of non-synonymous mutations (Ka) and the number of synonymous substitutions (Ks) of duplicated genes were calculated by DnaSP 6.0 [79]. The Ka/Ks ratios between duplicated genes and gene pairs were analyzed to determine the mode of selection. The duplication time (T, million years ago, MYA) was calculated as $T = Ks/2\lambda \times 10^{-6}$ ($\lambda = 1.5 \times 10^{-8}$) for *B. napus* [80].

Plant material and stress treatment

Brassica napus L. cv. *Xiangyou 15* seedlings (The seeds were provided by Key Laboratory of Crop Epigenetic Regulation and Development in Hunan Province, Hunan Agricultural University, and Dr. Yong Huang identified the plant material) were grown in a growth chamber at 22 °C chamber in a 16 h light/8 h dark photoperiod. One-month old plants with 4 true leaves were treated. For drought stress, the seedlings were grown without watering, and leaves were sampled at 0, 5, 10, and 15 days. For salt stress, seedlings were treated with 0, 100, 200, and 300 mM NaCl, and leaves were harvested 3 days after treatment. For high temperature stress, seedlings were grown in 40 °C, and leaves were harvested at 0, 12, 24, and 36 h after treatment. All harvested samples were immediately frozen in liquid nitrogen. Three independent biological replicates for each treatment were conducted.

RNA extraction and real-time quantitative PCR (RT-qPCR)

RNA extraction and real-time quantitative RT-PCR were conducted as described previously [81]. Total RNA was extracted from samples using a TRIzol reagent kit (Invitrogen, Carlsbad, CA, US) following manufacturer's instructions. Total RNA were reverse transcribed into cDNA using Revert Aid RT Kit (Thermo Fisher, USA). The primer pairs used for real-time PCR were designed using Beacon Designer 8.20 according to the CDS sequences of the JmjC genes in *B. nupas* (Additional file 4), and were synthesized by Generay Biotech (Generay, PRC). The real-time quantitative PCR was conducted using Fast Start Universal SYBR Green Master (ROX) (Roche, Switzerland) on a CF x 96 Real Time System (BIORAD). Each sample was run in triplicate. The expression levels of group-KDM5 genes were calculated with the $2^{-\Delta\Delta CT}$ method [82].

Abbreviations

α KG: α -ketoglutarate; ELF6: EARLY FLOWERING 6; FLC: *Flowering Locus C*; FT: *Flowering Locus T*; FYRC: F/Y-rich C terminus; FYRN: F/Y-rich N terminus; JmjC: Jumonji C; JmjN: Jumonji N; KDMs: histone lysine demethylases; KMTs: histone lysine methyltransferases; PHD: plant homeodomain; ZnF: zinc-finger

Declarations

Ethics approval and consent to participate

The field trial experiments in the current study were permitted by the local government in China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and material

All data generated or analyzed during this study are included in this published article and its Additional files. The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

XH contributed most of the experiments and drafted the manuscript; PJ, LB and TC contributed the sample and qRT-PCR; RY and HY conceived and directed the study; HY wrote the final version of the manuscript; All authors read and approved the final manuscript.

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Additional Material

Additional file 1: Detailed information of JmjC genes in *Brassica*

Sequences and information of JmjC genes and proteins came from <http://brassicadb.org/brad/>

Additional file 2: Duplicated JmjC genes of *B. rapa*

Ka and Ks were calculated by DnaSP 6.0; Cover of CDS and Identify of protein was checked by NCBI.

Additional file 3: NJ phylogenetic tree of KDM5

48 JmjC proteins sequences (*Arabidopsis* 6, *B. rapa* 11, Rice3, *B.napus* 21, *B.oleracea* 7) were aligned using ClustalW and the phylogenetic tree analysis was performed using MEGA 7.0. The tree was constructed with the following settings: Statistical Method as Neighbor-joining; Include Sites as Partial deletion option for total sequence analyses; Substitution Model: Poisson model; and Bootstrap test of 1000 replicates for internal branch reliability.

Additional file 4: Primers list of group-V in *B. napus*

Fig. 1 Chromosomal distribution of *Brassica* genes

Brassica genes (57 *B. napus*, 23 *B. oleracea* and 29 *B. rapa*) was mapped on chromosomes except eight scaffolds genes of *B. napus*: A. *B. napus* genes distribution of A-genomics, B. *B. rapa* genes distribution, C. *B. napus* genes distribution of C-genomics, D. *B. oleracea* genes distribution. The scale on the chromosome represents megabases (Mb).

