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The use of haploidy to develop plants that express several recessive traits using light-seeded canola (*Brassica napus*) as an example

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Summary. The use of haploidy to introgress recessive traits into *Brassica napus* canola is illustrated by describing the properties of doubled haploids obtained by microspore culture from crosses between a yellow-seeded rapeseed line (low erucic acid, high glucosinolate) and black-seeded canola. Of the 99 doubled haploid lines that were produced, 3 were yellow-seeded canola lines. This result was not significantly different than the predicted frequency of 1 in 64 for the homozygous recessive phenotype in a doubled haploid population segregating for six recessive genes. Thus, the study supports previous models of inheritance determined for yellow seededness and glucosinolate content in *Brassica napus*.

Also, since the chances of obtaining a plant with the same characteristics in a F_2 population are 1 in 4,096, the underscore results the advantages of using haploidy to introgress recessive traits into *Brassica napus* canola.

Key words: Haploidy – *Brassica napus* – Yellow-seeded canola

Introduction

The development of canola from rapeseed varieties of *B. napus* was accomplished in the early 1970s by combining the low erucic acid trait, which is determined by two recessive genes (Harvey and Downey 1964), with the low glucosinolate trait, which is determined by three recessive genes (Zhou and Liu 1987; Love 1988). Currently, there is interest in developing yellow-seeded canola lines that have a thinner seed coat and consequently higher oil and protein contents as well as lower fiber contents than black-seeded types (Shirzadegan and Robbelen 1985; Daun and DeClerq 1988). The yellow seed colour trait is also thought to be recessive in *B. napus*. Shirzadegan (1986) suggested that it is determined by recessive alleles at three loci such that: (1) a yellow seed occurs only when all three loci are in the homozygous recessive condition, (2) a brown seed occurs when a dominant allele occurs at either the Bl_2 or Bl_3 locus and the Bl_1 locus is in a homozygous recessive or a heterozygous condition, and (3) a black seed occurs when the Bl_1 locus is in a homozygous dominant condition.

The consequence of the recessive nature of the yellow seed coat trait is that it is expressed in a small fraction (1 in 64) of F_2 individuals from a cross between black-seeded and yellow-seeded types. A further complication in breeding for yellow-seededness is that the seed coat colour is determined by the genotype of the plant on which it develops because the testa is maternal tissue. Therefore, the seed coat colour does not indicate the genotype of the embryo it encloses. In order to combine the yellow-seeded trait with canola quality, extremely large populations of plants need to be screened and test crossed in a conventional breeding programme. The use of haploidy for this purpose can significantly reduce the number of plants that must be screened because recessive traits are not masked, genetic ratios are simpler and the genotypes of the seed coat and the embryo are identical after selfing in populations of doubled haploid homozygous plants.

In the present study the use of haploidy to create a canola quality *B. napus* line with light-coloured seed from a cross between a black-seeded canola and a yellow-seeded, low erucic acid, high glucosinolate line is described as an example of the utility of haploidy to produce lines combining several traits determined by recessive genes.

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Materials and methods

Plant material

The donor plants for microspore culture were F_1 hybrids obtained from crosses between the yellow-seeded rapeseed line (low erucic acid, high glucosinolate) and dark-seeded, highly embryogenic, canola (G231 and "Topas"). The donor plants were grown in an environmentally controlled growth room under a 16-h photoperiod (approximately 200 $\mu M\ m^{-2}\ s^{-1}$ at the bench surface) at 22 °C in the light and 18 °C in the dark.

Microspore culture techniques

Whole racemes with one or more open flowers were removed from the donor plants. Buds 4 mm or less in size were surface sterilized in a 5.7% hypochlorite solution for 10 min and used for microspore culture as described by Coventry et al. (1988).

Embryo culture and plant regeneration

After 3 weeks, normal microspore-derived embryos (Chuong and Beversdorf 1985) were transferred to solid B5H media containing B5 salts, 2% sucrose and 0.8% agar without hormones (Gamborg et al. 1968). The cultures were kept in the dark at 4 °C for 10 days and were subsequently transferred to a 23 °C controlled environment with a 16-h day photoperiod for 4 weeks. Plantlets that had developed roots and normal shoots were transferred to 15-cm^3 cells in nine-cell pack containing Metro Mix 240 (W. R. Grace and Co, Ajax, Ont.), covered with a clear plastic lid and placed in the growth room.

