Breeding of temporary all maintainer with purple leaf in oilseed rape (*Brassica napus* L.)

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Abstract

Mutants with purple leaf were discovered in the temporary all maintainer (TAM) line 12-204TAM of recessive epistatic genic male sterility (GMS) system in *Brassica napus* L. New TAM line with purple leaf, tagged with PL12-204TAM, was bred after 3 selfing generations of mutants and with 100% of sterility maintaining rate. The progenies of reciprocal crosses between green leaf line and PL12-204TAM all showed normal green leaves, and recurrent cross and F_2 progenies had 3:1 and 15:1 segregation rate of green leaf to purple leaf respectively, which indicated that two pairs of recessive nuclear genes controlled trait of purple leaf. Agronomic performance of PL12-204TAM was weak, but two hybrids derived from it and 12-148TAM line with green leaf, showed over-parent heterosis of 19.7-20.7% on yield. The results lay a good foundation of application research of purple leaf trait on the oilseed rape heterosis utilization as agronomic marker traits (AMT).

Key words: *Brsssica naups* L, Purple leaf, Agronomic marker trait, Recessive epistatic GMS, Temporary all maintainer, Mutant

Introduction

Heterosis utilization is one of effective ways to increase yield of oilseed rape (*Brassica naups* L.). The yields of the elite hybrids are higher by 20-30% than conventional varieties. The growing area of hybrids accounted for about 30% in 1998 and several systems of heterosis utilization have been developed in China, such as, Polima CMS, Shan2A CMS, two-line recessive genic male sterile (GMS), and three-line recessive epistatic GMS, and so on (Fu 2000).

Hybrid purity has much effect on yield. Yield decreased 0.2%-0.4% for each 1% purity drop when hybrid purity was from 75% to 95% (Chen et al. 2002). Purity of parent line is main factor influencing the hybrid purity. Hybrid purity decreased 4.1% for every 1% fall of mother line purity (Li 1993). Oilseed rape *Brassica napus* L. is cross pollinating crop, its natural crossing rate is about 30%. Parent line purity of hybrid is liable to decrease because of biological contamination during the reproduction of parent line. One of methods to increase parent line purity is to mark parent line with agronomic marker traits (hereinafter AMT).

AMTs are special traits which are remarkably distinct with the common traits, therefore can be used as genetic markers. Compared with molecular marker, AMT marker can mark either individual plants or special characteristic, and also has advantages of easy identification, low cost and high efficiency. Marking parent line of hybrid with AMT and breeding of AMT parent line, will benefit pulling out of non-AMT plants during reproduction of parent line and its hybrid according its AMT, therefore easily enhance purity and reproduction efficiency of parent line and hybrid (Wu et al.2005).

So far about ten AMTs in *Brassica napus* L. have been discovered, such as purple leaf, purple stem, yellow seedling, waxless leaf, white flower, apetalous flower and so on (Liu 2000, Wu et al. 2005). CMS line 702A with waxless leaf from "Nilla" in *Brassica naups* L. was developed, and 16 of 27 hybrids derived from it showed higher yields than check Zhongyou 821 by 2.2%-65.6% (Mo et al. 1999). The genic and cytoplasmic double MS line Z01-1A with the Chlorophyll-reduced (Cr) trait marker was bred, and 5 hybrid showed higher yield than check variety (Wang and Zhao 2003). In this paper, the inheritance of newly-found purple leaf from mutants, which were discovered in the 12-204TAM line with green leaf of recessive epistatic GMS system in *Brassica napus* L in 2002, was studied. New TAM line with purple leaf was developed and effect of purple leaf trait on the hybrid performance was evaluated.

Materials and methods

Plant materials: Recessive epistatic GMS line 9012A, G9012A and maintainer 12-148TAM, 12-204TAM in *Brassica napus* L are all of green leaf, while leaves of maintainer PL12-204TAM are purple. All lines mentioned were developed by Crop Research Institute, Anhui Academy of Agricultural Sciences, and were grown in the experiment field in Hefei during 2002-2005.

Inheritance analysis: PL12-204TAM line with purple leaf was used as male and female parent in the crosses with 12-148TAM, and was made cross with 9012A, G9012A. Partial F_1 plants of some crosses were selfed to obtain F_2 seed or were backcrossed to the PL12-204TAM parent line. All crosses were made by hand, subsequently, parental, F_1, F_2 and BC_1 seeds were sown in separate plots. Leaves color were investigated in winter. The data were analysed using χ^2 test to determine the goodness of fit to different genetic ratios in the F_1,F_2 and BC_1.

