Material breeding, heredity and utilization of purple-red leaf marker character in *Brassica napus* L.

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Abstract

We selected red rape (*B. campestris*, vegetable) with purple-red leaf as the female parent, the local variety "sinan white rape "(*B. campestris*) as the male parent to make cross combination, then used the F_1 as female parent, youyan 2(*Brassica* napus L.) as the male parent and recurrent parent to make the backcross hybrid. By using self-cross and orthoselection method, we successfully got the rape line (*Brassica* napus L.)with the vegetable character of purple-red leaf--zigan. The whole plant of this rape line is purple-red when it is in seeding stage. When it is growing up, the whole plant become green gradually. It is different at the time of becoming green among different lines. The latest time of becoming green is full–blossom stage. The heredity research results of purple-red leaf character showed: purple-red leaf character was controlled by one pair of dominant genes, it was dominant to green leaf character. Purple-red leaf is an important character of morphological markers, it can be used on the test of hybrid purity and genuineness. It can also be used to distinguish the false hybrid in seedling stage, and improve the production security.

Key words:*Brassica napus* L., purple-red leaf character, selection and breeding, heredity

Improvement and utilization of crop germ plasm are important components of crop breeding. Using genetic marker of crop trait is an important technique in modern breeding. Genetic marker is a special easily identified manifestation of germ plasm, which can be divided into morphological markers, cytological markers, biochemical markers, and molecular markers[1]. Morphological marker of leaf can be observed in period of crop lifecycle and identified early, so it can be used to test hybrid and variety purity, and protect variety rights and interests. Color and shape of leaf are primary components of leaf traits. In general, color expression of rape leaf is green, including deep and thin, and red leaf and purple-red leaf mean that the rape is in bad condition. But red leaf and purple-red leaf have advantages of visible, credible and easily identified etc. For example, leaf colors of purple-red rice, red rape (*Brassica* campestris) in vegetables and so on are very easy to identify at a glance.

Purple-red leaf trait of red rape was successfully transferred into *Brassica* napus L. by distant crossing, and we got the rape lines with purple-red leaf morphological marker in *Brassica* napus L.. The whole plant of these rape lines is purple-red when it is in seeding stage. When it is growing up, the whole plant becomes green gradually. It is different at the time of becoming green among different lines. The latest time of becoming green is full–blossom stage. We further researched heredity and utilization of this trait. Now we'll report selection, breeding and genetic study of purple-red leaf rape in order to provide information and reference for improvement and utilization of rape germ plasm.

1 Materials and Methods

1.1 Materials

Red rape (*B. campestris*, vegetable), Sinan White Rape(*Brassica* campestris L.), youyan 2(*Brassica* napus L.), hybridize offspring and Lines; Recessive nucleic male sterile lines (green leaf) (RNMS)3331A, 3448A, 3347A, 3363A, 432A, Qianyou 4A and CMS line 5023A ; green leaf strains III9, III112, III16, III3, III201, III189, 6508, 5188, 5187, 5189 and 5196 in *Brassica* napus.

1.2 Methods

The methods of hybridization, backcross, self-cross and test cross were used in this research. After we got the combinations of distant hybridization by crossing and recurrent crossing, we used self-cross and orthoselection method to select and breed the strains, and obtained the purple-red leaf rape lines in *Brassica* napus. When we studied on the heredity of purple-red leaf character, the purple-red leaf rape lines were used as one of the parents to cross with green leaf lines. We used the RNMS lines as female parents, the purple-red leaf rape lines as male parents to study the application of the purple-red leaf rape. According to the research purpose, different generation of the rape were planted and recorded. The purpose for heredity study was focused on the color of leaves in different generations, so we observed the proportion of purple-red and green leaves plants in different generations, and did the Chi-square test for fixed-ratio fit.

