Preliminary study on inheritance of an artificially resynthesized white flower line in *Brassica napus* L.

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Abstract

A group of white flower lines of the resynthesized *B.napus* have been developed from the crosses between *B.campestris* L. and *B. oleracea var. Alboglabra Bailey* collected in Southwestern China. A pure white flower line, 'HW243' (P_2), from the resynthesized *B.napus* was crossed reciprocally to a common *B.napus* cultivar 'Westar' (P_1). The F_1 hybrid of 'Westar×Hw243' (F_1) was selfed and backcrossed to both parents to obtain F_2, BC_1 and BC_2 populations, and the variation in flower color and segregation rule were investigated with the six populations P_1, P_2, F_1, F_2, BC_1 and BC_2. The results showed that the petal color of F_1 was milky white and there was no obvious difference in petal color between the reciprocal F_1 hybrids. This indicated that the white color of the petals in the line was controlled by nuclear genes without cytoplasmic effect and that the white color was incompletely dominant to yellow color. The petal colors in the F_2 population could be roughly classified into 3 classes, i.e. pure white, milky white and yellow. Based on the investigations of two seasons at two locations the ratio of plants with pure white, milky white and yellow flower in the F_2 populations was 1:11:4 (P=0.8-0.9); the ratio of plants with yellow flower and milky white flower in the BC_1 population was 1:1 (P=0.3-0.7), and the ratio of plants with milky white and white flower in the BC_2 population was 3:1 (P=0.5-0.7). It was supposed that the white flower character in this resynthesized line was controlled by two interacting duplicate genes which were incompletely dominant to the yellow color genes. It was presumed that the genotype of white flower parent was $W_1W_1W_2W_2$, the genotype of yellow flower parent was $w_1w_1w_2w_2$, and the genotype of F_1 was $W_1w_1W_2w_2$. The petal color of F_1 was milky white. In F_2, the genotype of pure white flower offspring was $W_1W_1W_2W_2$, taking a ratio of 1/16. The genotypes of yellow flower offspring were $w_1w_1w_2w_2$, $w_1w_1W_2w_2$ and $w_1w_1W_2W_2$, respectively, taking a ratio of 4/16. In the yellow color group, the homozygous w_1w_1 alleles were epistatic over the dominant W_2 alleles. The genotypes of milky white flower offspring were $W_1W_1W_2w_2$, $W_1W_1w_2w_2$, $W_1w_1W_2W_2$, $W_1w_1W_2w_2$ and $W_1w_1w_2w_2$, respectively, taking a ratio of 11/16. Nevertheless, a variation in whiteness in flower color was observed in the milky color group in F_2. It seemed that there was a gene-dosage effect on the whiteness of flower color, which appeared to be related to the number of dominant W_1 and W_2 alleles in the genome. Further investigations are necessary to fully understand the genetic rule of the white flower character.

Key words: *Brassica napus* L., white flower, inheritance

Introduction

The petal color of rapeseed was usually yellow. In recent years researchers have reported some different petal colors in rapeseed such as golden yellow, orange, orange yellow, fresh yellow, milky white and white, and so on. White flower may be used as a very good and useful morphological marker in rapeseed breeding and genetic studies. Sichuan Agricultural University Rapeseed Research Center has developed a group of white flowered resynthesized *B. napus* lines, which were derived from interspecific crosses between *B. campestris* L., or *B. chinenesis* L., and *B. oleracea var. Alboglabra* Bailey. The parent species in the wide crosses were all collected in the Southwestern regions of China. In this paper, a preliminary study on the genetic characteristics and rules of the white flower character is reported.

