Genetic analysis of flowering time and photoperiod sensitivity in rapeseed (*Brassica napus* L.)

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Abstract

One doubled haploid (DH) population used to perform the inheritance of flowering time and photoperiod sensitivity of spring *Brassica napus* in this study was produced from F_1 plants of the cross between Hyola401 (P_1) and Q2 (P_2), two spring *Brassica napus* cultivars exhibiting a low and high sensitivity to photoperiod, respectively. Days to flower (DTF) were investigated in the P_1, P_2 and DH population at Gansu with a long day and at both Guangdong and Wuhan with a short day in two years. Photoperiod sensitivity index (PSI) was calculated according to DTF obtained from Gansu and Guangdong. The means of DTF and PSI were analyzed by a mixed major gene and polygene inheritance model. The results showed that DTF at Gansu, Wuhan and Guangdong was controlled by 3, 2 and 2 pairs of major genes with additive effect, respectively, and plus polygenes with epistatic and additive effects, respectively. Heritability values of the major genes and polygenes at the three locations were 91.13% and 4.43%, 63.05% and 1.58%, and 62.02% and 4.43%, respectively. The PSI was conditioned by 2 pairs of major genes with epistatic recessive effect and polygenes. The heritability values of the major genes and polygenes were 50% and 37.5%, respectively. Other genetic parameters were also estimated. Therefore, it is speculated that both flowering time and photoperiod sensitivity in this DH population are controlled by 2 or over 2 pairs of major genes and polygenes, and different photoperiods resulted in different interaction models among flowering genes. The implications for *B. napus* breeding have been discussed.

Key words: *Brassica napus* L., flowering time, photoperiod sensitivity index, mixed major gene and polygene inheritance model, genetic analysis

Introduction

Brassica napus L. is one important oilcrop of *Brassica*. That it flowers early or late will seriously affect the harvesting time, field and planting adaptability. As we know, the major factor influencing the flowering time of spring *Brassica napus* L is photoperiod sensitivity. The relatively minor affecting factor is temperature. As a result, it is very important for practical breeding to research the genetic control of flowering time and photoperiod sensitivity of *Brassica napus* L. Until now, a lot of plant scientists from home and abroad have done much in inheritance characteristics and genetic control of flowering time and photoperiod sensitivity of *Brassica napus* L and other *Brassica* species(Grant & Beversdorf, 1985; Zaman, 1989; Guo & Gai, 1998; Guan & Gai, 1997; Gai et al, 1999; Teutonico & Osborn, 1994; Ferreira et al, 1995, Osborn et al, 1997; Bohuon et al, 1998; Camargo & Osborn, 1996; Lagercrantz et al, 1996). The results almost showed that flowering time in rapeseed was one quantitative trait conditioned by several major genes with polygenes. However, as for research on photoperiod sensitivity in rapeseed, it was seldom reported(Robert et al, 1998). Many important genes controlling these traits have been finely mapped or even cloned in model plant rice and *Arabidopsis*(Putterill et al, 1995, Yano et al, 2000; Yamamoto et al, 2000; Kojima et al, 2002). That genome project was completed sped up such research. The present paper studied the genetic control of flowering time and photoperiod sensitivity in one DH population derived from a cross of Hyola401 and Q2 of spring *Brassica napus* L by applying a mixed major and polygene inheritance model of plant quantitative trait.

Material and Methods

Plant materials and field experiments: The materials used in this study were two spring canola *B. napus* cultivars Hyola401 and Q2, exhibiting a low and high photoperiod sensitivity, respectively. Doubled haploid (DH) plants produced from F_1 plants of the cross Hyola401 × Q2 using the microspore culture developed by Shi and Liu (1993). DH population and their parents, were grown in the summer-autumn growing season in Hezheng (China, 2003 and 2004) with a 14.3 h day length and a 15.1°C average temperature and in the autumn-spring growing season in Zhaoqing (China, 2003 and 2004) with a 10.1 h day length and a 15.5°C average temperature and in Wuhan (China, 2003 and 2004) with a 10.4 h day length and a 15.4°C. At each location a randomized complete block design was used with two replications. Each DH line and parent comprising 40-45 individuals planted in two rows with 15 cm between plants and 30 cm between rows.

