GENETIC ANALYSIS OF SEED COLOR INSTABILITY IN YELLOW-SEEDED BRASSICA NAPUS L.

H.Z. Wang(1), H.L. Liu(2)

(1) Institute of Oil Crops Research, CAAS, Wuhan, China 430062
(2) Huazhong Agricultural University, Wuhan, China 430070

INTRODUCTION

The seed color of rapeseed is associated significantly with oil and protein contents and some other traits of seed. Investigations of heredity and variability of seed color become of importance and worthwhile to improvements of seed oil and protein contents by breeding yellow-seeded varieties.

The heredity of the seed color in Brassica napus was reported to be controlled by 3 alleles (Liu et al 1979; Shirzadegam 1986). However, long breeding for yellow-seeded B. napus for some time has indicated that it was extremely difficult to stabilize and purify the yellow-seeded trait (Olsson 1960; Liu 1987). Seed color of yellow-seeded B. napus resynthesized artificially did not breed true too (Chen 1988). In fact, the changeable seed color has become a puzzling problem in rapeseed breeding and research. The variability and instability of rapeseed seed color were studied anatomically, biochemically and genetically (Wang 1990). Results of genetic analysis in B. napus are reported.

MATERIALS AND METHODS

A variegated plant, 2178-25, was analyzed. This sample originated from a yellow-seeded mutant discovered by H.L. Liu in 1976. It had been selfed for 7 generations. The mutant derived from the cross of 75-53xYi-Bin38-175. These two parents were lines of two different black-seeded B. napus varieties. All other variegated plants tested were derived from the progenies of different generations of 2178-25. For example, Ly84-24 is the progeny of 2178-25 selfed for one generation; W85302, for 2 generations; W86001, for 3 generations; W87280, for 4 generations. Single plant of each line was selfed artificially and harvested and scored separately. An amplifier was employed to observe the seed color of each sample.

Additional genetic lines used in our researches were Hua-Yu 13 (black-seeded), G87007 (black-seeded), W87034 (black-seeded, with purple silique and purple stem as markers). These lines were totally obtained from the rapeseed germplasm stock in Huazhong Agricultural University.

RESULTS

Identification of Genetic Instability in B. napus

One isoline, Ly84-24, was used in 1984 in order to investigate the association of seed color to seed oil content, etc. Ly84-24 was a yellow-seeded line bred out by H.L. Liu through 7 years' selfing. Surprisingly the seed color of different plants in this line was discovered to be variant from yellow to black. From a total of 24 plants, 3 produced all black seeds, 5 produced a mixture of black and variegated yellow seeds, 16 produced all variegated seeds. Nevertheless, such markers as leaf shape, leaf color and stem color of those 3 black-seeded plants were identical to those of the other plants.

No black-seeded lines were planted in the yellow-seeded germplasm stock. The stock field had been rotated. The possibility of the mixing of stray plants could be ruled out. Lastly, Ly84-24 had been derived from artificially selfed seeds of the variegated plant 2178-25. It is impossible for the 3 plants to come from the outcrosses of 2178-25.

In order to verify that the black-seeded plants were derived from the germinal mutations of the variegated parent, selfed seeds of 2 progeny plants, variegated Ly84-24-4 and Ly84-24-7 were grown individually in the next season. After the scoring of the seed color of offspring plants, seed color variation from the black to yellow were still observed. Black-seeded plants in progenies of Ly84-24-4 accounted for 3.2% (2 to 62), while those for Ly84-24-7 accounted for 7.5% (5 to 67). These frequencies were more than ten thousand times of that of natural mutation. It could be concluded from here that the gene of (genes) involved in dark pigments syntheses in seed coat of B. napus was mutable.

