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Microspore mutagenesis in transgenic oilseed rape for the modification of fatty-acid composition

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Abstract

One of the first successful applications of the haploid embryogenic system in *Brassica* was aimed at mutagenesis and subsequent selection for desirable traits. A major advantage of the haploid embryogenic system for the selection of fatty-acid mutants is that analysis can be performed with only one of the two cotelydons while the remainder of the embryogenic tissue is available for plant regeneration. In the present study the applicability of the haploid technique of oilseed rape (*Brassica napus* L.) cv. 'Drakkar' transformed with the thioesterase gene *Cl*FatB4 from *Cuphea lanceolata* combined with *in vitro* mutagenesis for the selection of a high myristic-acid (C14:0) content and a lower ratio of palmitic acid/myristic acid (C16:0/C14:0) in the seed oil was tested. The embryogenic capability of isolated microspores varied between 0.03 and 2.6 embryos per bud. After ultraviolet-light (UV) treatment only 3 lines regenerated to plants. Applying an UV-dose of 0.005 J cm^{-2} resulted in 74 regenerants from which 23 % diploids were obtained. The fatty-acid composition of the different genotypes of the corresponding doubled-haploid lines displayed some changes but there was no clear evidence for their mutagenic nature. The offspring of nine additional mutagenised plants were studied and these results are presented.

Key words: *Brassica napus*, microspore, haploid production, fatty acid content, mutagenesis.

Introduction

The induction of haploid embryos from microspores in *Brassica* is recognised as the most rapid route to achieve homozygosity for the production of haploid and doubled haploid lines, which have considerable value in plant breeding. The doubled-haploid technique is an important tool for assembling desirable traits quickly into true breeding cultivars with minimal progeny numbers. For the efficient recovery of haploid embryos from *Brassica* one of the most important controlling factors is the genetic background of the donor plants (Ferrie, Keller 1995). This factor influences the frequency of embryogenesis, the quality of embryos and the way of plant regeneration (Kieffers et al. 1993; Ferrie et al. 1995).

One of the first attempts to exploit the haploid embryogenic system in *Brassica* was the selection for disease resistance (MacDonald et al. 1989; Ahmad et al. 1991). A major advantage of the haploid embryogenic system for the isolation of storage product mutants is that analysis can be performed using one of the two cotelydons, leaving the rest of the embryo for plant regeneration (Palmer, Keller 1999).

The aim of this project was to develop germplasm for the breeding of winter rapeseed with a high myristic-acid (C14:0) content and a lower palmitic-acid/myristic-acid (C16/C14) ratio in the seed oil by use of the haploid techniques combined with *in vitro* mutagenesis procedure and selection in the field.

Materials and methods

Six transgenic lines of oilseed rape (*Brassica napus* L.) cv. 'Drakkar' which had been transformed with the thioesterase gene *Cl*FatB4 (Martini et al. 1999) were grown in a growth chamber at a day/night temperature of 14/8 °C to ensure induction of flowering. The floral buds, in which microspores were in late uniculate stage, were collected and microspores were isolated and cultured as described by Lichter (1982). The buds were sterilised in 3 % NaClO and homogenised in a modified Lichter medium. After filtration microspores were collected by centrifugation, washed, diluted to 60 000 spores ml^{-1}. Then microspores were plated in 35 mm petri dishes and subjected to a pulse in the range of 10 to 180 seconds of different UV intensity (0.005; 0.02; 0.03 J cm^{-2}) in 'Biometra' UV equipment to induce mutation in microspore DNA. Embryogenesis was induced at 32 °C for 3 days in dark, then cultures were transferred to 25 °C for embryo development. After 4 weeks, embryos were transferred to solid media. The ploidy level of the regenerated plants was analysed using a flow cytometer (Cell Analyser CA-II, Partec, Münster, Germany). *In vitro* grown plants were transferred to the greenhouse, where they were propagated by bagging after colchicine treatment of the haploid plants. Seeds of each plant were harvested separately and formed a doubled-haploid line. Fatty-acid composition was analysed using one cotelydon (half-seed technique), growing the remaining embryo for seed production according to Thies (1971). Both the mean myristic-acid (C14:0) content and the mean palmitic-acid/myristic-acid (C16/C14) ratio were compared among lines using the F-test and the Tukey-test, respectively, using the respective procedures of the SAS/STAT® software, release 6.12 (SAS Institute Inc.).

