Generation of triploids of hop (*Humulus lupulus* L.)

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INTRODUCTION

Hop (*Humulus lupulus* L) is a dioecious perennial plant belonging to the family of Cannabinaceae. Lupulin glands in the female inflorescences produce acid resins and essential oils, which are mainly used as flavouring components in beer brewing (Skof et al. 2007). Production of triploid forms is a method widely used in hop breeding. The studies have shown that triploids are more vigorous, higher yielding and seedless compared to their diploid counterparts. Polish aromatic variety Sybilla is characterized by both a good flavour and high content of alpha acids in cones. Moreover, is one of the most promising varieties corresponding to both the demands of the brewing industry and the processors of hop. A major breeding goal has been to produce tetraploid plants (2n=4x=40) as well as triploid forms (2n=3x=30) similar to Sybilla in respect to morphological traits. We report the efficiency of the *in vitro* induction of tetraploid hops. The paper describes the success of triploid production, by crossing artificially induced tetraploids with diploid male plants.

MATERIALS AND METHODS

Triploids production - Apical buds of cultivar Sybilla excised from *in vitro*-grown cultures were transferred to MS liquid medium with 2% glucose containing colchicine at concentrations of 0,1%; 0,01%; 0,05%. Plant material was incubated at 25^{0}C on an orbital shaker for 24h, 48h and 72h (Roy et al.2001). A total of forty two shoot apices were used per treatment. Following the colchicine treatment, buds were transferred to shoot multiplication medium (Kremhaller 1989). After 14 days of post treatment growth, the viability of plants was estimated. Four-week old plants were placed on rooting medium. Colchicine-treated plants KG15/2010 and KG16/2010 which were proved to be tetraploid were crossed under controlled conditions with two diploid male parents D8 and D11. The obtained seeds were sown and plantlets were evaluated for the ploidy level by flow cytometry as well as mitotic chromosome count in somatic cells (Burns 1964).

Flow cytometry analysis - The DNA ploidy level was analyzed in colchicine treated plants as well as in plantlets obtained from tetraploid x diploid crossing. Nuclei were extracted by chopping leaf samples with 2 ml nuclear isolation medium supplemented with DAPI (2 ug/ml). For each sample, around 5000 nuclei were analyzed using a Beckman Coulter Cell Lab Quanta ™ SC flow cytometer equipped with an mercury arc lamp.

Cytology - Young leaves were soaked in a solution of oxychinoline and then fixed in the Carnoy solution according to a procedure developed by BURNS (1964) and acetocarmine stained preparations were made. Chromosome number was determined for 30 cells of each plant.

RESULTS

Colchicine treatment was found to affect the survival rate of buds (Tab. 1). Vitality of buds decreased together with increasing concentrations of colchicine and prolonged exposure times. The highest mortality was observed after a 72h exposure with 0,1% colchicine exposure Flow cytometry analysis of surviving plants showed that eigth of nine applied colchicine treatments generated tetraploids 2n=2x=40. Exposure to 0,1 % colchicine for 48 hours provided the best percentage of tetraploid plants (28,6%), followed by treatment with 0,05% for 48h. Appart from generating tetraploids, this technique generated mixoploid plants (2n=2x=20/40).

All crosses of tetraploid KG15/2010 and KG16/2010 with diploid parent generated seeds (Tab. 2). Flow cytometry analysis of the obtained seedlings revealed that from the total of 29 plants 27 were triploids (2n=3x=30), while two individuals were tetraploids. Chromosome counting confirmed the relationship beetwen chromosome complement and DNA ploidy level (Fig.2). The obtained triploids will be evaluated for their agronomic performance.

Table 1. Apical buds viability and the assesment of DNA ploidy level of hop (*Humulus lupulus)* cv. Sybilla treated with three colchicine concentrations and three exposure times

Colchicine treatment		Number of treated buds		Number of viable individuals (%) by ploidy level		
		Total	Dead (%)	Diploid 2x	Tetraploid 4x	Mixoploid 2x/4x
0,01 %	24h	42	4,8	71,5	0	28,5
	48h	42	4,8	58,4	8,7	32,9
	72h	42	9,5	47,4	13,0	39,6
0,05 %	24h	42	7,1	66,6	8,6	24,8
	48h	42	9,5	39,5	21,7	38,8
	72h	42	14,3	55,5	14,3	30,2
0,1%	24h	42	9,5	31,6	18,6	49,8
	48h	42	11,9	27,0	28,6	44,4
	72h	42	19,1	44,0	12,7	43,3

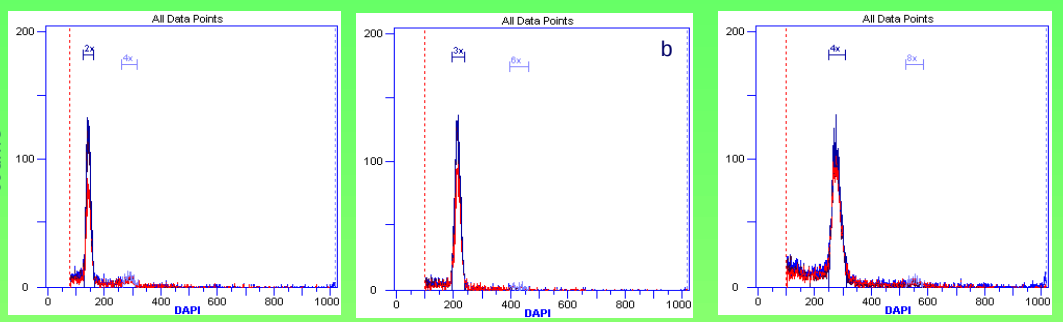

Fig. 1 Flow cytometry histograms of nuclei isolated from leaves of hop *Humulus lupulus*: a) diploid profile showing diploid (2x) and tetraploid (4x) peaks and b) triploid profile showing triploid (3x) and hexaploid (6x) peaks, c) tetraploid profile showing tetraploid (4x) and octaploid (8x) peaks.

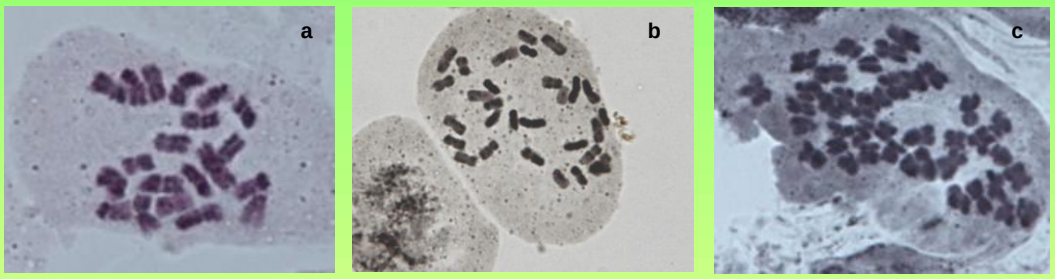

Fig. 2 Mitotic chromosomes of a) diploid 2n=2x=20 b) triploid 2n=3x=30 and c) tetraploid 2n=4x=40 plants of hop (*Humulus lupulus*)

Table 2. Numer of plantlets and the DNA ploidy level of hop obtained after tetraploids (2n=4x=40): KG15/2010, KG16/2010 crossing with diploid (2n=2x=20) male parents: D8 and D11

Genotype	Number of obtained Plantlets	Number of individuals by ploidy level	
		Triploid 3x	Tetraploid 4x
KG15/2010 x D8	12	12	0
KG16/2010 x D11	17	15	2
Total	29	27	2

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