Somatic Mutability of the Mutable Gene (Genes)

Somatic mutability of the unstable gene (genes) was reflected in the 2 aspects:

(a). The seeds of 2178-25, Ly84-24, Ly84-24-7 and their yellow-seeded offsprings were variegated rather than purely yellow, i.e. they were colored with numerous dark spots or stripes on the yellow or yellowish-brown background. The number of spots was different from each other with respect to different plants or different seeds on the same plant in some cases. Some seeds were heavily dotted with more than 60 spots per seeds while others were lightly dotted with less than 30 spots per seeds. These two types of seeds were distinguishable under an amplifier. The was scored as yellow (HD), yellow (heavily dotted), and the later as yellow (LD), yellow (lightly dotted). Striped seeds were colored with one to three dark stripes of different lengths. Each spot or stripe reflected one mutation event of that gene (genes).

Anatomical researches indicated that all stripes, spots at lightly dotted seeds, and a part of spots at heavily dotted seeds were originated from the variegation of palisade layer between dark cells (or tissues) and light tissues, which were determined by maternal genotypes. The other spots at heavily dotted seeds were derived from pigment synthesis in some aleurone cells, which were controlled by endosperm genotypes (Wang 1990, unpublished material). So somatic mutability may be reflected in both variegated palisade and dotted aleurone layer.

(b). Some plants descended from a variegated parent produced a mixture of black and variegated seeds. The proportion of black seed was various. It could be less than 1% to more than 90%. An entire branch of a plant or a silique on a branch might produce black seeds while the others produced variegated seeds. A single silique could produce both black and variegated seeds which contributed to most cases of color-mixed plants. These events showed that the time for somatic mutation is not consistent.

Black seeds from mixed plants and from revertant plant possessed the same seed coat structure as wild black seeds, i.e. possessed a fully dark-colored palisade layer. So one unstable allele may be involved in the instability of palisade color.

Genetic Analysis of the Mutable Gene (Genes) in Selfed Progenies

(a). Segregation of yellow (HD) and yellow (LD) seeds on the same plant: 2178-25, LY84-24-7 and some other progeny plants produced seeds which segregated 3:1 for yellow (HD): yellow (LD) (Table 1). This segregation pattern indicated that one allele was involved in the determination of dotting degree. Yellow (HD) was dominant to yellow (LD). Referring to the solution mentioned before, we may conclude the dominance of dotted aleurone to light-colored aleurone layer. The gene dotting the aleurone layer was named a11* (aleurone 1*). Its recessive allele was a11. It can also be concluded that aleurone dotting genes of 2178-25, Ly84-24-7 are heterozygous.

(b). Genetic studies of progeny plant of Ly84-24-7: A progeny row, W85302, was obtained from the growing of seeds from Ly84-24-7. In it there segregated yellow (HD), yellow (HD&LD), yellow (LD), mixed with black and variegated, and black-seeded plants. Seeds of the 5 types were planted and mature plants were scored for seed color (Table 2). Segregation results of different types were summarized as follows:

Offsprings of yellow (HD) segregated yellow (HD), mixed with black and variegated, and black-seeded plants. Among them black-seeded plants accounted for 15.4%. This demonstrated the tendency for the mutable allele to remain unstable in off generations. As no yellow (LD) plant appeared, the aleurone dotting allele of yellow (HD) plant should be homologous.

Progenies of yellow (HD&LD) continually segregated 5 types of plants, just as 2178-25 and LY84-24-7 did. The segregation of yellow (HD):yellow (HD&LD):yellow (LD) was 1:2:1. The mean rate of black-seeded plants was 9.8%.

Progenies of yellow (LD) segregated yellow (LD), mixed with black and variegated, and black-seeded plants. The mean frequency of black-seeded plant was 3.1%, less than that of yellow (HD). As no purely yellow-seeded neither yellow (HD) plants was discovered, the mutable palisade gene should be homologous, and their aleurone dotting gene be recessively homologous.

Segregation of progenies of mixed type belonged to one of the 3 patterns mentioned above. This indicated that black seeds on a mixed plant were derived from somatic mutations of the unstable palisade gene.

