Effect of *in vitro* and *in vivo* colchicine treatment on efficient production of doubled haploid plants of oilseed rape

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Development of doubled haploid (DH) plants through microspore culture is an important and efficient method of production of fully homozygous lines. This method has wide application in genetic and breeding programs. Haploid technology for *Brassica napus* is probably the most highly developed system among flowering plant species. Since microspore-derived plants originate from gametes, they have usually a single set of chromosomes, and in the vast majority they are haploids. In the case of oilseed rape, the rate of spontaneous chromosome doubling is low and range from 10% to 30% depending on the genotype. This number of diploids is unsatisfactory, therefore, the use of effective techniques for chromosome duplication such as application of antimitotic agents is necessary. Chromosome doubling agents can depolymerise microtubules and disrupt mitotic cell division (Hansen and Andersen 1996). Among agents promoting the production of DH plants (e.g., colchicine, oryzalin, trifluralin and amiprophos methyl), colchicine is the most commonly applied one because of its relative simplicity, water solubility and storage potential. Colchicine can be applied across several stages of the microspore culture process – from isolated microspores to the regenerated plants. The purpose of this study is to evaluate the effects of *in vitro* colchicine treatment into microspore culture and *in vivo* colchicine treatment of young plants and auxiliary shoots on the efficiency of diploidisation of different winter oilseed rape breeding materials.

In vitro colchicine treatment on microspores

The best stage to apply colchicine are freshly isolated microspores. The *in vitro* colchicine treatment of microspores does not cause any developmental delay and can improve induction of embryogenesis (**Figure 1**). In our study, colchicine added in vitro to the NLN medium with winter oilseed rape microspores immediately after isolation, induced a doubling of the chromosome number of haploid microspores from 30% to 95%, depending on the genotypes (**Table 1**).

Figure 1. Plant development after colchicine treatment of microspores

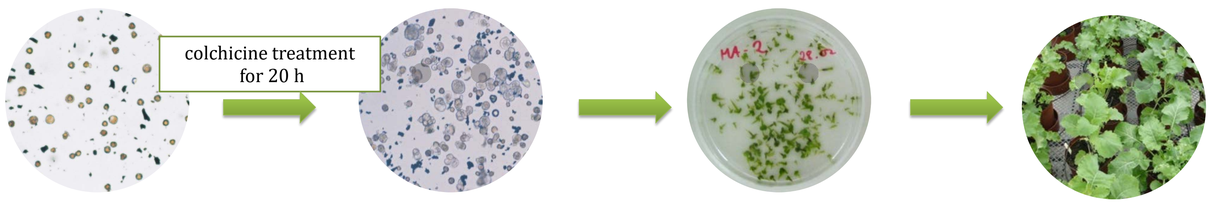


Table 1. Ploidy level of androgenic plants of winter oilseed rape developed from microspores treated in vitro with colchicine

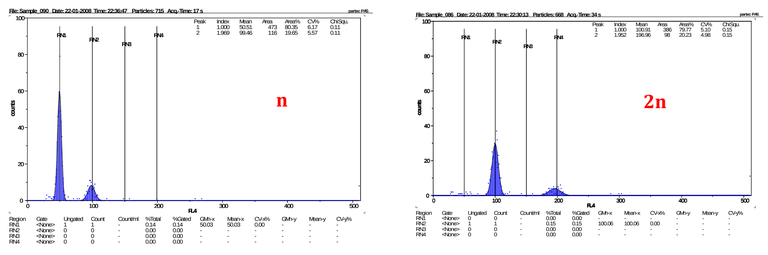
Hybrid	No of plants	Ploidy			% of duplication
		n	2n	other	
D 5	37	24	11	2	29,7
T	50	16	33	1	66,0
C	50	15	32	3	64,0
J	55	15	40	0	72,7
BK	108	53	52	3	48,1
K	100	21	75	4	75,0
KS17/14	134	2	127	5	94.8
KP10/14	57	8	40	9	70.2
KP30/14	28	2	22	6	78.6
12×12	129	53	76	0	58.9
12×17	109	45	74	0	67.9

In vivo colchicine treatment

However, sometimes the number of chromosomes must be doubling in the plants well developed for example by soaking the roots or shoots in a solution of colchicine. Although this methods are laborious, time consuming and result in ploidy chimeras, they are used to duplicate chromosomes in haploid plants developed from different explants subjected to mutagenesis or transformation, in the case of plants obtained through interspecific crosses for resynthesis of oilseed rape or where duplication of chromosomes *in vitro* gave unsatisfactory results.

Flow cytometry is very usefull to study ploidy in plant cells (**Figure 2**). Analysis is simple and is not problem to determine ploidy in plant sample from all parts of plant .

Figure 2. Flow cytometry histograms analysis



Colchicine treatment of young plants by soaking the roots

If cytometric analysis shows low level of chromosome doubling *in vitro*, colchicine has been applied on young plants of winter oilseed rape before or after vernalization. The roots of plants at the four- to six-leaf stages were dipped in a water colchicine solution for 22 h. After washing the roots, the plants were transferred back into soil (**Figure 3**). The efficiency of chromosome doubling was from 79% to 86% (**Table 2**).

Table 2. Ploidy level of androgenic plants of winter oilseed rape developed from colchicine treated plantlets

Hybrid	No of plants	Ploidy			% of duplication
		n	2n	other	
A1	90	11	74	5	82.2
A2	22	3	19	0	86.4
A3	100	19	79	2	79.0

Colchicine treatment of auxiliary shoots

The third method of doubling the number of chromosomes used in our laboratory is dipping auxiliary shoots in a water solution of colchicine. We use it in the absence of DNA ploidy analysis. From blooming haploid plants auxiliary shoots are taken and placed in a solution of colchicine for 22 hours. After washing away the shoots are placed in soil in for rooting within 2-3 weeks (**Figure 4**).

Population	No of genotypes	Serie	Ploidy				% of duplication
			1n	2n	n+2n	others	
12x12	53	I	15	34	3	1	64.2
		II	10	35	7	1	66.0
		III	20	30	2	1	56.6
12x17	45	I	10	29	5	1	64.4
		II	3	37	4	1	82.2
		III	6	38	0	1	84.4

Figure 1. Plant development from auxiliary shoots with colchicine treatment