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Inheritance of Seed Colour in Turnip Rape (*Brassica campestris* L.)

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Summary. The inheritance of seed colour was investigated in the progenies of crosses between seven yellow seeded forms and a brown seeded one of turnip rape (*Brassica campestris* L.). Seed colour differences were found to be determined in each case by one or two genes with epistatic effect. Moreover, independent inheritance of hilum colour was observed. Seed colour was predominantly but not completely determined by the maternal parent. In total, two genes for seed colour with epistatic (Br_1br_1; Br_6br_6) and four with hypostatic effect (Br_3br_3; Br_4br_4; Br_5br_5; Br_7br_7) as well as one gene for hilum colour (Br_8br_8) were distinguished. Two alleles for yellow seed colour (br_1^1 and br_1^2) were present in the locus Br_1br_1, whereas in the locus Br_6br_6 one allele was found for yellow seed colour (br_6^1) and another for light hilum colour (br_6^2). The possibilities of multiple allelism are discussed.

Key words: Mendelian analysis – Epistatic genes – Multiple allelism – Predetermination – Xenia – Turnip rape

Introduction

Apart from their usual brown or dark seeds, nearly all economically important *Brassica* species include genotypes which produce light brown to yellow seeds. These yellow seeded forms have been intensively bred for ever since their lower crude fibre and higher oil and protein content was reported (Jönsson and Bengtsson 1970; Stringam et al. 1974; Bechyne et al. 1979; Vangheesdaele and Fournier 1980). Work in this direction has been especially successful in Canada where the first partially yellow seeded turnip rape cultivar 'Candle' is now widely cultivated. Simultaneously, the inheritance of yellow seed colour has been investigated in *B. campestris* (Ahmed and Zuberi 1971; Stringam 1980) and *B. juncea* (Sun 1945; Heyn 1973). Already from the early investigations of Mohammad et al. (1942) it had been concluded that seed colour in turnip rape was determined by three genes and that two of them, Br_1br_1 and Br_2br_2, were epistatic to the third one, Br_3br_3. In the present experiments, seed colour inheritance was studied with seven yellow seeded turnip rape forms from different origins in order to determine the number and allelic relationships of the involved genes in this broad material.

Materials and Methods

The following *B. campestris* forms from the *Brassica* collection of the Institute in Göttingen were used (Table 1).

Plants were grown in the greenhouse and crossed by the usual techniques. For each cross combination only one mother and one father plant were used; these also were selfed to check seed colour inheritance in the progeny. Four plants of each cross combination were backcrossed with the involved yellow form as the male parent. One to four backcross (F_2') and F_2 plant populations were raised for segregation analysis. All F_2' and F_2 plants were also selfed by hand. A diallel cross was performed between seven yellow seeded turnip rape forms. Four F_1 and four or more F_2 plants were sown and selfed from each cross combination. F_2 segregation was analysed in three reciprocal cross combinations.

Table 1.

Collection No.	Subspecies or variety	Colour of	
		seed	hilum
2017^{l}	'Yellow Sarson'	yellow	light
2552^{l}	'Yellow Sarson'	yellow	light
2016^{l}	*oleifera*	yellow	light
2016^{d}	*oleifera*	yellow	dark
2018^{l}	*oleifera*	yellow	light
2020^{l}	*oleifera*	yellow	light
2020^{d}	*oleifera*	yellow	dark
2021^{l}	*oleifera*	yellow	light
2021^{d}	*oleifera*	yellow	dark
2009^{d}	*oleifera*	yellow	dark
d	*pekinensis*	brown	dark

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Fully mature seeds were harvested and five classes of seed colour were identified:

(1) brown (dark brown to medium brown);
(2) light brown;
(3) yellow + brown (plants with yellow to brown seeds. The yellow seeds may have light or dark hilum colour)
(4) yellow/dark (yellow with dark hilum colour)
(5) yellow/light (yellow with light hilum colour).

Some of the parental forms segregated for hilum colour at our initial investigations. From these, lines with light or dark hilum were selected for the following analyses. To ensure proper designation of hilum colour in the parental forms, the letters l (for light) and d (for dark) were added as an index to the collection number.

Data were analyzed using the Chi-square test. The homogeneity test was applied in all cases of cross combinations with more than one F_1 progeny; a significant difference was observed in one case only.

Genes were designated according to the nomenclature (*Br/br*) proposed by Mohammad et al. (1942).

