**RESEARCH ARTICLE**

**Differential Expression of Flowering Genes between Rapid- and Slow-Cycling *Brassica rapa***

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**ABSTRACT**  
Flowering time is a very important agronomic trait in *Brassica* crops and regulation of the time is one of major factor in the breeding program. To understand the control of flowering time in *Brassica rapa*, we have carried out Br300K microarray with two contrasting *Brassica* inbred lines, Rapid Cycling *B. rapa* (RCBr) as rapid cycling type and *B. rapa* ssp. *pekinensis* inbred line Chiifu as slow flowering phenotype. Reproductive process-related genes were specifically expressed in RCBr, whereas environmental stimuli-responsive genes in Chiifu. Flowering stimulating genes, such as *BrFT* and *BrSOC1*, were preferentially expressed in RCBr, while flowering repressing genes, such as *BrFLC* and *BrMAF4*, expressed in Chiifu. Several paralogues present in *B. rapa*, *BrFLCs* and *BrCOLs*, were expressed with paralog-specific pattern depending on flowering phenotypes: i.e., *BrFLC1* and *BrFLC2*, major floral repressors, were expressed in Chiifu, *BrFLC1/BrFLC5* in RCBr and *BrFLC3* in both plants. The expression of several flowering repressing genes was gradually decreased in RCBr growth, but increased in Chiifu growth. However, the expression of genes involved in photoperiodic flowering was no difference between these two plants under LD and SD conditions, indicating photoperiodic pathway is not major factor to distinguish fast vs. slow flowering in *B. rapa*. The mechanism underlined in the rapid or fast flowering of RCBr would be further elucidated in association with the controlling mechanism of its short life span.

**Keywords**  
Br300K microarray, Flowering time, RCBr, Chiifu, Photoperiod

**INTRODUCTION**

*Brassica rapa* is an important crop species of the genus *Brassica*, which is cultivated worldwide and includes a variety of vegetable crops such as Chinese cabbage, Pak-choi, turnip, and turnip green, and oil seed crops including turnip rape and sarson (Gómez-Campo and Prakash 1999). *B. rapa* ssp. *pekinensis* (Chinese cabbage) belongs to one of nine subspecies, which is characterized by requirement of long period of cold treatment for flowering. Rapid cycling *B. rapa* (RCBr), also known as Fast Plant, is a widely used model organism in biology education (Williams and Hill 1986; Musgrave 2000). RCBr was developed by selection of *B. rapa* for several traits, such as short time to flowering, rapid seed maturation, lack of seed dormancy, petite growth habit, and high female fertility (Williams and Hill 1986). The result is a plant with 10-generations per year. The earliness of flowering is an important trait for crops with higher seed yields (such as oilseed rape), whereas the lateness of flowering is a favourable trait for crops with high leafy head yields (such as Chinese cabbage) (Mao et al. 2014; Wang et al. 2014).

Flowering, a developmental process that transits vegetative stage to reproductive stage, is very important trait in Brassica species. Flowering in *Arabidopsis thaliana*, a model plant, is controlled by at least five genetically defined regulatory pathways: the photoperiod-dependent, vernalization-dependent, gibberellic acid (GA)-dependent, autonomous promotion, and FRIGIDA (FRI)-dependent pathways (Lee et al. 2000). Flowering time of the Chinese cabbage is largely controlled by vernalization and photoperiod (Kakizaki et al. 2011). Vernalization, exposure to
low temperature, represses FLOWERING LOCUS C (FLC) which is a central repressor of flowering (Helliwell et al. 2015). Plants with high FLC activity are late-flowering because FLC directly represses the expression of the floral inducers FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CO1 (SOC1) (Helliwell et al. 2006). Four copies of FLC have been reported in B. rapa, and five copies were identified in B. napus (Lin et al. 2005; Kim et al. 2006; 2007). A genetic-genomics approach revealed that BrFLC2 is a major regulator of flowering time in B. rapa (Xiao et al. 2013; 2014). Recently, Li et al. (2016) reported that mutation in FLC1 and FLC2 leading to production of truncated proteins was associated with shortness of flowering time in B. rapa. In photoperiodic flowering pathway, CONSTANS (CO) induces the day-length specific expression of FT in leaves (Putterill et al. 1995; Samach et al. 2000). Both CO and FT are mainly expressed in the vascular tissue of leaves (Takada and Goto 2003).

Although the regulation of flowering genes are important in Brassica breeding, information on RCBr with respect to gene expression and rapid flowering is still unclear. To get insight on flowering-control mechanism, we have applied Br300K microarray (Dong et al. 2013) using two contrast B. rapa inbred lines, RCBr as rapid cycling B. rapa and B. rapa ssp. pekinensis inbred line Chiifu as slow cycling B. rapa. Differentially expressed genes (DEGs) and major flowering genes were identified and characterized.

**MATERIALS AND METHODS**

**Plant materials**

Plant materials were rapid cycling Brassica rapa inbred line (RCBr24001) as an extremely early-flowering type and B. rapa ssp. pekinensis, inbred line Chiifu as slow or late flowering type. Plants were grown under either long day (LD; 16 hours light/8 hours dark) or short day (SD; 8 hours light/16 hours dark) conditions with 140±2 μl/m²/s light intensity at 22°C±5°C. For microarray, shoots were sampled at both cotyledon stage and five-leaves stage of RCBr (2 weeks old) and Chiifu (4 weeks old), and stored at −70°C until use. For expression study during RCBr development, RCBr and Chiifu (as control) were sampled until RCBr started flowering. To compare expression of several flowering genes, plants were grown under LD and SD conditions until 21 day after germination (DAG), and sampled at indicated time.

**RNA isolation and hybridization to the Br300K microarray GeneChip**

Shoot samples from cotyledon stage and five-leave stage were ground in liquid nitrogen, and equal amounts of powders were pooled. Total RNA was isolated from samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and further purified with a NucleoSpin RNA Clean-up Kit (Macherey-Nagel GmbH&Co. KG, Düren, Germany). For biological repeats, RNAs were extracted from two independent samples, and subjected to microarray experiment. Microarray experiments and subsequent analyses were performed as described in our previous report (Dong et al. 2013). To obtain insights regarding the putative biological functions and biochemical pathways of DEGs, we carried out enrichment analyses by searching Gene Ontology (GO) (Ashburner et al. 2000), agriGO (Du et al. 2010), and the Kyoto Encyclopedia of Genes and Genomes (Kanehisa et al. 2008).