2 Results and analysis

2.1 Selection and breeding process of purple-red leaf rape

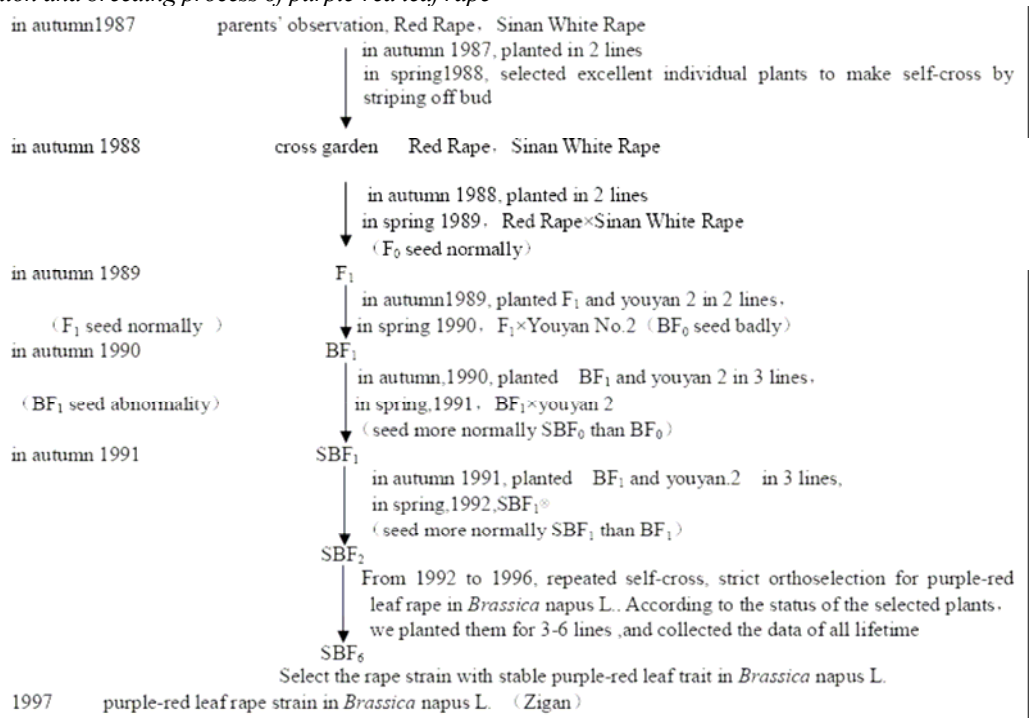

Fig 1 selection and breeding process of purple-red leaf rape in *Brassica* napus L. (Zigan)

2.1.1 Making combination We planted Red Rape and Sinan White Rape for two lines in autumn 1987, and made self-cross for them in spring 1988. Then We planted them in the same way in autumn 1988, and made combinations by using Red rape as female parent and Sinan white rape as male parent in spring 1989. We planted the combinations in autumn 1989, and observed that the leaf of them were purple-red and they gave seed normally. The F_1 generation and youyan 2 were used as female parent and male parent to make the hybrids in spring 1990, the hybrids were planted for two lines in autumn 1990, and the plants with purple-red leaf were used as female parent to make backcross with youyan 2 in spring 1991. The backcrosses were planted in three lines in autumn 1991.

2.1.2 Selection of offspring We began to do the selection of offspring from 1992. Every year we chose the good plants with purple-red leaf character, and made them self-cross,

the selected lines were planted for 3~6 lines. It was until 1997 the rape strain with stable purple-red leaf trait in *Brassica* napus L.- Zigan was selected and bred successfully.

2.2 Research for heredity of purple-red leaf character

2.2.1 Expression of F_1, F_2 and BC generations

The study results in table 1 showed that the leaf color of hybrid F_1 was always purple-red, no matter the purple-red leaf rape was used as male parent or female parent, it meant that purple-red color was dominant; the ratio of the purple-red leaf to the green in F_2 generation is 3:1. We used the F_1 generations whose female parent were RNMS as male parents to make the backcross with original RNMS, and got the ratio of the purple-red leaf to the green in BC_1 was 1:1. We made the self-cross for F_2 plants which were labeled purple-red leaf or green leaf respectively. Then we observed the F_3 generation for the leaf color. The results showed that the leaf colors of all plants in F_3 generation were green if the leaf color of plant in F_2 generation was green; but if the leaf color of plant in F_2 generation was purple-red, plants in F_3 generation had two kinds of phenomenon, one was the leaf colors of all plants were purple-red, the other was the leaf colors of plants were purple-red and green, and the ratio was 3:1. All the results above indicated that the purple-red leaf character of rape in *Brassica* napus L. was controlled by one pair of dominant genes.

