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Genome-wide Association Analysis of Flowering Time in *Brassica campestris*

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Abstract In order to locate candidate genes related to flowering time, and to provide a basis for genetic improvement and flowering time of *Brassica campestris*, a natural population composed of 110 *B. campestris* was used as research materials. Flowering investigation and resequencing were performed on them. The high-quality SNP set obtained by resequencing was used for population evolutionary tree analysis, principal component analysis, population genetic structure analysis and a genome-wide association analysis. The observation results showed there were wide differences in flowering time between different types of *B. campestris*. The results of population structure analysis showed that 110 materials could be divided into two subgroups. The distribution within each phylogenetic tree was relatively concentrated, and the distribution between different subgroups was closely related to the geographical origin of the material. Genome-wide association analysis revealed that the average LD of the whole *B. campestris* attenuated LD was 19 kb, and 4 signal sites significantly associated with the flowering schedule type were obtained. Functional annotation was performed on related genes in a certain region upstream and downstream of the physical location of the four associated candidate sites, and 9 candidate transcripts related to flowering time were screened. Further analysis revealed that 9 transcripts contained a total of 4 candidate genes, which were homologous genes of *Arabidopsis LOL1*, *CAT5* and *FAD8*. The results of this study could provide some theoretical basis and clues for obtaining candidate genes related to flowering time of *B. campestris* and using them to regulate flowering and genetic improvement.

Keywords *Brassica campestris*; Flowering time; Linkage disequilibrium; Genome-wide association study; Candidate gene

Brassica campestris is an important oil crop of the genus *Brassica* in Brassicaceae. Flowering time is an important agronomic trait in the production of *Brassica rapa*. The early or late flowering time has an important impact on its yield and quality. There are many genes regulating the flowering time of *Brassica campestris*, and the gene regulatory network is complex. Moreover, environmental factors have important effects on flowering time and gene expression. A large number of flowering genes have been found in *Brassica rapa*, and the functions of some flowering genes have been verified (Liu et al., 2019; Su et al., 2020). Xiao (2012) identified 144 candidate gene markers related to flowering time in a double haploid population of Chinese cabbage. 10 *QTLs* related to flowering time were detected under different growth conditions, and candidate genes such as *BrFLC2*, *SOC1* and *FT* were screened. Gong (2016) detected 2 *QTLs* controlling flowering of Chinese cabbage by genome-wide association analysis, which were in linkage groups A02 and A07, respectively. The closest gene to SNP-A026709555 on chromosome A02 is *Bra035633*, and the closest gene to SNP-A0711143455 on chromosome A07 is *Bra012139*. Both genes are not in the attenuation range, so other candidate genes related to flowering time located in the 2 *QTLs* attenuation regions need to be further found. Gao et al. (2017) re-sequenced 116 natural populations of *Brassica rapa*. And correlation analysis was conducted between resequencing results and flowering time of *Brassica rapa* under different conditions. 14 candidate genes related to flowering time of *Brassica rapa*, including *FUL*, *PHYB*, *FPF1* and *FT1*, were finally obtained through gene collinearity and gene function annotation between *Brassica rapa* and *Arabidopsis thaliana*. Wu et al. (2012) analyzed the association between resequencing results and flowering time traits of 159 *Brassica rapa* and found that an InDel mutation in *BrFLC2* gene was closely related to flowering time. Li et al. (2019) analyzed the molecular mechanism, evolution,

expression and function of the members of the important gene family MAF/FLC that controls flowering time in *Brassica rapa*. The results showed that the *AtMAFs* gene in *Arabidopsis thaliana* had high sequence similarity with *BrMAFs* gene in *Brassica rapa*, but their gene functions were different between the two species. In addition, the analysis of gene expression level confirmed the triplication event of *Brassica rapa* gene.

With the continuous development of biological sequencing technology, it is more and more common to explore the molecular mechanism of plant morphology and traits by resequencing technology, especially in major crops and vegetables (Cheng et al., 2016). Genome-wide association analysis (GWAS) using resequencing technology plays an important role in the analysis of complex quantitative traits in plants. At present, GWAS has been successfully applied in the association analysis of plants and traits. Association analysis is widely used in model plants of various families. Xuan et al. (2019) screened new candidate genes *BnaA.GL1.a*, *BnaC.SWEET4.a*, *BnaC.WAT1.a*, and *BnaC.WAT1.b* related to leaf trichome formation in *Brassica napus* by GWAS indicating that sugar and auxin signaling are involved in leaf trichome initiation. 31 soybean flowering time traits related candidate genes *Glyma10g36600* and *Glyma19g37890* were obtained by resequencing and genome-wide association analysis (Song, 2018).