Material and Methods

Experimental materials

The parental materials were a white-flowered resynthesized *B. napus* line, 'HW243', and a yellow-flowered variety, 'Westar'. The resynthesized white-flowered line 'HW243'was developed from a wide cross of *B. campestris* L. with *B. oleracea* L. *var. Alboglabra* Bailey, collected in Southwestern China. The petal color of 'HW243' is purely white and genetically stable. The petal color of 'Westar' is typically yellow. The materials were supplied by the Sichuan Agricultural University Rapeseed Research Center. In Spring, 2004, the reciprocal F_1 hybrids were obtained with the cross combination, 'Westar×Hw243'. In Spring, 2005, the flower color of the reciprocal F_1 hybrids was observed and the F_1 hybrid of 'Westar×Hw243' was selfed and backcrossed to both parents to obtain F_2, BC_1((Westar×HW243) ×Westar) and BC_2((Westar×HW243) ×HW243).

Methods

Field experiment In Autumn, 2004, the parental materials, 'Westar' (P_1) and 'HW243' (P_2), and the reciprocal F_1 hybrids were grown in the field. In Spring, 2005, the flower colors of P_1, P_2 and reciprocal F_1's were observed with 30 individual plants each. The flower color of the reciprocal F_1's was compared and the two parents were used as the reference at the full blossom stage. In Autumn, 2005, the six generations of the hybrid from Westar×Hw243, P_1, P_2, F_1, F_2, BC_1 and BC_2, were grown in the field for observations of the genetic segregation of flower color. All of the materials were grown on the experimental farm of Sichuan Agricultural University, Ya'an, Sichuan, China. P_1, P_2 and F_1 were 50 plants each; F_2 population was 600 plants; BC_1 and BC_2 were 300 plants, respectively. The field experiment was laid out in a randomized complete block design, with a row length of 2.5m and a row spacing of 40cm.Ten plants were grown in a row with spacing of 25cm. In Summer, 2006, the six generations, P_1, P_2, F_1, F_2, BC_1, BC_2, were grown in Kangding, a high mountainous region (altitude 2650m) in Western Sichuan. The segregation of flower colors was further investigated. P_1, P_2 and F_1 were 30 plants, F_2 was 250 plants, and BC_1, BC_2 were 150 plants, respectively. The cultivation was the same as the former.

Observation of flower color The petal colors were observed at full blossom stage with naked eyes. The fully and freshly opened flowers on the primary branches were used to observe the flower color. The time of observation was 10 o'clock in the morning with cloudless weather. Each plant was observed at the same, and flower color was scored into three classes. The flower colors were observed once again on the next day. If the flower color was scored differently, a third observation was made to confirm it. The white flower petals showed a slight pale yellow color in advance of full opening and quickly turned to pure white color after they fully opened. It is, therefore, important to observe the petal color with freshly and fully opened flowers.

Three types of flower color were classified in the segregating F_2 population, i.e., white, milky white and yellow, using the two parents as reference.

Statistics Chi square test was used to examine the significance of the segregating ratios in the F_2, BC_1, BC_2 hybrid offspring. The chi square values were calculated with following formulae.

When three types of flower color were classified in the population (F_2):

$$X^2 = \sum_{i=1}^{k} \frac{(O_i - E_i)^2}{E_i}, \quad df \geqslant 2$$

When two types of flower color were classified in the population (BC_1, BC_2):

$$X^2 = \sum_{i=1}^{k} \frac{(|O_i - E_i| - 0.5)^2}{E_i}, \quad df = 1$$

where O_i is the actual frequency observed, E_i is theoretical frequency, k is number of observations.

Results

The flower color of F_1 hybrid

Observations of the flower color in the reciprocal F_1hybrids in both 2005 and 2006 showed that there was no obvious difference in flower color between the reciprocal F_1 hybrids. The petal color of F_1 hybrids was milky white, slightly inclined to the white flower parent. This suggested that the flower color in the lines was controlled by nucleate genes, without cytoplasmic influence.

