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Breeding, development, and characteristics of the hop (*Humulus lupulus*) cultivar 'Nelson Sauvin'

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Abstract The breeding and development of the seedless triploid hop (*Humulus lupulus*) cultivar 'Nelson Sauvin' is described. 'Nelson Sauvin' was selected from a seedling population in 1987 and released for commercial production in 2000 following several years of replicated and large-scale semi-commercial trials. 'Nelson Sauvin' was primarily selected for its high alpha acids content, low seed content, low cohumulone content, and subsequently because 'Nelson Sauvin' imparts a unique "grape-like" flavour to beer. Storage trials showed that 'Nelson Sauvin' lost alpha acids more quickly than most other New Zealand-bred high alpha cultivars. 'Nelson Sauvin' has been grown commercially for three seasons mainly for its alternative flavour uses in beer.

Keywords hops; *Humulus lupulus*; breeding; cultivar; triploid; alpha acids; essential oils

INTRODUCTION

Hops (*Humulus lupulus* L.) are used in brewing to impart bitterness and aroma to fermented malt beverages. Bitterness is derived from the alpha acids contained in the resinous glands of the mature female inflorescences, called cones, and cultivars used primarily to provide bitterness are known as "high alpha types" (Neve 1991). Breeding of high alpha-type cultivars with a good agronomic performance has been one of the most important goals of the New Zealand hop breeding programme (Frost 1983). Cultivars released from the programme since the early 1960s have enabled the hop industry to expand from one of domestic supply to predominantly export (Beatson & Inglis 1999). The New Zealand breeding programme has concentrated on developing a range of seedless cultivars with a triploid chromosome constitution (Beatson 1993). Tetraploid (2n = 4x = 40) parents are crossed to diploid (2n = 2x = 20) parents to produce triploid (2n = 3x = 30) seedlings.

In the last two decades, brewers have become more specific in their requirements for the amount and type of alpha and beta acids, as well as the qualitative and quantitative differences of essential oil profiles of hop cultivars (Moir 2000; Kaltner et al. 2001). This paper describes the breeding, development, and main characteristics of a new high alpha-type cultivar, with unique flavour properties, for the New Zealand hop industry.

MATERIALS AND METHODS

Plant material

In 1984–85, open pollinated seeds were obtained from the tetraploid female parent, 'Smoothcone'. Seeds were stratified and germinated following the procedures of Haunold & Zimmermann (1974). One hundred seedlings from this population were raised in a glasshouse and grown on in a plant nursery facility during 1985–86. They were then planted in the field in early spring of 1986, along with single plant replicates of control cultivars 'Green Bullet', 'Super Alpha', and 'Sticklebract', which were assigned at random to the seedling rows. Plant spacing was 1.2 m (within rows) × 2.5 m (between rows).

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In March 1987, 44 seedlings from this population were selected for harvesting. 'Nelson Sauvin', which then had the code number "85-03-06", was chosen from this population primarily for its high alpha acids content (15.1%) and low seed content (1.7%) (Table 1). Selection 85-03-06 was relocated to a non-replicated plot in winter 1987, where its chemical and agronomic performance was observed for five seasons starting in the 1987–88 season. Results during this period confirmed the characteristics of high alpha acids and low seed content. At this stage, 85-03-06 was cytologically verified as a triploid seedling ($2n = 3x = 30$). In anticipation of its commercial potential, 85-03-06 was vegetatively propagated by soft-tip procedures (Haunold 1980) during the 1992–93 season for a replicated trial and during the 1995–96 season for a large-scale brewing trial.

Trial design

To evaluate its commercial potential more extensively, 85-03-06 was included in a 4-replicate randomised complete block design (RCB) trial along with 12 other promising triploid hop selections. Control cultivars included for comparative purposes were the New Zealand bred cultivars 'Green Bullet', 'Pacific Gem', and 'Super Alpha', as well as the cultivar 'Nugget', bred in the United States. The RCB trial had a plot size of five plants with a plant spacing of 1.2 m (within row) × 2.5 m (between row). For each plot, measurements for a range of agronomic and chemistry characters were made (see Measurements section). The RCB trial was conducted over two growing seasons, in 1993–94 and 1994–95.