Out of 5 black-seeded plants, 2 essentially bred true for black, 3 segregated 3:1 for black:variegated plant. These segregations showed that one or two germinal cells were reverted. The appearance of a few variegated plants in offsprings of those 2 black-seeded plants implied the instability of the reverted allele.

Genotypes of those types of plant become apparent based on results described above and the fact that only one allele was involved in the variation between black and variegated palisade (Table 3). With B11 (black palisade 1) for black palisade gene, b11* (*represents mutable) for variegated palisade allele, along with a11* and a11, genotypes of those 5 types were: yellow (HD), b11*/b11*, a11*/a11*; yellow (HD&LD), b11*/b11*, a11*/a11; yellow (LD), b11*/b11*, a11/a11; mixed, one of three above; black B11/-,--. The mutable allele b11* possessed both somatic and germinal instabilities. As for somatic, it leads to variegated palisade and mix-colored seeds on the same plant. As for germinal, it results in the high frequencies of black-seeded plant in the progeny of its variegated plant, some times with the presence of a11*.

Germinal Revertion Frequencies of Yellow (HD) and Yellow (LD) Ones

Germinal revertion rates were estimated on half of the frequencies of black-seeded plant in progenies of variegated plant. Two types of plant, yellow (HD) and Yellow (LD) from W85302 were tested for 3 generations (Tables 4 and 5). Rates of black-seeded plant in offsprings of yellow (HD) were 10% and 22%, meanly about 16.4% (Table 4). The rates of that of yellow (LD) were 0% to 7%, meanly about 3.2% (Table 5). These data indicated the minimal germinal frequency of about 8.2% for yellow (HD), and about 1.6% in germinal cells, depending on whether or not there existed a11*. The frequency in different generations was essentially stable.

Tests for the Stability of the Reverted B11

Among 46 black-seeded plants from the progeny of W85302-3, and 65 from that of W85302-6, 19 and 36 were tested for the stability of the reverted B11 (Table 6). Of 19 plants from W85-302-3, 12 produced progenies segregating 3:1 for black-seeded: variegated plants, 5 produced all black-seeded progenies, the other 2, however, produced progenies segregating most black-seeded plants and a few variegated mutants. Of 36 plants from W85302-6, 19 produced all black-seeded plant offsprings, while the other 17 produced progenies segregating most black-seeded plants and a few variegated ones. These evidences again proved that the reverted B11 could easily mutated to b11* in return. Variegated plants could be produced in its progeny rows with the rate of approximately 1.9%.

Behavior of b11* in Crossing Tests

With yellow (HD) and yellow (LD) plants as pollen givers, they were cross-pollinated to black-seeded materials with wild genotypes. Teach F_1 plant produced one F_2 crowd. F_2 data for seed color segregation were listed in Table 6.

Plants in F_2 rows from crosses of wild black x yellow (LD) segregated in a 3:1 ratio for black-seeded:variegated plants. No purely yellow-seeded plant was discovered in these progenies. Plants in most F_2 rows from crosses of wild black x yellow (HD) also segregated in a 3:1 ratio for black-seeded:variegated plant. These segregations suggested that only one allele was involved in the variation of palisade color.

Unexpectedly, three abnormal F_2 progenies were observed. One from the cross of G87034-1 x W87280-1 and another from G87034-2 x W87280-2 bred true for black-seeded character. No variegated plants were produced in these two F_2 progenies. Nevertheless, markers as purple stem and purple silique showed that these two F_2 were derived truly from the hybridization of that two parents. These results suggested that the germinal cells of their variegated parents had mutated from b11* to B11. So the male germinal reversion frequency of yellow (HD) may be as high as 10%.