Traits measurements: DTF was recorded as the number of days from the sowing date to the date when 50% of the plants in one DH line or parent had at least one open flower. The mean DTF of each DH line was used for QTL analysis. The degree of PS of each DH line or parent was calculated by the delay in DTF in Zhaoqing as compared to DTF in Hezheng.

Genetic analysis: The mean of flowering time and photoperiod sensitivity index of p_1, p_2 and DH population in two years were used to do genetic analysis of the two traits by applying one mixed major and polygene inheritance model (Gai & Wang, 1998; Hu & Zhang, 1998; Zhang et al, 2001; Gai et al, 2003). This model included seven main types from A to G and was divided into 37 kinds of genetic models in this study. A, B C, D, E, F and G means one major gene model, two major gene

model, polygene model, one major gene plus polygene model, two major gene plus polygene model, three major gene model and three major gene plus polygene model, respectively. Distribution parameters in mixed model were estimated by max-likelihood and iterated expectation and conditional maximization (IECM). The best-fit model was selected through discriminating Akaike's information criterion (AIC), testing likelihood ratio (LR) and examining one group of suitability parameters (U_1^2, U_2^2, U_3^2, nW^2, D_n).

Results

Variance analysis showed that genotype, year and environment affected flowering time of DH population significantly ($p \leqslant 0.001$). Among the three factors, environment had the largest effect. In two way interactions, genotype × environment also exhibited a significant influence on flowering time ($p \leqslant 0.001$). However, there was no significant interaction between genotype and year ($p=0.74$). As a consequence, genotype displayed a special adaptability for environment. In other words, different environments (photoperiod) were the main external factors conditioning flowering time of spring canola *Brassica napus* (Table 1).

Table 1 The ANOVA of DTF in DH population

Source	*DF*	*MS*	*F* Value	*P*
G	148	1162.68	14.99***	⩽0.001
Y	1	39920.22	514.64***	⩽0.001
E	2	234203.31	3019.29***	⩽0.001
G×Y	148	70.573	0.91	0.74
G×E	296	229.034	2.95***	⩽0.001

Notes: *** represents significance at 0.001 probability level. G, Y and E represent genotype, year and environment, respectively.

The best-fit models for flowering time in different locations and for photoperiod sensitivity index were selected by methods introduced in material and methods (Table 2). The 3 pairs of major genes with additive-epistasis effect plus polygenes model (G-1), 2 pairs of major genes with additive-epistasis effect plus polygenes model (E-1-1) and 2 pairs of major genes with additive effect plus polygenes model (E-1-6) were the best-fit models for Hezheng, Wuhan and Zhaoqing, respectively. The inheritance of photoperiod sensitivity accorded with 2 pairs of major genes with additive-epistasis effect plus polygenes model. Flowering time of *Brassica napus* also was conferred by polygenes besides major genes under whether short day or long day. Additionally, photoperiod also was 1 quantitative trait controlled by major genes and polygenes simultaneously.

Table 2 Select lower AIC values of DTF and PSI in DH population from cross of Hyola401 × Q2

Location	Model	AIC value	Location	Model	AIC value
Hezheng	F-1	1201.839	Zhaoqing	G-2	1490.637
	G-1	1205.339		E-1-6	1494.515
	G-0	1207.309		E-1-7	1495.505
	E-1-0	1225.979		E-1-8	1495.505
	E-2-0	1228.039		G-3	1495.738
Wuhan	E-1-1	1465.257	PSI(photoperiod sensitivity index)	E-1-5	-59.5278
	E-1-5	1465.286		F-1	-58.6578
	E-1-4	1468.612		E-1-1	-57.8004
	E-2-4	1468.773		E-1-0	-57.6333
	E-2-5	1468.773		E-2-3	-57.5927

