

Use of doubled haploid lines in Polish rapeseed breeding

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INTRODUCTION

The microspore culture involves protocol to produce with high efficiency homozygous plants. The use of androgenesis in vitro admits pure lines production from heterozygous parents in one generation.

The haploidization of rapeseed has been obtained by anther culture (Keller and Armstrong, 1977) or isolated microspores (Polsoni, Kott and Beversdorf, 1988). The doubled haploid lines of *Brassica napus* have been received in the following stages:

- induction of embryos from microspores,
- plants regeneration from embryos,
- seeds collection from spontaneous diploids or haploids doubled by colchicine treatment.

In Oil Plant Department in Poznań for a few years over three thousands of homozygous lines from a wide range of donor plants have been obtained.

In this paper the results of investigation on doubled haploids (DH) produced from two rapeseed hybrids have been described.

MATERIAL AND METHODS

The donor plants of hybrid 3859/86 (C) of double low winter rapeseed and the hybrid 4488/87 (Z2) with genes determining the heritability of yellow-seeded characters were grown in the natural conditions.

Anthers were cultured according to Keller and Armstrong (1978) but shoots were regenerated directly from embryos, when they were grown on B5 medium supplemented with kinetin (10^{-4} M). Rooting stage was initiated by further transfer to MS medium with addition of 5 mg/l IBA (Nałęczyńska, 1991).

After artificial vernalization plantlets were grown in the greenhouse. Spontaneous diploids were bagged to produce self pollination seeds by while haploid plants underwent colchicine treatment. For this purpose two or three secondary axillary shoots were cut from each haploid and placed into 0,05% colchicine solution in 1,5% DMSO for 18 hs at 25°C in the dark. Then cuttings were rooted in pots with soil (Nałęczyńska and Cegielska, 1984).

Seeds of DH lines in A₁ generation were multiplied on the microplots of 1 m² area. Preliminary selection of doubled haploids for morphological and biochemical characteristics were performed at this stage. Oil and glucosinolates content in

seeds were also estimated. In DH lines developed from hybrid Z2 the forms with yellow or light brown color of seed coat were chosen exclusively. Homozygosity of winter rapeseed lines was tested especially by spring sowing method (Nałęczńska and Krzymański, 1985).

Previously selected 78 DH lines obtained from hybrid C were tested in A₃ generation in field trial on the plots of 10 m² area in four replications. The double low Polish variety Mar was used as a standard. The following characters were recorded: seed yield (dt/ha), oil content (%), alkenylglucosinolate level (uM/g f.f.d.m.), 1000 seed weight (g), length of pods (cm) and number of seeds per pod.

RESULTS AND DISCUSSION

Results of haploidization by means of androgenesis in vitro of seven rapeseed hybrids were published previously (Nałęczńska, 1991; Nałęczńska and Cegielska, 1991). Frequencies of embryo production and plant regeneration obtained in present study from two donor hybrids (C and Z2) are given in table 1.

It is well known that the efficiency of embryogenesis by anther culture is lower than in the isolated microspores technique used by other authors (Siebel and Pauls, 1989). However, the number of embryos obtained in our laboratory from all tested donor hybrids was sufficient for the breeding purposes. It could be the advantage of anther culture method that significant part of regenerated plants consists of spontaneous diploids what is not observed in plants produced by means of isolated microspores. Possibility to obtain the seeds from each haploid is real by using method of colchicine treatment but this is labor and time consuming.

Besides diploids and haploids sometimes other forms were observed. They were characterised by flowers with normal morphology but neither pods nor seeds were developed. Especially a lot of such plants were noted among lines received from donor hybrid Z2. Probably the above deformations were caused by disturbances during meiosis. Only seeds of a few lines with yellow or light brown seed coat were taken to further breeding. At present the inheritance of color of these selected seeds have been investigated.

In contradiction to DH lines from hybrid Z2 all doubled haploids obtained from the hybrid C were tested in later study. First comparison of these lines under microplots conditions was done in A₂ generation. The excellent morphological interline uniformity was found which was contrasted strongly with the variation between the lines. Just a few segregating lines were discarded.

DH lines with superior agronomic performance including long pods with great number of seeds, with glucosinolate level lower than 5 uM/g f.f.d.m. and high content of fat were used in further investigations. After such preliminary selection on microplots 78 DH lines were chosen at least to the next experiments.

Seeds from selfed plants of selected lines in A₃ generation were sown on the field. Comparative results of this experiment concerning the yield of seeds, fat and glucosinolates content are presented on fig.1,2, and 3.

The extreme value of seeds yield obtained in 78 DH lines ranged from 12,0 dt/ha to 27,4 dt/ha compared with yield of Mar variety equal 21,9 dt/ha (fig.1). The

variability in fat content was significant and ranged from 40 to 50 per cent. Under the trial conditions the fat content of 71 DH lines exceeded 44 per cent obtained by standard variety (fig.2).

