

# **Inheritance of Seed Coat Color Genes of *Brassica napus* (L) and Tagging the Genes Using SRAP Molecular Markers**

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## **Introduction**

Yellow seed coat color is more desirable in *B. napus*. The yellow-seeded varieties have a thinner seed coat, higher protein and lower fiber content over dark-seeded varieties resulting higher oil content in the seed which increases the financial return.

Seed coat color is mainly controlled by the maternal genotype. Different authors have been reported that seed coat color in *B. napus* is mostly controlled by three genes and yellow seed coat color produced only when all three seed coat color genes are in homozygous recessive condition.

Breeding effort in canola/rapeseed would be greatly facilitated through the development of molecular markers for genes controlling the seed coat color trait.

## **Objectives**

The objectives of this study were to find out the inheritance pattern of the seed coat color genes and to develop the SRAP molecular markers for individual seed coat color genes from a multi-gene family for marker assisted selection for seed coat color in the breeding program.

## **Materials and methods**

Three pure breeding spring type yellow seeded *B. napus* lines (SRYS-1, SRYS-2, SRYS-3) were obtained from Norddeutsche Pflanzenzucht (NPZ) Lembke, Hohenlieth, Germany and four different selfed homozygous black-seeded spring type varieties, Sentry (canola type), MillenniUM03 (high erucic acid rapeseed), Holly-276 (high oleic and low linolenic acid content), Allons (low linolenic acid content) and one black seeded DH (double haploid) line, all developed at the Department of Plant Science, University of Manitoba, were used in this research. The crosses and reciprocal crosses were done between yellow-seeded and black-seeded parents. The F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> plants were grown in green house of the Department of Plant Science, University of Manitoba. Backcross seeds were produced by crossing the F<sub>1</sub> plant with the respective yellow seeded parental line. Inheritance of seed coat color was studied in the F<sub>2</sub> (i.e. in F<sub>3</sub> seeds) and BC<sub>1</sub>S<sub>1</sub> populations.

## **DNA extraction and SRAP molecular marker**

DNA was extracted using a modified version of the CTAB method according to Li & Quiros (2001). SRAP is a PCR-based marker system with two primers, a forward primer of 17 bases

and a reverse primer of 18 bases. These are labeled with [ $\gamma^{33}\text{P}$ ]-ATP for amplification of genomic DNA. DNA amplification is using the protocol of Li & Quiros (2001) and analysis is continuing using an ABI 3100 gene analyzer.

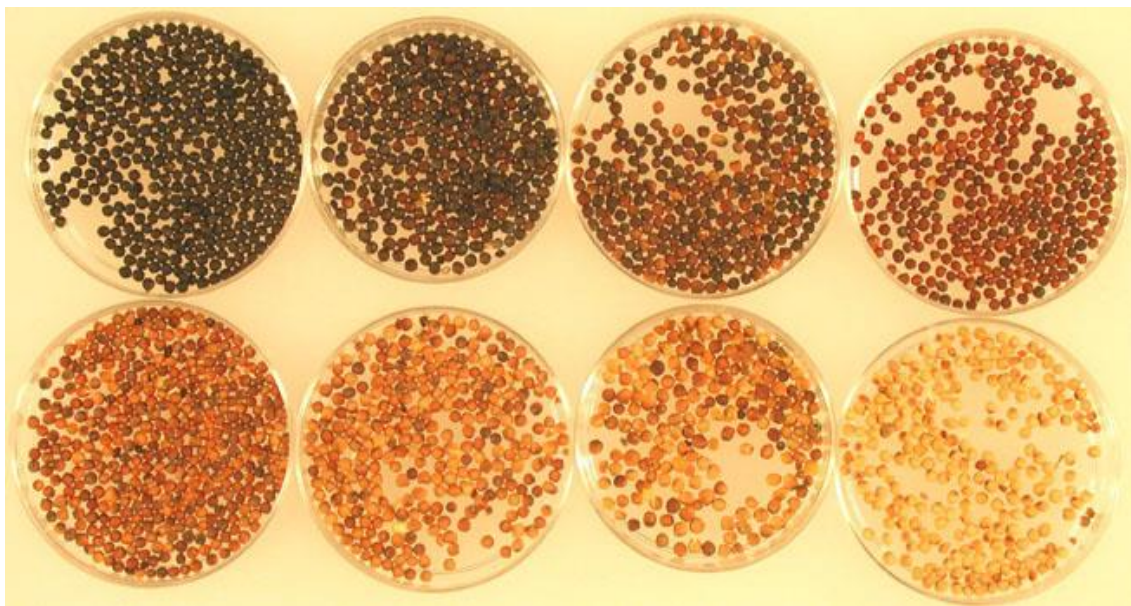
## Results and Discussion

### Seed coat color in F<sub>1</sub>

Since seed color in *Brassica* is determined primarily by the genotype of maternal tissue, i.e. the testa, all F<sub>1</sub> seeds exhibited the same color as the female parent when black seeded parents were used as maternal parent. However, when the yellow seeded lines were used as maternal parent and black seeded parents were used as pollen source, the F<sub>1</sub> seed coat color turned dull yellow/ or yellowish brown (Fig: 1). These results indicated that pollen grains from the black seeded parent had a zenia effect on these yellow seeded parental lines.