In Hezheng, 3 pairs of major genes contributed small effect on flowering time. However, the interactions between them (i_{ab}, i_{ac}, i_{bc}) all decreased flowering time by 1.3 d to 2.3 d. The heritability of major genes was 91.13%, the largest one among the 3 locations. This implied that there were many major genes with full expression conferring flowering time under long day condition. The heritability of polygenes was only 4.43%. In Wuhan, 2 pairs of major genes offered the same large, but contrary effect (-22.01 and 22.19). The interaction between them (i_{ab}) increased flowering time by 14.3 d. The heritability of major genes was 63.05%. The effective number of polygenes (K_g) was 4.77. This indicated that polygenes expressed more fully under short day condition than under long day condition. But its heritability was 1.58%. In Zhaoqing, 2 pairs of major genes had the same effect and increased flowering time by 15 d. But their interaction increased it by 35.9 d. The heritability of major genes was 62.02%. That of polygenes was 22.17%, the largest one in three locations. As for photoperiod sensitivity, there was small difference between 2 pairs of major genes. Their interaction was not tested. The heritability of major genes was 50%, which showed that photoperiod sensitivity was affected by environmental factors largely. That of polygenes was 37.5%. But the effective number of polygenes (K_g) was 0.12 (Table 3).

Discussion

DH population was obtained by microspore culture, but mutations of some important genes probably occurred because microspore was very sensitive to culture mediun components especially colchicines, temperature, photoperiod and so on. Therefore, the flowering time some of plants displayed difference deviated from expectation. In addition to this, there were

less than 30 plants in each plot, which maybe resulted in unstable flowering time, relatively early or late.

At present, there were some reports on classification of quantitative traits such as yellow seed (Rahman, 2001) and *Sclerotinia sclerotiorum* (Lid) (Zhou, 1994) in *Brassica napus*. As far as DH population in this study was concerned, Flowering time and photoperiod sensitivity displayed continuous, but non-normal distributions (Chart omitted). So how to get a scientific classification need further study.

Temperature was the second important factor affecting flowering time of spring rape besides photoperiod.

Table 3 Estimates of genetic parameters of DTF and PSI in DH population from cross of Hyola401 × Q2

Location	1st order para.									2nd order para.						
	M	d_a	d_b	d_c	i_{ab}	i_{ac}	i_{bc}	i_{abc}	[d]	σ_p^2	σ_{pg}^2	σ_{mg}^2	σ_e^2	h_{mg}^2(%)	h_{pg}^2(%)	K_g
Hezheng(G-1)	61.69	0.35	-0.21	-2.28	-2.21	-0.13	-1.25	7.29	-2.5	87.12	3.86	79.39	3.87	91.13	4.43	1.62
Wuhan(E-1-1)	141.45	22.19	-22.01		14.32				-6.5	562.36	8.86	354.58	198.92	63.05	1.58	4.77
Zhaoqing(E-1-6)	94.71	15.05	15.05		-35.87				-7	490.48	111.39	304.19	74.9	62.02	22.71	0.44
PSI(E-1-5)	0.47	0.2	0.16						-0.06	0.08	0.03	0.04	0.01	50	37.5	0.12

Notes: The best-fit genetic model in the brackets.

Therefore, it resulted in a large difference of major genes' effect between Wuhan and Zhaoqing though with a small photoperiod difference (0.3 h). There was a small difference for the heritability of major genes between Wuhan and Zhaoqing, but a large difference for that of polygenes, which implied that the major genes controlling flowering time were mainly affected by photoperiod and that the polygenes were still influenced by temperature besides photoperiod. Additionally, the coefficient of variation (CV) of flowering time was 10%, 18% and 22% in Hezheng, Wuhan and Zhaoqing, respectively. As a consequence, DH population displayed much trimmer for flowering time under long day condition than under short day condition. In other words, it was better to identify the photoperiod sensitivity of DH lines under short day than long day. This study analyzed the primary genetic characteristics of flowering time and photoperiod sensitivity of spring canola *Brassica napus*. But that how many genes controlled these two traits on earth need to perfect inheritance models and carry out further molecular biology analysis such as quantitative trait loci (QTL) mapping.

Conclusion

The present study made a detailed analysis for number of major genes, genes effect, gene interaction, number of effective polygenes and so on by utilizing the mixed major and polygenes inheritance model. The results showed that flowering time and photoperiod sensitivity mainly were conditioned by over two pairs of major genes and modified by polygenes. This not only set up a good base for QTL mapping of genes controlling flowering time and photoperiod sensitivity of *Brassica napus*, but also supplied a valuable guidance for breeding insensitive *Brassica napus* varieties with broad geography adaptability.

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