Anther Culture as a Breeding Tool in Rape
II. Progeny Analyses of Androgenetic Lines and Induced Mutants from Haploid Cultures

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Summary. Progeny analysis of androgenetic plants from inbred rape-seed (Brassica napus) shows that selective growth of microspores can occur in cultured anthers. The property of privileged growth in culture seems to be linked to such characters as flowering time and seed glucosinolate content which can be analyzed in regenerated plants. This type of selection and the fact that more variability is visible in regenerants from different microspores than in the progeny of the highly inbred anther donor line, demonstrates the higher degree of homozygosity in the doubled amphihaploids of B. napus. Furthermore, it is shown that haploid genomes of rape may be mutable. Thus it is possible to obtain several different homozygous lines from a single microspore. A system of haploid embryoids arising from single cells of the primary microspore regenerant has also been used to produce experimentally induced mutants. It is demonstrated that recessive mutations can be obtained in a homozygous state in doubled haploid regenerants from mutagenized haploid single cells.

Key words: Anther culture – Brassica napus – Mutagenesis – Rape-seed

Introduction

Rape-seed (Brassica napus) is the most productive oil plant of the northern temperate zone. Its economic value could be further increased if high yielding oil- and forage-types could be developed which have low contents of toxic glucosinolates. This would make possible the extensive use of the high quality seed protein and of the green fodder. Furthermore, Brassica napus is one of the very few important crop plants which can be successfully established in tissue culture and probably one of the first examples of crop improvement by the combination of classical plant breeding methods and modern in vitro-techniques (Hoffmann 1980).

Many of these in vitro-techniques have recently been described. The production of haploid plants of androgenetic origin has been reported by several authors (Thomas and Wenzel 1975; Wenzel et al. 1977; Keller and Armstrong 1977, 1978; Hansson 1978; Renard and Dosba 1980; Lichter 1981). Regeneration in stem explants has been shown by Kartha et al. (1974) in diploid tissue and by Stringham (1977) in haploids. Furthermore, stem embryogenesis has been induced from somatic embryoids (Thomas et al. 1976) and this system of mass regeneration of single tissue cells has been utilized in mutagenesis (Sacristán and Hoffmann 1979). Plant production from callus cultures of different origin and from isolated protoplasts has also been obtained for both ploidy levels (Kartha et al. 1974b; Thomas et al. 1976; Stringham 1979; Sacristán 1981). An improved protoplast method has been described by Kohlenbach et al. (1982). Brassica napus protoplasts have been used in fusion experiments for the successful creation of hybrid cells (Kartha et al. 1974a; Hoffmann et al. 1980).

In this paper we report the results from several field tests with androgenetic doubled haploids, including the analysis of biochemical quality characters, and the production of homozygous mutant populations from mutagenized haploid tissue cultures.
Materials and Methods

The anther donor material of *Brassica napus*, the anther culture technique and plant production from androgenetic embryos have been described previously (Wenzel et al. 1977). Only the method of diploidization was altered. Instead of applying colchicine with small cotton wool plugs to debudded axils of leaves, the colchicine (0.2% aqueous solution) was injected into the buds with a 0.45 mm needle. The volume depended on the receptivity of the tissue. This method is very simple and efficient: in an experiment with 21 stable haploid plants, e.g., a haploid genotype which did not show spontaneous chromosome doubling for many years, we dispensed 306 injections and eventually obtained 31 sprouts with seeds. The average success rate was probably higher but was not regularly monitored. In one case chromosome doubling could not be induced at all.

Field tests have been carried out from 1977 to 1980 at Ladenburg. Selfing and crossing were performed either in isolation chambers or by bagging the flowers. The material was precultured in Jiffy strips and transplanted in early April. The test plots had a size of 150×150 cm. The distance between the plants was 30 cm, which resulted in 16 plants per plot.