In addition, GWAS plays an important role in screening candidate genes related to flowering time traits in maize (Li et al., 2020), candidate genes related to seminal root length of maize seedlings under drought stress (Guo et al., 2019), candidate genes related to seed density within per silique and its related traits in *Brassica napus* (Ren et al., 2018), and candidate genes controlling continuous storage root formation and bulking in potato (Bararyenya et al., 2020). The study on flowering time is of great value for the genetic breeding of yield and quality of *Brassica napus*. In this study, 110 *Brassica campestris* collected nationwide were used as the research objects. The high-quality SNP polymorphism markers of 110 materials were obtained by resequencing. Combined with the flowering schedule type and its significantly associated SNP, the flowering time related candidate genes were determined according to the linkage disequilibrium intensity and gene annotation, which provided effective molecular markers and theoretical basis for the genetic breeding of early or late flowering materials.

1 Results and Analysis

1.1 Obtain of resequencing data

After strict filtering of the sequencing data, high-quality sequencing data were obtained. The total amount of sequencing data was 300.297 G, and the amount of high-quality data was 299.751 G. The genome size was 351 063 200 bp (excluding N), and the GC content was 36.83 %. The average alignment rate of population samples was 97.25%. The average sequencing depth of genome was 6.55 (only considering the sequence with alignment quality greater than 0), and the average coverage of samples was 83.85%. A total of 6 619 760 SNP loci were detected by SAMTOOLS software. After further strict filtration, a total of 140 164 high-quality SNP loci were finally obtained for subsequent analysis.

1.2 Flowering schedule type of *Brassica campestris*

The flowering time of 110 *Brassica campestris* was observed and recorded. The results showed that the flowering time of *Brassica campestris* from different sources was quite different (Table 1), and the variation range of flowering time was 122~156 d. Some materials from Sichuan, Yunnan, Tibet and Fujian flowered earlier, the flowering time was about 125 d, while some materials from Jiangsu and Qinghai flowered later, and the flowering time was more than 150 d. There were 24 materials with flowering time range of 122~125 d, 36 materials with flowering time range of 126~132 d, 25 materials with flowering time of 133~143 d, 16 materials with flowering time of 144~151 d, and 6 materials with the latest flowering time of 152-156 d. The flowering time of 110 materials showed partial normal distribution (Figure 1).

