

Microspore-derived segregating doubled-haploid lines in *Brassica napus* and *B. oleracea*

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Microspore-derived doubled-haploid lines have been produced from approximately 20 oilseed rape cultivars (80% of the lines we have attempted to microspore-culture). About half of the microspore-derived embryos developed into plants directly and plants were produced from the remaining embryos by inducing new shoots in tissue-culture. The ploidy level of the microspore derived plants was determined cytologically by examining mitotic nuclei from axillary shoots. Most plants were haploid and colchicine treatment was used to produce fertile diploid (and occasional tetraploid) shoots. Between 5% and 30% of the microspore-derived lines (depending on the cultivar being cultured) had doubled spontaneously. Self-seed from microspore derived plants is being assessed in field plots to see whether it performs in a similar way to parental seed.

F₁ plants from a pair of reciprocal crosses between a doubled-haploid winter rape line and a doubled-haploid spring rape line and from a second pair of reciprocal crosses between the same winter rape line and a resynthesized *B. napus* line have been microspore-cultured to obtain four sets of segregating doubled haploid lines (Fig. 10). *B. napus* (n=19) is an amphidiploid species formed from the hybridization of *B. oleracea* (n=9) and *B. rapa* (n=10). The resynthesized *B. napus* was produced by crossing *B. oleracea* ssp. *alboglabra* with *B. rapa* ssp. *chinensis*. These East-Asian subspecies of the two diploid parents are as phylogenetically distinct as is possible from the European subspecies, which were the diploid ancestors of oilseed rape.

Microspore-culture of seven F₁ plants from the cross between the winter doubled-haploid line and the resynthesized line yielded 418 microspore-derived lines and ten F₁ plants from the reciprocal cross yielded 637 microspore-derived lines. We plan to maintain 200 lines from each cross. A similar number of microspore-derived embryos have been cultured from F₁ plants from the reciprocal crosses between the doubled-haploid winter-rape line and the doubled-haploid spring-rape line.

We have also derived completely homozygous lines from two subspecies of *B. oleracea* with short generation times, namely, ssp. *alboglabra* and ssp. *italica*. These microspore-derived lines are currently being assessed for vigour and uniformity. We plan to use them not only as the parents of a *B. oleracea* RFLP mapping cross but also as recurrent parents in a variety of backcrossing programmes.

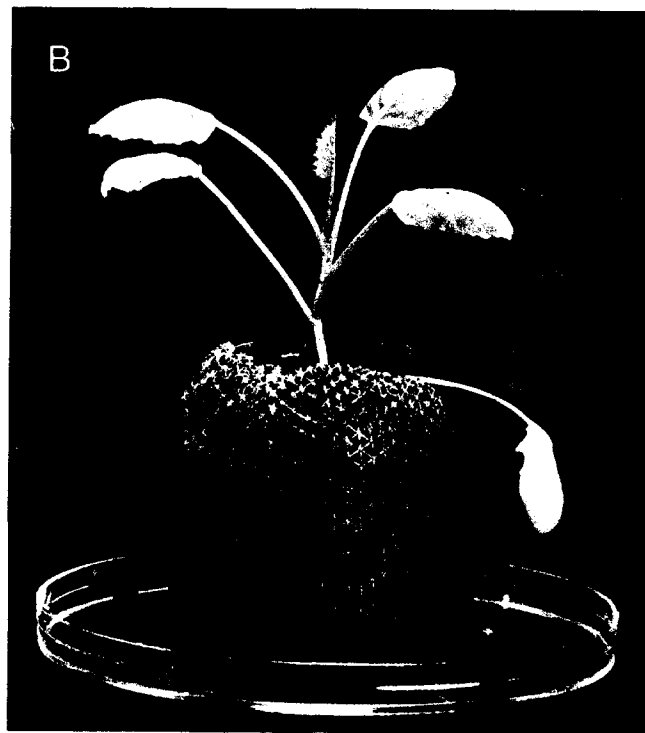


Fig. 10 Haploid oilseed rape (*B. napus*) plants derived from microspore-culture: A, embryos after two weeks of culture; B, young plants, ready to be transferred to the greenhouse, after seven weeks of culture.