**Reverse transcription polymerase chain reaction (RT-PCR) analysis**

Total RNA was isolated using TRIzol reagent (Invitrogen), and the total RNA (1 μg) from each sample was subjected to the first-strand cDNA synthesis using the Ace-α kit with the Oligo(dT) primers (Toyobo, Osaka, Japan). Complementary DNA was diluted 10-fold and 1 μl of diluted cDNA was used in a 20-μl PCR mixture. RT-PCR primers were listed in Table 1, and primers for BrACT2 (B. rapa Actin 2) used as control are 5′-GAA-CCGGGTGCTCCTCAGGA-3′ (forward) and 5′-ATGGTACCCGGAATGGTCAAGGC-3′ (reverse). To design paralog specific primer, expressed sequence tag (EST)-sequences were subjected to BRAD (http://brassicadb.org/brad/; V1.5), and all sequences were then aligned to figure out polymorphism. A standard PCR was performed with a 5-minute denaturation at 94°C, followed by 25
cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 90 seconds. PCR products were analyzed following electrophoresis through a 1.2% agarose gel.

## RESULTS

### Overall microarray

Among 47,553 EST clones deposited on Br300K microarray, 40,047 (84%) showed over 500 in probe intensity (PI), which means their transcripts could be detected by regular RT-PCR (Supplementary Table 1). In addition, 8,542 clones were not annotated with Arabidopsis gene locus (18% and expressed as ‘no_hit_found’, HNF). Estimated number of genes in *B. rapa* ssp. *pekinesis* inbred line Chiifu and RCBr will be 44,710: 41,173 from BRAD (http://brassicadb.org/brad/; V1.5) (Wang et al. 2011), and 3,537 novel genes recently identified by deep RNA-Seq (Devisetty et al. 2014). Our microarray included more number of EST clones than the estimated numbers, but actual coverage of unigenes will be below 80% due to overlapped EST clones.

### Differentially or specifically expressed genes

Genes showing RCBr-specific expression were defined as those genes with PI values greater than 1,000 in RCBr but less than 500 in Chiifu (Supplementary Table 2). Chiifu-specific expression genes were selected with same strategy as RCBr (Supplementary Table 3). RCBr- and Chiifu-specific expression genes were 541 and 1,037, respectively. Among them, 178 (33%) and 446 (43%) genes from RCBr and Chiifu, respectively, were NHF: i.e., no Arabidopsis counterpart to be present. These NHFs will be Brassica specific genes (or orphan genes). Only 363 and 591 genes from RCBr and Chiifu, respectively, were annotated with Arabidopsis genome data. RCBr-specific genes included many flower-stimulating genes and transposable elements. On the other hand, Chiifu-specific genes included several floral repressor genes, such as *FLC* and *MAF4*.

To obtain insights regarding the putative biological functions and biochemical pathways of DEGs, we carried out functional annotation and enrichment analysis. The results revealed that many DEGs were involved in various biological processes and metabolic pathways, with a significant enrichment in the category of flower development.
out GO enrichment analyses by searching agriGO (Du et al. 2010) using RCBr- (Supplementary Table 2) or Chiifu-induced genes (Supplementary Table 3). With the threshold value ($P$-value) below 0.05, 51 and 159 GO items were significantly enriched by the RCBr- and Chiifu-induced genes, respectively (Fig. 1). GO items related reproductive processes were only significantly represented by RCBr-induced genes, such as gametophyte development, flower development and cell differentiation. On the other hand, GO items related to stress response or environment stimulus were only significantly represented by Chiifu-induced genes. There are 27 GO Cellular component items were significantly represented in Chiifu-induced genes, and most of them related membrane structure, no such items were represented in RCBr-induced genes.

**Transcription factors (TFs)**

Since TFs play roles as a master controller for plant growth and development, differentially expressed TF genes were analyzed (Table 2). In this analysis, we omitted TFs classified as flowering genes and then summarized as three categories; highly expressed-top 20 genes in both RCBr and Chiifu, RCBr-specific and Chiifu-specific genes. Ethylene-responsive TF RAP2-4 ($RAP2.4$) was the most highly expressed TF in both plants with over 40,000 in PI values. Genes expressed over two fold in RCBr and Chiifu were 19 and 20, respectively; representative genes were *CAULIFLOWER (CAL)* and *SHINE2 (SHN2)* for RCBr, and *KNOTTED1-LIKE HOMEobox GENE 5 (KNAT5)* and *CURLY LEAF (CLF)* for Chiifu.
### Table 2. Transcription factors whose expressions levels were top 20 in both RCBr and Chiifu, and over two-fold in RCBr or Chiifu.

