# Utilisation of Doubled Haploids in Winter Oilseed Rape (*Brassica napus* L.) Breeding

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**Abstract:** A survey of development and prospects of the utilisation of doubled haploid techniques in rapeseed breeding in the world and in the Czech Republic is presented. The first utilisation of spontaneously occurred haploids from *Brassica napus* in breeding programmes is described. The development of techniques of anther and later microspore culture is outlined. Special emphasis is given to the practical use of doubled haploids for the improvement of the effectiveness of breeding new productive cultivars. Some partial results of evaluation of yield parameters and resistance to important diseases in the obtained doubled haploid lines of winter oilseed rape are shown. The literary review and present results indicate, that the doubled haploid technique can be effectively used for the development of homozygous oilseed rape lines as an alternative to conventional methods.

**Keywords**: winter oilseed rape; doubled haploid; homozygous line; breeding

Doubled haploids (DH) are presently used in breeding of a number of crop species. This method enables breeders to develop completely homozygous genotypes from heterozygous parents in one single generation. Doubled haploids allow to fix recombinant gametes directly as fertile homozygous lines. Time saving is the most obvious advantage, because yield and other traits can be tested much earlier than with conventional lines. DH lines in oilseed rape are commonly produced from $F_1$ hybrids by means of chromosome doubling. Every doubled haploid produced could be a potential cultivar. Yield tests begin usually in the $R_3$ generation, and field selection is used to identify lines with the desirable combinations of characters. The doubled haploid method reduces the time needed to develop and to release new cultivars by approx. 2–4 years in comparison with conventional techniques.

The objective of this work was to survey the utilisation of doubled haploids in oilseed rape breeding and to present some results obtained with this method in the Czech Republic.

## Spontaneous haploids

First attempts of utilising *Brassica napus* L. haploids in breeding programmes were reported by THOMPSON (1983). In the beginning some naturally occurring haploid plants found in various cultivars were used for the development of completely homozygous DH lines by colchicine treatment. With this method, for example, the spring rape cultivar Maris Haplona was derived from a naturally occurring haploid in the Canadian cultivar Oro (THOMPSON 1979). Haplona reached an approx. 11% higher oil yield than the parental cultivar. It became the main spring rape cultivar in the UK from 1976 until 1978 and was also widely grown in Denmark. It was the first commercially grown agricultural crop variety in the world derived directly from a haploid.

However, the frequency of naturally occurring haploids, especially in winter types, is very low.

## *In vitro* androgenesis

The possibility to produce haploids in *B. napus* from anther culture (WENZEL *et al*. 1977; KELLER & ARMSTRONG 1978) and later from microspore culture (LICHTER 1982) provided the breeders with a new tool for breeding improved cultivars. The method of anther cultures was successfully applied in the Research Institute of Crop Production Prague-Ruzyně (RICP) in several cultivars and breeding lines of winter rape (VYVADILOVÁ *et al*. 1987).

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The microspore culture technique, which has recently been gradually optimised, is now routinely used on large scale due to higher efficiency of embryo production, compared with anther culture.

## Possibilities and advantages of doubled haploid utilisation

Except significant shortening of the breeding cycle this method is fully compatible with other biotechnological approaches such as mutation, *in vitro* selection, cryopreservation and gene manipulation techniques. Responsiveness to microspore culture of 35 genotypes, including seven winter and four spring cultivars, eight $F_1$ hybrids of various breeding materials, eight DH lines originated from anther cultures and eight self-incompatible lines, was in RICP first investigated at the beginning of 90s (VYVADILOVÁ & ZELENKOVÁ 1992). Pollen embryogenesis with resulting haploid plants was obtained in 12 genotypes. The embryo development from microspores stopped in other genotypes at different stages. Various methods of diploidisation of the obtained haploid plants were also verified (VYVADILOVÁ *et al*. 1993). The method of microspore culture is now optimised for practical use to the extent, that enough regenerants can be obtained from any genotype.

