

Improving Australian Hop Varieties: Polyploid Breeding and Mutagenesis

Anthony Koutoulis¹, Grey Leggett² and Aina Price¹

¹ School of Plant Science, Private Bag 55, University of Tasmania, Hobart TAS 7001, Australia (Anthony.Koutoulis@utas.edu.au)

² Hop Products Australia, GPO Box 104, Hobart, TAS 7001, Australia

Introduction

The Australian hop breeding program develops new bitter and aroma varieties that possess characteristics meeting the exacting requirements of brewers. Two different strategies have been employed to obtain improved hop varieties: polyploid breeding and mutagenesis.

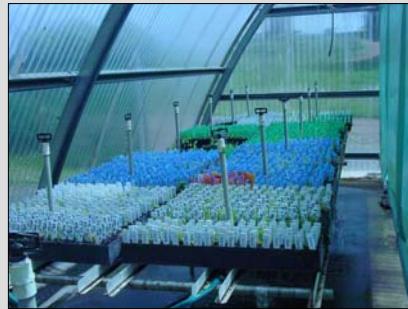
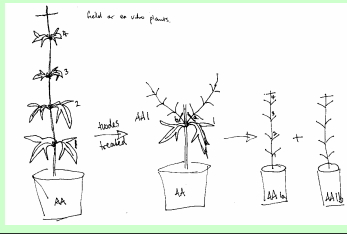
Polyploid breeding was used to obtain a breeding population of polyploid plants. The aim is to produce triploid hop varieties, usually resulting from a female tetraploid x male diploid cross. Traditionally, female tetraploids have been generated asexually using a genome-doubling agent, such as colchicine. Polyploid breeding involved identifying sexually-derived tetraploids by analyzing progeny from female triploids using flow cytometry. This approach has also permitted the identification of pentaploids as well as diploids and triploids.

The mutagenesis strategy involved exposing axillary buds of hop to the mutagenic agent ethyl methanesulfonate (EMS), with the aim of generating lesions at the DNA level. A number of protocols have been employed including EMS exposure to (i) *ex vitro* material, (ii) *ex vitro* material subsequently placed *in vitro* and (iii) *in vitro* material. Over 6000 EMS-treated plants have been generated to date and many have been transferred to the field for evaluation. The aim of this research is to obtain genetically stable plants with improved characteristics.

Mutagenesis

Ex vitro treatment

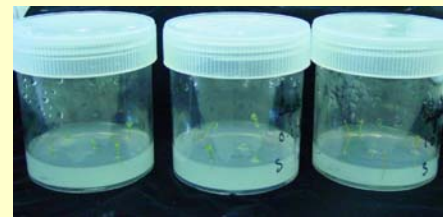
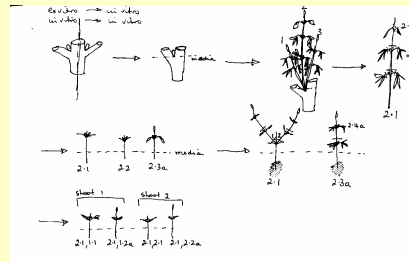
Axillary buds of field plants were exposed to EMS in either a phosphate buffer or lanolin paste. The apical buds were removed to promote growth through the treated buds. Cuttings were propagated from the shoots that developed from these EMS treated buds and planted out to the field.



Acclimatisation of *in vitro*-treated Symphony plants on mist bench at Bushy Park, Tasmania, Oct-Nov 2004

In vitro treatment

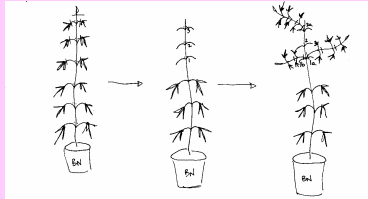
Explants of either *ex vitro* or *in vitro* material were treated with various concentrations of EMS for 1h, 5h or 25 h and placed on media. Plants were subcultured twice and planted out.



Symphony, *in vitro* treatment, exposed to EMS for 25h
Day 0: Placed on media

3-node treatment

The top three buds were treated with EMS, and the leaves, apical and lower buds removed. Cuttings were propagated from the resulting shoots.

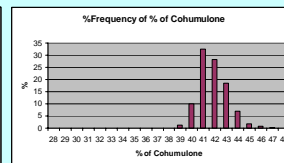
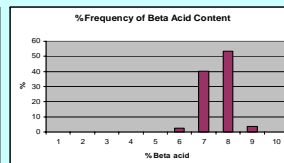
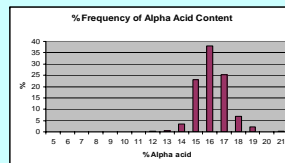


Right: Symphony bines propagated from plants exposed to 1.5% EMS, 3-node treatment.



Results

Following EMS treatment in 2003 using the *ex vitro* and 3-node approaches, 423 plants were analysed by HPLC in 2005. The graphs display the frequency distribution of alpha acid, beta acid and cohumulone content. At this stage, we have yet to identify an individual plant that exhibits a significantly different chemical profile within the population. We have another 2 trials of EMS-treated plants using the *in vitro* approach that will be analysed for the first time in 2006 (~5700 plants).



Polyploid Breeding

Breeding Methods

Hop (*Humulus lupulus* L.) is dioecious, which means that female and male flowers are borne on separate plants. New varieties arise from the seed produced from the hybridization of female and male plants. The female and male plants are similar in growth habit, but differ markedly in their floral structure. This therefore has an impact on breeding strategies.



Hop plants with sleeves in order to carry out controlled pollinations

The normal genetic state of hop is to have two sets of chromosomes ($2n=20$). The Australian hop-breeding program focuses on the generation of triploid (3 sets of chromosomes, $3n=30$) females. Traditionally, triploids were generated by crossing a male diploid ($2n=20$) with a female tetraploid ($4n=40$), the latter of which had been asexually-derived using a genome-doubling agent, such as colchicine (Roy et al. 2001).

Polyploid hop breeding has been an on-going activity in Australia for some time and, recently, this activity has been expedited with the incorporation of flow cytometric evaluation of ploidy levels (Roy et al. 2001). Triploid hops are relatively seedless; however, a small percentage of viable seed is produced. Haunold (1970) suggested that triploid x diploid crosses would produce a significant number of tetraploids. We have used flow cytometry to quickly identify sexually-derived tetraploids from triploids, in a similar way to work by Beatson et al. (2001 & 2003). This approach provides a genetically diverse population of tetraploids and triploids (Haunold 1970, Beatson et al. 2001 & 2003) for use in the breeding program.

Ploidy of progeny derived from crosses of triploid cultivars

	Total	2X	2X-3X	3X	4X	5X
2001 19 families	247	116 47.0%	0%	77 31.2%	53 21.5%	1 0.4%
2002 8 families	248	77 31.0%	0%	137 55.2%	34 13.7%	0%
2003 24 families	161	92 57.1%	3 1.9%	50 31.1%	15 9.3%	1 0.6%
2004 17 families	373	149 40%	0%	147 39%	72 19%	5 1%

Sex of triploid progeny by ploidy level (incomplete data)

	Total	2X	2X-3X	3X	4X	5X
Male	71	42	-	22	7	-
Female	282	120	-	112	50	-
Hermaphrodite	73	4	-	45	24	-