| Classification | At_Locus | Gene annotation | Br_SEQ_ID | Probe intensity | Fold change |
|----------------|----------|-----------------|-----------|-----------------|-------------|
| Top 20 in both | AT1G78080 | RAP2-4 (Ethylene-responsive transcription factor RAP2 4) | Brapa_ESTC021276 | 40264 | 40257 | 1.0 | 1.0 |
| RCBr and Chiifu | AT3G54810 | BME3/BME3-ZF (BLUE MICROPLYLAR EN33) | Brapa_ESTC043727 | 38658 | 39227 | 1.0 | 1.0 |
| Chiifu | AT1G51210 | ATERF4/ERF4 (ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 4) | Brapa_ESTC011149 | 38808 | 38636 | 1.0 | 1.0 |
| | AT1G65440 | GTB1 (GLOBAL transcription factor GROUP B1) | Brapa_ESTC043584 | 33003 | 38201 | 0.9 | 1.2 |
| | AT5G9820 | ZAT12 | Brapa_ESTC009473 | 34297 | 37970 | 0.9 | 1.1 |
| | AT1G28370 | ATERF11/ERF11 (ERF domain protein 11) | Brapa_ESTC013565 | 37200 | 36359 | 1.0 | 1.0 |
| | AT1G20696 | HMG83 (HIGH MOBILITY GROUP B 3) | Brapa_ESTC008254 | 34777 | 35905 | 1.0 | 1.0 |
| | ATIG08840 | WRKY40 (WRKY DNA-binding protein 40) | Brapa_ESTC014413 | 35869 | 33203 | 1.1 | 0.9 |
| | AT4G34410 | AP2 domain-containing transcription factor, putative | Brapa_ESTC044912 | 33099 | 31364 | 1.0 | 0.9 |
| RCBr specific | AT5G21120 | EL2 (ETHYLENE-INSENSITIVE3-LIKE 2) | Brapa_ESTC037616 | 1460 | 116 | 12.6 |
| | AT2G45680 | TCP family transcription factor, putative | Brapa_ESTC042103 | 3852 | 377 | 10.2 |
| | AT1G26310 | CAL (CAULIFLOWER) | Brapa_ESTC07036 | 2570 | 287 | 9.0 |
| | AT1G15050 | IAA34 (indoleacetic acid-induced protein 34) | Brapa_ESTC035350 | 2023 | 250 | 8.1 |
| | AT1G67690 | GT2 (GT2) | Brapa_ESTC039899 | 1178 | 199 | 5.9 |
| | AT5G25390 | SHN2 (Ethylene-responsive transcription factor SHINE3) | Brapa_ESTC032163 | 1763 | 307 | 5.7 |
| | AT1G73830 | BEE3 (BR-ENHANCED EXPRESSION 3) | Brapa_ESTC019151 | 2796 | 496 | 5.6 |
| | AT5G22290 | ANAC089 (Arabidopsis NAC domain containing protein 89) | Brapa_ESTC021310 | 1579 | 281 | 5.6 |
| | AT5G24036 | AP2 domain-containing transcription factor, putative | Brapa_ESTC040454 | 1104 | 301 | 3.7 |
| | AT1G69560 | MYB105 (myb domain protein 105) | Brapa_ESTC025442 | 1817 | 419 | 4.3 |
| | AT5G15310 | AtMYB16 (myb domain protein 16) | Brapa_ESTC025442 | 1817 | 419 | 4.3 |
| | AT1G20530 | Unknown (similar to hsp-related transcription factor -like) | Brapa_ESTC000221 | 1921 | 497 | 3.9 |
| | AT5G60990 | AMYB34 | Brapa_ESTC026773 | 1226 | 326 | 3.8 |
| | AT1G7590 | CCAAT-binding transcription factor (CBF-B/NF-YA) family protein | Brapa_ESTC003250 | 1089 | 302 | 3.6 |
| | AT4G33280 | AP2/B3-like transcription factor | Brapa_ESTC044866 | 1607 | 480 | 3.3 |
| | AT2G03960 | Similar to AGL102 | Brapa_ESTC019197 | 1325 | 401 | 3.3 |
| | AT3G12720 | AtMYB67/AtMY3 (myb domain protein 67) | Brapa_ESTC042873 | 1358 | 467 | 2.9 |
| | AT5G06839 | bZIP family transcription factor | Brapa_ESTC045391 | 1162 | 456 | 2.5 |
| | AT1G432280 | IAA29 (indoleacetic acid-induced protein 29) | Brapa_ESTC035154 | 1126 | 472 | 2.4 |
| | AT1G15360 | SHN1/WNI (SHINE1/WAX INDUCER 1) | Brapa_ESTC070404 | 1064 | 464 | 2.3 |
| Chiifu specific | AT5G32040 | KNAT5 (KNOTTED-LIKE HOMEBOX GENE 5) | Brapa_ESTC012634 | 44 | 18085 | . | 413.2 |
| | AT5G51190 | AP2 domain-containing transcription factor, putative | Brapa_ESTC013139 | 145 | 12449 | . | 85.7 |
| | AT4G01550 | ANAC069 (Arabidopsis NAC domain containing protein 69) | Brapa_ESTC007789 | 95 | 7576 | . | 80.0 |
| | AT3G50260 | CEJ1 (COORDINATEDLY REGULATED BY ETHYLENE AND JASMONE 1) | Brapa_ESTC030959 | 17 | 1102 | . | 66.0 |
| | AT5G60890 | AMYB34 | Brapa_ESTC040043 | 408 | 22870 | . | 56.1 |
| | AT2G22430 | ATHB6 (ARABIDOPSIS THALIANA HOMEBOX PROTEIN 6) | Brapa_ESTC04579 | 323 | 13848 | . | 42.9 |
| | AT2G46970 | PIL1 (PHOTOCYCLE INTERACTING FACTOR 3-LIKE 1) | Brapa_ESTC042137 | 108 | 3570 | . | 33.1 |
| | AT2G42780 | similar to Os06g0169000 | Brapa_ESTC050862 | 49 | 1577 | . | 32.1 |
| | AT1G49950 | ATRIB1/CTB1 (TELOMERE REPEAT BINDING FACTOR 1) | Brapa_ESTC051029 | 270 | 4988 | . | 18.5 |
| | AT4G12020 | MAPKKK11 | Brapa_ESTC039087 | 149 | 2540 | . | 17.1 |
| | AT5G9820 | ZAT12 | Brapa_ESTC006145 | 100 | 1379 | . | 13.0 |
| | AT4G33180 | WRYK18 (WRKY DNA-binding protein 18) | Brapa_ESTC001172 | 142 | 3971 | . | 12.4 |
| | AT3G27810 | AtMYB21 (MYB DOMAIN PROTEIN 21) | Brapa_ESTC080810 | 445 | 4910 | . | 11.0 |
| | AT5G61150 | HDG1 (HOMEODOMAIN GLABROUS1) | Brapa_ESTC022251 | 201 | 1813 | . | 9.0 |
| | AT5G28920 | ATHB34 (ARABIDOPSIS THALIANA HOMEBOX PROTEIN 34) | Brapa_ESTC051903 | 418 | 3716 | . | 8.9 |
| | AT5G1100 | Transcription factor | Brapa_ESTC052968 | 361 | 2986 | . | 8.3 |
| | AT2G23380 | CLF (CURLY LEAF) | Brapa_ESTC048306 | 138 | 1075 | . | 7.8 |
| | AT1G71030 | AtMYB32 (Arabidopsis myb-like 2) | Brapa_ESTC021415 | 249 | 1765 | . | 7.1 |
| | AT5G16820 | HSFA1B | Brapa_ESTC063030 | 481 | 1465 | . | 3.0 |
| | AT5G61590 | AP2 domain-containing transcription factor family protein | Brapa_ESTC021781 | 465 | 1249 | . | 2.7 |

2R/C and C/R indicate the expression ratio in RCBr over Chiifu and Chiifu over RCBr, respectively. Flowering-related TF genes were omitted in this Table.
Flowering genes

Genes listed in Table 3 were selected from 196 flowering-related genes on Br300K microarray: 26, 9 and 23 genes for up-regulated in RCBr, in Chiifu and both, respectively. Genes (26 genes) up-regulated in RCBr included most flowering-promoting genes, such as FT and SOC1. On the other hand, genes (9) up-regulated in Chiifu included floral repressors, such as FLC and MAF4. Twenty-three genes were highly expressed in both RCBr and Chiifu, which included DWARF AND DELAYED FLOWERING 1 (DDF1) and EARLY FLOWERING 3 (ELF3).