Table 1 Information and flowering time of *Brassica campestris*

Field code	Name of material	Collection place	Flowering time (d)	Field code	Name of material	Collection place	Flowering time (d)
7 503	Ganyuhuangyacai	Jiangsu	150	7 521	Gushihuaziyoucai	Henan	141
7 504	Guannanbaicai	Jiangsu	150	7 522	Shangchengxiaohuangyoucai	Henan	141
7 505	Kunshanhuangcaizi	Jiangsu	150	7 523	Luoshantuyoucai	Henan	141
7 506	Kunshanhuangzicai	Jiangsu	150	7 524	Jinxiansanyuehuang	Jiangxi	124
7 507	Lianshuibaicai	Jiangsu	156	7 525	Baojinghuayoucai	Hunan	130
7 508	Aijiaohuang	Jiangsu	142	7 526	Haiziyoucai	Guizhou	127
7 509	Mayeheicai	Jiangsu	150	7 527	Longquanyoucai	Guizhou	127
7 510	Xiaoroucai	Jiangsu	152	7 528	Xiaosonglinyoucai	Guizhou	127
7 511	Siyuebai	Jiangsu	154	7 529	Ziyundayoucai	Guizhou	127
7 512	Chehacai	Jiangsu	156	7 530	Gaopingguanshanyoucai	Guizhou	127
7 513	Lvyexiangbianbaicai	Jiangsu	144	7 531	Ganlongbaiyoucai	Guizhou	127
7 514	Xiangqingcai	Jiangsu	156	7 532	Yanhebaiyoucai	Guizhou	127
7 515	Suzhouqing	Jiangsu	141	7 533	Shaziyoucai	Guizhou	126
7 516	Yizhengwucai	Jiangsu	143	7 534	Zhenfengyoucai	Guizhou	126
7 517	Guangshansiyuehuang	Henan	137	7 535	Wangbianyoucai	Guizhou	126
7 518	Guangshantuyoucai	Henan	132	7 536	Sansuibendihuangyoucai	Guizhou	126
7 519	Guangshanhuayoucai	Henan	137	7 537	Kaiyangtianyoucai	Guizhou	127
7 520	Pingqiaoxiaoyoucai	Henan	138	7 538	Xixianheiyoucai	Henan	156
7 539	Jianghuangzhong	Zhejiang	131	7 558	Damulusikang	Xinjiang	127
7 540	Gaoqizhong	Zhejiang	148	7 559	Xiaoriqi	Neironggu	127
7 541	Denglongzhong	Zhejiang	133	7 560	Xiaohuangyoucai	Neimenggu	127
7 542	Wuxintuhuangzi	Zhejiang	150	7 561	span	Canada	127
7 543	Deqingjincaizi	Zhejiang	143	7 562	Polar	Canada	127
7 544	Xiashihuangzi	Zhejiang	143	7 563	Hexianyoucai	Anhui	148
7 545	Haiyanhuangcaizi	Zhejiang	143	7 567	Hja.Vankka	Fenlan	133
7 546	Changgenbai	Zhejiang	143	7 568	Hja 96337	Fenlan	148
7 547	Hangzhouhuangshanzi	Zhejiang	153	7 569	Yinhuang	Zhejiang	135
7 548	Shaoxinhuayoucai	Zhejiang	132	7 570	Suzhoujidanbai	Jiangsu	134
7 549	Zhenyuanminzhuyoucai	Yunnan	142	7 571	Wuxiancangcaizi	Jiangsu	136
7 550	Yuanyangheicaizi	Yunnan	129	7 572	Wuxianyinghuacaizi	Jiangsu	134
7 551	Fuzhouyoucai	Fujian	125	7 573	Kunshanmaquedan	Jiangsu	143
7 552	Fuqingaijiaoyoucai	Fujian	125	7 574	Kunshanaiqilvzhouwu	Jiangsu	131
7 553	Xiapuaijiaoyoucai	Fujian	125	7 575	Tailunsiyuehuang	Jiangsu	141
7 554	Wulumuqiheiyoucai	Xinjiang	125	7 576	Changshusanyuehuang	Jiangsu	134
7 555	Qitaiheiyoucai	Xinjiang	125	7 577	Wujiangjiangmeiwu	Jiangsu	129
7 556	Kuchezaoshuyoucai	Xinjiang	126	7 578	Jiashanheiyoucai	Anhui	129
7 557	Baichengheiyoucai	Xinjiang	127	7 579	Xunxixiaoyoucai	Hubei	129
7 580	Changshahuangyoucai	Hunan	129	7 599	Q-101	Xizang	126
7 581	Hukouyihao	Jiangxi	127	7 600	Q-102	Xizang	147
7 582	Guangchangheicaizi	Jiangxi	127	7 601	Q103-5	Xizang	124
7 583	Xiezuoyihao	Sichuan	124	7 602	Q-105	Xizang	124
7 584	Xindutizixuan	Sichuan	124	7 603	Q-107	Xizang	134
7 585	Xiaohuangyoucai	Guizhou	124	7 604	Q-109	Xizang	124
7 586	Zhenkanhongyoucai	Yunnna	124	7 605	Q-110	Xizang	124
7 587	Yunxianaijiaoyoucai	Yunnna	124	7 606	Q-114	Xizang	122
7 588	Jiangchengheicaizi	Yunnna	124	7 607	Qinghai-1	Qinghai	151
7 589	Zhenyuanxintangheihongyoucai	Yunnna	124	7 608	Qinghai-2	Qinghai	150
7 591	Q-25-7	Xizang	124	7 609	Qinghai-3	Qinghai	150
7 592	Q27 -4	Xizang	124	7 610	Qinghai-4	Qinghai	150
7 593	Q56-3	Xizang	139	7 611	Qinghai-5	Qinghai	150
7 594	Q-67 -1	Xizang	125	7 612	Qinghai-6	Qinghai	150
7 595	Q-80-3	Xizang	148	7 613	Qinghai-7	Qinghai	150
7 596	Q84-2	Xizang	126	7 614	Qinghai-8	Qinghai	124
7 597	Q-91	Xizang	133	7 615	Haoyou11hao	Qinghai	122
7 598	Q-100	Xizang	126	7 616	Qingyou241	Qinghai	122

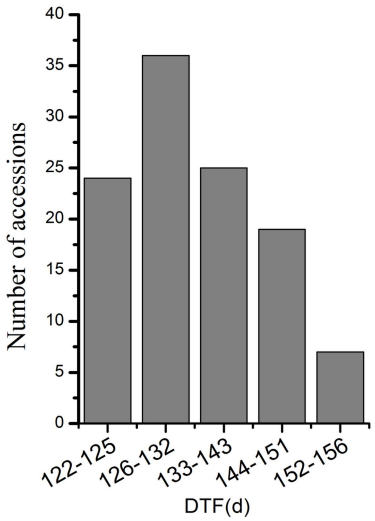

Figure 1 The frequency distribution of flowering time of 110 *Brassica campestris* accessions