Results

Reciprocal Crosses Between Seven Yellow Seeded and One Dark Seeded Turnip Rape Form

In all the different cross combinations between yellow seeded and dark seeded forms, F_1 plants produced brown seeds. Brown seed colour thus is dominant over yellow.

For the two 'Yellow Sarson' forms 2017^l and 2552^l with light hilum colour, the F_2 data exhibit a ratio of 12 : 1 : 2 : 1, indicating a two gene model of inheritance (Br_1/br_1, Br_3/br_3) with epistatic effect (Table 2). In class (3) with Br_3/br_3 heterozygous, phenotypic expression of seed colour was characteristically variable: in the same plant and even the same silique, seeds developed every gradation from yellow to brown. In the backcross with 2552^l, only three classes were observed, confirming the presence of both recessive alleles br_1 and br_3 in this 'Yellow Sarson'. Low accordance with expectation was revealed by the Chi-square test for the cross $d \times 2017^l$; but this was the only case among all our cross combinations in which the homogeneity test showed significant differences between F_1 progenies.

The turnip rapes of the *oleifera* group Nos. 2009, 2016, 2020, and 2021 are self-incompatible. This required a very careful check of homozygosity for the parents. As to 2021 and 2016, lines with light and dark coloured hilum, respectively, were used in the crosses (Table 3). In the F_2 from the cross $2021^l \times d$, four seed phenotypes segregated in a ratio of 48 : 12 : 3 : 1; after backcrossing, the corresponding F_2' ratio was 4 : 2 : 1 : 1. This implies three genes with epistatic effect. In the cross $2016^l \times d$ only a digenic F_2 ratio of 12 brown (1) : 3 yellow/dark (4) : 1 yellow/light (5) and a F_2' ratio of 2 brown (1) : 1 yellow/dark (4) : 1 yellow/light (5) was observed. In this cross, the light brown seed colour which occurred in all other combinations was lacking. In $d \times 2021^d$ and $d \times 2016^d$, where the yellow seeded paternal parent had a dark hilum, no segregation for hilum colour was observed (Table 3). With line 2020^d the F_2's of the reciprocal combinations showed the light brown class reduced and the yellow one increased in number, giving a ratio of 12 : 1 : 3 instead of 12 : 3 : 1. After backcross, light brown seeds were absent. Obviously light brown seeds are only produced when the epistatic gene is homozygous recessive and the hypostatic one homozygous dominant, but yellow seeds appear when the hypostatic gene is heterozygous or homozygous recessive. Light brown seeds, therefore, are absent in the backcross.

In the cross progeny $2021^d \times d$ the relatively high Chi-square value resulted from the low number of plants with brown seeds and too many with light brown and with yellow/dark seeds. In $d \times 2016^d$ also too many plants with light brown seeds were observed. In the F_2'

Table 2. Segregation of seed colour in F_2 and F_2' (backcross) progenies of combinations between two yellow seeded 'Yellow Sarson' forms (2017^l, 2552^l) and one dark seeded turnip rape (d). For legend of seed colour classes see Materials and Methods

Cross combination	Segregation in seed colour					χ^2	P
	Observed plants						
	brown (1)	light brown (2)	yellow + brown (3)	yellow/light (5)	Expected ratio		
$2017^l \times d$	57	3	6	4	12:1:2:1	1.71	0.64
$2552^l \times d$	48	5	7	3	12:1:2:1	0.62	0.89
$d \times 2017^l$	41	4	12	4	12:1:2:1	3.02	0.38
$d \times 2552^l$	54	4	8	4	12:1:2:1	0.17	0.98
$(2552^l \times d) \times 2552^l$	25		11	13	2:1:1	0.18	0.92
$(d \times 2552^l) \times 2552^l$	23		12	9	2:1:1	0.50	0.78

Table 3. Segregation of seed colour in F_2 and F_2' (backcross) progenies of combinations between three yellow seeded (2021, 2016, 2020) and one dark seeded turnip rape form (d)

