DOUBLED HAPLOID PRODUCTION AND FIELD EXPERIMENTS WITH HOMOZYGOUS LINES OF RAPESEED

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INTRODUCTION

Androgenetic induction of haploids is the most economic way to produce homozygotes in rapeseed (Polsoni et al. 1988) and numerous other crops. Presently, the embryo production and plant regeneration frequencies in anther and microspore cultures of rapeseed are sufficient for their routine use in practical breeding programs. Up to now we have concentrated our work on the improvement of haploid plant production and the subsequent testing of androgenic doubled haploid (DH) lines in field trials.

MATERIALS AND METHODS

The donor plants of one variety (Jet Neuf) and six hybrid lines (M-1, M-2, RB, RC, Z1 and Z2) of double low winter rapeseed were grown in a natural field environment. Buds at the stage of uninucleate microspores were sterilized with 70% ethanol for 1-2 min. and rinsed twice with autoclaved water. Intact anthers were placed on B_5 medium according to the method of Keller and Armstrong (1977), incubated in the dark at 35°C for 2 days and then at 25°C until embryos emerged .

The regeneration of plants was done in two ways. First, embryos were planted on B_5 medium according to Keller and Armstrong (1977), and after that, abnormal hypocotyl explants were transferred several times on hormone-free Murashige and Skoog (MS) medium until whole plants were obtained. In the second procedure, shoots were regenerated directly from embryos when they were cultured on B_5 medium supplemented with kinetin (10^{-4}M), and roots were initiated by further transfer to MS medium with addition of 5 mg IBA/l (Naleczynska 1991).

Young plants were vernalized by exposing them to 4oC for 7 weeks under an 8-hour photoperiod. Spontaneous diploids were bagged to produce selfed seeds while haploid plants underwent colchicine treatment. For this purpose two or three secondary auxiliary shoots were cut from each haploid and placed into 0.05% colchicine solution in 1.5% DMSO for 18 hrs. at 25°C in the dark. Cuttings were then rooted in soil (Naleczynska and Cegielska 1984). Using this technique seeds were obtained from each colchicine-treated unfertile plant.

All doubled haploid lines were sown in small plots and individual plants were evaluated for several morphological and biochemical characters in the A_2 generation. At this stage the unproductive lines and noticeable heterozygotes were discarded. The selected DH lines in A_3 and further generations were tested in field experiments. To date 150 DH lines produced from the same double low winter rapeseed F1's have been investigated. The experiments were performed at one location with two replications for the A_3 generation and four replications for the A_4 generation in $5m^2$ and $10m^2$ plots respectively. The following characters were recorded and evaluated: seed yield (q/ha), oil content (%), alkenyl glucosinolate content (μM/g defatted meal),

1000 seed weight (g), length of pods (cm), number of seeds per pod and plant height (cm). The double low variety Bolko was used as a control. Oil content of the seed was determined by NMR and glucosinolate content using gas chromatography (Byczynska and Krzymanski 1981).

RESULTS AND DISCUSSION

A total of 3,770 anthers collected from seven donor genotypes of rapeseed were cultured, and 4,903 embryos, ranging from globular to cotyledonary stages, were obtained. In the genotypes examined the yield of androgenesis varied from 45.1 to 221.0 embryos per 100 anthers. The frequency of embryo initiation also varied greatly between individual anther platings. The observed differences could not be interpreted because the donor plants were grown under uncontrolled field conditions. In the present study the embryo production frequencies obtained by anther culture were lower than by the microspore culture technique used by other authors (Polsoni et al. 1988; Siebel and Pauls 1989). However, the number of embryos obtained from all tested genotypes was enough for our breeding purposes.

It is well known that for embryo development transfer to another medium is required. When the embryos were plated on the B_5 medium according to the Keller and Armstrong method (1977), only a small fraction of plants generated directly while the majority underwent somatic embryogenesis. Whole plant production occurred after one to seven subcultures of abnormal hypocotyl explants on MS hormone-free medium (Table 1). By using this procedure many explants died with only about 35% plant regeneration obtained. Much better results were achieved when primary embryos were plated on B_5 medium with a high level of kinetin. On this medium in the first transfer, shoots were initiated from about 70% of planted embryos (Table 2). The rest of the undifferentiated explants produced shoots when they were transplanted the second time on the same medium. These data confirmed the influence of cytokinins on organ regeneration of somatic embryos cultured in vitro as described by Loh et al. (1983).

From the seven experimental genotypes, 4,936 androgenic plants were obtained. Observation of these plants revealed that 45.6% were haploids, 40.7% were spontaneous diploids and 13.7% were other forms such as mixoploids, non-flowering dwarfs, etc.