The segregation of flower color in the hybrid offspring

The field investigations on segregation of flower color were carried in two different seasons (Spring and Summer) at two locations (Ya'an and Kangding) in 2006. The results were summarized in table 1. In the segregating populations F_2, BC_1 and BC_2 the flower colors could be roughly classified into three types, i.e., pure white, milky white and yellow. In the F_2 hybrid offspring all of the three kinds of flower color were observed. The ratio of pure white, milky white and yellow flowered plants was 1:11:4 (P=0.8-0.9). In the BC_1 population, two types of flower color, i.e., milky white and yellow were observed. The ratio of the two types of plants was 1:1 (P=0.3-0.7). In the BC_2 population, still two types of flower color were observed, i.e., milky white and pure white. The ratio of the two types of plants was 3:1 (P=0.5-0.7). The results from different seasons and locations were consistent, although the growing conditions were entirely different (table1).

Table 1 Segregation of flower colors in the six generations of Westar×HW243

Time	Populations	Number of plants			Total plants	Expect ratio	χ^2	P
		White	Milky	Yellow				
March, 2006(Ya'an)	P_1			46	46			
	P_2	49			49			
	F_1		42		42			
	F_2	37	395	150	582	1:11:4	0.216	0.8-0.9
	BC_1		144	131	275	1:1	0.524	0.3-0.5
	BC_2	78	215		293	1:3	0.329	0.5-0.7
July, 2006(Kang ding)	P_1			27	27			
	P_2	30			30			
	F_1		29		29			
	F_2	17	168	60	245	1:11:4	0.213	0.8-0.9
	BC_1		69	75	144	1:1	0.174	0.5-0.7
	BC_2	33	115		148	1:3	0.441	0.5-0.7

The model of inheritance for the white flower character

According to the segregation ratios of the petal color in table 1, it can be speculated that the white flower character in the studied material was controlled by two pairs of interacting nucleate genes. The white flower color was incompletely dominant to the yellow color. It is presumed that the genotype of the white flower parent was $W_1W_1W_2W_2$, the genotype of the yellow flower parent was $w_1w_1w_2w_2$ and the genotype of the F_1 hybrid was $W_1w_1W_2w_2$ (Fig. 1 A).

In the F_2 offspring, the genotype of the pure white offspring was $W_1W_1W_2W_2$, taking a ratio of 1/16. The genotype of the yellow flower offspring was $w_1w_1w_2w_2$, $w_1w_1W_2w_2$ or $w_1w_1W_2W_2$, taking a ratio of 4/16. In the yellow flowered offspring the homozygous recessive gene pair w_1w_1 acted epistatically over the dominant gene W_2, restricting the expression of the W_2 allele at the other locus. The genotypes $W_1W_1W_2w_2$, $W_1W_1w_2w_2$, $W_1w_1W_2W_2$, $W_1w_1W_2w_2$, $W_1w_1w_2w_2$ were all milky white flowered, taking a ratio of 11/16(Fig 1-A).

In the BC_1 population, the genotype of yellow flowered hybrid was $w_1w_1w_2$_, taking a ratio of 1/2. The genotype of milky flowered hybrid was $W_1w_1w_2$_, taking a ratio of 1/2. The ratio of the two types was 1:1 (Fig 1-B). In the BC_2 population, the genotypes of the milky flowered hybrid were $W_1W_1W_2w_2$ and $W_1w_1W_2$_, taking a ratio of 3/4. The genotype of pure white flowered hybrid was $W_1W_1W_2W_2$, taking a ratio of 1/4. The ratio of the two types was 3:1 (Fig 1-C).

Discussion

In this study the genetic rule of the white flower color in an artificially resynthesized *Brassica napus* line was investigated and analyzed with a cross between a common yellow flowered cultivar and the white flowered line. The results showed that the white flower color was controlled by two pairs of duplicate, incompletely dominant genes, which also interacted with a recessive epistatic gene (w_1w_1). Three types of flower color were observed in the segregating hybrid offspring, i.e., pure white, milky white and yellow. This was consistent with the report of Liu Houli (1985) and Zhang Jifu (2000). However, the intermediate type, milky white, varied in whiteness. This may be related to the number of dominant W_1 or W_2 alleles in the genetic background. It seemed that the more dominant W_1 or W_2 alleles in the background, the whiter the flower color. Further studies are necessary to investigate the actions of the white flower genes.