After the small plot RCB trial, 85-03-06 was forwarded to a larger-scale semi-commercial trial evaluation, where its chemical and agronomic performance was compared with New Zealand-bred high alpha cultivars, over seven seasons, starting in 1996–97. The primary aim was to supply up to 100 kg of hops for pilot brewing trials to be conducted by commercial brewery interests. The plot size for 85-03-06 was 225 plants and plant spacing was 1.2 m (within row) × 2.5 m (between row).

Storage trials

Six-month storage trials were conducted over six seasons on non-replicated samples of 85-03-06 taken from the large-scale semi-commercial plot, in which its chemical performance was compared to four commercial cultivars 'Green Bullet', 'Pacific Gem', 'Southern Cross', and 'Super Alpha'. Storage trials were conducted on cone samples, where each storage trial entry was compressed into cartons at a density of 200 kg/m^3, and held at a constant temperature of 20°C for 6 months. For each entry, the chemical characteristics of stored hops were compared to that of freshly harvested hops. Quantitative changes for each character were expressed as the percentage remaining after 6 months in storage. The hop storage index (HSI) (see Measurements section) was also calculated on 6-month storage samples. For the data analysis, years were regarded as replicates.

Measurements

For each plot of the RCB trial, cone fresh weights were measured at maturity following machine harvesting. Dry matter content was determined from a c.1.5 kg cone subsample taken from each RCB trial plot at harvest. For the large-scale brewing trial plots, between 4 and 6 subsamples were taken for dry matter content. All cone samples were dried at 60°C in forced air kilns until moisture content was reduced to c. 10%. Yield (kg/ha) of dried hops was then calculated for each plot. Following equilibration under ambient conditions, the dried samples were then frozen at –20°C in sealed plastic bags for subsequent determination of chemical constituents. Alpha (% w/w) and beta (% w/w) acids, calculated on a dry weight basis (i.e., 0% moisture) and HSI were determined by the modified toluene method (ASBC Method Hops-6A 1996) using a spectrophotometer

Table 1 Original seedling performance of *Humulus lupulus* cultivar 'Nelson Sauvin' (85-03-06) in comparison to control cultivars 'Super Alpha', 'Green Bullet', and 'Sticklebract'. (HSI, hop storage index.)

Name	Yield (kg green weight/plant)	Alpha acids (%)*	Beta acids (%)*	HSI	Seed content (%)
85-03-06	2.73	15.1	8.4	0.21	1.7
Super Alpha	2.18	12.8	8.2	0.21	2.5
Green Bullet	3.52	13.2	7.3	0.22	1.0
Sticklebract	2.62	13.7	7.8	0.22	1.0

*"As is" moisture basis.

to measure absorptions at 275, 325, and 355 nm. Derived formulae were then used to obtain alpha acids, beta acids, and HSI values for each plot. Seed content (% w/w) of dried cone samples was measured using standard procedures for hops (Analytica-EBC Method 7.3 1998). Alpha acids were measured by Lead Conductance Value (LCV) Method 7.4 (Analytica-EBC 1998). Total oil content (ml/100 g) was determined by steam distillation procedures (ASBC Method 13 1996). Essential oil components were estimated by standard gas chromatography (GC) methods for hops (Forster & Nickerson 1985). High-pressure liquid chromatography (HPLC) (Analytica-EBC Method 7.7 1998) was also used to measure the amount of alpha and beta acid types. From these values, the proportions of cohumulone and colupulone of alpha and beta acids (respectively) were calculated.

Statistical analysis

For the RCB trial, the data were analysed for each year separately and combined over the 2 years to examine the importance of genotype × environment interaction. For the purposes of this paper, the combined data were statistically analysed over years by SAS Version 8 (The SAS Institute, Cary, NC, United States 2000) using the GLM procedure.

For the large-scale trial and the storage trial, data was analysed over years by GLM procedure of SAS.

RESULTS AND DISCUSSION

Small plot replicated trials

The performance of 'Nelson Sauvin' (85-03-06) in the 2-year RCB trial (1994 and 1995 harvests) is presented in Table 2, along with other commercial high alpha-type cultivars and promising seedling selections. 'Nelson Sauvin' had a commercially acceptable yield (2060 kg/ha) and a low seed content (1.4%), whereas its alpha acids content determined by LCV and spectrophotometry of 13–15% was similar to that of commercial cultivars included in the study. 'Nelson Sauvin' had a beta acid content similar to commercial cultivar 'Green Bullet', but

Table 2 Performance of 'Nelson Sauvin' (85-03-06) hop (*Humulus lupulus*) in comparison to other selections and commercial cultivars in a replicated small plot trial series (2-year means). (LCV, lead conductance value; HSI, hop storage index; –, no value, non-replicated data.)