1.3 Analysis of population structure and genome-wide linkage disequilibrium

140 164 high-quality SNP loci obtained by resequencing were used to analyze the population structure of 110 materials. The results showed that △K had the maximum change when K=2 (Figure 2A). At the same time, the population phylogenetic tree analysis of 110 materials showed that the 110 *Brassica campestris* populations could be divided into two subgroups. The small subgroup included 28 materials, mainly from spring and part of semi-winter rapeseed in Tibet, Qinghai and Jiangsu, and the large subgroup included 82 materials, mainly from semi-winter rapeseed lines in China (Figure 2B). The population evolutionary tree analysis of 110 *Brassica campestris* materials was performed, and it was found that the internal distribution of each subgroup was relatively concentrated, and the distribution between the two subgroups was also closely related to the geographical origin of the material. In addition, the population genetic structure and principal component analysis (PCA) were carried out, and the results verified the phylogenetic tree results, that was appropriate to divide 110 population materials into two subgroups, and the materials with similar geographical locations had close genetic relationship. And clustered together in the figure, the farther the geographical distance was, the greater the genetic background difference was (Figure 2C; Figure 2D; Table 2).

Genome-wide linkage disequilibrium analysis (Figure 2E) was performed using 140 164 high-quality SNP loci combined with flowering time of 110 *B. campestris* materials. The results showed that when R2 decreased from 0.167 to 0.08, the corresponding physical length was 19 kb. Therefore, the average genome-wide LD of *B. campestris* was 19 kb.

The GEMMA software was used to perform genome-wide association analysis on the flowering time of the population using the mixed linear model, and the linkage strength analysis was performed on the obtained signal points. When -log10(P) > 5, 4 signal sites significantly associated with flowering schedule type were obtained, among which 3 were located on chromosome A05, mutated from base C to T, 1 was located on chromosome A10, mutated from base G to A (Figure 3; Table 3).

1.4 Candidate gene prediction

The flowering time candidate sites were determined according to the significantly associated SNP sites and linkage disequilibrium interval, and the candidate genes related to flowering time in the site were further predicted. 9 candidate transcripts were screened from 4 significant association sites. Further analysis of the 9 transcripts showed that the 9 transcripts corresponded to 4 candidate genes, namely *Bra010171*, *Bra036954*, *Bra030175*, and *Bra030176*, which corresponded to At1g32540 (*LOL1*), At2g34960 (*CAT5*), At5g05580 (*FAD8*), and At1g74530 (unknown) genes in *Arabidopsis thaliana*. Whether these 9 transcripts and 4 candidate genes are related to flowering time will be further verified (Table 4).