Another F_2 progeny from the cross of G87034-3 x W87280-1 segregated a new type of mutant, i.e. purely yellow-seeded plant. Of 59 F_2 plants 2 possessed purely yellow seeds on which there was not any spot or striple. The appearance of purely yellow-seeded plant showed that b11* of the parent had mutated to a new recessive allele which results in purely yellow seeds. This allele was termed as r-b11 (recessive b11). Apart from purely yellow-seeded plants, there also segregated a large proportion of black-seeded and variegated plants. The segregation ratio of that F_2 progeny as 12:3:1 for

black-seeded:variegated:purely yellow-seeded plants. This pattern suggested a two elements model for the controlling of variegated palisade in B. napus.

DISCUSSION

Association of the Unstable b11* to a Transposable Element

Variegated somatic tissue along with high germinal reversion frequencies is typical for a transposable element inserted allele (Doring and Starlinger 1986). The most common way to recognize a transposable element is when the element lowers or obliterates the expression of a gene, but reverts frequently to nonmutant phenotype (Freeling 1984). The unstable B. napus allele, b11*, is just a such case. This allele is recessive to B11 but can reverted frequently to B11, i.e., b11* is genetically unstable. Researches in other plants indicate that all unstable alleles analyzed molecularly are resulted from insersions of transposable elements (Fedoroff 1983; Bonas and Sommer 1984; Martin et al 1985; Brown and Mattes 1989). So b11* is very likely to be resulted from the insertion of a transposable element in B11. Such a element could be characterized as below:

(a). It was an autonomous element. The reason that only one allele was involved in the variation of variegated palisade. This element was termed as Tbn1 (Transposon Brassica napus 1). However, Tbn1 could occasionally mutate to - a two elements system which still control the instability of the palisade color. The production of r-b11 along with the 12:3:1 ratio of that F_2 progeny has proved this conclusion.

(b). The timing for Tbn1 to transpose was not consistent. It could transpose very early or extremely late during plant development course. If it transposed early, fully black-seeded blanches were derived. When it transpose very late, small dark dots were scattered on the palisade layer.

(c). Other evidences for the transposition of Tbn1: the excision of Tbn1 at the very beginning of ovule development. Each stripe represented one excision event. Apart from continuous strips, discontinued stripes were also discovered. There may be one to four yellow blockades in one such discontinued dark stripe. Yellow blockades indicated that the recovered ability of pigment synthesis in that piece of tissue was obliterated again. Such events suggested the re-insersion in B11 of the excised Tbn1. Excision and re-insersion activities of Tbn1 were also proved by the instabilities of germinal reverted plants. So the re-insersion behavior was characteristic to Tbn1.

REFERENCES

BONAS, U. SOMMER, H. et al. 1984. The transposable element Tam1 of Antirrhinum majus is 17 kb long. Mol. Gen. Genet. 194: 138-143.

BROWN, J.J, MATTES, M.G. et al. 1989. Molecular characterization of rDt, a maize transposon of the "Dotted" controlling element system. Mol. Gen. Genet. 215: 239-244.

CHEN, B.Y., HEENEEN, S.K., JONSSON, R. 1988. Resynthesis of Brassica napus L. through interspecific hybridization between B. alboglabra Bailey and B. campestris L. with special emphasis on seed color. Plant Breed. 101: 52-59.

DORING, H.P., STARLINGER, P. 1986. Molecular genetics of transposable elements in plants. Ann. Rev. Genet. 20: 175-220.

FREELING, M. 1984. Plant transposable elements and insertion frequencies. Ann. Rev. Plant Physiol. 35: 271-298.

SHIRZADEGAM, M. 1984. Inheritence of seed color in Brassica napus L. Z. Pflanzensuchtg. 96: 140-146.

WANG, H.Z. 1990. Studies on variability and instability of seed color in rapeseed (Ph.D. thesis). In Huazhong Agricultural University, Wuhan, China.