Interestingly, expression of several flowering genes

Table 3. Flowering-related genes which were selected from 196 flowering genes on Br300K microarray.

| Classification | At_Locus | Gene annotation | Br_SEQ_ID | Probe intensity | Fold change |
|----------------|----------|-----------------|-----------|----------------|-------------|
| Up-regulated genes in RCBr | AT5G20240 | PI (PISTILLATA) | Brapa_ESTC028268 | 4123 | 43 | 95.0 |
| | AT5G15800 | SEP1 (SEPALLATA1)/AGL2 | Brapa_ESTC025908 | 1629 | 32 | 50.6 |
| | AT1G69120 | AP1 (APETALA1)/AGL4 | Brapa_ESTC011597 | 2267 | 94 | 24.2 |
| | AT3G02310 | SEP2 (SEPALLATA2) | Brapa_ESTC034571 | 5018 | 200 | 25.0 |
| | AT3G54340 | AP3 (APETALA 3) | Brapa_ESTC010485 | 1242 | 197 | 6.3 |
| | AT4G18960 | AG (AGAMOUS) | Brapa_ESTC008198 | 1927 | 93 | 20.6 |
| | AT2G45650 | AGL6 (AGAMOUS LIKE-6) | Brapa_ESTC010831 | 2371 | 253 | 9.4 |
| | AT1G65480 | FT (FLOWERING LOCUS T) | Brapa_ESTC010942 | 1086 | 112 | 9.7 |
| | AT3G07650 | COL9 (CONSTANS-LIKE 9) | Brapa_ESTC019941 | 2432 | 462 | 5.3 |
| | AT2G45660 | SOC1/AGL20 (AGAMOUS-LIKE 20) | Brapa_ESTC011095 | 14264 | 3600 | 3.9 |
| | AT5G69010 | FUL/AGL8 (AGAMOUS-LIKE 8) | Brapa_ESTC017129 | 22280 | 5691 | 3.9 |
| | AT5G24860 | PPFI (FLOWERING PROMOTING FACTOR 1) | Brapa_ESTC018052 | 4399 | 1195 | 3.7 |
| | AT5G21650 | TFL1 (TERMINAL FLOWER 1) | Brapa_ESTC010942 | 1086 | 112 | 9.7 |
| | AT2G03960 | Similar to AGL102 | Brapa_ESTC034579 | 1985 | 278 | 7.1 |
| | AT3G21320 | Similar to ELF3 (EARLY FLOWERING 3) | Brapa_ESTC010485 | 2902 | 138 | 21.0 |
| | AT2G43010 | PIF4 (PHYTOCHROME INTERACTING FACTOR 4) | Brapa_ESTC017129 | 22280 | 5691 | 3.9 |
| | AT2G45830 | DTA2 (DOWNSTREAM TARGET OF AGL15) | Brapa_ESTC018123 | 2371 | 253 | 9.4 |
| | AT5G15850 | COL1 (CONSTANS-LIKE 1) | Brapa_ESTC010942 | 1086 | 112 | 9.7 |
| | AT3G59060 | PIF5/ PIL6 (PHYTOCHROME-INTERACTING FACTOR 5) | Brapa_ESTC019197 | 1325 | 401 | 3.3 |
| | AT1G71692 | AGL12 (AGAMOUS-LIKE 12) | Brapa_ESTC018786 | 4340 | 1879 | 2.3 |
| | AT5G10140 | FLC (FLOWERING LOCUS C)/AGL25/FLC5 | Brapa_ESTC017216 | 8885 | 4014 | 2.2 |
| | AT5G1790 | AGL15 (AGAMOUS-LIKE 15) | Brapa_ESTC010865 | 5636 | 2491 | 2.3 |
| | AT2G33350 | Similar to zinc finger CONSTANS-related | Brapa_ESTC041607 | 1624 | 809 | 2.0 |

2) R/C and C/R indicate the expression ratio in RCBr over Chiifu and Chiifu over RCBr, respectively.
Table 3. Continued.

