Application of new biotechnologies in *Brassica napus* L. resynthesis

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Oilseed rape (*Brassica napus* L., AACC, 2n = 38) originates from a spontaneous interspecific hybridization between turnip rape (*Brassica rapa* L., AA, 2n = 20), and cabbage (*Brassica oleracea* L., CC, 2n = 18). The current oilseed rape gene pool has relatively narrow genetic diversity, resulting from a limited geographical area of cultivation, selection during the formation and subsequent improvement of this species (most of the current 00-quality cultivars derive from common ancestors). For this reason, there is a need to introduce a new genetic variation to the breeding material. One strategy for broadening the genetic base of oilseed rape germplasm is to exploit the diploid progenitor species of *B. napus*, specifically *B. rapa* and *B. oleracea*. Both of these species possess extensive variability in morphology and agronomic characteristics, and represent a valuable resource for improving the pathogen and pest resistance, tolerance of abiotic stress, and heterosis Thus, the resynthesis of a new *B. napus* from interspecific crosses between the original ancestor species has been aimed to increase the genetic variation in this species. The development of new biotechnologies like *in vitro* culture (including *in vitro* pollination, embryo rescue) and molecular techniques for detection of valuable genotypes, allow a much broader and targeted approach.

In the present study, resynthesized (RS) oilseed rape was obtained by reciprocal crosses between *B. rapa* and *B. oleracea* by 1) *in vivo* pollination where 51 RS oilseed rape were obtained, and 2) by *in vitro* placental pollination where 30 RS oilseed rape were obtained. Two subspecies of *B. oleracea* and three subspecies of *B. rapa* were used in the hybridization process. In earlier work, prezygotic incompatibility barriers between genetically distant species were reduced by applying *in vitro* placental pollination, whereas postzygotic barriers were avoided by an *in vitro* culture of enlarged ovules with embryos. In this study, ovules were isolated from pistils or ovaries from 7 to 15 days after pollination (dap). Only ovules isolated after 12-15 dap were suitable for further development. The average efficiency of obtaining new oilseed rape plants from enlarged ovules was around 6.1% in *in vivo* pollination, and 6.8% in *in vitro* pollination. To confirm their hybrid genotype, all plants were tested for nuclear DNA content via flow cytometry. An analysis of leaf samples showed that the received hybrids were amphihaploid (n = 19). The number of chromosomes was doubled using colchicine. Further cytogenetic studies of these hybrids are being conducted. The phenotyping analysis of RS *Brassica napus* plants indicated their large morphological diversity (leaf shape and/or a color; flower size). For measurements of pollen fertility, pollen grains were stained with 1% acetocarmine solution. Well-filled pollen grains with stained nuclei were regarded as fertile, while unstained pollen grains were counted as sterile. Pollen fertility of the analyzed resynthesized plants ranged from 49.5% to 92.6%. Genetic similarity of resynthesized *B. napus*, their parental lines, and different cultivars of winter oilseed rape, were determined by AFLP-PCR using 10 fluorescently labeled primer combinations. The dendrogram based on AFLP markers showed that resynthesized lines of *B. napus* formed a group genetically distinct from the compared cultivars of winter oilseed rape.

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