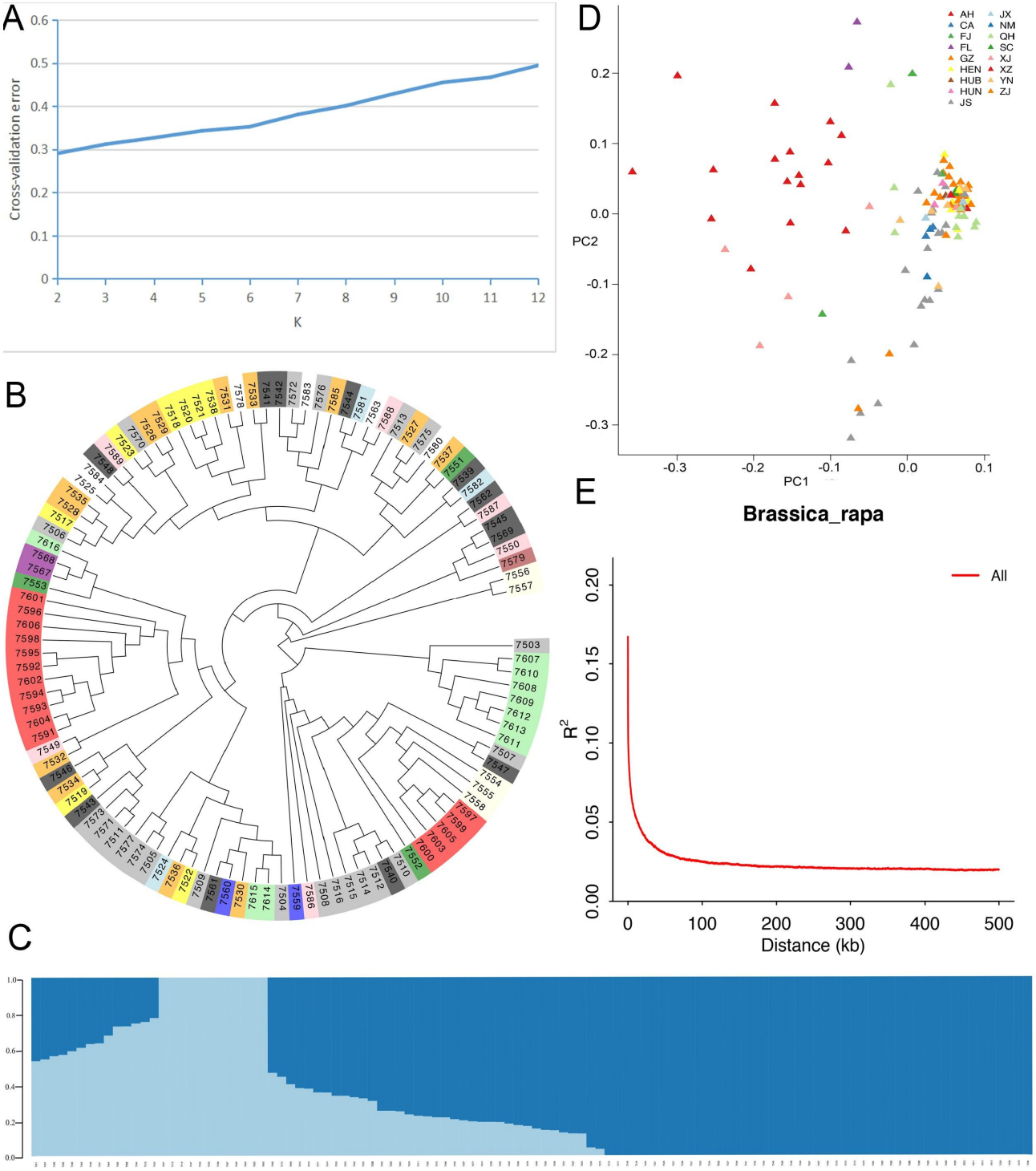

Figure 2 Population structure of 110 *Brassica campestris* materials

Note: A: Estimation of the number of sub-populations of 110 *Brassica campestris* L. materials; B: Neighbor-joining tree of the 110 *B. campestris* L. materials; C: Population structure of 110 *B. campestris* L. materials based on SNP markers; D: PCA of 110 *B. campestris* L. materials; E: The genome-wide LD decay in *B. campestris* L.