Results and discussion

The overall comparison of the six transgenic lines of cv. 'Drakkar' reflected great variation of lines in embryogenic capability from 0.03 to 2.6 embryos per bud. Different effects on embryogenic response have been observed in many species of *Brassicaceae* (Phippen, Ockendon 1990; Ferrie et al. 1995). After UV treatment, only three of the six transgenic lines of cv. 'Drakkar' regenerated to homozygous plants for use in breeding programs. Altogether, after UV irradiation (0.005 J cm^{-2}) 74 regenerants developed, from which 23 % spontaneous diploids were obtained. Similar results were obtained with spontaneous chromosome doubling by Hansen and Anderson (1996) for the cv. 'Topas'.

In the first step, the fatty-acid composition of different genotypes of these doubled haploid lines displayed some changes, but there was no clear evidence of their mutagenic nature.

The offspring of nine plants were studied, which originated from two different transgenic lines #6 and #39. Seven plants from #6 were identified in the *in vitro* phase as haploid (1n) and one (6E) as diploid (2n), respectively. The two plants of #39 (39A, 39B) were haploid (1n). The haploid plants were doubled by colchicine treatment and all plants

Table 1. Fatty acid composition in the offspring of regenerated oilseed rape plants resulting from UV mutagenesis of microspores from transgenic lines. *, F-test between #39 A, B: $F_{obs.}$ = 1.48 (C14:0%), 1.49 (C16/C14); $F_{tab.(P = 0.95; 26/37 FG)}$ = 1.86. **, Tukey-test of # 6 A-G. Means followed by an identical letter are not significantly different (P = 0.95). [a], *in vitro* plants; [b], number of analysed seeds. C.V., coefficient of variation

Offspring	Ploidy[a]	N[b]	C14:0 (%)			C16:0/C14:0 ratio		
			Mean	Significance	C.V. (%)	Mean	Significance	C.V. (%)
39A	1n	27	19.9	n.s.*	23.0	1.15	n.s.*	16.6
39B	1n	38	19.6	n.s.*	19.1	1.12	n.s.*	20.8
6A	1n	25	21.6	a**	17.9	1.04	be**	15.4
6B	1n	5	17.5	ac**	23.1	1.27	ade**	16.3
6C	1n	9	20.5	a**	16.3	1.47	ac**	14.8
6D	1n	9	23.8	a**	16.7	1.12	bd**	15.2
6E	2n	10	19.3	ac**	18.1	1.35	ad**	14.0
6F	1n	16	20.1	a**	17.5	1.13	bd**	19.4
6G	1n	6	14.5	bc**	29.2	1.23	ade**	22.6

were propagated in the greenhouse by bagging. The C14:0 and the C16:0 contents of the seeds were analysed by half-seed technique. The 80 seeds of #6 varied between 9.7 and 33.3 % C14:0 and the C16/C14 ratio ranged from 0.83 to 1.78. The mean C14:0 content of the seven lines of #6 varied between 14.5 and 23.8 %. There were significant differences between the lines. The 65 seeds of #39 displayed a C14:0 content between 7.3 and 29.6 % and a C16/C14 ratio ranging from 0.84 to 1.96. The two lines of #39 did not show any significant differences in the C14:0 content (Table 1). The number of seeds displaying a C16/C14 quotient < 1.0 was 18 (22 %) in #6, and 19 (29 %) in #39, respectively. The coefficient of variability between the seeds of the same plant was in all cases relatively high. Particularly considering that all but one regenerated *in vitro* plants displayed a haploid DNA content, the colchicine-induced diploids should be homogeneous in their characters. The variability may be due to modifying influences of the culture conditions. The first generation after *in vitro* regeneration and colchicine treatment often shows delayed flowering and seed ripening. Fifty-two half-seed individuals of the 145 analysed seeds were selected on the basis of a high C14:0 content and low C16/C14 ratio, respectively. Grown in a greenhouse, they showed a normal and homogenous development. The fatty-acid analysis of their offspring will show whether the effect of environmental influences on plant development is an appropriate explanation for the observed variability.

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