Genotype	Yield (kg/ha)*	Seed content (%)	LCV (%)‡	Alpha acids (%)‡	Beta acids (%)‡	HSI	Alpha/ beta acid ratio	Cohumulone content (%)†
Nelson Sauvin	2060	1.4	13.6	15.0	7.0	0.21	2.23	25
75-05	1752	2.6	12.4	13.1	6.7	0.22	1.98	40
75-19	1600	2.2	11.0	12.0	4.9	0.22	2.43	38
83-12-11	1739	2.2	10.0	10.4	6.4	0.22	1.63	39
86-03-03	1626	2.2	13.7	14.2	7.5	0.23	1.89	30
89-06-37	1719	2.3	12.9	13.5	7.2	0.23	1.87	32
90-06-13	2421	1.6	10.0	10.3	7.4	0.22	1.40	29
90-07-04	2955	3.2	10.2	11.0	4.5	0.23	2.50	36
90-10-03	2399	2.6	10.1	11.1	6.0	0.24	1.89	46
90-10-39	2489	2.2	9.9	9.9	5.2	0.23	1.93	36
90-12-23	2726	1.4	9.5	10.4	6.4	0.23	1.63	45
90-13-39	1818	1.1	10.3	10.7	4.4	0.23	2.45	29
90-14-35	1682	1.6	11.7	12.1	7.5	0.21	1.59	38
Green Bullet	1999	0.9	13.2	13.9	6.7	0.22	2.07	42
Nugget	1749	11.7	13.8	14.5	5.4	0.24	2.70	27
Pacific Gem	2270	1.7	15.2	16.1	8.5	0.22	1.89	42
Super Alpha	2383	1.6	14.9	15.6	8.3	0.22	1.89	38
$LSD_{(P = 0.05)}$	585	1.2	1.9	2.0	1.1	0.02	0.28	–

*1995 data only.
†Cohumulone (HPLC) tests completed at Washington State University, on a bulked (non-replicated) sample for each entry.
‡Dry weight basis.

Table 3 Performance of hop (*Humulus lupulus*) cultivar 'Nelson Sauvin' (85-03-06) in large-scale plots in comparison with four commercial cultivars (7-year means).

Cultivar	Yield (kg/ha)	Seed content (%)	Alpha acids (HPLC) (%)§	Beta acids (HPLC) (%)§	Cohumulone (% of total alpha acids)	Colupulone (% of total beta acids)	Essential oils (ml/100 g)§	Myrcene (%)	Caryophyllene (%)	Farnesene (%)	Humulene (%)	Oxidation products* (%)	Floral-estery products† (%)	Citrus-piney products‡ (%)	Other compounds (%)	Humulene/caryophyllene ratio
Nelson Sauvin	2129	1.6	14.3	7.1	24	47	1.9	58.5	5.5	0.2	18.6	2.0	1.3	5.2	8.4	3.17
Green Bullet	2520	1.1	14.2	6.9	42	67	1.6	65.1	4.9	0.0	14.0	1.2	1.4	5.8	7.2	2.87
Pacific Gem	2656	1.3	15.3	8.0	38	65	2.1	57.8	6.0	0.2	16.1	1.4	1.3	7.0	9.8	2.76
Super Alpha	2235	1.8	13.6	8.2	37	63	2.3	59.0	5.6	0.2	17.8	1.6	1.6	5.8	8.2	3.23
Southern Cross	2316	1.0	13.1	5.5	26	51	2.1	63.6	3.8	4.8	11.5	1.1	1.3	5.3	8.0	3.08
SE_{mean}	155	0.2	0.3	0.2	0.4	0.4	0.1	1.6	0.2	0.4	0.8	0.2	0.2	0.3	0.6	0.17
Confidence $limit_{(95\%)}$	310	0.3	0.7	0.5	0.9	0.8	0.2	3.3	0.5	0.7	1.6	0.4	0.3	0.6	1.2	0.33

*Oxidation products of humulene and caryophyllene.
†Linalool, geranyl acetate, geranyl isobutyrate, and geraniol.
‡Limonene, δ-cadenine, γ-cadenine, muurolene, and selinene.
§Dry weight basis.

Table 4 Performance of 'Nelson Sauvin' (85-03-06) hop (*Humulus lupulus*) in comparison to four commercial cultivars after 6 months of storage at 20°C (means over 6 years). (HSI, hop storage index.)