Table 2 Color information of 110 *Brassica campestris* samples in the figures

Field code	Collection place	Background color	Field code	Collection place	Background color	Field code	Collection place	Background color
7 503	Jiangsu	Grey	7 540	Zhejiang	Black	7 580	Hunan	White
7 504	Jiangsu	Grey	7 541	Zhejiang	Black	7 581	Jiangxi	Lightblue
7 505	Jiangsu	Grey	7 542	Zhejiang	Black	7 582	Jiangxi	Lightblue
7 506	Jiangsu	Grey	7 543	Zhejiang	Black	7 583	Sichuan	White
7 507	Jiangsu	Grey	7 544	Zhejiang	Black	7 584	Sichuang	White
7 508	Jiangsu	Grey	7 545	Zhejiang	Black	7 585	Guizhou	Orange
7 509	Jiangsu	Grey	7 546	Zhejiang	Black	7 586	Yunnan	Pink
7 510	Jiangsu	Grey	7 547	Zhejiang	Black	7 587	Yunnan	Pink
7 511	Jiangsu	Grey	7 548	Zhejiang	Black	7 588	Yunnan	Pink
7 512	Jiangsu	Grey	7 549	Yunnan	Pink	7 589	Yunnan	Pink
7 513	Jiangsu	Grey	7 550	Yunnan	Pink	7 591	Xizang	Red
7 514	Jiangsu	Grey	7 551	Fujian	Green	7 592	Xizang	Red
7 515	Jiangsu	Grey	7 552	Fujian	Green	7 593	Xizang	Red
7 516	Jiangsu	Grey	7 553	Fujian	Green	7 594	Xizang	Red
7 517	Henan	Yellow	7 554	Xinjiang	White	7 595	Xizang	Red
7 518	Henan	Yellow	7 555	Xinjiang	White	7 596	Xizang	Red
7 519	Henan	Yellow	7 556	Xinjiang	White	7 597	Xizang	Red
7 520	Henan	Yellow	7 557	Xinjiang	White	7 598	Xizang	Red
7 521	Henan	Yellow	7 558	Xinjiang	White	7 599	Xizang	Red
7 522	Henan	Yellow	7 559	Neimeng	Blue	7 600	Xizang	Red
7 523	Henan	Yellow	7 560	Neimeng	Blue	7 601	Xizang	Red
7 524	Jiangxi	Lightblue	7 561	Canada	Black	7 602	Xizang	Red
7 525	Hunan	White	7 562	Canada	Black	7 603	Xizang	Red
7 526	Guizhou	Orange	7 563	Anhui	White	7 604	Xizang	Red
7 527	Guizhou	Orange	7 567	Finland	Purple	7 605	Xizang	Red
7 528	Guizhou	Orange	7 568	Finland	Purple	7 606	Xizang	Red
7 529	Guizhou	Orange	7 569	Zhejiang	Black	7 607	Qinghai	Lightgreen
7 530	Guizhou	Orange	7 57 0	Jiangsu	Grey	7 608	Qinghai	Lightgreen
7 531	Guizhou	Orange	7 571	Jiangsu	Grey	7 609	Qinghai	Lightgreen
7 532	Guizhou	Orange	7 572	Jiangsu	Grey	7 610	Qinghai	Lightgreen
7 533	Guizhou	Orange	7 573	Jiangsu	Grey	7 611	Qinghai	Lightgreen
7 534	Guizhou	Orange	7 574	Jiangsu	Grey	7 612	Qinghai	Lightgreen
7 535	Guizhou	Orange	7 575	Jiangsu	Grey	7 613	Qinghai	Lightgreen
7 536	Guizhou	Orange	7 576	Jiangsu	Grey	7 614	Qinghai	Lightgreen
7 537	Guizhou	Orange	7 577	Jiangsu	Grey	7 615	Qinghai	Lightgreen
7 538	Henan	Yellow	7 578	Anhui	White	7 616	Qinghai	Lightgreen
7 539	Zhejiang	Black	7 579	Hubei	Brown			

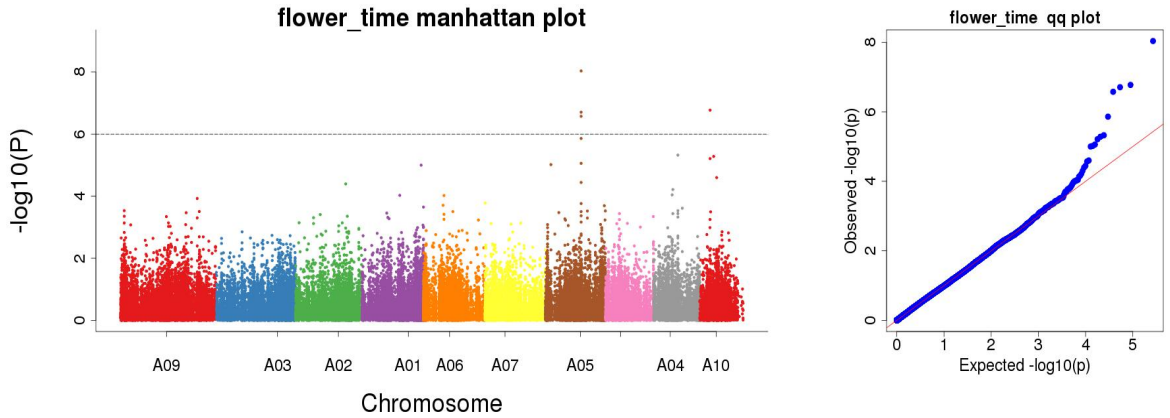

Figure 3 Manhattan plot and qq plot of genome-wide association study for flowering time in *B. campestris*

Table 3 The strong association signals and functional annotation

Chromosome	Location	Adjacent gene	-log (p_value)	Minimum allele frequency	Distance	Base mutation
A05	16 943 383	BraA05g023100.3C	8.04	0.3 364	18 653	C→T
A05	16 943 901	BraA05g023110.3C	6.58	0.4 454	-7 228	G→A
A05	16 945 137	BraA05g023120.3C	6.71	0.2 629	-12 449	T→A
A10	5 004 502	BraA10g007660.3C	6.77	0.0 561	13 115	G→A
A10	5 004 502	BraA10g007670.3C	6.77	0.0 561	11 533	G→A
A10	5 004 502	BraA10g007680.3C	6.77	0.0 561	9 524	G→A
A10	5 004 502	BraA10g007690.3C	6.77	0.0 561	6 586	G→A
A10	5 004 502	BraA10g007700.3C	6.77	0.0 561	-2 773	G→A
A10	5 004 502	BraA10g007710.3C	6.77	0.0 561	-9 912	G→A

