Inheritance study and utilization of Leaf-on-leaf marker character in *Brassica napus* L.

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Abstract

The leaf-on-leaf in *Brassica napus* L. is an easy to identify morphological marker character during seedling stage. The main expression is that a couple of leaflets growing on its normal leaf. It has the great research and utilization value on rapeseed genetics and breeding. We investigated its growth location, size, genetic mechanism and relations with the nuclear male sterile genes. The results showed: 1~5 leaf-on-leaf leaflets grown on the face of vein every normal leaf, the size of leaflet ranged 0.05~4.20m^2. The leaf-on-leaf character was dominant relative to the non leaf-on-leaf and it was controlled by two genes in *B. napus.* The new recessive male sterile line of leaf-on-leaf was obtained through backcross breeding with the recessive male sterile line 98-116A and the leaf-on-leaf line T2632 as parents. The leaf-on-leaf male sterile line has the advantage of higher sterile plants ratio, and its sterile plants ratio had reached above 90% and higher observably than 98-116A' sterile plant ratio. Research result showed that its genetic mechanism was different from normal rapeseed recessive male sterile lines. The main aim in this paper was to summarize and evaluate the investigated results and utilization foreground about leaf-on-leaf in *B. napus L.*

Key words: *Brassica napus*, leaf-on-leaf character, morphological marker, genetic mechanism, nuclear male sterility

Rapeseed is similar to other crops, with a lot of specific and evident characters, which are so-called genetic properties. Some of these specific properties follow the Mondel's genetic laws in its genetical pathway. These characters can be inherited and expressed stably in its offspring, without beeningt affected by environmental conditions. So it has more significance in rapeseed genetical and shape marker research. These characters can be also regarded as important indication characters in heterosis utilization of rapeseed, such as petal color (white petal), no petal, no wax powder, purple stem and aubergine leaf characters [1,2,3,4,5]. The rapeseed leaf mutant material had been discovered in hybrid offspring materials of *B. napus* L, and it was called rapeseed leaf-on-leaf material. There are a couple of leaflets (no handle) on normal leaf face and its expression is very evident at seedling stage[1]. The leaf shape investigation and genetic research results had identified the leaf-on-leaf character has the properties that can be stably inherited and not affected by environmental conditions. In addition it can be expressed with a certain genetic ratio in the offspring [2,6]. The backcross breeding work of new recessive male sterile line of leaf-on-leaf between 98-116A and T2632 began from 2000. The rapeseed leaf-on-leaf genes had been introduced into original recessive unclear male sterile line through backcross breeding approach. Now we had obtained the genetic stable recessive male sterile line of leaf-on-leaf (T2632A). It has a significant property, which is high sterile plant ratio in its populations. The sterile plant ratio of some lines of among recessive male sterile lines of leaf-on-leaf can reach above 90%, but general sterile plant ratio of recessive unclear male sterile line is only 50%. So this male sterile material will play a role in the future two-line heterosis utilization and studies in *B. napus* L.

1. Discovery and genetic study of Leaf-on-leaf material in *B. napus* L.

1.1 Discovery of leaf-on-leaf material

The leaf-on-leaf plants were discovered in the hybrid progenies and resource material in *B. napus*. There are a couple of leaflets (non handle) on normal leaf surface and its expression is very evident at seedling stage[1]. It was named the leaf-on-leaf. Selection of the plants with typical leaf-on-leaf property was taken and with continuous selfed pollination to produce progenies. At the same time, we investigated the numbers, growth place and size of leaf-on-leaf in *B. napus*. The stable leaf-on-leaf lines were obtained.

