Efficient production of doubled haploid *Brassica napus* plants by colchicine treatment of microspores

C. Möllers, M.C.M. Iqbal* & G. Röbbelen

Institute of Agronomy and Plant Breeding, Georg-August University, Von Siebold-Str. 8, D-37075 Göttingen, Germany

* *Present address: Plant Biotechnology Program, Institute of Fundamental Studies, Hautane, Kandy, Srilanka*

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Summary

The effect of colchicine on isolated microspore cultures of *Brassica napus* was evaluated in order to combine a positive effect of colchicine on the induction of embryogenesis with the possibility to induce chromosome doubling at an early developmental stage, thus avoiding the production of haploid or chimeric plants. Colchicine was added to the culture medium immediately after isolation of *B. napus* microspores. The cultures were incubated from 6 to 72 h with various concentrations of colchicine. Samples were taken from the regenerating embryoids after 6 weeks for ploidy determination by flow-cytometry.

The highest diploidization rate was obtained after a 24 h treatment of microspores with 50 mg/l colchicine, leading to 80–90% diploid embroids. A concentration of 100 mg/l colchicine applied for the same duration resulted in a lower diploidization rate (76–80%). Treatment durations of 6 h were not long enough to induce a high rate of diploidization, whereas the application of 10 mg/l for 72 h was also very effective.

A sample of the plants regenerated from the colchicine treated microspores was transferred to the greenhouse. The plants looked similar to normal diploid rapeseed plants and showed reasonable pod and seed set. Thus, an additional generation for seed increase in the greenhouse is rendered unnecessary. The advantage of applying a minimum volume of colchicine under controlled *in vitro* conditions means a considerable saving of time and labour in DH-breeding programs.