The advantages of using doubled haploids in oilseed rape breeding consist primarily in the substantially reduced time to produce and release cultivars. Normally, 8–10 years are required to develop a new cultivar using traditional procedures. The DH method reduces that time by about 2–4 years but its cost is sometimes twice higher than in conventional breeding (FRAUEN 1994). Besides this, the probability of selecting progeny exceeding the performance of parents is higher than with other breeding methods. Doubled haploids segregate much simpler in the progeny of crosses, since their population consists entirely of true breeding individuals. Regarding just one locus, for example, only one-half of a $F_2$ population is true breeding, compared with 100% of DH from $F_1$ microspores. The DH method opens also a unique way for the fixation of hybrid performance in homozygous lines, which would avoid all problems with the production of hybrid seeds (MALUSZYNSKI *et al*. 2001). The DH method using microspore cultures is routinely used in many *Brassica napus* breeding programs for both line and hybrid cultivars (STRINGAM *et al*. 1999).

## Evaluation and practical exploitation of doubled haploids

The agronomic performance of 6 DH lines, selected from 22 DH lines after preliminary evaluation, were tested in field trials. They were derived from the winter oilseed rape cultivar Darmor through anther cultures at the RICP. The seed yield of the tested DH lines did not differ significantly from the standard cultivar Ceres (KUČERA *et al*. 1993).

Doubled haploids were also used successfully at the RICP to stabilise self-incompatibility (SI) in DH lines, that originated from $F_1$ hybrids of SI × self-compatible (SC) genotypes. The expected ratio of SI to SC regenerants is approximately 1:1. However, a considerable shift

Table 1. Characteristics of selected SI regenerants of rapeseed genotype OP 23 (derived from $F_1$ hybrid of AIK Tandem 6/85 × 2051)

| No. of plant | Number of seed per pod | | Rs | Number of chromosomes in PMC | Notice |
|---|---|---|---|---|---|
| | SF | SF NaCl | | | |
| OP 23/2 | 0.3 | 3.5 | 9.51 | 15, 19 | |
| OP 23/3 | 0.0 | 11.4 | 0.00 | 19 | |
| OP 23/4 | 0.5 | 6.9 | 7.27 | 11, 12, 15, 17 | aneuploid |
| OP 23/5 | 2.0 | 15.4 | 12.95 | 15, 19 | |
| OP 23/6 | 0.6 | 7.5 | 7.52 | 19 | |
| OP 23/8 | 1.0 | 7.8 | 12.82 | 19, 15, 17 | |
| OP 23/10 | 0.1 | 2.5 | 4.05 | 19 | |
| OP 23/11 | 0.5 | 2.8 | 17.86 | indeterminate | |
| OP 23/13 | 1.2 | 2.2 | 57.41 | 15, 19 | low seed set |
| OP 23/18 | 1.1 | 5.7 | 18.76 | 15, 19 | |
| OP 23/22 | 0.0 | 10.7 | 0.00 | indeterminate | |

SF = mean number of seeds after self-pollination in opened flowers
SF NaCl = mean number of seeds after self-pollination with NaCl application
Rs = relative number of seeds (SF/SF NaCl × 100)

to self-compatibility has been observed, probably due to gametic selection against SI genotypes during the microspore culture (KUČERA *et al*. 1996a). The possibility to combine self-incompatibility and double zero quality was also investigated. About 250 DH regenerants were derived from four $F_1$ hybrids of self-incompatible, high glucosinolate lines and self-compatible donors of 00 quality. The number of highly SI regenerants ranged from 10 to 20%. This confirmed the previously observed gametic selection against SI. The glucosinolate content in individual plants ranged from 20 to 87 µmol per g of seeds. Table 1 shows some characteristics of selected DH regenerants classified as SI, that were derived from one $F_1$ combination. These results indicate the possibility of obtaining DH lines, that combine recessively determined traits, if a large amount of regenerants from $F_1$ hybrids is used (KUČERA *et al*. 1996b, 1999). Doubled haploids were also used to stabilise the seed colour and locule number in interspecific hybrids of *Brassica rapa* × *B. oleracea*. Although the fixation of ploidy was not completely achieved, individual regenerants with *Brassica napus* and intermediate habit and chromosome numbers higher than in *B. rapa* were found. They could be used for breeding of yellow-seeded rape (VYVADILOVÁ *et al*. 1999).