Cross combination	Hilum colour of the yellow seeded parent	Segregation in seed colour					χ^2	P
		Observed plants				Expected ratio		
		brown (1)	light brown (2)	yellow/ dark (4)	yellow/ light (5)			
$2021^l \times d$	light	94	35	10	2	48:12:3:1	5.84	0.12
$(2021^l \times d) \times 2021^l$	light	23	12	6	5	4:2:1:1	0.13	0.98
$d \times 2021^d$	dark	113	22	9		12:3:1	1.16	0.56
$(d \times 2021^d) \times 2021^d$	dark	25	20	7		2:1:1	6.58	0.03
$2016^l \times d$	light	134		38	10	12:3:1	0.65	0.72
$(2016^l \times d) \times 2016^l$	light	26		13	13	2:1:1	0.00	1.00
$d \times 2016^d$	dark	31	15	1		12:3:1	6.14	0.04
$(d \times 2016^d) \times 2016^d$	dark	19	7	14		2:1:1	2.55	0.28
$2020^d \times d$	dark	110	6	28		12:1:3	1.07	0.58
$(2020^d \times d)\ 2020^d$	dark	16		10		1:1	1.38	0.24
$d \times 2020^d$	dark	33	3	5		12:1:3	1.18	0.54
$(d \times 2020^d) \times 2020^d$	dark	20		9		1:1	4.17	0.04

Table 4. Segregation of seed colour in F_2 and F_2' (backcross) progenies of combinations between the yellow seeded (2009^d) and the dark seeded turnip rape (d)

Cross combination	Segregation in seed colour				χ^2	P
	Observed plants			Expected ratio		
	brown (1)	light brown (2)	yellow/dark (4)			
$2009^d \times d$	111	30	12	12:3:1	0.80	0.66
$d \times 2009^d$	121	24	10	12:3:1	1.09	0.58
$(2009^d \times d) \times 2009^d$	34	15	14	2:1:1	0.43	0.81

of $d \times 2021^d$ and $d \times 2020^d$ the number of plants with yellow seeds was too low (Table 2).

The turnip rape 2009^d forms yellow seed with dark hilum exclusively. After crossing with the dark seeded turnip rape (d), the F_2 plants developed seeds in the ratio of 12 brown (1) : 3 light brown (2) : 1 yellow/dark (4); the backcross ratio was 2:1:1. The Chi-square tests showed good agreement with the assumption of two genes with epistatic effect (Table 4).

Diallel Crosses Between Seven Yellow Seeded Turnip Rape Forms

The seven turnip rape forms used can be divided into three groups: (a) two 'Yellow Sarson', 2017^l and 2552^l with yellow seed and light hilum, (b) the *oleifera* forms 2016, 2018, 2020, and 2021, from which yellow seeded lines with light and dark hilum had been selected, and (c) the *oleifera* form 2009^d, in which only yellow seed with dark hilum was found.

From a diallel set of combinations (Table 5), the F_1's between the two Yellow Sarson forms 2017^l and 2552^l gave only yellow seed colour in both forms, which is therefore, controlled by the same genes. After crossing the four oleifera forms in group (b), the F_1 plants developed yellow seeds with light hilum colour provided both parents also produced yellow seed with light hilum colour. If one of the parents had yellow seeds with a dark coloured hilum, the F_1 always produced yellow seeds with dark coloured hilum. This indicates that the epistatic gene in these forms is the same and that dark hilum colour is dominant to light hilum colour. In crosses between 'Yellow Sarson' and the *oleifera* forms from the (b) group, yellow + brown seeds (class (3) phenotype) were formed on F_1 plants independent of whether the *oleifera* forms used had yellow seeds with dark or light hilum colour. Consequently,

Table 5. Seed colour of F_1 plants from diallel crosses between seven yellow seeded turnip rape forms. Seed colour classes (in brakkets) are characterized in Materials and Methods