Fig. 2 Phylogenetic tree of JmjC domain proteins

The Phylogenetic tree included 21 JmjC domain-containing proteins from *Arabidopsis thaliana*, 19 from *Oryza sativa*, 29 from *Brassica rapa*, 23 from *Brassica oleracea* and 65 from *Brassica napus*. The JmjC domain proteins can be grouped into 7 groups based on the phylogenetic tree and domain organization. Different colors show different groups. JmjC domain protein sequences were aligned using ClustalW, and the phylogenetic tree analysis was performed using MEGA7.0. The trees were constructed with the following settings: tree inference as neighbor-joining; include sites as pairwise deletion option for total sequences analysis; substitution model as p-distance.

Fig. 3 The schematic diagrams of Group-KDM4/JHDM3

A. Phylogeny tree, B. domain organization, C. Gene structure, D. Logos analysis of JmjC domain.

Fig. 4 The schematic diagrams of Group-KDM5A/B

A/E. Phylogeny tree, B/F. domain organization, C/G. Gene structure, D/H. Logos analysis of JmjC domain.

Fig. 5 The schematic diagrams of Group-JmjC domain-only A/B

A/E. Phylogeny tree, B/F. domain organization, C/G. Gene structure, D/H. Logos analysis of JmjC domain.

Fig. 6 The schematic diagrams of Group-JMJD6

A. Phylogeny tree, B. domain organization, C. Gene structure, D. Logos analysis of JmjC domain.

Fig. 7 The schematic diagrams of Group- KDM3&JHDM2

A. Phylogeny tree, B. domain organization, C. Gene structure, D. Logos analysis of JmjC domain.

Fig. 8 Expression of *B. naups* KDM5 subfamily in response to drought, high temperature or NaCl stresses

Many of BnJMJ14-19 genes involved in drought, high temperature or NaCl stress response. The error bars depict SD, an asterisk represent corresponding gene significantly up- or down-regulated by Student's t test between the treatment and the control ($0.01 < P < 0.05$), two represent ($p < 0.01$).

Figures

Figure 1

Chromosomal distribution of Brassica genes (57 *B. napus*, 23 *B. oleracea* and 29 *B. rapa*) was mapped on chromosomes except eight scaffolds genes of *B. napus*: A. *B. napus* genes distribution of A-genomics, B. *B. rapa* genes distribution, C. *B. napus* genes distribution of C-genomics, D. *B. oleracea* genes distribution. The scale on the chromosome represents megabases (Mb).

Figure 2

Phylogenetic tree of JmjC domain proteins The Phylogenetic tree included 21 JmjC domain-containing proteins from *Arabidopsis thaliana*, 19 from *Oryza sativa*, 29 from *Brassica rapa*, 23 from *Brassica oleracea* and 65 from *Brassica napus*. The JmjC domain proteins can be grouped into 7 groups based on the phylogenetic tree and domain organization. Different colors show different groups. JmjC domain protein sequences were aligned using ClustalW, and the phylogenetic tree analysis was performed using

MEGA7.0. The trees were constructed with the following settings: tree inference as neighbor-joining; include sites as pairwise deletion option for total sequences analysis; substitution model as p-distance.

Figure 3

The schematic diagrams of Group-KDM4/JHDM3 A. Phylogeny tree, B. domain organization, C. Gene structure, D. Logos analysis of JmjC domain.

Figure 4

The schematic diagrams of Group-KDM5A/B A/E. Phylogeny tree, B/F. domain organization, C/G. Gene structure, D/H. Logos analysis of JmjC domain.

Group-JmjC domain-only A

Group-JmjC domain-only B

Figure 5

he schematic diagrams of Group-JmjC domain-only A/B A/E. Phylogeny tree, B/F. domain organization, C/G. Gene structure, D/H. Logos analysis of JmjC domain.

Figure 6

The schematic diagrams of Group-JMJD6 A. Phylogeny tree, B. domain organization, C. Gene structure, D. Logos analysis of JmjC domain.

Figure 7

The schematic diagrams of Group- KDM3&JHDM2 A. Phylogeny tree, B. domain organization, C. Gene structure, D. Logos analysis of JmjC domain.

Figure 8

Expression of *B. naupis* KDM5 subfamily in response to drought, high temperature or NaCl stresses Many of BnJM14-19 genes involved in drought, high temperature or NaCl stress response. The error bars depict SD, an asterisk represent corresponding gene significantly up- or down-regulated by Student's t test between the treatment and the control ($0.01 < P < 0.05$), two represent ($p < 0.01$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile3NJphylogenetictreeofKDM5.jpg](#)
- [Additionalfile1DetailsofJmjCgenesinBrassica.xlsx](#)
- [Additionalfile2JmjCgenesduplicatedgenesofB.rapa.xlsx](#)
- [Additionalfile4PrimerslistofgroupVinB.napus.xlsx](#)