2.2.2 Heredity pattern of purple-red leaf character in *Brassica* napus L.

According to the performance of the purple-red leaf gene, we supposed that the dominant gene which controlled the purple-red leaves character was HH, and green leaf was hh, then we got the heredity mode as follow:

2.3 Discussion the utilization of purple-red leaf character in heterosis

By using 432AB and Qianyou 4AB (both have green leaves) which are nucleic male sterile lines as female parents and the strains with purple-red leaf character as male parents, We got sixteen hybrids in isolation place, but we didn't take the fertile plant out of 432AB and Qianyou 4AB. Identification for these hybrids in field showed that it was possible to completely distinguish the false hybrid from true by purple-red leaf in the seedling stage; but proportion of true hybrid was low because we didn't take the fertile plant out. This may due to the fertile plants were closer to the sterile plants than the strains, and the

sterile plants was likely to accept the pollen of the fertile plants. The pollen competition abilities of purple-red leaf material were different according to the distance to female plants and the place they were planted. The results from table 2 showed that(1) the hybrid proportion of 432AB which was planted at the top of mountain was 56.8%, but the hybrid proportion of Qianyou 4AB which was planted at the foot of mountain was only 23.6%,the reason for the results may be the wind power was more powerful at the top of mountain than at the foot;(2) the proportions of hybrids in different position were different, the shorter the distance between the male parent and the female parent was, the higher the proportion of hybrid was.

Table 1 Expression about the purple-red leaf character of the hybrid and F_2

Combination	F_1	F_2			Combination	F_1	F_2		
		Plants No. of observed	Plants No. of fitting 3:1	Exception			Plants No. of observed	Plants No. of fitting 3:1	Exception
3331A×P.007	P	3	3		01P.16×5188	P	3	3	
3331A×P.024	P	2	2		01P.16×5187	P	3	3	
3331A×P.026	P	2	2		01P.16×5189	P	3	3	
3448A×P.007	T.P	2	2		00P.19×III112	P	5	5	
3448A×P.024	T.P	2	2		00P.19×5196	P	4	4	
3448A×P.026	T.P	1	1		00P.19×III16	P	2	2	
3363A×P.007	P	3	3		00P.19×III3	P	4	4	
3363A×P.024	P	2	2		02III206×P.001	P	2	2	
3347A×P.024	T.P	1	1		02III206×P.013	P	4	3	1
3347A×P.026	T.P	3	3		02P.001×III201	P	2	2	
3347A×P.026	T.P	2	2		02P.036×III189	P	2	2	
5023A×P.026	D.P	2	2		02P.036×III189	P	2	2	
01P.16×III9	P	3	3		P.036×02III201	P	5	4	1
01P.16×6508	P	4	4						

Note: M: purple-red; T.M: Thin purple-red; D.M: Deep purple-red

3 Brief Summary and Discussion

3.1 We selected red rape(*B. campestris*) with purple-red leaf as the female parent, the local variety "sinan rape "(*B. campestris*) as the male parent to make cross combination, then used the F_1 as female parent, youyan 2(*Brassica* napus L.) as the male parent and recurrent parent to make the backcross hybrid. By using self-cross and orthoselection method, we successfully got the rape line (*Brassica* napus L.)with the vegetable character of purple-red leaf--Zigan. The whole plant of this rape line is purple-red when it is in seeding stage. When it is growing up, the whole plant become green gradually. It is different at the time of becoming green among different lines. The latest time of becoming green is full–blossom stage. The purple-red leaf is an important genetic character in *Brassica* napus L..