| Classification | At_Locus | Gene annotation | Brp_seq_ID | Probe intensity | Fold change |
|----------------|----------|-----------------|------------|----------------|-------------|
| ATG5G10140     | FLC (FLOWERING LOCUS C)/AGL25 (FLC1, FLC2) | Brapa_ESTC016530 | 53 | 1504 | 28.3 |
|                |          |                 | Brapa_ESTC012276 | 6738 | 13035 | 2.0 |
|                |          |                 | Brapa_ESTC013135 | 393 | 8499 | 21.6 |
|                |          |                 | Brapa_ESTC046597 | 986 | 4411 | 4.5 |
|                |          |                 | Brapa_ESTC013362 | 3209 | 11579 | 3.6 |
|                |          |                 | Brapa_ESTC046600 | 300 | 1051 | 3.5 |
|                |          |                 | Brapa_ESTC046598 | 3151 | 9784 | 3.1 |
| ATG6G5070      | FLC4/MAF4 (MADS AFFECTING FLOWERING 4) | Brapa_ESTC040293 | 206 | 2363 | 11.5 |
|                |          |                 | Brapa_ESTC020902 | 368 | 1517 | 4.1 |
|                |          |                 | Brapa_ESTC020193 | 1290 | 4174 | 3.2 |
|                |          |                 | Brapa_ESTC014042 | 276 | 776 | 2.8 |
|                |          |                 | Brapa_ESTC022419 | 1327 | 3363 | 2.5 |
|                |          |                 | Brapa_ESTC026673 | 2332 | 5556 | 2.4 |
|                |          |                 | Brapa_ESTC021222 | 2876 | 5626 | 2.0 |
| ATG3G7590      | AGL18 (AGAMOUS-LIKE 18) | Brapa_ESTC039504 | 30466 | 29212 | 1.0 |
|                |          |                 | Brapa_ESTC002858 | 19586 | 15570 | 1.3 |
|                |          |                 | Brapa_ESTC041284 | 26959 | 22606 | 1.2 |
|                |          |                 | Brapa_ESTC041298 | 22936 | 16177 | 1.4 |
|                |          |                 | Brapa_ESTC015252 | 19828 | 20034 | 1.0 |
|                |          |                 | Brapa_ESTC047777 | 23968 | 21573 | 1.0 |
|                |          |                 | Brapa_ESTC014320 | 23538 | 18005 | 1.3 |
|                |          |                 | Brapa_ESTC050176 | 8869 | 9304 | 1.0 |
|                |          |                 | Brapa_ESTC041116 | 18874 | 17799 | 1.0 |
|                |          |                 | Brapa_ESTC042608 | 21959 | 17640 | 1.2 |
|                |          |                 | Brapa_ESTC001982 | 19200 | 13055 | 1.5 |
|                |          |                 | Brapa_ESTC011117 | 19001 | 27161 | 0.7 |
|                |          |                 | Brapa_ESTC014037 | 15458 | 24927 | 0.6 |
|                |          |                 | Brapa_ESTC027269 | 13996 | 19872 | 0.7 |
|                |          |                 | Brapa_ESTC013303 | 17486 | 16533 | 1.1 |
|                |          |                 | Brapa_ESTC021830 | 13579 | 14983 | 0.9 |
|                |          |                 | Brapa_ESTC039504 | 26959 | 22606 | 1.2 |
|                |          |                 | Brapa_ESTC041284 | 22936 | 16177 | 1.4 |
|                |          |                 | Brapa_ESTC015252 | 19828 | 20034 | 1.0 |
|                |          |                 | Brapa_ESTC047777 | 23968 | 21573 | 1.0 |
|                |          |                 | Brapa_ESTC014320 | 23538 | 18005 | 1.3 |
|                |          |                 | Brapa_ESTC050176 | 8869 | 9304 | 1.0 |
|                |          |                 | Brapa_ESTC041116 | 18874 | 17799 | 1.0 |
|                |          |                 | Brapa_ESTC042608 | 21959 | 17640 | 1.2 |
|                |          |                 | Brapa_ESTC001982 | 19200 | 13055 | 1.5 |
|                |          |                 | Brapa_ESTC011117 | 19001 | 27161 | 0.7 |
|                |          |                 | Brapa_ESTC014037 | 15458 | 24927 | 0.6 |
|                |          |                 | Brapa_ESTC027269 | 13996 | 19872 | 0.7 |
|                |          |                 | Brapa_ESTC013303 | 17486 | 16533 | 1.1 |
|                |          |                 | Brapa_ESTC021830 | 13579 | 14983 | 0.9 |
|                |          |                 | Brapa_ESTC042641 | 14327 | 8484 | 1.7 |
|                |          |                 | Brapa_ESTC004485 | 15388 | 12464 | 1.2 |
|                |          |                 | Brapa_ESTC042284 | 12694 | 10565 | 1.2 |
|                |          |                 | Brapa_ESTC021115 | 15190 | 10154 | 1.5 |
|                |          |                 | Brapa_ESTC000396 | 14927 | 10695 | 1.4 |
|                |          |                 | Brapa_ESTC042798 | 14472 | 8979 | 1.6 |
|                |          |                 | Brapa_ESTC051649 | 12413 | 8766 | 1.4 |
|                |          |                 | Brapa_ESTC021535 | 12256 | 7067 | 1.7 |
|                |          |                 | Brapa_ESTC025316 | 8692 | 4979 | 1.7 |
|                |          |                 | Brapa_ESTC049114 | 7570 | 2054 | 3.5 |
|                |          |                 | Brapa_ESTC049113 | 7031 | 5176 | 1.4 |
|                |          |                 | Brapa_ESTC006144 | 6478 | 9542 | 0.7 |
|                |          |                 | Brapa_ESTC044575 | 14100 | 11058 | 1.3 |
|                |          |                 | Brapa_ESTC006396 | 12278 | 10830 | 1.1 |
|                |          |                 | Brapa_ESTC009046 | 8400 | 10613 | 0.8 |
|                |          |                 | Brapa_ESTC034160 | 10951 | 9936 | 1.1 |
|                |          |                 | Brapa_ESTC007967 | 7301 | 8061 | 0.9 |
|                |          |                 | Brapa_ESTC012323 | 4120 | 5899 | 0.7 |
|                |          |                 | Brapa_ESTC013840 | 3821 | 4959 | 0.8 |
|                |          |                 | Brapa_ESTC023000 | 2272 | 1607 | 1.4 |
|                |          |                 | Brapa_ESTC015151 | 8990 | 9444 | 1.0 |
|                |          |                 | Brapa_ESTC034801 | 9787 | 7499 | 1.3 |
|                |          |                 | Brapa_ESTC038745 | 9602 | 6902 | 1.4 |
|                |          |                 | Brapa_ESTC023094 | 8429 | 13088 | 0.6 |
|                |          |                 | Brapa_ESTC027935 | 6642 | 4190 | 1.6 |
|                |          |                 | Brapa_ESTC013309 | 9416 | 16434 | 0.6 |

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showed paralog-specific pattern: i.e., BrFLC1 (Bra009055; Brapa_ESTC016533) and BrFLC2 (Bra028599; Brapa_ESTC012276) in Chiifu, BrFLCL/BrFLC5 (FLC-Like, Bra022771; Brapa_ESTC017216 and Brapa_ESTC027067) in RCBR, and BrFLC3 (Bra006051; Brapa_ESTC013303 and Brapa_ESTC021830) in both plants. CONSTANS-LIKE 9 (COL9) was also paralog specific expression: Brapa_ESTC027877 (Bra029666; BrCOL9-2) and Brapa_ESTC051650 (Bra001264; BrCOL9-3) for up-regulated ones in RCBR, but rest (most of them are BrCOL9-1; Bra040020) in both plants. These paralog-specific expressions of flowering genes were highlight of this study.

**Gibberellin metabolism-related genes**

Since gibberellins (GAs) control flowering in concert with cytokinin (Matías-Hernández et al. 2016), the expression of GA-metabolism-related genes were examined (Table 4). Most genes involved in increase of the GA activity were up-regulated in RCBR; GA3OX1/GA4 (GA requiring 4) and GA20OX3 (Gibberellin 20 oxidase 3). Only GA2OX2 (Gibberelin 2-beta-dioxygenase) expression was high in Chiifu, but its function has not been identified besides its involvement in GA catabolism.

### Confirmation of selected gene expression

Fig. 2 showed genes whose expression was decreased during the development of RCBR, while increased in Chiifu. These included MADS AFFECTING FLOWERING 4 (FCL4/MAF4), FLC2, FLOWERING LOCUS D (FLD), REDUCED VERNALIZATION RESPONSE 1 (VRN1), REDUCED VERNALIZATION RESPONSE 2 (VRN2, Polycomb group), METHYL-CPG-BINDING DOMAIN 9 (MBD9) and KNAT5. Most of them are known to be floral repressors. The expression of these genes was gradually increased in Chiifu even though this period belongs to be very early developmental stages.