Seeds collected from spontaneous diploids and from haploids doubled by colchicine treatment were sown in the field and individual plants (generation A_2) were evaluated for several morphological characters such as leaf shape, flower shape and colour, plant habit, number of branches, pod length, seed weight, seeds per pod and date of flowering. Doubled haploids manifested excellent morphological uniformity within individual plant progenies, and only a very few lines showed segregation. On the other hand there were major differences between lines. The observed range of variation of several characteristics confirmed the supposition (Snape et al. 1986) that the doubled haploid populations of rapeseed originated from a random sample of the parental gametes. At this stage of breeding all DH lines which carried negative characters such as poor vigour of plant, short pods, small seeds, prolonged cycle of vegetation, etc. could be eliminated. The number of discarded lines was dependent on the donor plant genotype, and in the material examined the proportion discarded varied from 27% for hybrid M-1 to about 90%

for hybrids Z1 and Z2.

A preliminary estimation of agronomic value was performed in field experiments with A_3 and A_4 generations of 150 DH lines derived from the rapeseed hybrid M-1. Heterozygotes or other genetically unstable forms were not found in the plant materials tested. The range of variation in seed yield and oil content are presented in Figures 1 and 2. Among the 150 DH lines evaluated for yield, the extreme value was from 25.6 dt/ha to 60.7 dt/ha in comparison with 44.4 dt/ha obtained for the control variety Bolko. Sixty-three DH lines were similar or higher yielding than the check variety. The oil content in the androgenic lines ranged from 40.0% to 52.2% compared to 47.9% for the Bolko check.

Alkenyl glucosinolate content of the DH lines was examined. The variability in total content of these compounds is shown in Figure 3. Only two of the 150 lines analyzed had relatively high glucosinolate levels, namely 10 and 26.6 µM/g ffm while 110 were low or very low in glucosinolate and varied in content between 0.7 to 5.0 µM/g ffm.

In the investigated DH lines the range in yield components observed was as follows: 1,000 seed weight 3.5 to 7.8 g, length of pods 4.8 to 8.0 cm, seeds per pod 6.0 to 29.0, and plant height 137 to 185 cm.

Of the 150 doubled haploid lines evaluated in the field several were identified which combine high yield of seed and oil with a very low glucosinolate content. However, much of the information available at present is of a preliminary nature. There is a need to repeat and to expand these studies.

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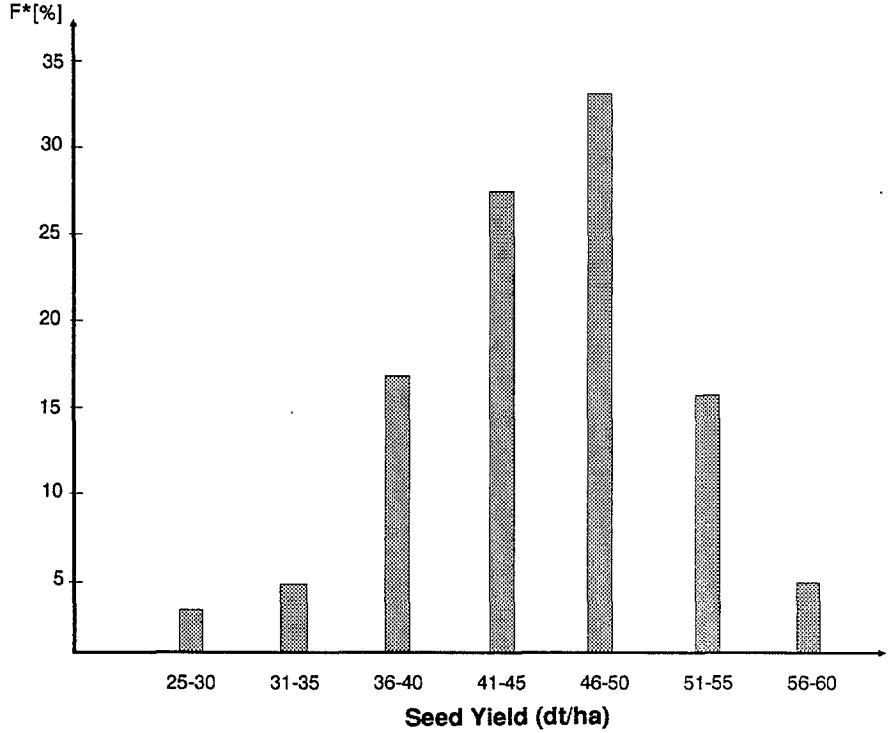
Table 1. Regeneration of androgenetic plants derived from rapeseed hybrids M-1 and M-2 on B5 medium and after several transfers of secondary embryos on the MS medium.