Glucosinolates content in seeds ask for special attention because high yielded varieties of rapeseed with very low level of these compounds are not only a good row material for oil production but also their meal can be used as a feed for monogastric animals.

In presented work it was noticed that 77 lines from 78 investigated had glucosinolate level lower than 17,4 uM/g f.f.d.m. ascertained for Mar variety (fig.3). It seems interested that 43 DH lines produced from hybrid C possessed glucosinolate level lower than 5 uM/g f.f.d.m.

The possibility of full expression of recessive genes by doubled haploids is very convenient in breeding of maintaining lines for gene-cytoplasmic male sterility CMS pol. Details of such experiments were described in the separate paper (Bartkowiak-Broda et al., in press).

These results confirmed our earlier reports that the application of anther culture allow for quick selection of homozygous forms which can be useful in rapeseed breeding programs. However, it is obviously that number of the best lines depends on a great extent on the genetic potential of donor plants.

Table 1.

The embryogenesis in anther culture, the ploidy level of plant and DH lines regeneration.

Donor	Efficiency of embryos/100 anthers	Ploidy of plants regenerated %			Number obtained DH lines
		haploids	diploids	other forms	
Hybrid C	221,0	52,1	44,6	3,3	567
Hybrid Z2	172,9	43,6	25,9	30,5*	209

* - part of plants derived from hybrid Z2 developed without seeds

REFERENCES

1. Bartkowiak-Broda I., Popławska W., Górska-Paukszta M., Gazecka-Michalska B., Liersch A.: Investigations on possibility of CMS pol utilization for winter rapeseed hybrids production. In press in Bull. GCIRC.
2. Keller W.A., Armstrong K.C.: Embryogenesis and plant regeneration in Brassica napus anther cultures. Can.J.Bot. 1977, 55: 1383-1388.
3. Nałęczyńska A.: Zastosowanie podwojonych haploidów w hodowli rzepaku (The application of doubled haploids in rapeseed breeding). Hod.Rośl.Aklim.i Nasien. 1991, 35: 3-40.

4. Nałęczńska A., Cegielska T.: Doubled haploids production in *Brassica napus* L. by in vitro androgenesis. *Genet. Polon.* 1984, 25: 271-276.
5. Nałęczńska A., Cegielska T.: Doubled haploids and field experiments with homozygous lines of rapeseed. *Proc. 8th Inter. Rapeseed Congress in Saskatoon.* 1991, 1488-1491.
6. Nałęczńska A., Krzymański J.: Siew wiosenny jako metoda testowania homozygotyczności podwojonych haploidów rzepaku ozimego (Spring sowing as a method for testing of homozygosity of winter oilseed rape doubled haploids). *Wyniki Badań nad Rzepakiem Ozimym IHAR Radzików.* 1985, 59-64.
7. Polsoni P.M., Kott S.L. and Beversdorf W.D.: Large-scale microspore culture technique for mutation-selection studies in *Brassica napus*. *Can.J.Bot.* 1988, 66: 1681-1685.
8. Siebel J., Pauls K.P.: A comparison of anther and microspore culture as a breeding tool in *Brassica napus*. *Theor. Appl. Genet.* 1989. 78: 473-479.

Fig.1.