1.2 Genetic study results of leaf-on-leaf in B. napus L

Using conventional genetic principle and methods, crossing between the leaf-on-leaf character line T2632 and the non leaf-on-leaf character lines, such as Zhongshuang 2, Zhongshuang 4 and Qianyoushuangdi 2. F_1 seeds of six cross combination were obtained and they were self-pollinated by bag isolation to autogamy and collected F_2 hybrid seeds respectively. On the side we selected to backcross between leaf-on-leaf plants (female parents) and respectively three non leaf-on-leaf lines (Zhongshuang 2, Zhongshuang 4 and Qianyoushuangdi 2) as backcross male parents, and obtained backcross offspring seeds BC_1 and BC_2. We investigated the segregation ratio of F_2, BC_1 and BC_2 offspring populations between leaf-on-leaf and non leaf-on-leaf plants.

The results indicated: the phenotypes of all plants in F_1 generation expressed the leaf-on-leaf property whatever in positive and reverse cross. So it was confirmed that the leaf-on-leaf character is dominant to non leaf-on-leaf in *B. napus*. And it was controlled by nuclear genes. In addition, it was identified that it was controlled by two pairs of independent genes through the test of F_2 and BC_1, BC_2 generation[2,6]. The genetic segregation ratio between leaf-on-leaf and non leaf-on-leaf accorded with the law of inheritance in F_2, BC_1 and BC_2 generation populations (table 1).

Table 1:The genetic segregation ratio of plants leaves in backcrossing BC_1, BC_2 and selfing F_2 generation

Generation	Combination	Cross No	Plants	Leaf-on-leaf No.	Non leaf-on-leaf No.	Practice ratio	Theoretics ratio	X^2_c
BC_1	T2632/qianyoushuangdi 2// qianyoushuangdi 2	3	203	112	91	1.23:1	1:1	1.970
			175	136	39	3.49:1	3:1	0.550
			198	101	97	1.04:1	1:1	0.045
	T2632/zhongshuang 4//zhongshuang 4	3	156	69	87	0.79:1	1:1	1.853
			168	90	78	1.15:1	1:1	0.720
			192	87	105	0.83:1	1:1	1.505
	T2632/ zhongshuang 2// zhongshuang 2	3	153	86	67	1.28:1	1:1	2.118
			163	125	38	3.29:1	3:1	0.167
			143	65	78	0.83:1	1:1	1.007
BC_2	T2632/ qianyoushuangdi 2// qianyoushuangdi 2 /// Qianyoushuangdi,2	3	175	126	49	2.57:1	3:1	0.688
			153	86	67	1.28:1	1:1	2.118
			162	90	72	1.25:1	1:1	1.784
	T2632/ zhongshuang.4// zhongshuang.4/// zhongshuang.4	3	135	58	77	0.75:1	1:1	2.400
			147	68	79	0.86:1	1:1	0.680
			129	71	58	1.22:1	1:1	1.116
	T2632/ zhongshuang 2// zhongshuang.2/// zhongshuang.2	3	158	85	73	1.16:1	1:1	0.766
			154	69	85	0.81:1	1:1	1.461
			126	52	74	0.70:1	1:1	3.500
F_2	T2632(leaf-on-leaf)/ qianyoushuangdi 2(non leaf-on-leaf)	2	1259	928	331	2.80:1	3:1	0.017
			1360	1274	86	14.81:1	15:1	0.003
	T2632(leaf-on-leaf)/ zhongshuang.4(non leaf-on-leaf)	2	1049	798	251	3.18:1	3:1	0.588
			1345	981	364	2.69:1	3:1	2.944
	T2632(leaf-on-leaf)/ zhongshuang 2(non leaf-on-leaf)	2	1186	1115	71	15.70:1	15:1	0.099
			1247	953	294	3.24:1	3:1	1.273

$X^2_{0.05,1}$=3.84

1.3 Genetic hypothesis and validate of leaf-on-leaf property in B. napus *L.*

The leaf-on-leaf character was controlled by two pairs of independent dominant genes in *B. napus*. We presumed that genes controlling leaf-on-leaf trait in *Brassica napus* were dominant genes T_1T_2, and the genes of controlling non leaf-on-leaf character were recessive genes t_1t_2. So the genotypes of expressing leaf-on-leaf character should be $T_1T_1T_2T_2$, $T_1T_1t_2t_2$, $t_1t_1T_2T_2$, T_1-T_2-, T_1--- and --T_2-, but the genotype expressing non leaf-on-leaf character was $t_1t_1t_2t_2$. The phenotypes of all plants in F_1 generation expressed leaf-on-leaf character, and its genotype was $T_1t_1T_2t_2$.