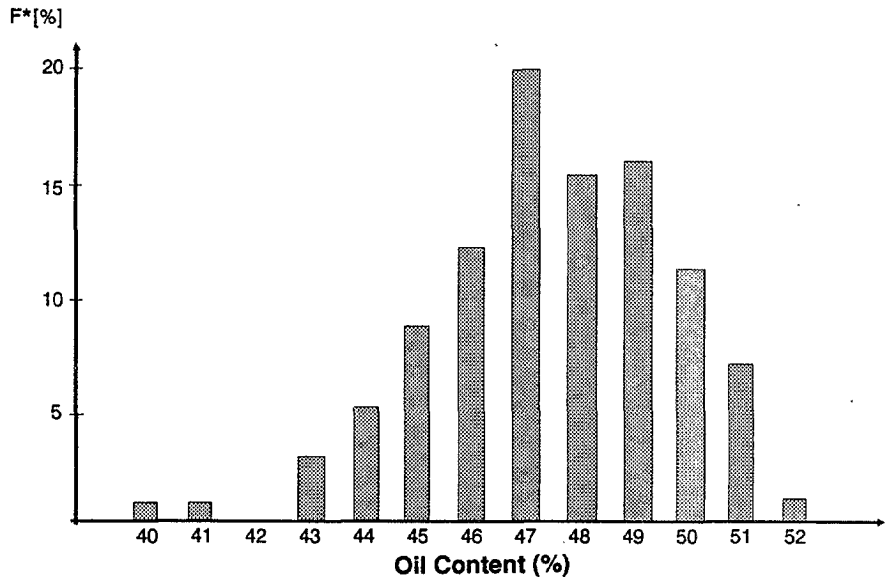
Donor Plant	Number of Embryos	Number of Regenerated Lines* (%) On the Medium					Frequency
		B_5	MSx1**	MSx2	MSx3	MSx4-7	(%)***
Hybrid M-1	559	27 (13.0)	30 (14.5)	45 (21.7)	33 (16.0)	72 (34.8)	207 (37.0)
Hybrid M-2	412	28 (20.0)	24 (17.1)	18 (12.9)	33 (23.6)	37 (26.4)	140 (34.0)

* The term "line" is used to denote a plant or plants derived from a single microspore.
** Number of transfers to MS medium.
*** Number of lines per 100 cultured embryos.

Table 2. Regeneration of androgentic plants derived from rapeseed hybrid RB and RC on the B_5 medium with kinetin.

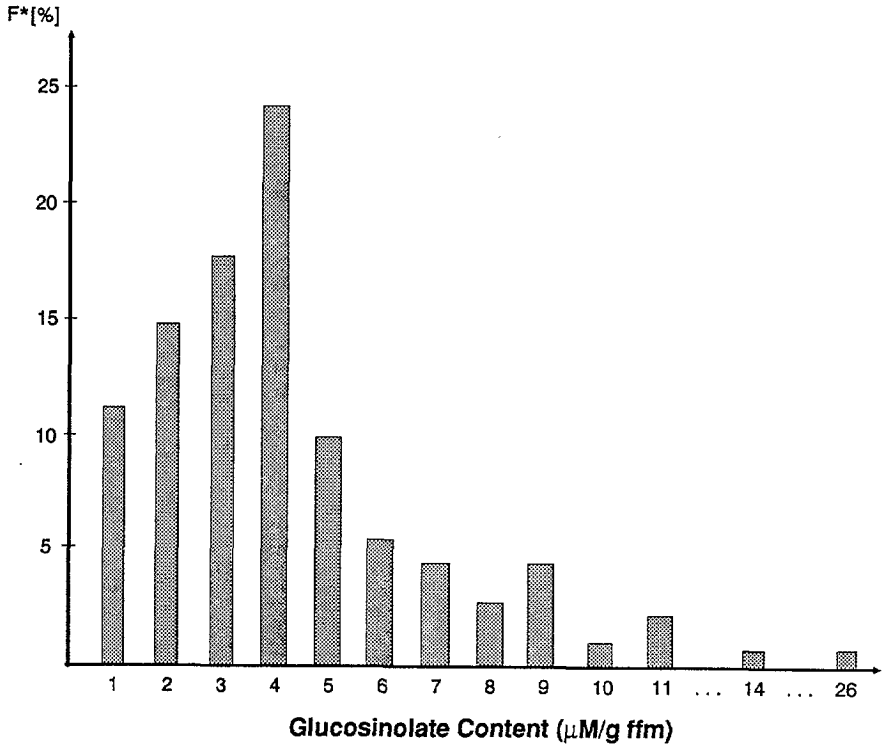
Donor Plant	Number of Embryos	Number of Embryos with Shoots on the Medium B_5 + Kinetin (%)		Frequency (%)
		Transfer 1	Transfer 2	
Hybrid RB	221	140 (66.0)	52 (24.5)	192 (90.6)
Hybrid RC	290	210 (72.4)	40 (13.8)	250 (86.2)





* Frequency

Figure 1. Frequency distribution of seed yield and oil content in 150 DH lines derived from rapeseed hybrid M-1.



* Frequency

Figure 2. Glucosinolate content in seed of 150 DH lines derived from rapeseed hybrid M-1.