Colchicine doubling and seed production

At flowering, the floral morphology of the regenerates was examined to determine the ploidy. Spontaneous diploids were bagged to produce selfed seed. The haploids were treated with colchicine. The roots of the newly flowering plants were washed and immersed in 0.34% colchicine solution for 3 h. The roots of the treated plants were rinsed, and the plants were potted in nine-cell (approximately 15 cm^3) flats containing Metro Mix. After a week of recovery, the plants were cut back to new growth and watered as required. Doubled sectors were bagged to produce selfed seed.

Seed colour analysis

The diploid lines underwent a seed increase either in 15-cm-diameter pots in the growth room or in the field. Seed colour was scored on the healthy doubled haploid plants at or near complete seed maturity. Three colour classes were observed, and seed was categorized as either black, brown or yellow.

Glucosinolate analysis

Seeds from the selfed doubled haploid plants were tested for approximate glucosinolate levels with TES-TAPE (Eli Lily Canada Inc). The quantity of glucosinolates in the yellow-seeded low-glucosinolate lines was confirmed by gas-liquid chromatography as decribed by Thies (1976).

Results

The doubled haploid lines obtained from the F_1 s of light-seeded rapeseed (low erucic acid, high glucoinolate) and dark-seeded canola had the complete range of seed colours that would be expected (Table 1). In total, 99 doubled haploids were produced that gave seed of sufficient quality to classify their colour. The 11:27:66 ratio of yellow: brown: black-seeded lines is significantly different than the 12:37:50 ratio that was expected based on the model of inheritance proposed by Shirzadegan (1986) (Table 2).

Pure yellow as well as brown lines were obtained by microspore culture of the yellow-seeded parent. A total of 21 doubled haploid lines were produced from these experiments. The ratio of yellow-seeded to brown-seeded lines in this population was 6 to 15.

Three light-seeded canola quality lines were recovered in the population of 99 doubled haploids produced from the light-seeded rapeseed by dark-seeded canola cross (Table 3). This frequency was not significantly different than the expected frequency of 1 in 64 for an androgenic

Table 1. Seed colour frequencies in a doubled haploid population produced by microspore culture from crosses between yellow-seeded rapeseed (high glucosinolate, low erucic acid) and black-seeded canola

Seed coat colour	Observed	Expected	X^2
Black	66	50	13.0**
Brown	29	37	
Yellow	4	12	

** Significant at the 1% level of probability

[a] Based on Shirzadegan (1986)

Table 2. Postulated genotypes and seed colours and expected ratios for androgenic lines and F_2 lines obtained from a cross between yellow-seeded and black-seed *B. napus*

Genotype	Seed coat colour	Expected ratios in F_2 and androgenic lines[a]
F_2 lines		
$Bl_1\ Bl_1\ -\ -\ -\ -$	black	16
$Bl_1\ bl_1\ Bl_2\ -\ -\ -$ $Bl_1\ bl_1\ -\ -\ Bl_3\ -$ $bl_1\ bl_1\ Bl_2\ -\ -\ -$ $bl_1\ bl_1\ -\ -\ Bl_3\ -$	brown	47
$bl_1\ bl_1\ bl_2\ bl_2\ bl_3\ bl_3$	yellow	1
Androgenic lines		
$Bl_1\ Bl_1\ Bl_2\ Bl_2\ Bl_3\ Bl_3$ $Bl_1\ Bl_1\ Bl_2\ Bl_2\ bl_3\ bl_3$ $Bl_1\ Bl_1\ bl_2\ bl_2\ Bl_3\ Bl_3$ $Bl_1\ Bl_1\ bl_2\ bl_2\ bl_3\ bl_3$	black	4
$bl_1\ bl_1\ Bl_2\ Bl_2\ Bl_3\ Bl_3$ $bl_1\ bl_1\ Bl_2\ Bl_2\ bl_3\ bl_3$ $bl_1\ bl_1\ bl_2\ bl_2\ Bl_3\ Bl_3$	brown	3
$bl_1\ bl_1\ bl_2\ bl_2\ bl_3\ bl_3$	yellow	1