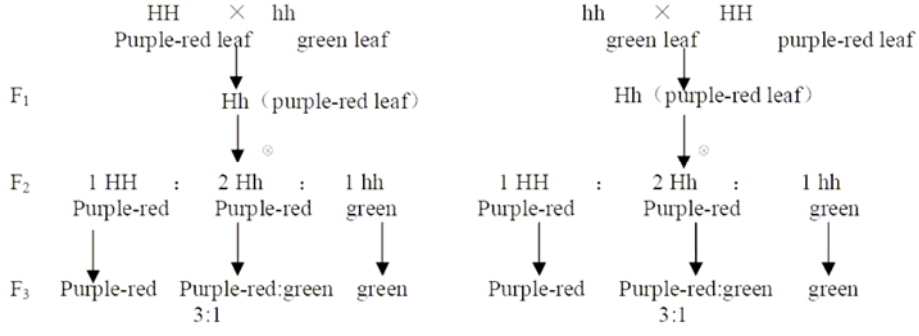

Fig. 2 Heredity mode for leaves colour of hybrid F_1 and its offspring

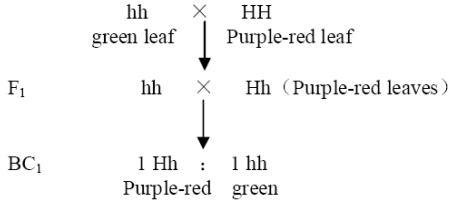

Fig. 3 Heredity mode of backcross offspring

3.2 Through the breeding procedure of Zigan, we found that the combination couldn't be done successfully by using youyan 2 (*Brassica* napus L.) mated Red rape (*B. campestris*)directly, we had to use the local variety "sinan white rape " (*B. campestris*) as the bridge parent, and use the youyan 2 as backcross parent to do backcross. Only in this way, we improved the fertility of offspring. This phenomenon maybe relate with its evolution and the relative.

3.3 The heredity research results of purple-red leaf character showed that purple-red leaf character of rape in *Brassica* napus L. was controlled by one pair of dominant genes, it was dominant to green leaf character.

3.4 In utilization of rape heterosis, it was easy to identify purity of hybrid in its early time by choosing purple-red leaf character as male parent. When we use RNMS as the female parent to produce the hybrid, we must take the fertile plant out, only in this way we can increase the hybrid proportion. Purple-red leaf character could be used to test of hybrid purity and genuineness, and distinguish the false hybrid in seedling stage, and improve the production security.

3.5 During the investigation of hybrid leaf color, we found that the leaves of hybrids which had different female parents and the same purple-red leaf male parent were purple-red, but the degrees of purple-red were different, some of the hybrids had deep purple-red leaf while the others had purple-red leaf or thin purple-red leaf; and the time for leaf color changing from purple-red to green were different, some were at the stage of bolting, some were at the stage of flowering, and the latest time were full–blossom stage. How to explain this phenomenon? We should do more research on physiology of purple-red leaf character.

3.5 We may put the genes of purple-red and male sterility together by biology technology, and get all the male sterile plant with purple-red leaf, so we could take out all the fertile plants at the seedling stage, and transplant only purple-red leaf plants to field during the seed production. In this way we could ensure the hybrid quality. If this becomes true, the purple-red character would be more useful and valuable.

Table 2 Proportion of hybrid by using purple-red leaf material

Planted code	F_1					Planted code	F_1				
	Combination	No. of Green leaf plant	No. of Purple-red leaf plant	Total	proportion of the purple-red %		Combination	No. of Green leaf plant	No. of Purple-red leaf plant	Total	proportion of the purple-red %
03-62	432AB	134	176	310	56.8	03-63	Qianyou 4AB	155	48	203	23.6
03-70	432A×19-1	61	98	159	61.6	03-64	Qiany4AB×13-1	165	39	204	19.1
03-71	432A×19-2	81	68	149	45.6	03-65	Qiany 4AB×13-2	167	35	202	17.3
03-72	432A×19-3	77	47	124	37.9	03-66	Qiany4AB×13-3	164	32	196	16.3
03-73	432A×19-4	86	67	153	43.8	03-67	Qiany4AB×13-4	172	28	200	14.0
03-74	432A×19-5	96	46	142	32. 4	03-68	Qiany 4AB×13-6	158	18	176	10.2
03-75	432A×19-8	78	63	141	44.7	03-69	Qiany 4AB×13-7	160	33	193	17.1
03-76	432A×19-9	112	59	171	34.5	Average					15.67
03-77	432A×19-10	97	42	139	30.2						
Average					41.34						

Note: (1)Female parent is green leaf, male parent is purple-red leaf. It did not remove the fertile plant in female parent.
(2)432AB is the mixed-sample of the peak, Qianyou4AB is the mixed-sample of the foot.
(3)432AB×19-1 is the first row beside the male line, 432AB×19-2 is the second row beside the male line,and the rest may be deduced by analogy.