### Seed coat color inheritance

The F<sub>2</sub> progenies varied from black to dark brown, light brown, brown, yellowish brown, dark yellow, yellow (Fig 2), indicating an incomplete dominance of the black color (Shirzadegan 1986).



### Goodness of fit:

To test the segregating populations for goodness of fit to expected Mendelian ratios, the yellow seeded F<sub>2</sub> progenies were pooled in one group and all other non-yellow types of seeds were pooled in another group. The total pooled data fit a 63:1 ratio, indicating three gene inheritance ( $\chi^2 = 2.272$ ,  $P = 0.1-0.2$ ), but did not fit a 255:1 segregation ratio which would indicate a four gene inheritance (Table 1).

Pooled data from five backcross populations were studied. The total pooled backcross data fit a 7:1 segregation ratio, indicating three gene inheritance ( $\chi^2 = 1.961$ ,  $P = 0.1-0.2$ ), but did not

fit a 15:1 segregation ratio for four gene inheritance (Table 1). Therefore, the results suggested that seed coat color of *B. napus* is controlled by three genes with black seed coat color dominant over yellow seed coat color.

Table 1: Segregation of seed coat color in F<sub>2</sub> families and backcross populations of black-seeded and yellow-seeded *Brassica napus* crosses.

Populations	Black/brown /partially yellow	Pure Yellow seeds	Segregation ratio			
			63:1		255:1	
			$\chi^2$	P	$\chi^2$	P
<u>F<sub>2</sub> Populations</u>						
DHBS-126 x SRYS-1	209	1	3.307	0.05-0.1	0.039	0.7-0.9
Sentry x SRYS-2	194	3	0.500		6.490	
Holly-276 x SRYS-2				0.3-0.5		<0.05
MillenniUM03 x SRYS-3	180	3	0.007		7.332	
Allon x SRYS-3						<0.01
Total	292	2	1.487	>0.95	0.634	
	215	2	0.579	0.2-0.3	1.572	0.3-0.5
	1090	11	2.272	0.3-0.5	10.48	0.2-0.3
				0.1-0.2		<0.01
<u>Backcross populations</u>						
(DHBS-126 x SRYS-1) x SRYS-1	43	6	$\chi^2$	P	$\chi^2$	P
(Sentry x SRYS-2) X SRYS-2	107	17	0.003	>0.95	3.006	0.05-0.1
(Holly-276 x SRYS-2) x SRYS-2	59	10	0.166	0.5-0.7	11.78	<0.001
(MillenniUM03x SRYS-3)xSRYS-3	176	17	0.250		8.001	
(Allon x SRYS-3) x SRYS-3	131	11	2.405	0.5-0.7	2.048	<0.01
Total	516	61	2.934	0.1-0.2	0.543	0.1-0.2
			1.961		18.93	0.3-0.5
				0.05-0.1		<0.001
				0.1-		

### **Molecular marker linked to the seed color gene**

Bulked segregant analysis (BSA) is being used to identify markers closely linked to the seed coat color genes. The development of molecular markers for the seed coat color genes continues.

Figure 3. Analysis of PCR products obtained using different SRAP primer combinations on yellow seeded and black seeded parental and segregating populations of *B. napus*.

The markers, once developed, will be tested to the F<sub>3</sub> progenies (i.e. F<sub>4</sub> seeds) showing a 3:1 segregation ratio indicating individual genes for seed coat color are segregating (Fig 3).

### **Conclusions**

Seed coat color in black seeded *B. napus* is determined by the genotype of the maternal tissue when it is selfed or crossed with a yellow parent. However, the yellow seeded parental lines used in this study, when used as females in crosses with black seeded parents produced F<sub>1</sub> progeny with seed coats which turned dull yellow / or yellowish brown. In contrast, when the yellow seed lines used in this study were selfed, they produced only pure breeding yellow seed.

In *B. napus*, seed coat color is controlled by three genes and black is dominant over yellow seed coat color.

SRAP molecular markers for the yellow seed coat color genes are being identified using bulk segregant analysis. These markers will be used in F<sub>4</sub> segregating populations to identify single gene loci affecting seed coat color from a multi-gene family.

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