♀ No.	♂ No.	2017^{l}	2552^{l}	2016^{l}	2018^{l}	2020^{d}	2021^{d}	2009^{d}
	Seed colour of parents[a]	yellow/ light (5)	yellow/ llight (5)	yellow/ light (5)	yellow/ light (5)	yellow/ dark (4)	yellow/ dark (4)	yellow/ dark (4)
2017^{l}	yellow/ light (5)		yellow/ light (5)	yellow + brown (3)	yellow + brown (3)	yellow + brown (3)	yellow + brown (3)	brown (1)
2552^{l}	yellow/ light (5)	yellow/ light (5)		yellow + brown (3)	yellow + brown (3)	yellow + brown (3)	yellow + brown (3)	brown (1)
2016^{l}	yellow/ light (5)	yellow + brown (3)	yellow + brown (3)		yellow/ light (5)	yellow/ dark (4)[b]	yellow/ dark (4)	brown (1)
2018^{l}	yellow/ light (5)	yellow + brown (3)	yellow + brown (3)	yellow/ light (5)		yellow/ dark (4)	yellow/ dark (4)	brown (1)
2020^{d}	yellow/ dark (4)	yellow + brown (3)	yellow + brown (3)	yellow/ dark (4)	yellow/ dark (4)		yellow/ dark (4)[c]	brown (1)
2021^{d}	yellow/ dark (4)	yellow + brown (3)	yellow + brown (3)	yellow/ dark (4)	yellow/ dark (4)	yellow/ dark (4)		brown (1) + light brown (2)[d]
2009^{d}	yellow/ dark (4)	brown (1)	brown (1)	brown (1)	brown (1)	brown (1)	brown (1) + light brown (2)[d]	

[a] Seed colour/hilum colour
[b] Mother plant with dark hilum colour
[c] Mother plant with light hilum colour
[d] The parent 2021 produced yellow seeds with dark and light hilum colour on the same plant

Table 6. Segregation of seed colour in F_2 of three reciprocal crosses between yellow seeded turnip rape forms with dark (d) and light (l) hilum colour, respectively

Cross combination	Plants with seed colour					χ^2	P
	brown (1)	light brown (2)	yellow + brown (3)	yellow/ dark (4)	yellow/ light (5)		
$2009^{d} \times 2016^{l}$	37	13		13	4	1.85	0.60
$2016^{l} \times 2009^{d}$	36	13		15	5	1.52	0.68
Expected ratio	144	36		60	16		
$2009^{d} \times 2017^{l}$	31	14	8	4	4	1.30	0.86
$2017^{l} \times 2009^{d}$	37	13	6	5	5	0.72	0.95
Expected ratio	144	57	24	15	16		
(F_1 = brown (1) seed)							
$2009^{d} \times 2021^{d}$	12	5		2		0.37	0.83
Expected ratio	144	81		31			
(F_1 = light brown (2) seed)							
$2009^{d} \times 2021^{l}$	7	35		11	1	1.62	0.66
$2021^{l} \times 2009^{d}$	11	39		10	0	2.39	0.49
Expected ratio	48	147		57	4		

the epistatic gene in these forms must be the same and the hypostatic ones must be different. Crosses between 2009 (group c) and all other turnip rape forms gave only brown seeded F_1's. Therefore, this form 2009^{d} carries another epistatic gene for seed colour. There was only one exception: Obviously, the plant of 2021^{ld} which was used for both reciprocal cross combinations, was heterozygous in the third gene since it produced yellow seeds with light and with dark hilum colour, respectively. For time reasons, it was not yet possible to repeat this cross with homozygous parents. When the obtained F_1 seeds were used for F_2 analysis, 4 F_1

Table 7. Colour of seeds (see classification (1) – (5) in Materials and Methods) produced by pollination of seven yellow seeded turnip rape forms with a dark seeded one

Cross-combination	Seed colour of the yellow seeded parent	Colour of cross seed
$2017^{l}\times d$	yellow/light (5)	brownish; between (2) and (4)
$2552^{l}\times d$	yellow/light (5)	brownish; between (2) and (4)
$2016^{l}\times d$	yellow/light (5)	yellow/dark (4)
$2018^{l}\times d$	yellow/light (5)	brownish; between (2) and (4)
$2020^{d}\times d$	yellow/dark (4)	brownish; between (2) and (4)
$2021^{l}\times d$	yellow/light (5)	light brown (2)
$2009^{d}\times d$	yellow/dark (4)	brownish between (2) and (4)

plants developed brown seeds and 4 developed light brown seeds in the combination of $2021^{ld}\times 2009^{d}$ while 4 plants produced brown and 6 plants light brown seeds in the reciprocal combination.

Segregation in F_2 was studied for three reciprocal crosses; data are given in Table 6. For each of the first two reciprocal combinations, expectation was calculated for two independent genes (one gene from each form with epistatic and one with hypostatic effect) which resulted in a significant correspondence with the observed plant numbers. From the third reciprocal cross $2009^{d}\times 2021^{ld}$ a part of F_1 plants developed brown (1) and another part light brown seeds (2). Only 19 brown seeded F_1 plants were tested in F_2. In the progenies of the light brown seeded F_1 plants, however, no explainable segregation pattern was identified.