The genetic segregation pattern from F_1 to F_2 hybrid generation

$T_1T_1T_2T_2$ (leaf-on-leaf) × $t_1t_1t_2t_2$ (non leaf-on-leaf)

↓ (Zhongshuang .2,4 and Qianyoushuangdi 2)

F_1 $T_1t_1T_2t_2$ (leaf-on-leaf)

↓ ⊗

F_2 (T_1_T_2_ 、 T_1––– 、––T_2–) : $t_1t_1t_2t_2$ T_1–t_2t_2 : $t_1t_1t_2t_2$ $t_1t_1T_2$– : $t_1t_1t_2t_2$

15 (leaf-on-leaf): 1 (non leaf-onleaf) 3 (leaf-on-leaf): 1 (non leaf-on-leaf) 3 (leaf-on-leaf): 1 (non leaf-on-leaf)

The genetic segregation pattern of backcross offspring

F_1 $T_1t_1T_2t_2$ (leaf-on-leaf) × $t_1t_1t_2t_2$ (non leaf-on-leaf Zhongshuang No.2,4 and Qianyoushuangdi No..2)

↓

BC_{11}: $T_1t_1T_2t_2$ $T_1t_1t_2t_2$ $t_1t_1T_2t_2$: $t_1t_1t_2t_2$

leaf-on-leaf: non leaf-on-leaf=3: 1

$T_1t_1T_2t_2$ (leaf-on-leaf) × $t_1t_1t_2t_2$ $T_1t_1t_2t_2$ (leaf-on-leaf) × $t_1t_1t_2t_2$ $t_1t_1T_2t_2$ (leaf-on-leaf) × $t_1t_1t_2t_2$

↓ ↓ ↓

BC_2: $T_1t_1T_2t_2$ $T_1t_1t_2t_2$ $t_1t_1T_2t_2$: $t_1t_1t_2t_2$ $T_1t_1t_2t_2$: $t_1t_1t_2t_2$ $t_1t_1T_2t_2$: $t_1t_1t_2t_2$

leaf-on-leaf: non leaf-on-leaf=3: 1 leaf-on-leaf: non leaf-on-leaf=1: 1 leaf-on-leaf: non leaf-on-leaf=1: 1

Note: The backcross male parents were still Zhongshuang No.2,4 and Qianyoushuangdi No..2 in BC_2. There were two segregation ratio between leaf-on-leaf and non leaf-on-leaf plants in BC_2 generation.

In the six F_2 populations, there were two genetic segregation ratio between leaf-on-leaf and non leaf-on-leaf. They were 15 (leaf-on-leaf) : 1 (non leaf-on-leaf) and 3 (leaf-on-leaf) :1 (non leaf-on-leaf). Selecting the leaf-on-leaf plants in F_1 generation to backcross with Zhonghuang 2, Zhongshuang 4 and Qanyoushuangdi 2 (non leaf-on-leaf), respectively. We had obtained 18 backcross offspring and observed the form characters of seedling leaves. There were two genetic segregation ratio (3 leaf-on-leaf : 1 non leaf-on-leaf; 1 leaf-on-leaf : 1 non leaf-on-leaf) between leaf-on-leaf and non leaf-on-leaf in these backcross populations. These results derived from the genetic tests and analysis of crossed, selfed and backcrossed populations between leaf-on-leaf and non leaf-on-leaf had identified that leaf-on-leaf property was controlled by two pairs of dominant genes, and accorded with the Mondel's independent genetic law of two pairs of genes[2,6].