Photoperiod is important for controlling flowering of *Brassica* species. To elucidate its association, several TF genes and flowering-related genes were selected and their expressions were examined during a day under LD and SD conditions. Plants grown for 21 DAG under LD and SD were sampled at indicated time (Fig. 3A). *B. rapa*

| At_Locus   | Gene annotation                                                                 | Prove intensity Br_SEQ_ID | Fold change R/C | C/R |
|------------|----------------------------------------------------------------------------------|---------------------------|-----------------|-----|
| AT1G30040  | GA2OX2 (Gibberelin 2-beta-dioxygenase)                                            | Brapa_ESTC040202          | 1319            | 6104 | 0.2 | 4.6 |
| AT1G02400  | GA2OX6/DTA1 (Gibberellin 2-OXIDASE 6)                                              | Brapa_ESTC015031          | 16              | 1969 | 0.0 | 126.4 |
| AT4G21200  | GA2OX8 (Gibberellin 2-OXIDASE 8)                                                   | Brapa_ESTC015242          | 1076            | 8386 | 1.3 | 0.8 |
| AT1G15550  | GA3OX1/GA4 (GA requiring 4)                                                        | Brapa_ESTC024684          | 431             | 665  | 0.6 | 1.5 |
| AT5G07200  | GA20OX3/YAP169 (Gibberellin 20 oxidase 3)                                          | Brapa_ESTC015062          | 423             | 624  | 0.7 | 1.5 |
| AT5G14920  | Gibberellin-regulated family protein                                               | Brapa_ESTC027777          | 554             | 164  | 3.4 | 0.3 |
| AT5G59845  | Gibberellin-regulated family protein                                               | Brapa_ESTC029845          | 4761            | 183  | 26.1 | 0.0 |
| AT5G74670  | Gibberellin-responsive protein, putative                                            | Brapa_ESTC037822          | 507             | 20   | 25.9 | 0.0 |
| AT1G22690  | Gibberellin-responsive protein, putative                                            | Brapa_ESTC037994          | 4417            | 3177 | 1.4 | 0.7 |
| AT5G51310  | Gibberellin 20-oxidase-related                                                     | Brapa_ESTC024407          | 6879            | 6685 | 1.0 | 1.0 |
| AT3G10185  | Gibberellin-regulated GASA/GAST/Snakin family protein                              | Brapa_ESTC010071          | 5161            | 3227 | 1.6 | 0.6 |
| AT4G26420  | GAMT1 | Gibberellin carboxyl-O-methyltransferase                                             | Brapa_ESTC003796          | 5081            | 1538 | 3.3 | 0.3 |
| AT5G51310  | Gibberellin 20-oxidase-related                                                     | Brapa_ESTC046793          | 859             | 1135 | 0.8 | 1.3 |
| AT5G6300   | GAMT2 | Gibberellin carboxyl-O-methyltransferase                                             | Brapa_ESTC036747          | 4237            | 5060 | 0.8 | 1.2 |

Table 4. Genes related to gibberellic acid metabolism.

$R/C$ and $C/R$ indicate the expression ratio in RCBR over Chiifu and Chiifu over RCBR, respectively.
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Fig. 2. Genes whose expression was decreased in RCBr, while increased in Chiifu. RCBr and Chiifu were grown in long-day condition for 19 days and shoot was sampled at 4th hour of light period.

Fig. 3. Expression of flowering- and photoperiod pathway-related genes. (A) Photoperiod for plant growth and sampling time a day. (B) Genes showing differential expression in a microarray. (C) Constance (CO) and its paralogs. 
BrCCA1 was used as control for circadian rhythm. 
LD: long-day condition, SD: short-day condition.

CIRCADIAN CLOCK ASSOCIATED 1 (BrCCA1) was used as a control for the circadian clock. The expression of BrFLC2 was high in Chiifu, but not detected in RCBr. Individual paralogues of BrSEP1 (SEPALLATA1/AGL2) were specific for either plant (Fig. 3B). However, photoperiodic pathway integrator, CO and its relatives (COLs) were expressed with similar patterns in both plants
(Fig. 3C). Only BrCOL2-1 was expressed in Chiifu. Taken together, there was no photoperiodic- dependent expression, but the expression of BrFLC2, BrSEP1s and BrCOL2-1 was plant-specific. These daily expression patterns was not agreement with microarray data, due to sampling time difference.
DISCUSSION

Because of striking difference in periods required for flowering between RCBr and B. rapa ssp. pekinensis, inbred line Chiifu, normalization of developmental stages of these two plants would be almost impossible. Therefore, identification of master gene(s) for controlling flowering time would be also very difficult. However, microarray data in this study showed remarkable difference in the expression of flowering-related genes between two plants; high expression of flowering promoting genes in RCBr, whereas high expression of floral repressor genes in Chiifu. DEGs between RCBr and Chiifu included many NHFs with respect to BlastN against Arabidopsis gene ID, suggesting that these would be Brassica species-specific genes. That can be supported by that most sequences were matched to the public genome data or NCBI data for B. napus and B. oleracea. For example, Brapa_ESTC051261 was the most highly expressed gene (937 fold) in Chiifu compared with RCBr. As result of BlastN in RCBr, this clone was matched to 100% with B. napus uncharacterized LOC106444620, ncRNA and 90% with B. oleracea var. oleracea uncharacterized LOC106327354. Brapa_ESTC033111 was the most highly expressed gene (937 fold) in Chiifu compared with RCBr. As result of BlastN in RCBr, this clone was matched to 100% with B. napus uncharacterized LOC106444620, ncRNA and 90% with B. oleracea var. oleracea uncharacterized LOC106330763. This finding strongly suggests that functional study on these Brassica-specific genes will be very important for understanding evolution of Brassicaceae crops.

Analysis of DEGs

GO enrichment analysis with DEGs showed distinct difference between RCBr, very early flowering phenotype, and Chiifu, a slow or late flowering phenotype (Fig. 1). Flowering process-related genes were specifically induced in RCBr, whereas temperature and other environment stress tolerant-related genes were expressed in Chiifu. Some general biological processes were conserved in both RCBr and Chiifu. This result might imply that Chiifu is not ready to flower yet. However, it was ruled out whether this difference is due to the difference in developmental stages during whole life cycle.

Expression of TF genes

TFs control the expression of a large number genes, thereby exerting big impact on plant morphogenesis. As shown in Table 2, many TFs were highly expressed in both plants, some were specifically expressed in RCBr or in Chiifu. Based on current knowledge, it was hard to find out the specific association with growth and development for highly expressed TFs in both plants; i.e., RAP2.4, the most highly expressed gene, is known to promote leaf senescence (Xu et al. 2010). However, several genes among RCBr-induced TFs seemed to be closely related to early flowering phenotype. TCP family determines in the height of inflorescence shoot (Davière et al. 2014), which might be related to short height of RCBr. SHN2 is known to be related to flower organ (Shi et al. 2011), and CAULIFLOWER (CAL) is closely related to another MADS box gene APETALA1 (AP1) and control floral meristem development (Alvarez-Buylla et al. 2000).