Paternal Influences on Seed Colour Expression

Heyn (1973) first observed brownish seeds in a yellow seeded mother after pollination with a dark seeded father plant. In the present investigation, seeds developed in this way were also shown to be brownish, or they at least possessed a dark hilum (Table 7). The same was true after pollination between different yellow seeded forms carrying different genes for seed colour (Table 8). Seed colour on a yellow seeded mother is thus influenced by the heterozygous embryo developed after pollination with a dark seeded father plant. In the F_2 of the cross 'Yellow Sarson'×dark seeded turnip rape, even after selfing seeds with varying shades and colour intensities were produced within the same silique according to their respective genotype. The same was sometimes observed in the F_2 plants from crosses with other yellow seeded turnip rape forms, although in these cases it was not possible to classify the seeds into distinct colour groups. In F_1 progenies which segregated for hilum colour, plants with yellow seeds exhibited dark as well as light hilum

Table 8. Colour of seeds (see classification (1) – (5) in Materials and Methods) produced by diallel pollinations between different yellow seeded turnip rape forms

♀ No.	♂ No.	2017^{l}	2552^{l}	2016^{l}	2018^{l}	2021^{d}	2009^{d}
	Seed colour of the parents[a]	yellow/light (5)	yellow/light (5)	yellow/light (5)	yellow/light (5)	yellow/dark (4)	yellow/dark (4)
2017^{l}	yellow/light (5)		yellow/light (5)	brownish; between (2) and (4)	brownish; between (2) and (4)	brownish; between (2) and (4)	brownish; between (2) and (4)
2552^{l}	yellow/light (5)	yellow/light (5)		brownish; between (2) and (4)	brownish; between (2) and (4)	brownish; between (2) and (4)	brownish; between (2) and (4)
2016^{l}	yellow/light (5)	light brown (2)	brownish; between (2) and (4)		yellow/light (5)	yellow/dark (4)	yellow/dark (4)
2018^{l}	yellow/light (5)	light brown (2)	brownish; between (2) and (4)	yellow/light (5)		yellow/dark (4)	brownish; between (2) and (4)
2021^{d}	yellow/dark (4)	brownish; between (2) and (4)	light brown (2)	yellow/dark (4)	yellow/light (5)[b]		brownish; between (2) and (4)[c]
2009^{d}	yellow/dark (4)	brownish; between (2) and (4)	light brown (2)	brownish; between (2) and (4)	yellow/dark (4)	brownish; between (2) and (4)[c]	

[a] Seed colour/hilum colour

[b] Mother plant with light hilum colour

[c] The parent 2021 produced yellow seeds with dark and light hilum colour on the same plant

colours. In F_1 of crosses between 'Yellow Sarson' and the turnip rape forms 2016, 2018, 2020, and 2021, on the same plant and in the same silique yellow + brown seeds (3) were produced.

Discussion

Predetermination of Seed Colour

Seed pigment is deposited in the palisade and the parenchyma layer of the testa in *Brassica* species (Viehoever et al. 1920; Vaughan 1956; Vaughan et al. 1963; Vaughan 1970; Stringam et al. 1974). The testa originates from the integuments, that is from the maternal tissue. Therefore, the maternal genotype plays the dominating role in seed colour determination.

Heyn (1973) observed for the first time that seed colour in turnip rape is not solely controlled by the mother plant. In his experiment, 'Yellow Sarson' was pollinated with a dark seeded *Brassica japonica* and later, a second time, with its own pollen. The harvested seeds had rather different colours. After sowing, the brownish seeds gave hybrids with fern leaves (this marker gene from *Brassica japonica* is dominant) without exception, while from yellow seeds the offspring was apparently a selfed one with normal leaves. Similarly, Jönsson (1977) questioned the exclusively maternal inheritance of *Brassica* seed colour since he also observed differently coloured (from yellow to black) seeds within the same plant of turnip rape. Other authors who have investigated seed colour inheritance in *B. campestris* (Mohammad et al. 1942; Ahmed and Zuberi 1971; Stringam 1980) have not mentioned this problem. In crosses between a light seeded synthetic *B. napus* B-24 and another light seeded rapeseed form, No. 151, Schwetka (1981) described the variation of seed colour in single F_2 plants to an extent not normally found in turnip rape. In other families, for example the *Papilionaceae*, testa conditioned seed colours are inherited exclusively through the mother as expected (Sirks 1931; Owen 1928; Prakken 1970; Wilson and Huston 1980; Gorz et al. 1975).