		Remaining (%)*										
Cultivar	HSI	Alpha acids	Beta acids	Essential oils	Myrcene	Caryophyllene	Farnesene	Humulene	Oxidation products†	Floral-estery products‡	Citrus-piney products§	Other products
Nelson Sauvin	0.68	53	38	38	5	68	45	76	392	153	71	88
Green Bullet	0.42	72	76	38	11	87	87	94	363	106	65	77
Pacific Gem	0.46	68	70	35	6	75	68	77	381	127	57	57
Super Alpha	0.47	66	70	38	8	78	47	79	295	102	63	75
Southern Cross	0.63	55	49	34	10	72	25	74	374	123	66	83
SE_{mean}	0.02	1.8	2.0	2.8	2.0	7.3	13.1	7.1	54.9	19.1	7.1	10.3
Confidence $limit_{(95\%)}$	0.05	3.7	4.0	5.6	4.1	14.5	26.2	14.2	109.8	38.2	14.2	20.6

*Percentage of the original remaining.
†Oxidation products of humulene and caryophyllene.
‡Linalool, geranyl acetate, geranyl isobutyrate, and geraniol.
§Limonene, δ-cadenine, γ-cadenine, muurolene, and selinene.

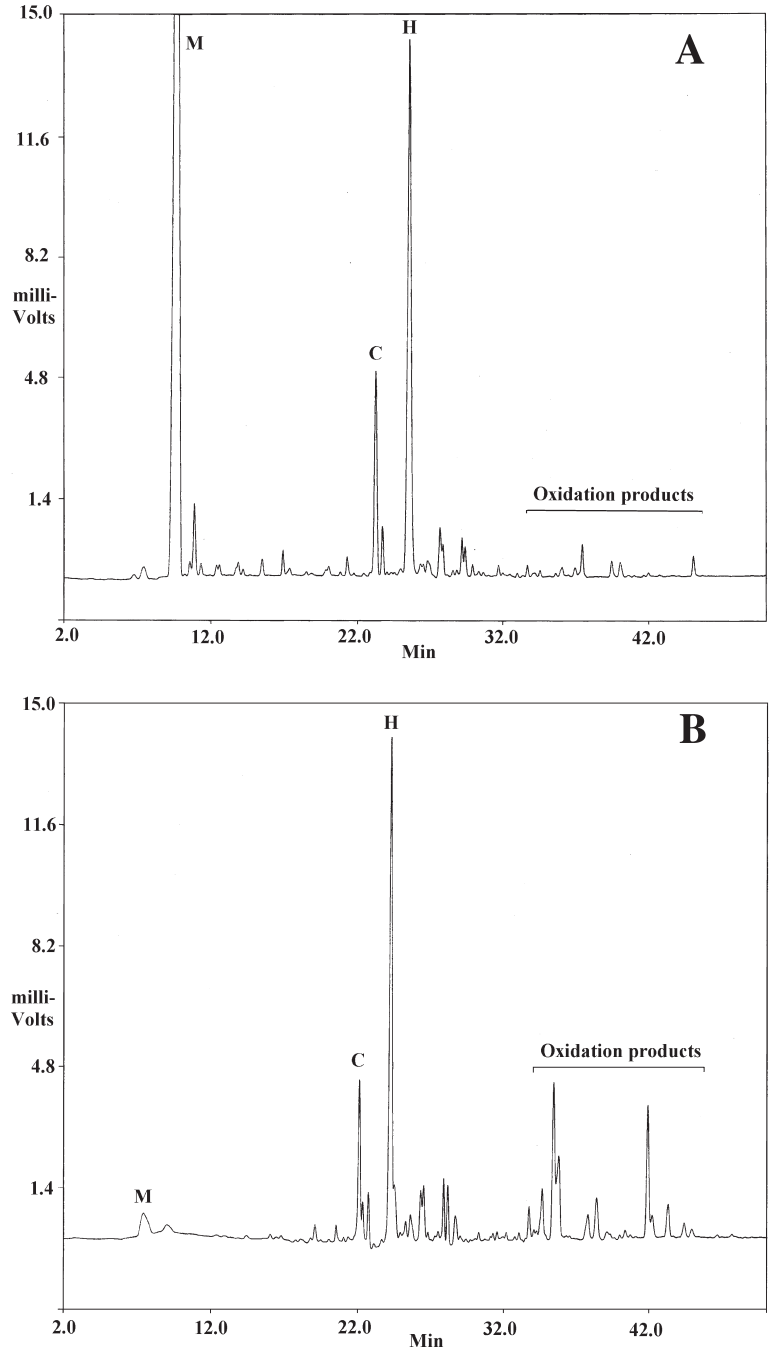

Fig. 1 Gas chromatographic essential oil profiles of 'Nelson Sauvin' hops (*Humulus lupulus*) (M, myrcene; C, caryophyllene; H, humulene; and Oxidation products, of humulene and caryophyllene.) **A**, at harvest and **B**, 6-month storage at 20°C.

lower than both 'Pacific Gem' and 'Super Alpha', which is reflected in its higher alpha/beta acids ratio. 'Nelson Sauvin' also had a low cohumulone content of 25%, similar to the internationally accepted cultivar 'Nugget' (Table 2). Literature suggests that a high cohumulone content imparts a harsher bitterness to beer flavour (Rigby 1972) and high content has also been implicated in poor head retention of beers (Diffor et al 1978; Wackerbauer & Balzer 1993).

All of the other selections shown in Table 2 were eliminated from further commercial consideration based on a combination of yield, alpha acids, seed content, and cohumulone content. Under New Zealand growing conditions, it is common practice to use males in commercial gardens to stimulate the yield of triploid (female) cultivars. The diploid cultivar 'Nugget', bred in the United States, as expected under these growing conditions, had an unacceptably high seed content, where seeds are considered undesirable in the brewing process (Neve 1991).

Large-scale trials

Combined results (over 7 years) for the evaluation of several commercially important characters are presented in Table 3. The results confirm the low cohumulone content of 'Nelson Sauvin' with a 7-year average of 24%. Yield of 'Nelson Sauvin' was moderate at 2129 kg/ha compared with the best performing commercial cultivar 'Pacific Gem' (2656 kg/ha). Likewise its alpha acids were 1% lower than 'Pacific Gem', but were comparable to