Photometrical analysis of the total glucosinolate content was carried out after Lein (1972a). Determination of the content of single glucosinolates was performed using the gas chromatography method of Thies (1977). The fatty acid composition of some samples was measured according to the method of Thies (1971). Usually, the appearance of erucic acid was only controlled by thin layer chromatography. The extraction of fatty acids was performed as described by Thies (1971) and samples were run on paraffin impregnated cellulose plates (Merck 5716) in acetic acid. Staining of the fatty acids was carried out in an iodine vapor.

Photometrical estimation of myrosinase (thioglucoside glucohydrolase, E.C. 3.2.3.1.) activity was performed as described by Lein (1972), and analysis of the multiple forms of the enzyme was carried out according to the method of Davis (1964) using a disc acrylamide gel system. The separation system used was No 6 of Maurer (1968) and the electrode buffer was borate-NaOH at pH 8.3. Staining of myrosinase isozymes was performed as described by MacGibbon and Allison (1970) with sinigrin. Additionally, we used glucoiberin in our experiments. As an early screening, a quick and simple test was carried out with callus or tissue pieces from stem embryo cultures. Homogenates from about 100 mg of material in 1 ml M/15 phosphate buffer, pH 7, were centrifuged and mixed with 1 mg ascorbic acid and 5 mg glucotropaeolin. The presence of myrosinase was indicated by an increase in turbidity caused by the precipitation of benzylisothiocyanate.

For the induction of mutants, stem embryogenic tissue of stable haploid lines (ca. 2 cm² expansion) was mutagenized. The treatment was such that, on average, out of 10 pieces mutagenized only one plantlet regenerated. For the X-irradiation we used the Philips Radifluor 120. The dose was 120 Gy at 120 kV and 5 mA. EMS (ethyl methane sulfonate) was applied as a suspension of 1 g per 100 ml (1 h) and ethidiumbromide as a 2.5 µg per 100 ml solution (3 h). To avoid chimerism only secondary embryos derived from surviving cells were isolated, propagated and used for seed production.

In the experimental series reported here a total of 66 plants were grown from 78 androgenetic macroscopic structures. From these, 59 plants were analyzed in the field: 45 were descended from "homozygous" anther donor lines (inbred line 24/72 and the commercial varieties 'Tower' and 'Egra') and 14 from F₁ hybrids. Later on, by carefully monitoring all culture parameters and the genetic background of the donor material, the induction rate of the embryos could be steadily increased (Fig. 5; see also Lichter 1981) in new experimental series. These data will be published elsewhere.

Results

Derivatives from Homozygous Anther Donor Plants

After selfing, 36 A₁ plants produced homozygous progeny (referred to as the A₂ generation); the offspring of the other 9 plants produced heterozygous A₂ populations. Most of these heterozygous progenies were rather vigorous and parental phenotypes were included. The variability amongst the lines was, however, much more distinct than the variability in progenies of the selfed anther donor lines, which shows that these plants were not of somatic origin. Twenty-eight of the 36 non-segregating lines showed a similar phenotype as the parent, while 8 revealed growth depression and partial sterility. Amongst the latter heterozygosity could not be completely excluded but vigorous plants were missing (in contrary to the 9 heterozygous populations). Between the 28 vigorous lines clear differences concerning leaf shape (Wenzel et al. 1977) and colour, flower type, pod shape and size were detected. These variations were much more distinct than differences within the progeny of selfed anther donor material, demonstrating quite a high amount of residual heterozygosity in classical inbred material. All 28 androgenetic homozygotes flowered, on average, 1 week later than the parental plants (Fig. 1), which indicated that during microspore culture selection took place.

Derivatives from F₁ Anther Donor Plants

The offspring of the self-fertilized anther donor plants (F₂) showed strong segregation. From 14 microspore-derived plants only 7 gave a homogeneous progeny (A₂) while another 7 produced a heterogeneous A₂. Of the former lines, 4 showed growth depression and poor seed set while the other 3 revealed vigorous growth. Two of these three were comparable with the donor F₁, and the remaining one was even superior. Amongst 200 plants of the segregating donor F₂ no plant

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1 The instrument is not well suited for this load because the cooling system is not efficient enough for the long irradiation time which is necessary to obtain the wanted dose.