Results of this work indicate that seed colour in turnip rape is not completely determined through the mother plant, but that the embryo also influences the colour development in the testa. This was confirmed by the brownish colour of seeds which originated on yellow seeded turnip rape from pollination of dark seeded forms, as well as by the observed colour variations of seeds from crosses between yellow seeded turnip rape forms with different genes for seed colour. But influence of the embryo on seed colour has not only been recorded in changes from yellow to brownish but from light brown to yellow as well. The latter case was observed in F_2 and F_2' (backcross) plants from crosses between 'Yellow Sarson' and the dark seeded turnip rape form which produced yellow + brown seeds (group (3)): these are homozygous recessive with respect to the epistatic gene and heterozygous with respect to the hypostatic gene for seed colour. From selfing of such plants, seeds in a 1 : 3 segregation ratio carry the hypostatic gene in the homozygous recessive condition and yellow/light seeds (5) develop through the influence of such embryos. Finally, all the F_1 plants from crosses between 'Yellow Sarson' and the turnip rape forms 2016, 2018, 2020 and 2021 also developed yellow + brown seeds (3). These forms carry the same or similar alleles for yellow seed colour with epistatic effects. Therefore the different hypostatic alleles, especially the alleles br_3br_3, can lead to the development of yellow/light seeds (5) on the selfed F_1 plants provided they are in the homozygous recessive condition.

Xenia are the result of a direct influence of the pollinator on the developing seeds. They are often displayed in the endosperm and aleurone colours of maize and in the embryo colours of some species of *Papilionaceae.* In *Brassica* species, seed colour development does not take place in the embryo nor in the endosperm which is normally reduced to a single aleurone layer. The testa colour in turnip rape results from condensed polyphenols, i.e. polymers of leucocyanidins (Leung et al. 1979) which are found in the palisade and partially in the parenchyme layers. Theander et al. (1977) determined a considerably lower amount of these polyphenols in the seed coat of yellow as compared to dark seeded turnip rape. Since such high molecular polyphenols are not transportable, the occurrence of xenia can only be explained by the diffusion of precursors or enzymes from the embryo into the testa, where the coloured polyphenols can then be synthesized.

Obviously, xenia are only expressed when the seed colour is inherited as a digenic or trigenic trait with epistatic effect. In *B. juncea,* where seed colours are determined by two completely dominant genes (Heyn 1973; Vera et al. 1979), no cases were found in the F_2 of different coloured seeds on the same plant and in the same silique; all plants had brown seeds with one dominant allele. No brownish seeds occurred on the yellow seeded mother after pollination with yellow or brown seeded forms. Likewise, it was not possible in *B. carinata* to differentiate between crossed and selfed seeds on the yellow seeded mother after crosses between yellow and brown seeded genotypes (Schwetka unpublished results).

Inheritance of Seed Colour

Earlier investigations on seed colour inheritance in turnip rape have been generally performed with one yellow seeded form only, and exclusively seed colour was considered. In the present work seven different yellow seeded turnip rape forms were investigated simultaneously and it has been possible to also identify genes for hilum colour.

The results of the former investigations agree well with some data of our investigations. Ahmed and Zuberi (1971) found a monogenic inheritance of seed colour and full dominance of brown over yellow in *B. campestris* var. 'Toria'. The same observation has been made in this work in the cross $2016^1 \times d$. In their first investigation on seed colour inheritance in *B. campestris* var. 'Yellow Sarson', Mohammad et al. (1942) assumed three independent genes for seed colour: Br_1br_1; Br_2br_2, Br_3br_3. In the event of dominance at each of the three loci, the plants produced brown, red-brown and yellow-brown seeds, respectively, provided the other loci were recessive. Yellow seeds were formed when all three loci were

Table 9. Interpretation of segregation for seed colour (see classification (1)–(5) in Materials and Methods) in F_2 after crosses between six yellow and one dark seeded turnip rape. Alleles with epistatic effect are underlined. Recessive alleles of Br_6/br_6^2 and Br_8/br_8 determine development of light hilum