Table 4 Candidate genes for flowering time of *Brassica campestris*

Transcript_ID	Corresponding candidate gene	Corresponding *Arabidopsis*	*Arabidopsis* gene annotations
BraA05g023100.3C	no	NA	NA
BraA05g023110.3C	no	NA	NA
BraA05g023120.3C	Bra010 171	At1g32 540,	*LOL1*(*LSD ONE LIKE 1*), DNA
BraA10g007660.3C	Bra036 954	At2g34 960,	*CAT5* (*CATIONIC AMINO ACID*
BraA10g007670.3C	Bra036 954 and Bra030 175	At2g34 960 and At5g05 580	*CAT5* and *FAD8* (*FATTY ACID*
BraA10g007680.3C	no	NA	NA
BraA10g007690.3C	Bra030 176	At1g74 530	Unknown protein
BraA10g007700.3C	no	NA	NA
BraA10g007710.3C	no	NA	NA

2 Discussion

Flowering time is an important agronomic trait affecting the yield and quality of *Brassica rapa*. There are many factors affecting the flowering time of *Brassica rapa*, including the complex gene regulatory network in plants and the external environmental factors. Previous studies have shown that the differential expression of *BrFLCs* (*BrFLC1*, *BrFLC2*, *BrFLC3*, *BrFLC4*, and *BrFLC5*) gene leads to the difference of flowering time in Brassica crops. Multi-copy *BrFLCs* genes regulate flowering time by the interaction of additive effects. The higher the level of *FLC* transcripts, the longer the flowering time of plants. The results showed that 5 natural antisense transcripts of *BrFLC2* were induced to express and the expression of 4 *BrFLC* genes decreased gradually during vernalization of Chinese cabbage Bre. In addition, the results showed that transgenic plants overexpressing natural antisense transcripts of *BrFLC2* did not require vernalization at low temperature, which confirmed that natural antisense transcripts of *BrFLC2* could change the growth cycle of Chinese cabbage (Li et al., 2016). Li (2019) found that 2 insertions/deletions (76-bp) in *BnFLC-C2* promoter region of *Brassica napus* were closely related to flowering time. Zhang (2018) found that the point mutation of *BcFLC1* gene in WuCai (*Brassica campestris* L.) could affect its interaction with *SVP* gene, further affecting the expression of downstream *FT* and *SOC1* genes, resulting in the late bolting phenotype of WuCai (*Brassica campestris* L.). Cheng (2017) et al. summarized 6 pathways regulating flowering time in *Arabidopsis thaliana*, and pointed out that *FCA*, *FLD*, *FLK*, *FPA*, *FVE*, *FY* and *LD* were involved in the autonomous flowering pathway, in which the flowering suppressor gene *FLC* played an important role in the autonomous flowering pathway. In recent years, there are more and more studies on agronomic traits of crops using genome-wide association analysis. Gao et al. (2017) used 116 natural populations of *Brassica rapa* as research materials. Resequencing technology and GWAS analysis were used to locate candidate genes related to flowering time of *Brassica rapa*, and 33 significant correlation signals related to flowering time were identified. Finally, 14 candidate genes related to flowering time of *Brassica rapa* were preliminarily identified, including *Bra022475*, *Bra029347*, *Bra030284*, *Bra022192*, and *Bra024337*, which were homologous genes of Arabidopsis *FT1*, *FUL*, *GRP7*, *PHYB*, and *HYS1*. In this study, 110 natural populations of *Brassica campestris* were used as the research objects to investigate their flowering time and resequencing the natural population. The obtained SNP markers were used for GWAS analysis, and 4 signal sites significantly