those of other cultivars tested. The essential oil profiles presented in Table 3 showed that 'Nelson Sauvin' produced only a trace of farnesene whereas it had similar amounts of the main hydrocarbon terpenoid compounds (myrcene, caryophyllene, and humulene) to 'Green Bullet', 'Super Alpha', and 'Pacific Gem'. However, 'Nelson Sauvin' does have some unique (and as yet unidentified) flavour properties described as "grape-like" (Graves et al. 2002). 'Nelson Sauvin' therefore offers alternative flavour uses for beers.

Storage trials

Comparison of the storage properties of 'Nelson Sauvin' with commercial high alpha cultivars is presented in Table 4. The trials conducted over 6 years show that 'Nelson Sauvin' does not retain its alpha and beta acids as well as the other commercial cultivars, with the exception of 'Southern Cross'. This is reflected in the lower alpha (53%) and beta (38%) acids remaining after 6 months of storage and its higher HSI value of 0.68. However, there did not appear to be any corresponding difference between 'Nelson Sauvin' and commercial cultivars for loss of total oils content, with all cultivars losing between 60% and 70%. Table 4 shows that there was a larger increase in the "floral-estery" group of compounds (linalool, geranyl acetate, geranyl isobutyrate, and geraniol) for 'Nelson Sauvin' in comparison to the other four cultivars. Fig. 1 shows the GC profile comparison for 'Nelson Sauvin' stored for 6 months at 20°C with that of freshly harvested hops. In the comparative chromatographs, myrcene drops in content from 60% to <10% of the oils profile, whereas oxidation products of humulene and caryophyllene increase in content from <2% to 16% of the oils profile. The fate of compound changes of 'Nelson Sauvin' during storage in relation to the grape-like flavour in beer brewed with 'Nelson Sauvin' hops warrants further investigation.

Cultivar release

85-03-06 was released in 2000 as cultivar 'Nelson Sauvin' and is now in commercial production, where it is grown for its alternative flavour uses in beer. Although data are not presented in this paper, 'Nelson Sauvin' is a late ripening cultivar with a small, compact cone. Its most distinguishing vegetative feature is its purple-red spring shoot production and dark red vine colour when fully grown. 'Nelson Sauvin' currently occupies 2% of the New Zealand hop producing area, where during the three seasons of commercial production on 15 farms, it has produced a moderate yield of 1550 kg/ha and had alpha acids of 13%.

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