Maintaining rate determination: To investigate the sterility rate of F_1 progeny from cross between 9012A and PL12-204TAM, which was maintaining rate of PL12-204TAM.

Effect of purple leaf trait on the hybrid performance: A randomized block design was used in this experiment, with two replications for each treatment. All together there were 6 treatments, including 2 hybrids and their 4 parent plants, among which 2 mother plants from 12-148TAM and 2 father plants from PL12-204TAM. 5-rows plot size was 2m×1.67m (3.34m^2). Row distance and plant distance were 0.33m, 0.13m respectively. Direct sowing was carried out and a density of 22.5 plants·m^{-2} was set 50 days after emergence. At harvest, 10 plants were chosen at random from middle 3 rows for each plot for agronomic evaluation. The average from all together 20 plants for each treatment was used to evaluate the effect of purple leaf trait on the hybrid performance.

Results

Breeding of PL12-204TAM with purple leaf: In the winter of 2002, 56 mutants with purple leaf trait were discovered in the population of 228 individual plants from a selfing progeny of 12-204TAM (plot number: TM084). The mutant rate was 24.6%. The performances of mutants were poor. New line with purple leaf, tagged with PL12-204TAM, was bred after 3 selfing generations of mutants. The expression of purple color began on the cotyledon. PL12-204TA showed stable purple leaves in winter, and then purple color degree declined with time in spring, to almost normal green during the stem elongation and bud period. Except for weakness, the performance of PL12-204TM was very similar to its wild type 12-204TAM (Fig.1).

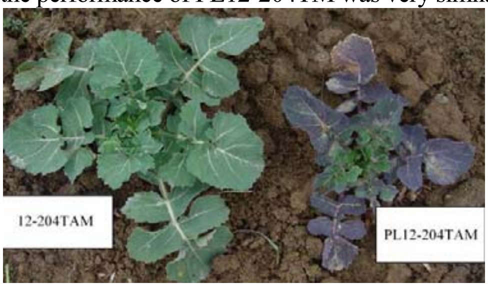

Fig. 1:Seedling performances of 12-204TAM and PL12-204TAM with purple leaf

In 2004, crosses between 9012A and individual plants from PL12-204TAM were made, and maintaining rate results were in Table 1. Most maintaining rates were 100%, except the plot 05TM413 with 97.3%, possibly due to the biological contamination during cross making. The results indicated that the maintaining ability of mutants was kept during the mutation, that is, a spontaneous mutation.

Table 1: Maintaining rates of PL12-204TAM plants with purple leaf

Plot number	Fertile plants	Sterile plants	Maintaining rate (%)
05TM403	0	28	100
05TM407	0	33	100
05TM412	0	35	100
05TM413	1	36	97.3

Inheritance of purple leaf trait: Irrespective of whether PL12-204TAM line was used as the male or female parent in crosses with normal green leaf line 12-148TAM. All F_1 plants were of green leaf, and no intermediate type was observed (Table 2). In the F_2 and BC_1 progeny, the segregating rates of green leaf to purple leaf were 15:1(χ^2=0.048), 3:1(χ^2=0.027) respectively. These results showed that two pairs of recessive nuclear genes controlled the purple leaf trait, and cytoplasmic factor didn't cause any effect on the expression of purple leaf trait in PL12-204TAM.

Table 2: Segregation ratios of green leaf to purple leaf in F_1, BC_1 and F_2 generations

Cross	Generation	Green leaf	Purple leaf	G/P	E	χ^2	P
9012A×PL12-204TAM	F_1	33	0				
	BC1	150	48	3.125:1	3:1	0.027	0.9-0.75
G9012A×PL12-204TAM	F_1	48	0				
12-148TAM×PL12-204TAM	F_1	25	0				
	F_2	293	18	16.278:1	15:1	0.048	0.9-0.75
PL12-204TAM×12-148TAM	F_1	21	0				

Performance of PL12-204TAM and influence of its purple leaf trait on the hybrid: The agronomic performances of selfing progeny of two parent plants from PL12-204TAM-1, PL12-204TAM-2 were weak with average height of 76.0-84.3cm, 4-5 first-order branches, no second-order branches, and low average seed yield per plant of 0.5-0.6g. But two hybrids derived from PL12-204TAM and 12-148TAM line had over-parent heterosis of 19.7-20.7% on seed yield per plant, 13.2-19.3% on the plant height, and 29.8-50.3% on number of seeds per pod (Table 3).