associated with flowering schedule type were identified. A total of 9 candidate transcripts were selected from these 4 significant association sites. 4 candidate genes related to flowering time of *Brassica campestris* were obtained through gene collinearity and gene function annotation between B. *Brassica campestris* and *Arabidopsis thaliana*, which were homologous genes of *Arabidopsis thaliana LOL1*, *CAT5*, and *FAD8*. It has been reported that *LOL1* gene may be involved in the response of *Fagopyrum tataricum* to abiotic stresses such as high concentration of salicylic acid, UV-B irradiation, and cold stress (Gao et al., 2015). Epple et al. (2003) identified two *LSD1*-like genes *AtLOL1* and *AtLOL2* in *Arabidopsis thaliana*. The results showed that *AtLSD1* and *AtLOL1* regulated the accumulation of Cu-ZnSOD by antagonism to determine the fate of cells. Yeh et al. (2011) found similar results by functional study of *LSD1*. The results showed that these *LSD1* zinc finger proteins had regulatory functions on allergic cell death, plant spectral disease resistance, and abiotic environmental stresses such as low temperature and long sunshine. The candidate gene *Bra036954* identified in this study is a homologous gene of *CAT5* in *Arabidopsis thaliana*. *CAT5* is a basic amino acid transporter with high affinity on the cell membrane, and its function may be to reabsorb the leaked amino acids at the leaf edge (Su et al., 2004; Zhao et al., 2012). The homologous gene corresponding to the candidate gene *Bra030175* is the fatty acid desaturase gene *FAD8*. It has been reported that the *FAD* gene family is not only an important gene regulating plant fatty acid composition, but also involved in the regulation of plant cold stress, heat stress and drought stress response (Tovuu et al., 2016; Zhang et al., 2018). The functions of the 4 candidate genes identified by us in flowering time have not been reported. Therefore, whether the 4 candidate genes related to flowering time of *Brassica campestris* obtained in this study are involved in flowering regulation remains to be further identified.

3 Materials and Methods

3.1 Materials and reagents

A total of 110 test materials *Brassica campestris* were widely collected from various regions in China and abroad. The 110 materials were natural populations, whose names, field numbers, geographical sources and flowering time were recorded in Table 1. And all materials were planted in the open field at the base of Zhuanghang Comprehensive Experiment Station of Shanghai Academy of Agricultural Sciences (Altitude 3.3 m, 121°38′ E, 30°88′ N).

3.2 Investigation on flowering time

The flowering time from sowing to 1/4 number of plants is considered to be the first flowering stage, and the time required for each material from sowing to first flowering is recorded as its day to flowering (DTF). The flowering time of 110 *Brassica campestris* materials was observed and recorded at flowering stage.

3.3 Genome-wide resequencing

The young leaves of 110 *Brassica campestris* materials at seedling stage were selected, and 3 typical plants of each material were selected as 3 biological replicates. The DNA of each sample was extracted by CTAB method. Illumina PE150 sequencing method (Illumina Inc., USA) was used to perform double-terminal resequencing of 110 sample DNA, resulting in a 150 bp double-terminal sequence (Reads) with a total sequencing data of 300.297 G. The raw data obtained by sequencing are strictly filtered to obtain high-quality sequencing data. The effective high-quality sequencing data were compared to the reference genome of Chinese cabbage by BWA software (Li and Durbin, 2009), and the repeat sequences were removed by SAMTOOLS software (Li et al., 2009), and the SNP polymorphism of the population was detected. Then, the Bayesian model was used to detect the polymorphic loci in the population, and high-quality SNPs were obtained through the 3 strict filtering conditions of Q20 quality control, SNP site spacing requirements and SNP coverage depth requirements. Finally, the strong annotation function of ANNOVAR (Wang et al., 2010) was used to annotate the SNPs detection results.

3.4 Population genetic diversity analysis

The high-quality SNPs data obtained by resequencing were used for population evolutionary tree analysis, principal component analysis, and population genetic structure analysis of 110 *Brassica campestris* materials. Neighbor method was used to construct population evolutionary tree, and the leading value is calculated 1000 times. The guiding value was obtained after 1000 calculations. Population principal component analysis was used

to draw the PCA distribution map by GCTA software and R software. Population genetic structure analysis was carried out by PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/), and the results were presented in the form of population genetic structure map.

3.5 Genome-wide linkage disequilibrium analysis

Haploview software was used for genome-wide linkage disequilibrium analysis of filtered high-quality SNP set sites.

3.6 Genome-wide association analysis

GEMMA software (http://www.xzlab.org/software.html) and mixed linear model were used to perform association analysis of flowering time traits in 110 *Brassica campestris* natural populations. According to the significance of association, potential candidate SNPs related to flowering time traits were screened out. Transcripts or genes in a certain area of upstream and downstream of the chromosome position of candidate SNPs were screened and functional analysis was conducted to predict candidate genes that may be related to flowering time of *Brassica campestris*.

Authors' contributions

JJX conceived of this review, wrote the first draft, and carried out sequencing analysis and florescence observation and statistics. LYL, JMY, and ZJF were responsible for consulting and sorting out relevant documents, as well as sequencing data analysis. ZXR, WWR, and SC revised the manuscript. ZJY, and YLY were corresponding authors, who were responsible for this review. All authors read and approved the final manuscript.

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