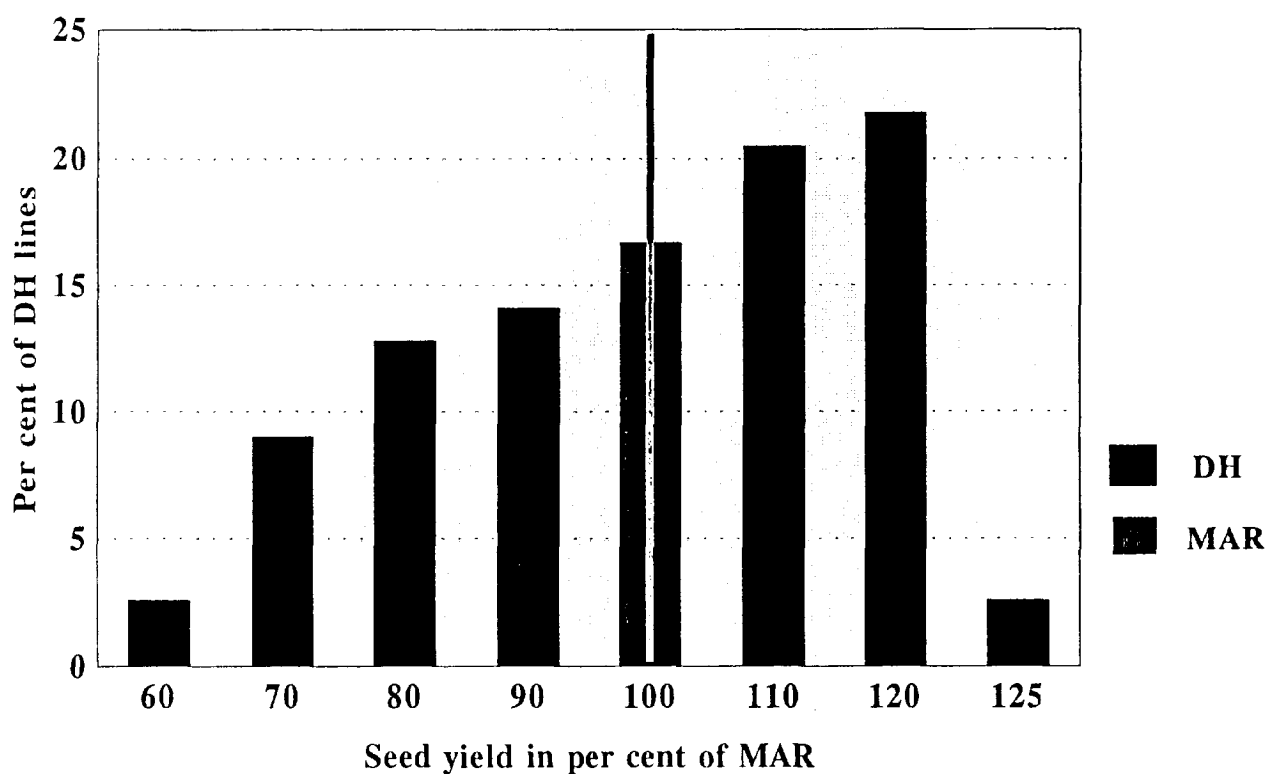


Fig.2.

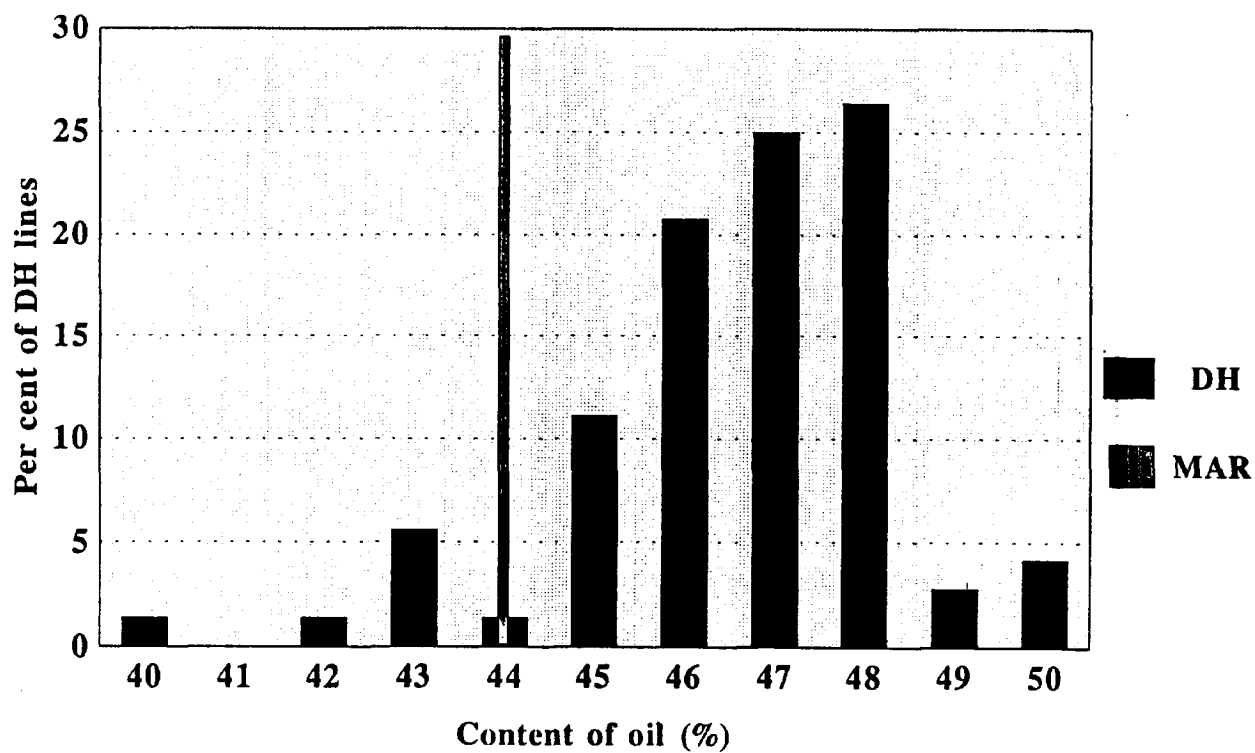


Fig.3.