Cross-combination	Yellow seeded parent								Seed colour			
	Hilum colour	Assumed genotype							brown (1)	light brown (2)	yellow/dark (4)	yellow/light (5)
'Yellow Sarson' × d and reciprocal	light	$\underline{br_1^1 br_1^1}$	$br_3 br_3$	+ +	+ +	+ +	+ +	+ +	12	3[a]		1
2021^{l} × d	light	$\underline{br_1^1 br_1^1}$	+ +	$br_4 br_4$	+ +	$br_6^2 br_6^2$	+ +	+ +	48	12	3	1
d × 2021^{d}	dark	$\underline{br_1^1 br_1^1}$	+ +	$br_4 br_4$	+ +	+ +	+ +	+ +	12	3	1	
2016^{l} × d	light	$\underline{br_1^2 br_1^2}$	+ +	+ +	+ +	+ +	+ +	$br_8 br_8$	12		3	1
d × 2016^{d}	dark	$\underline{br_1^1 br_1^1}$	+ +	$br_4 br_4$	+ +	+ +	+ +	+ +	12	3	1	
2020^{d} × d and reciprocal	dark	$\underline{br_1^1 br_1^1}$	+ +	+ +	$br_5 br_5$	+ +	+ +	+ +	12	1	3	
2009^{d} × d and reciprocal	dark	+ +	+ +	+ +	+ +	$\underline{br_6^1 br_6^1}$	$br_7 br_7$	+ +	12	3	1	

[a] The classes light brown (2) and yellow + brown (3) are combined

homozygous recessive. The authors concluded that Br_1 and Br_2 were dominant to Br_3. Later Stringam (1980) confirmed the two independent genes br_1 and br_3 for yellow seed colour, although a deficit of yellow seeded plants was present in the F_2 and the backcross progenies of his experiment. Stringam tried to explain this low frequency of recessives by the presence of minor genes for seed colour, which he expected to condition some additional brown pigment. Such an observation was not made in our investigations. It is possible that Stringam did not give proper consideration to the merely partial maternal predetermination of seed colour in this species, since he let the segregating generation flower openly in the field. Jönsson (1975) concluded from his segregation results that at least three genes are responsible for seed colour in *B. campestris* spp. *oleifera*.

From the present analyses it is similarly evident that several genes influence the production of seed colour and that multiple alleles are present at certain gene loci. Our assumptions have been summarized in Table 9. In order to retain the nomenclature of Mohammad et al. (1942) and Stringam (1980) genes for seed and hilum colour have been designated with the symbol *br*. Following to the recommendations of the "International Committee on Genetic Symbols and Nomenclature" (1957) we have designated the individual genes in the order of their discovery: br_1 and br_3 to br_8. Different alleles of the same gene have been marked by using different indices: br_1^1, br_1^2 and br_6^1, br_6^2.

The analyses of our segregation results (Table 9) from crosses between six yellow seeded and one brown seeded turnip rape forms, without consideration of the segregation of hilum colour, clearly shows a monogenic inheritance pattern only in the cross 2016^{l} × d and a digenic inheritance with epistatic effect in all other cases. Therefore, it could be assumed, that in the combination 2016^{l} × d, another gene for yellow seed colour is present and is not the same epistatic gene as in the other crosses. But when this line was crossed with 'Yellow Sarson', the F_1 plants produced yellow + brown seeds [group (3)]. After crossing with the other *oleifera* lines, except line 2009, the F_1 plants produced yellow seeds with light or dark coloured hilum, whenever the other parent had yellow seeds with corresponding hilum colour. Therefore, it can be concluded that different alleles of the same gene and not different genes are involved; otherwise the F_1 plants from these crosses would have produced brown seeds. It may be assumed, that there are two different alleles with epistatic effect for yellow seed colour in the gene locus Br_1, i.e. br_1^1 in the two 'Yellow Sarson' forms, in the *oleifera* forms 2020 and 2021, and in the yellow seeded line from the cross d × 2016^{d}, and br_1^2 in the yellow seeded line from the cross 2016^{l} × d. From the results of the latter cross, dominance of br_1^1 over br_1^2 is evident. Yellow seeds would have been formed even when br_1^2 alone was in the homozygous recessive condition and the other gene loci are occupied by dominant alleles. The F_1 plants from a cross between line 2016 carrying this allele and 'Yellow Sarson' formed yellow + brown seeds (3). These seeds were of the type as produced in F_2 plants from crosses between 'Yellow Sarson' and the dark seeded turnip rape form which were homozygous recessive for $br_1^1 br_1^1$ and heterozygous for $Br_3 br_3$. Therefore, in this case the effect of br_1^2 must be suppressed by br_1^1, so that Br_3 can become functional. Following Stringam (1980) the mutated hypostatic gene in 'Yellow Sarson' was named br_3. A comparison of the segregation results in Table 2 with those in Table 3 reveals that br_3 conditions yellow seed coat and light hilum colour, as well.