As is evident from the Czech Official Trials, some foreign DH lines derived from winter oilseed rape are highly competitive in comparison with other cultivars, even hybrids. Conquest, a double haploid spring cultivar derived from the $F_1$ of Quantum × RU4, was released in Canada (STRINGAM *et al*. 2001) and another winter rape DH line, cultivar Mohican from the U.K., was registered in the Czech Republic in 2000. But there are also some doubts concerning the effectiveness of using DH technology in practical breeding (NIEMIROWICZ-SZCZYTT 1997). DEWAN *et al*. (1998) noted the evident inbreeding depression resulting in a low seed yield in some *Brassica rapa* DH lines. Our previous work (KUČERA *et al*. 1996b) proved a high variability in some specific traits, particularly in glucosinolate content between the DH regenerants derived from one donor plant of winter oilseed rape.

Table 2. Yield of selected DH lines and conventionally bred lines in the field trials 1999/2000

| Genotype | Generation | Yield | | % of standard |
|---|---|---|---|---|
| | | (t/ha) | of standard | |
| OP 1014 | DH | 7.45 | 5.26 | 142 |
| OP 1032 | $F_7$ | 6.57 | 5.26 | 125 |
| OP 1021 | DH | 6.41 | 5.26 | 122 |
| OP 1018 | DH | 6.32 | 5.26 | 120 |
| OP 1043 | $F_9$ | 6.28 | 5.26 | 119 |
| OP 1011 | DH | 6.27 | 5.26 | 119 |
| OP 1120 | $F_8$ | 6.05 | 5.26 | 115 |
| OP 482 | DH | 4.32 | 2.91 | 148 |

Standard = Odila

Table 3. Mean yield of DH lines in comparison with conventionally bred lines (CBL) and standard cultivars (C) in the locations Humpolec, Chlumec, Slapy and Kujavy in 2000/2001

| | | Homogeneous groups | | | Mean yield | % of standard | |
|---|---|---|---|---|---|---|---|
| | | variants | locality | locality × variants | (t/ha) | Rasmus | Odila |
| Variants | Controls (C) | a | | | 4.85 | * | * |
| | Conventionally bred lines (CBL) | a | | | 4.60 | 89.2 | 101.1 |
| | Doubled haploids (DH) | a | | | 4.56 | 88.4 | 100.2 |
| Locality × variants | Humpolec × DH | | | a | 6.04 | 93.1 | 110.2 |
| | Humpolec × C | | a | ab | 5.99 | * | * |
| | Humpolec × CBL | | | bc | 5.80 | 89.4 | 105.8 |
| | Chlumec × C | | | cd | 5.17 | * | * |
| | Chlumec × DH | | b | de | 4.77 | 90.2 | 94.5 |
| | Chlumec × CBL | | | e | 4.71 | 89.0 | 93.3 |
| | Slapy × C | | | defg | 4.53 | * | * |
| | Slapy × CBL | | c | f | 4.46 | 95.7 | 101.6 |
| | Slapy × DH | | | g | 4.13 | 88.6 | 94.1 |
| | Kujavy × C | | | gh | 3.72 | * | * |
| | Kujavy × CBL | | d | h | 3.43 | 82.1 | 104.9 |
| | Kujavy × DH | | | h | 3.28 | 78.5 | 100.3 |

Duncan $P = 0.05$; * Mean yield (t/ha) of controls in total: Rasmus: 5.16, Odila: 4.55