Regarding to genes induced in Chiifu, most TFs appeared to be related to plant growth and development as follows. KNAT5 regulates photomorphogenic responses and represses late steps in gibberellin biosynthesis. (Hay et al. 2002). AP2 encoding a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 TF family may be related to the ethylene-singalling pathway. AtMYB34 regulates indole glucosinolate homeostasis (Celenza et al. 2005). ATHB6, a target of the protein phosphatase ABI1, regulates negatively ABA responses in Arabidopsis (Himmelbach et al. 2002). Only CLF, a repressor of AG function (Goodrich et al. 1997), is related to flowering; it suppresses flowering by inducing misexpression of floral promoting genes (Lopez-Vernaza et al. 2012)

Expression of GA metabolism-related genes

GA content is associated with fertility (Plackett et al. 2012). GA 20-oxidase (GA20ox) regulates GA content (Yamaguchi 2008), and there are five GA20ox genes in Arabidopsis, GA20ox1, GA20ox2, GA20ox3, GA20ox4, and GA20ox5 (Hedden et al. 2002). Among them, GA20ox3 functions almost entirely redundantly with GA20ox1 and GA20ox2 at most developmental stages, including the floral transition, thereby these genes control
floral organ growth and anther development (Plackett et al. 2012). In our results (Table 4), GA20OX3 was highly expressed in RCBr, suggesting the possible function in flowering. In addition, several GA responsive genes were up-regulated in RCBr, but their role in flowering have to be solved. Only one gene, GA2OX2 (Gibberellin 2-beta-dioxygenase) was up-regulated in Chiifu, but the function of this gene has not been identified yet.

**Expression of flowering-related genes**

Regarding to flowering-related genes, several flowering-promoting genes, such as PISTILLATA (PI), SEP1 and AP1, were up-regulated in RCBr, whereas floral repressors, such FLC and MAF4, were up-regulated in Chiifu (Table 3). These results can partially support why these two plants exhibit contrasting flowering phenotypes, rapid vs. slow. Interestingly, several genes contain paralogues; FLC, COL9 and SOC1. Schranz et al. (2002) and Franks et al. (2015) reported presence of four FLC paralogues in *B. rapa* (BrFLC1, BrFLC2, BrFLC3, and BrFLC5). A genetic-genomics approach revealed that BrFLC2 is a major regulator of flowering time in *B. rapa* (Wu et al. 2012; Xiao et al. 2013; 2014). Recently, Li et al. (2016) reported that early flowering phenotypes of *B. rapa* is related to be nonfunctional BrFLCs which are truncated form by premature termination, but slow or late flowering types contain functional or intact forms of BrFLC1, BrFLC2, and BrFLC3. In addition, Song et al. (2015) found out that expression of only one or some of paralogues is closely related to photoperiodic flowering in *B. rapa*. All these facts might be similar to our study: i.e., BrFLC2 were up-regulated in Chiifu, a slow or late flowering phenotype. However, BrFLC5 was up-regulated in RCBr, suggesting the another possible role of FLC besides flowering control.

RT-PCR results of selected genes demonstrated that the expression of flowering repressor genes was gradually decreased in RCBr, while gradually increased in Chiifu (Fig. 2). These included BrMBD9, BrVRN1, BrVRN2, BrFLC, and BrFLD. MBD9 controls flowering time by modulating gene expression through DNA methylation and histone acetylation (Yaish et al. 2009). VRN1 and VRN2 affect vernalization response of late flowering plants (Chandler et al. 1996). FLD (Flowering Locus D) encodes a plant ortholog of the human Lys-Specific Demethylase 1 (LSD1) protein. FLD functions in histone H3K4 demethylation and H3/H4 deacetylation to repress the expression of FLC (Jiang et al. 2007; Yu et al. 2011). Our data well agree with previous findings.

It was expected that photoperiodic flowering signaling-related genes are differentially expressed between RCBr and Chiifu, but no significant difference was observed (Fig. 3). Special attention paid to COL9 (CONSTANS-LIKE 9) which delays flowering by down-regulation of expression of CO, FT, and SOC1 (Cheng and Wang 2005). However, three paralogues showed similar expression patterns between two plants.

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**REFERENCES**

Alvarez-Buylla ER, Pelaz S, Liljegren SJ, Gold SE, Burgeff C, Ditta GS, et al. 2000. An ancestral MADS-box gene duplication occurred before the divergence of plants and animals. Proc. Natl. Acad. Sci. U.S.A. 10: 5328-5333.

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al.; The Gene Ontology Consortium. 2000. Gene ontology: tool for the unification of biology. Nat. Genet. 25: 25-29.

Celenza JL, Quiel JA, Smolen GA, Merrikh H, Silvestro AR, Normanly J, et al. 2005. The *Arabidopsis* ATR1 Myb transcription factor controls indolic glucosinolate homeostasis. Plant Physiol. 137: 253-262.

Chandler J, Wilson A, Dean C. 1996. *Arabidopsis* mutants showing an altered response to vernalization. Plant J. 10: 637-644.

Cheng XF, Wang ZY. 2005. Overexpression of COL9, a CONSTANS-LIKE gene, delays flowering by reducing expression of CO and FT in *Arabidopsis* thaliana. Plant J. 43: 758-768.

Davière JM, Wild M, Regnault T, Baumberger N, Eisler H,
Genschik P, et al. 2014. Class I TCP-DELLAR interactions in inflorescence shoot apex determine plant height. Curr. Biol. 24: 1923-1928.

Devisetty UK, Covington MF, Tat AV, Lekkala S, Maloof JN. 2014. Polymorphism identification and improved genome annotation of Brassica rapa through deep RNA sequencing. G3 (Bethesda) 4: 2065-2078.

Dong X, Feng H, Xu M, Lee J, Kim YK, Lim YP, et al. 2013. Comprehensive analysis of genic male sterility-related genes in Brassica rapa using a newly developed Br300K oligomeric chip. PLoS One 8: e72178.

Dong X, Feng H, Xu M, Lee J, Kim YK, Lim YP, et al. 2014. Class I TCP-DELLAR interactions in inflorescence shoot apex determine plant height. Curr. Biol. 24: 1923-1928.

Devisetty UK, Covington MF, Tat AV, Lekkala S, Maloof JN. 2014. Polymorphism identification and improved genome annotation of Brassica rapa through deep RNA sequencing. G3 (Bethesda) 4: 2065-2078.

Dong X, Feng H, Xu M, Lee J, Kim YK, Lim YP, et al. 2013. Comprehensive analysis of genic male sterility-related genes in Brassica rapa using a newly developed Br300K oligomeric chip. PLoS One 8: e72178.

Du Z, Zhou X, Ling Y, Zhang Z, Su Z. 2010. agriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res. 38: W64-70.