[a] As per Shirzadegan (1986)

Table 3. Comparison of the frequency of yellow-seeded canola in androgenic and F_2 populations from a cross between yellow-seeded rapeseed (low erucic acid, high glucosinolate) and black-seeded canola

Population	Expected frequency	Observed frequency	X^2
F_2	$1/2^{2n}$ [a] = 1/4,096	ND	ND
Androgenic	$1/2^{n}$ [a] = 1/64	3/99	1.52 NS

ND, Not done; NS, not significant at 5% level of probability
[a] n = number of recessive genes = 6

population segregating for six recessive genes. The glucosinolate and erucic acid levels in these plant ranged from 7.91–21.27 μM/g and 0.20–0.84%, respectively. The expected frequency of occurrence of the yellow-seeded canola in a F_2 population from the same cross is 1 in 4,096 (Table 3). The overall ratio of low glucosinolate to high glucosinolate doubled haploid lines was 1:3.8.

Discussion

Considerable advances have been made in haploidy since the first plants were produced by anther culture. In particular, very dramatic improvements in efficiency and expansions in applicability to a wider range of genotypes have led to the widespread use of anther and microspore culture for the production of homozygous lines of *B. napus* (Foroughi-Wehr and Wenzel 1989). The most often cited advantage of haploidy is the time-saving that accures from using haploidy to fix traits in a homozygous state. However, recent utilization of haploid tissues for protoplast fusion (Chuong et al. 1988), in vitro selection (Swanson et al. 1988, 1989) and microinjection to create transgenics (Neuhaus et al. 1987) have emphasized that haploidy can be used in a variety of ways for plant improvement. In addition, it has been shown that there are advantages to using doubled haploid populations to examine the inheritance of important traits in *B. napus* like fatty acid levels (Siebel and Pauls 1989a) and glucosinolates (Siebel and Pauls 1989b). Genetic ratios and in doubled haploid populations are much simpler and the differences between classes are more distinct because heterozygous genotypes are not present (Choo et al. 1985).

The advantages of simpler genetics in doubled haploid populations are most apparent when examining traits determined by recessive genes. This aspect should make haploidy particularly applicable to canola breeding because the important quality traits, namely low levels of erucic acid and glucosinolates, are recessive. In particular, because the frequency of the homozygous, multiple-recessive class is much greater in an adrogenic than a F_2 population, less effort is required to produce a higher number of canola plants that express the yellow-seeded traits by haploidy than by conventional crossing methods. For example, the chances of obtaining a plant that is a yellow-seeded canola from the cross used in the present study is 1 in 4,096 in a F_2 population (Table 3). By producing doubled haploid lines from the same cross the chance of obtaining a yellow-seeded canola plant increases to 1 in 64.

In addition, since the results of the present study did not differ significantly from what was predicted, they support previous models of inheritance determined for glucosinolate content and yellow-seededness. The fact that the yellow-seeded class was under-represented in the doubled haploid population obtained in the present study (only 4 out of 99 plants were yellow-seeded instead of 1 in 8 as predicted by Shirzadegan) (Table 2) can probably be attributed to residual heterozygosity in the yellow-seeded parent. This suggestion is supported by the results of the microspore culture performed on the yellow-seeded parent. The doubled haploids that were obtained from this material had a range of colours from brown to yellow rather than being all yellow. However, it should be noted that unless a good quantity of seed is available (approximately 200 mg), it can be difficult to classify its colour, even with seed produced by selfing doubled haploid lines. This is because of the relatively large effect that the environment has on this trait (Shirzadegan 1986). In particular, imperfectly formed seeds will often be darker in colour.

The biggest complication in breeding for the yellow-seeded trait in *B. napus* is the fact that the seed coat and the embryo have different genotypes when cross pollination occurs. Consequently, the genotype of the F_2 generation, with respect to seed coat colour, can only be determined by examining the colour of the seed it produces after selfing. Thus, the second important advantage of employing haploidy with *B. napus* populations in which the yellow-seed trait is segregating is that the colour of the seed produced on a doubled haploid line, after selfing, is a true indication of the genotype of the embryo.

Overall, the results illustrate the general principle that there are considerable advantages to using haploidy to introgress recessive traits into canola.

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