Table 3: Agronomic performances of TAM line PL12-204TAM and effect of purple leaf on hybrid derived from it and green leaf line

Hybrid or parent line	Plant height (cm)	Number of first-order branches	Number of second-order branches	Number of pods per plant	Number of seeds per pod	1000-seed weight(g)	Seed yield per plant (g)
PL12-204TAM-1*	84.3	4.0	0.0	48.0	7.5		0.5
12-148TAM-1×PL12-204TAM-1	145.0	7.8	3.0	348.5	16.3	3.4	10.5
12-148TAM-1*	121.5	10.8	5.5	361.1	10.8	4.1	8.7
Over-parent heterosis	19.3	-27.9	-45.5	-3.5	50.3	-17.0	20.7
PL12-204TAM-2*	76.0	5.0	0.0	52.0	6.5		0.6
12-148TAM-2×PL12-204TAM-2	137.7	9.0	3.7	383.7	18.4	3.9	16.1
12-148TAM-2*	121.6	10.5	5.8	434.5	14.2	3.9	13.4
Over-parent heterosis	13.2	-14.3	-36.2	-11.7	29.8	-0.1	19.7

*:-1,-2: Individual plant number of parent line.

Discussion

In the heterosis utilization system of three-line recessive epistatic GMS, two-purpose line and TAM line produce all sterile line in small scale, which later together with restorers makes hybrid, dramatically saves time and labour during the hybrid production based on the two-line recessive GMS system where about 50% of fertile plants from mother line will be pulled out, therefore greatly increased hybrid production efficiency and hybrid purity. Besides high efficiency, compared with other systems, three-line recessive epistatic GMS holds following advantages: wide range of restores, which make it easy to select combination with high heterosis, high stability and completeness of sterility, high hybrid production yield with low risk, and are being widely applied in the hybrid production now (Chen et al. 1998, Sun et al. 2002, Zhang et al. 2004, Wang et al. 2004).

TAM is the most important parent line in the system of three-line recessive epistatic GMS, which contribute to the high efficiency during hybrid seed production. So the TAM purity is vital to the hybrid seed purity and hybrid seed production efficiency. While maintaining character in TAM is controlled by 3 pairs of recessive genes (Chen et al. 1998), which can't be identified according to its morphological traits, therefore purity of TAM declines quickly during reproduction. Marking TAM with AMT marker is good way to solve this problem. A TAM line with milk white flower which was controlled by one pair of recessive genes was reported (Wu et al. 2006). In this paper a TAM with purple leaf, PL12-204TAM has been developed. And two hybrids derived from PL12-204TAM with purple leaf and 12-148TAM with green leaf, showed over-parent heterosis of 19.7-20.7% on yield. The results show much application potential of the purple leaf trait in the oilseed rape heterosis utilization as AMT marker.

Inheritances of several leaf color mutants were studied so far. Red leaf trait of 9512 line was controlled by one pair of dominant genes (Tu and Fu 2001), which was the same as inheritance of aubergine leaf trait of Zi001, Zi013 from distance crossing between *Brassica napus* L. and vegetable with aubergine leaf trait (Huang et al. 2005). While purple leaf trait 191-407 line was controlled by one incomplete dominant gene (Li et al. 1997). The inheritance of purple leaf trait of PL12-204TAM was different, which was controlled by two pairs of recessive nuclear genes.

Only in winter purple leaf trait in this study was very stable. In autumn, it was light purple, and purple color degree declined with time in spring, to almost normal green during the stem elongation and bud period. Similar phenomena were also observed in the study of purple leaf or red leaf trait (Li et al. 1997, Tu and Fu 2001). Purple leaf trait didn't appear when PL12-204 TAM line was planted in room condition (no direct sunshine) or in the climate chamber. The results indicated that light and temperature possible affected the expression of the purple leaf trait. Ultraviolet can destroy the cell pigment synthesis system, and change cell pigment composition, lead to the change of leaf color (Hollosy 2002). Further study need to be carried out to test if the color change in PL12-204 TAM is something to do with ultraviolet light in the sunshine or not.

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