2. Genetic integration of leaf-on-leaf trait into nuclear recessive male sterile material and study on fertility relation in *B. napus* L

2.1 The genetic transfer-breeding process of leaf-on-leaf male sterile line

Using the sterile plant of 98-116A (double-low nuclear recessive male sterile line in *B. napus*) as female parent and continuously backcrossed with leaf-on-leaf line T2632. T2632 was also double-low leaf-on-leaf line and this character was stably transmitted, all plants in its population had leaf-on-leaf property. There were all fertile leaf-on-leaf plants in F_1 and BC_1 generation. Selecting the strong leaf-on-leaf fertile plants in BC_1 and self with bags. The segregation population where there were sterile and fertile plants would be appeared after these plants were selfed for two generations. We continued to select the leaf-on-leaf fertile and to self in the segregation populations. The ratio of leaf-on-leaf sterile plants would be improved quickly along with the selfed generations were added. The same time qualities of rapeseeds should be tested and selected good qualities to self in each generation. The new leaf-on-leaf double-low male sterile line T2632A obtained after screen and test of 4~5 selfed generations. Its sterile ratio could achieve above 90% and much higher than 98~116A[7,8]. This is very unusual and interesting genetic phenomenon that we can't explain with traditional genetic principle.

2.2 F_1 fertilities compare between sterile lines T2632A, 98-116A and testcross parents

We observed the sterility restoring results of F_1 population among the test hybrids between T2632A, 98-116A and each test variety (Table 2). These results indicated that three testcross varieties could fully restore the sterility of T2632A and the F_1 generation plants fertility ratio reached 100%. The testcross varieties were Zhongshang 7, Huyou 12 and Zheshuang 72 respectively. While all testcross varieties (8 varieties) could restore the sterility of 98-116A and all plants of cross hybrids (F_1) expressed fertility. The restoring ratio also reached 100%. It illustrated the testcross varieties were the restoring lines of non leaf-on-leaf recessive male sterile line 98-116A. The results also identified that sterile restoring mechanism had changed possibly because the leaf-on-leaf trait had been introduced into non leaf-on-leaf recessive male sterile material in *B. napus*.

3. Application perspective and outlook of leaf-on-leaf trait in B. *napus*

It had been identified by genetic tests and the law of inheritance that leaf-on-leaf trait in *B. napus* was controlled by two pairs of independent dominant genes. And it has expressed the evident morphological characteristic at seedling stage. So it can be regarded as the important form marker trait for genetical basic research and heterosis utilization in *B. napus*. In the heterosis research and application in *B. napus*, the leaf-on-leaf trait can be introduced into restoring and male sterile lines by cross and backcross approach. So F_1 hybrid can express the leaf-on-leaf character at seedling stage. According to this property, we are able to distinguish the real or fake hybrids, and ensure seeds qualities, reduce producing cost and improve seed production in the hybrid producing process. It will play a significant role on the two-lines heterosis utilization in *B. napus*.

Table 2 The F_1 fertility comparison among sterile lines T2632A, 98-116A and testcross parents

Parents (sterile lines)	Crossing parent	No. combination	Sterility expression (F_1)		
			Fertile	Sterile plants	Restoring ratio (%) plants
T2632A	Zhongshuang 4	3	144	35	80.45
T2632A	Zhongshuang 5	3	122	5	96.83
T2632A	Zhongshuang 7	2	96	0	100.00
T2632A	Huyou 12	2	77	0	100.00
T2632A	Huyou 15	2	74	6	92.50
T2632A	Suyou 1	3	34	100	25.37
T2632A	Zheshuang 72	3	151	0	100.00
T2632A	Huashuang 3	2	83	6	93.26
98-116A	Zhongshuang 4	3	137	0	100.00
98-116A	Zhongshuang 5	5	243	0	100.00
98-116A	Zhongshuang 7	5	204	0	100.00
98-116A	Huyou 12	4	179	0	100.00
98-116A	Huyou 15	7	247	0	100.00
98-116A	Suyou 1	2	85	0	100.00
98-116A	Zheshuang 72	3	149	0	100.00
98-116A	Huashuang 3	2	98	0	100.00

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