Table 4. Evaluation of resistance against important diseases

| *Phoma lingam* | | *Sclerotinia sclerotiorum* | | *Alternaria brassicae* | |
|---|---|---|---|---|---|
| Genotype | resistance degree* | genotype | resistance degree | genotype | resistance degree |
| OP 1011 | 6.56 | Rasmus | 6.88 | Odila | 6.88 |
| OP 482 | 6.50 | Odila | 6.75 | OP 1014 | 6.75 |
| Odila | 6.25 | OP 1014 | 6.69 | OP 1021 | 6.63 |
| Rasmus | 6.25 | OP 1021 | 6.56 | Rasmus | 6.50 |
| OP 1018 | 6.13 | OP 1011 | 6.19 | OP 1018 | 6.44 |
| OP 1021 | 6.13 | OP 482 | 5.94 | OP 1011 | 6.31 |
| OP 1014 | 5.94 | OP 1018 | 5.75 | OP 482 | 6.19 |

* on 1–9 scale (9 – without symptoms)

Further studies were undertaken to provide more information regarding the comparison of agronomic performance, seed quality and resistance against important diseases of DH lines and traditionally bred winter rape breeding lines. Twenty four DH lines derived from various $F_1$ hybrid combinations and selected after preliminary seed quality tests were evaluated for agronomic performance and disease resistance in a preliminary field trial with two replicates at the locality Opava in 1999/2000. Table 2 shows the yield of the five selected DH lines in comparison with some advanced conventionally bred lines and the standard cultivar Odila. The five DH lines were also included into trials at four localities with 4 replicates of 10 $m^2$ plots in 2000/2001. They were compared with 27 conventionally bred lines originating from three breeding stations and with the registered cultivars Odila and Rasmus as standards. The results of yield evaluation are in Table 3. The obtained data were statistically evaluated by analysis of variance (ANOVA). To test the differences between the lines the Duncan test was used ($P = 0.05$) .

Although the DH lines seemed to differ in yield to some extent, the differences were statistically not significant, both within and across localities. The DH lines yielded 100% relative to the standard Odila and 88% relative to the high yielding foreign cultivar Rasmus. The results of testing resistance against *Phoma lingam*, *Sclerotinia sclerotiorum* and *Alternaria brassicae* are given in Table 4. Disease resistance parameters were demonstrated by means of a nine point scale. Although slight differences between DH lines and standard cultivars were noticed in resistance against individual diseases, there was no indication of a general increase in susceptibility of the DH lines due to a possible inbreeding depression.

## CONCLUSION

It can be concluded, that breeding based on the production of DH lines is as effective as conventional breeding with 6–8 generations of inbreeding. But the possibility to obtain the most productive DH lines with desirable agronomic performance depends on the availability of suitable initial breeding materials with genes determining favourable traits and characterised by a good combining ability.

## References


DEWAN D.B., RAKOW G., DOWNEY R.K. (1998): Growth and yield of doubled haploid lines of oilseed *Brassica rapa*. Can. J. Plant Sci., **78**: 537–544.

FRAUEN M. (1994): Use of biotechnology in breeding new varieties of winter oilseed rape in Germany. Bul. GCIRC, **10**: 16–29.

KELLER W. A., ARMSTRONG K.C. (1978): High frequency production of microspore derived plants from *Brassica napus* anther cultures. Z. Pflanzenzücht., **80**: 100–108.

KUČERA V., VYVADILOVÁ M., TOMÁŠKOVÁ D., HAVEL J. (1996a): Tvorba autoinkompatibilních linií řepky ozimé pomocí dihaploidního systému. Czech J. Genet. Plant Breed., **32**: 1–8.

KUČERA V., VYVADILOVÁ M., TOMÁŠKOVÁ D., HAVEL J. (1996b): Segregation of specific traits in doubled haploid regenerants of winter oilseed rape. Czech J. Genet. Plant Breed., **32**: 241–248.

KUČERA V., VYVADILOVÁ M., ZELENKOVÁ S., DEVERA S. (1993): Variabilita hospodářských znaků dihaploidních linií ozimé řepky odvozených z odrůdy Darmor. Rostl. Výr., **39**: 421–427.