Table 1. Segregation of yellow (HD) and yellow (LD) seeds

	Number of Seeds on the Same Plant				
Samples	Total	Yellow (HD)	Yellow (LD)	Striped	p (3:1)
2178-25	101	74	19	8	0.50-0.25
Ly84-24-7	288	193	71	24	0.50-0.25
W85302-8	292	186	75	27	0.25-0.10
-11	298	201	64	33	0.75-0.50
-16	294	195	69	30	0.75-0.50
-17	257	178	53	26	0.50-0.25
-19	324	210	78	36	0.50-0.25

Table 2. Segergation of progeny plants of W85302

	Number of Plants in the Next Generation's Progenies					
Phenotypes Sample	Total	Yellow (HD)	Yellow (HD&LD)	Yellow (LD)	Yellow Mixed	Bl.
Yellow (HD)						
W85302-1	79	39			28	12
-2	77	35			34	8
-5	67	31			25	11
-20	76	32			29	15
Yellow (HD&LD)						
W85302-8	68	8	24	12	17	7
-11	61	9	18	11	19	4
-16	64	9	23	11	13	8
-17	67	7	21	14	20	5
-19	67	10	17	11	21	8
Yellow(LD)						
W85302-7	63			47	13	3
-10	65			52	11	2
-15	67			49	16	2
-28	62			51	10	1
Mixed						
W85302-4	66	10	16	12	22	6
-9	64			53	8	3
-13	68	9	18	6	20	15
-18	74	36			24	14
-21	72	40			25	7
Black						
W85302-3	62			13	3	46
-6	67				2	65

Table 3. Revertion frequency of yellow (HD) plants

Sample	Black	Total	Rate (%)	Sample	Black	Total	Rate (%)
W85302-1	12	79	7.6	W85302-2	8	77	5.2
-5	11	67	8.2	-20	15	76	9.7
W86001-3	11	63	8.7	W87280-1	9	77	5.8
-5	14	66	10.6	-2	12	68	8.8
-9	17	77	11.0	-3	14	75	9.3
-10	14	76	9.2	-5	10	78	6.4
-13	10	62	8.1	-7	9	66	6.8
-14	11	65	8.5	-9	10	71	7.0
-15	14	72	9.7	-10	12	69	8.7
-18	11	70	7.8	-13	10	64	7.8

Table 4. Revertion frequency of yellow (LD) plants

Sample	Black	Total	Rate (%)	Sample	Black	Total	Rate (%)
W85302-7	3	63	2.4	W85302-15	2	67	1.5
-10	2	65	1.5	-28	1	62	0.8
W86014-1	2	59	1.7	W87256-1	2	64	1.6
-3	1	69	0.7	-2	0	62	0
-5	3	69	2.2	-3	3	64	2.3
-6	0	57	0	-4	2	61	1.6
-7	2	64	1.6	-6	4	68	2.9
-8	4	60	3.1	-9	2	59	1.7
-10	2	60	1.7	-10	0	63	0
-11	3	63	2.4	-13	2	66	1.5

Table 5. Test of seed color stability of reverted plant

	Plant Number	Total	Number of Seeds on the Same Plant			
Parent		Rows	NRPVP	Dotted	Mixed	Black-seeded
W85302-3	12	12	12	113	38	489
	7	7	2	2	1	315
W85302-6	36	36	17	13	22	1621

NRPVP: Number of rows producing varietgated plants.

Table 6. Segregation in F2 of crosses of Black x Dotted plants

Cross	Cross Number	Number of Plants in F2					
		Total	Black	Mixed	Dotted	Purely	Ratio
Hua-Yul3-xLy84-24-4	6	296	224	13	59	0	3:1
G87007-2xW87256-2	8	374	282	18	67	0	3:1
G87034-2xW87256-2	8	346	266	15	70	0	3:1
G87007-1xW87280-2	12	852	655	94	103	0	3:1
G87034-1xW87280-1	9	590	456	52	82	0	3:1
G87034-1xW87280-1	1	58	58	0	0	0	
G87034-4xW87280-1	9	619	477	56	86	0	3:1
G87034-3xW87280-1	1	59	46	4	7	2	12:3:1
G87034-2xW87280-2	8	636	491	63	82	0	3:1
G87034-2xW87280-2	1	76	76	0	0	0	