Another hypostatic gene is present in the yellow seeded form 2021 and the yellow seeded line from the cross d×2016^d. It is proposed that this gene be designated br_4. When br_1^1 is homozygous recessive and br_4 heterozygous or homozygous dominant, light brown seeds (2) appear. When both genes are homozygous recessive, yellow/dark (4) seeds are formed.

The third hypostatic gene, designated as br_5, is present in the yellow seeded form 2020. When br_1^1 is homozygous recessive and Br_5 homozygous dominant, seed colour is light brown. When both genes are homozygous recessive, as well as when br_1^1 is homozygous recessive and Br_5br_5 heterozygous, yellow seeds with dark coloured hilum are produced. Therefore the allele Br_5 is not expressed in this case, giving a F_2 ratio of 12 : 1 : 3 instead of 12 : 3 : 1 as in the other crosses.

Brown seeds were produced in the F_1 plants of a cross between the *oleifera* form 2009^d and the remaining yellow seeded forms with the exception of the cross with 2021. This means that a different epistatic and hypostatic gene for yellow seeds must be present in this forms. It is proposed to call the epistatic gene br_6 and the hypostatic gene br_7. The name br_8 is proposed for the hilum gene in the line 2016^l.

In total, we, therefore, assume seven different genes to be involved in the inheritance of seed and hilum colour. Five genes control only seed colour and one only hilum colour, while one gene conditions both seed and hilum colour pleiotropically. Multiple allelism appears in the cases br_1^1 and br_1^2. The genes br_4, br_5 and br_8 can also be allelic with each other but they can not be allelic to the gene br_3. If this were so, F_1 plants from crosses between 'Yellow Sarson' and the *oleifera* forms, except 2009, should produce yellow seeds. Likewise br_7 can not be allelic to br_3, br_4, and br_8. Otherwise, no plants with light brown (2) or yellow + brown seeds (3) could have been present in the F_2 of the crosses 2009^d×2017^l, 2009^d×2021^{ld}, and 2009^d×2016^l.

In the F_1 of the reciprocal crosses 2009^d×2016^l and 2009^d×2017^l all plants developed only brown seeds and the F_2's segregated as expected. The results were different in the case of the reciprocal cross 2009^d× 2021^{ld} (Table 6), in which obviously the parental plant 2021^{ld} was heterozygous for the hilum gene. Here, some F_1 plants developed uniform brown and some uniform light brown seeds. It can be assumed that brown seeds were produced, when the effective gametes from 2021^d were recessive in the alleles br_1^1 and br_4 and dominant in the hilum allele. But where the hilum allele also was recessive light brown seeds were formed. Accordingly, the epistatic gene br_6 of 2009^d is allelic to the hilum gene in 2021^l; hence the designation of these alleles is br_6^1 and br_6^2. This assumption is supported by the segregation results in F_2 (Table 6). In the brown seeded progeny the Chi-square test revealed significant agreement (P = 0.83). In the light brown seeded progeny the observed segregation was the expectation for the alleles br_6^1 and br_6^2. Nevertheless direct proof in support of the given assumption is lacking. This could be obtained by crossing the form 2009^d with the two homozygous yellow seeded lines 2021^l and 2021^d. These lines must be homozygous recessive or homozygous dominant with respect to the hilum alleles (here known as br_6^2 and Br_6). At present the number of plants in the progeny of brown or light brown seeded forms used for the segregation analysis was too small. This may explain that in one segregating progeny the seed phenotype yellow/light (5) did not occur at all. In such case the Chi-square test can not be used adequately.

In the form 2016^l the hilum gene br_8 must be different from the one in the form 2021^l since the allele with epistatic effect of the form 2009^d is localized at the gene locus of the latter. Otherwise, the F_1 plants from the cross 2009^d×2016^l would produce light brown seeds (2) and from the cross 2021^l×2018^l yellow/light seeds (5) since yellow/light seeds (5) were also found after crossing 2016^l×2018^l (Table 5).

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