KUČERA V., VYVADILOVÁ M., TOMÁŠKOVÁ D. (1999): Development of self-incompatible double low winter oilseed rape lines by means of doubled haploid system. In: Proc. 10th Int. Rapeseed Congr., September 26–29, 1999 Canberra, Australia: 250 (CD ROM).

LICHTER R. (1982): Induction of haploid plants from isolated pollen of *Brassica napus*. Z. Pflanzenphysiol., **105**: 427–434.

MALUSZYNSKI M., SZAREJKO I., BARRIGA P., BALCERZYKA A. (2001): Heterosis in crop mutant crosses and pro-

duction of high yielding lines using doubled haploid systems. Euphytica, **120**: 387–398.

NIEMIROWICZ-SZCZYTT K. (1997): Excessive homozygosity in doubled haploids – advatages and disadvantages for plant breeding and fundamental research. Acta Physiol., **19**: 155–167.

STRINGAM G.R., DEGENHARDT D.F., THIAGARAJAH M.R., BANSAL V.K. (1999): Q2 summer rape. Can. J. Plant Sci., **79**: 597–598.

STRINGAM G. R., DEGENHARDT D.F., THIAGARAJAH M.R., BANSAL V.K. (2001): Conquest summer rape. Can. J. Plant Sci., **81**: 107–108.

THOMPSON K.F. (1979): Superior performance of two homozygous diploid lines from naturally occurring polyhaploids in oilseed rape (*Brassica napus*). Euphytica, **28**: 127–135.

THOMPSON K.F. (1983): Breeding winter oilseed rape, *Brassica napus.* Adv. Appl. Biol., **7**: 1–104.

VYVADILOVÁ M., TOMÁŠKOVÁ D., BECHYNĚ M., KUČERA V. (1999): The use of doubled haploids to stabilise yellow-seedness in oilseed rape (*Brassica napus*). Czech J. Genet. Plant Breed., **35**: 7–9.

VYVADILOVÁ M., ZELENKOVÁ S. (1992): Responsiveness in microspore cultures of some cultivars and Czech breeding materials of rapeseed (*Brassica napus* L.). Genet. a Šlecht., **28**: 243–252.

VYVADILOVÁ M., ZELENKOVÁ S., TOMÁŠKOVÁ D. (1987): Anther cultures of *Brassica napus* L. Scientia Agricult. Bohemoslov., **19**: 267 -273.

VYVADILOVÁ M., ZELENKOVÁ S., TOMÁŠKOVÁ D., KOŠNER J. (1993): Diploidizace a cytologická kontrola haploidů *Brassica napus* L. Rostl. Výr., **39**: 129–137.

WENZEL G., HOFFMAN F., THOMAS E. (1977): Anther culture as a breeding tool in rape. Z. Pfl.-Zücht., **78**: 149–155.

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**Abstrakt**

KUČERA V., VYVADILOVÁ M., KLÍMA M. (2002): **Využití dihaploidů ve šlechtění řepky ozimé** (*Brassica napus L.*). Czech J. Genet. Plant Breed., **38**: 50–54.

V práci je podán přehled vývoje a perspektivy využití systému dihaploidů ve šlechtění řepky v zahraničí a v České republice od využití spontánních haploidů přes prašníkové až po mikrosporové kultury. Zvláštní důraz je kladen na praktické využití dihaploidů pro zvýšení efektivnosti šlechtění nových výkonných odrůd. Jsou uvedeny dílčí výsledky hodnocení výnosu a odolnosti k houbovým chorobám dosud získaných vybraných dihaploidních linií ozimé řepky. Literární údaje a dosavadní výsledky naznačují, že technika dihaploidů může být efektivní alternativou konvenčních šlechtitelských postupů při tvorbě homozygotních linií ozimé řepky.

**Klíčová slova**: ozimá řepka; dihaploid; homozygotní linie; šlechtění

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