Franks SJ, Perez-Sweeney B, Strahl M, Nowogrodzki A, Weber JJ, Lalchan R, et al. 2015. Variation in the flowering time orthologs BrFLC and BrSOC1 in a natural population of Brassica rapa. Peer J. 3: e1339.

Gómez-Campo C, Prakash S. 1999. Origin and domestication, p. 33-58. In: C. Gómez-Campo (ed.). Biology of Brassica coenospecies. Elsevier, Amsterdam.

Goodrich J, Puangsomlee P, Martin M, Long D, Meyerowitz EM, Coupland G. 1997. A Polycomb-group gene regulates homeotic gene expression in Arabidopsis. Nature 386: 44-51.

Hay A, Kaur H, Phillips A, Hedden P, Hake S, Tsiantis M. 2002. The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans. Curr. Biol. 12: 1557-1565.

Hedden P, Phillips AL, Rojas MC, Carrera E, Tudzynski B. 2001. Gibberellin biosynthesis in plants and fungi: a case of convergent evolution? J. Plant Growth Regul. 20: 319-331.

Hellwell CA, Anderssen RS, Robertson M, Finnegan EJ. 2015. How is FLC repression initiated by cold? Trends Plant Sci. 20: 76-82.

Hellwell CA, Wood CC, Robertson M, James Peacock W, 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.

Himmelbach A, Hoffmann T, Leube M, Höhener B, Grill E. 2002. Homeodomain protein ATHB6 is a target of the protein phosphatase ABI1 and regulates hormone responses in Arabidopsis. EMBO J. 21: 3029-3038.

Jiang D, Yang W, He Y, Amasino RM. 2007. Arabidopsis relatives of the human lysine-specific demethylase 1 repress the expression of FWA and FLOWERING LOCUS C and thus promote the floral transition. Plant Cell 19: 2975-2987.

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 like Nou 6 gou. Breed. Sci. 61: 151-159.

Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, et al. 2008. KEGG for linking genomes to life and the environment. Nucleic Acids Res. 36: D480-484.

Kim JS, Chung TY, King GJ, Jin M, Yang TJ, Jin YM, et al. 2006. A sequence-tagged linkage map of Brassica rapa. Genetics 174: 29-39.

Kim SY, Park BS, Kwon SJ, Kim J, Lim MH, Park YD, et al. 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.

Lee H, Suh SS, Park E, Cho E, Ahn JH, Kim SG, et al. 2000. The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in Arabidopsis. Genes Dev. 14: 2366-2376.

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 SJ, 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.

Lopez-Vernaza M, Yang S, Müller R, Thorpe F, de Leau E, Goodrich J. 2012. Antagonistic roles of SEPALLATA3, FT and FLC genes as targets of the polycomb group gene CURLY LEAF. PLoS One 7: e30715.

Mao F, Wu F, Yu X, Bai J, Zhong W, He Y. 2014. MicroRNA319a-targeted Brassica rapa ssp. pekinensis TCP genes modulate head shape in Chinese cabbage by differential cell division arrest in leaf regions. Plant Physiol. 164: 710-720.

Matías-Hernández L, Aguilar-Jaramillo AE, Cigliano RA, Sanseverino W, Pelaz S. 2016. Flowering and trichome development share hormonal and transcription factor regulation. J. Exp. Bot. 67: 1209-1219.
Musgrave ME. 2000. Realizing the potential of rapid-cycling *Brassica* as a model system for use in plant biology research. J. Plant Growth Regul. 19: 314-325.

Plackett AR, Powers SJ, Fernandez-Garcia N, Urbanova T, Takebayashi Y, Seo M, *et al.* 2012. Analysis of the developmental roles of the *Arabidopsis* gibberellin 20-oxidases demonstrates that GA20ox1, -2, and -3 are the dominant paralogs. Plant Cell 24: 941-960.

Putterill J, Robson F, Lee K, Simon R, Coupland G. 1995. The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80: 847-857.

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, *et al.* 2000. Distinct roles of *CONSTANS* target genes in reproductive development of *Arabidopsis*. Science 288: 1613-1616.

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.

Shi JX, Malitsky S, De Oliveira S, Branigan C, Franke RB, Schreiber L, *et al.* 2011. SHINE transcription factors act redundantly to pattern the archetypal surface of *Arabidopsis* flower organs. PLoS Genet. 7: e1001388.

Song X, Duan W, Huang Z, Liu G, Wu P, Liu T, *et al.* 2015. Comprehensive analysis of the flowering genes in Chinese cabbage and examination of evolutionary pattern of CO-like genes in plant kingdom. Sci. Rep. 5: 14631.

Takada S, Goto K. 2003. Terminal *flwr2*, an *Arabidopsis* homolog of heterochromatin protein1, counteracts the activation of floweringlocus T by constans in the vascular tissues of leaves to regulate flowering time. Plant Cell 15: 2856-2865.

Wang X, Wang H, Wang J, Sun R, Wu J, Liu S, *et al.* 2011. The genome of the mesopolyploid crop species *Brassica rapa*. Nat. Genet. 43: 1035-1039.

Wang Y, Wu F, Bai J, He Y. 2014. BrpSPL9 (*Brassica rapa* ssp. *pekinesis* SPL9) controls the earliness of heading time in Chinese cabbage. Plant Biotechnol. J. 12: 312-321.

Williams PH, Hill CB. 1986. Rapid-cycling populations of *brassica*. Science 232: 1385-1389.

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

Xiao D, Wang H, Basnet RK, Zhao J, Lin K, Hou X, *et al.* 2014. Genetic dissection of leaf development in *Brassica rapa* using genetical genomics approach. Plant Physiol. 164: 1309-1325.

Xiao D, Zhao JJ, Hou XL, Basnet RK, Carpio DPD, Zhang NW, *et al.* 2013. The *Brassica rapa* FLC homologue *FLC2* is a key regulatory of flowering time, identified through transcriptional co-expression networks. J. Exp. Bot. 64: 4503-4516.

Xu H, Wang X, Chen J. 2010. Overexpression of the Rap2.4f transcriptional factor in *Arabidopsis* promotes leaf senescence. Sci. China Life Sci. 53: 1221-1226.

Yaish MW, Peng M, Rothstein SJ. 2009. AtMBD9 modulates *Arabidopsis* development through the dual epigenetic pathways of DNA methylation and histone acetylation. Plant J. 59: 123-135.

Yamaguchi S. 2008. Gibberellin metabolism and its regulation. Annu. Rev. Plant Biol. 59: 225-251.

Yu CW, Liu X, Luo M, Chen C, Lin X, Tian G, *et al.* 2011. HISTONE DEACETYLASE 6 interacts with FLOWERING LOCUS D and regulates flowering in *Arabidopsis*. Plant Physiol. 156: 173-184.