As for the segregation of white flower character in the F_2 population, Chen (1987), Qi cunkou et al., (1992) and Zhang jifu(2000) observed a segregation ratio of white flower: yellow flower to be 3:1. They all believed that the white flower characteristics were controlled by a pair of incompletely dominant gene. In the study of Zhang jifu et al., (2000), the ratio of white flower: milky flower: yellow flower plants were 1:2:1. They considered that under conditions where white flower was not completely dominant to yellow flower, pure white flower and milky white flower were observed as white flower, thus resulting in to a ratio of 3:1. According to this consideration, the ratio in the present study could be seen as the same, i.e., (1+11):4=3:1. But we observed a segregation ratio of 1:11:4, in stead of 1:2:1. This may be a result from the difference in parental materials. In the study of Qi cunkou et al., (1992), the actual numbers of milky white flowered plants in F_2 population were not listed.

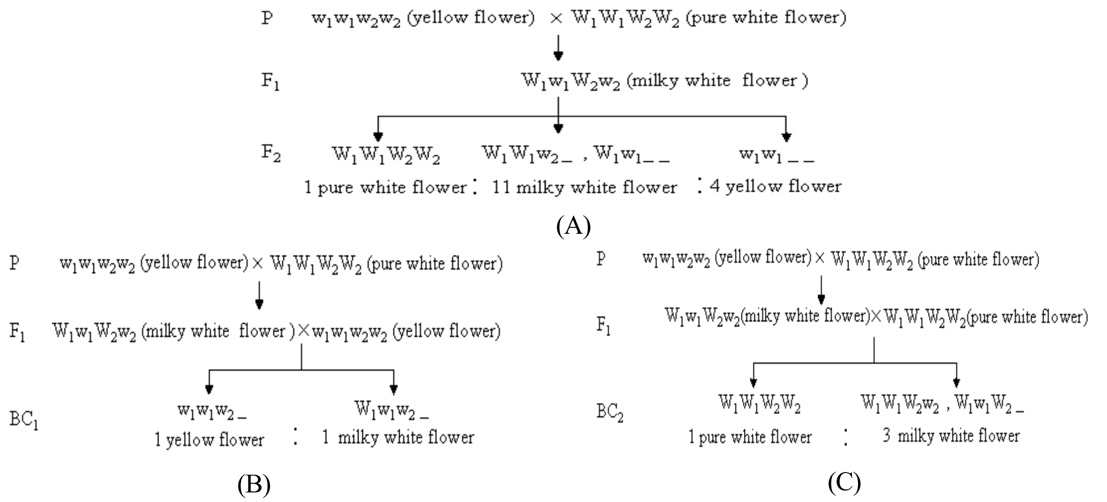

Fig 1 The segregation model of flower color in F_2 (A), BC1(B), BC2(C) generations

The complexity in genetic behaviors of the white flower characteristics may be derived from the different genetic origin of the parent materials. Kato et al., (1976) obtained white petaled *B.napus* lines through interspecific hybridization between *B. campestris* L. and *R. sativa* L. Heyn (1977) obtained white flowered lines in *B.napus* from hybrid of *Raphanobrassica*. Chen et al., (1987, 1990) obtained white flowered *B. napus* lines "No 4003" and "No 7076" from the interspecific hybrids of *B. campestris* L. and *B. oleracea var. alboglabra*. The white flower materials in the present study were originated from the interspecific hybrid of *B. chinensis* (from Eastern Tibet) and the Yunnan *B.oleracea var. alboglabra*. The parental materials used in this study had a completely different genetic background from the materials used in the other studies. Therefore, different genetic characteristics may be shown in the hybrid offspring.

In the present study only the F_2, BC_1 and BC_2 hybrid populations were used to observe the segregation model and the genetic rule of the white flower character. It was, however, not enough to prove the genetic rule and the segregations in F_3 generation are necessarily investigated. In 2006, we grown the $F_{2:3}$, the $BC_{1:2}$, and the $BC_{2:2}$ families for further observations. These further observations will help us to thoroughly understand the genetic rule of the white flower character of the resynthesized line.

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