New hop variety Agnus as the result of breeding process innovation in the Czech Republic

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ABSTRACT

In the 90th, a methodology of hop breeding was innovated in the Czech Republic. Registration of a new variety Agnus in 2001 represents the result of the innovation mentioned above. Agnus variety is the first high-alpha hop in the assortment of Czech hop varieties. It contents 11–15% w/w of α-bitter acids and 5–7.5% w/w of β-bitter acids. The yield usually exceeds 2 t.ha^{-1}. Cultivation of Agnus is tested in various localities within hop growing areas. Simultaneously brewing tests in pilot and full scale are performed in five Czech breweries.

Keywords: hops; hop breeding; hop resins; hop oils; zoning; beer

Hop breeding had been largely aimed at mass selection in original vegetation of Saaz semi-early red-bine hop (Saaz) in the Czech Republic for a long time (Nesvadba et al. 1999). The hybridisation has been utilised in breeding programme from the beginning of 50th, but not before the 90th, it has become the main breeding method (Beránek 1996). The aim of hybridisation is to get new varieties with better quality parameters compared to the present ones. First Czech hybrid varieties Bor and Sládek were registered in 1994 after more than 25 years of crossing; Premiant was registered in 1996 after twenty years of testing. Variety Sládek belongs to the group of aroma hops, Bor and Premiant are typical representatives of bitter or dual purpose hops (Vent 1999). During the 90th, Czech hop industry passed through substantial reorganisation. Up to 1996, Saaz hop has been solely cultivated in the Czech Republic. After that year, hybrid varieties Bor, Sládek and Premiant have begun to be cultivated on a large scale (Fric 1998). The result of this change in breeding strategy at the beginning of the 90th is a new hybrid variety Agnus, registered in 2001 (Nesvadba et al. 2001).

MATERIAL AND METHODS

Suitable parental components have to be chosen for crossing of new prospective genotypes (Nesvadba et al. 1999). A part of a female plant is isolated before flowering to prevent open pollination. The pollen from selected male plant is transferred on flowers of a female plant at the stage of full developed flowers. Isolator is removed alter blossoming passes and when hop plant does not receive pollen. Pollinated hop cones were picked up at the stage of biological ripeness (14–21 days after technical ripeness). Seeds were kept in a cold place at the temperature of about + 7°C and were put into soil next year at the beginning of February (Nesvadba 2001). Acquired seedlings were evaluated for two years. The selected ones were introduced into a hybrid nursery and observed for the subsequent 8–10 years. After that time best hybrids were cultivated in a special nursery and later the best ones were introduced to field trials and simultaneously applied for state varietal tests (Beránek and Rígr 1990). According to this schedule breeding period of a new variety took over 20 years. Breeding material was evaluated according to hop classifier (Rígr and Fáberová 2000). Yield, contents and composition of hop resins and hop oils were analysed every year. Susceptibility to fungal diseases (powder mildew, downy mildew) and other pest's infestation was recorded. Farming technology aspects were thoroughly evaluated as well.

The contents and composition of hop resins were tested according to EBC 7.7 and EBC 7.5 analytical methods, resp. (Analytica EBC 1997). First method determines specific contents of α-bitter and β-bitter acids by liquid chromatography. Bitter acids were extracted from hops by a mixture of diethylether-methanol. Analyses were performed on SHIMADZU LC 10 liquid chromatograph. Analytical column Nucleosil RP C_{18}, 5 μm, 250 × 4 mm was used for separation. Mobile phase consisted of methanol-water-85% phosphoric acid (850:190:5 v/v/v) mixture, flow 0.8 ml.min^{-1}. Diode array detector was used for detection at the wavelength of 314 nm. Contents of total, soft and hard resins were determined by gravimetric analysis according to EBC 7.5 method, which is based on different solubility of hop resins components in various organic solvents (diethylether, *n*-hexane). β-Fraction and lead conductance value (LCV) are other parameters determined by this method.

Hop oils were isolated from hops by steam-distillation method. Analysis was performed by gas chromatography on DB5 column (30 m × 0.25 mm × 0.25 μm) with temperature programme at the range of 60–250°C (Krofta 2002). Chromatography analyses were performed on VARIAN 3400 gas chromatograph and FINNIGAN ITD 800 mass detector. Carrier gas helium 1.0 ml.min^{-1}, split injection 1:50.

The choice of suitable localities for cultivation of new variety is an integral part of the breeding process. Eight field trials in all growing areas were established for this purpose. Individual localities differ in altitude (200–430 m above sea level), type of soil and weather conditions.

Saaz area – Blšany, Stekník, Nesuchyně, Kolešovice
Úštěk area – Horní Počaply, Liběšice
Tršice area – Velká Bystřice

Breeding process was completed by brewing tests performed in pilot and full scale. First brewing tests with Agnus variety were performed in a pilot brewery, which is situated at Hop Research Institute in Žatec. Since the year 2000 brewing quality of Agnus variety has been tested in five Czech breweries – Pilsner Urquell, King's brewery Krušovice, Bernard Humpolec brewery, Drinks Union and Louny breweries.

RESULTS AND DISCUSSION

Agnus variety is a result of hop breeding process innovation in the Czech Republic. Its family tree is very complex (Figure 1). Genetic base is formed by Northern Brewer, Fuggle and Saaz varieties. During breeding period open pollination was used three times and mutation twice (ethylmethylsulphonate – mutation agent). Crossing of parent components Sm 2933 (female) × 82/6 (male) was performed in 1989. Acquired seeds were sowed next year. In 1991, the progenies reached full performance. Prospective hybrids were chosen for testing in successive years. Agnus variety had been recorded under breeding code 4587. Since 1992, it has been cultivated in a hybrid nursery. Based on good yield and high α-acids contents, hybrid 4587 was inserted in higher stage of breeding process – a control nursery in 1996. Since 1998, it has been evaluated within a framework of state variety tests performed by Central Checking and Testing Institute for Agriculture. Finally, it was registered under the name Agnus in 2001 (registered number OOZ/27/10021/PHA-/1214/2001), 13 years after crossing of parental hop plants.

The contents and composition of hop resins and bitter acids of Agnus variety are summarised in Table 1. The data were acquired from raw hops in the period 1998–2001. Hop samples were collected from variety and zoning tests, established in all hop-growing areas. The results show, that contents of total resins is at the level of about 30% w/w. Contents of α-bitter acids is in the range of 12–16% w/w (based on dry matter). It ranks Agnus to the category of high alpha hops. These parameters are by 25–30% higher in comparison to first Czech hybrid varieties Bor and Premiant. Agnus is comparable to foreign hop varieties like Nugget, Magnum, Columbus and Target. Compared to foreign varieties Agnus contains more β-bitter acids and therefore α/β-acids ratio moves mostly at the value of 2.0. This value is substantially lower than value 3.0 found in some foreign hops mentioned above. Cohumulone ratio in Agnus is mostly higher than 30% rel. and in maximal values it can reach the limit up to 39% rel. Higher cohumulone ratio corresponds with higher colupulone ratio, that in upper limit reaches value about 60% rel. Soft resins form approximately 90% of total resins, the reminder falls to hard resins. Contents of total resins in Agnus hops are positively influenced by relatively high amount of β-fraction. Its amount is comparable to α-acids content. The share of β-fraction in total resins is approximately 45 ± 5% rel. Typical values for contents and composition of hop resins and bitter acids in Agnus are shown in Table 2.

Contents and detailed hop oils composition determined in Agnus hops samples from 2000 crop are summarised in Table 3. Contents of hop oils are usually higher than 2.0%

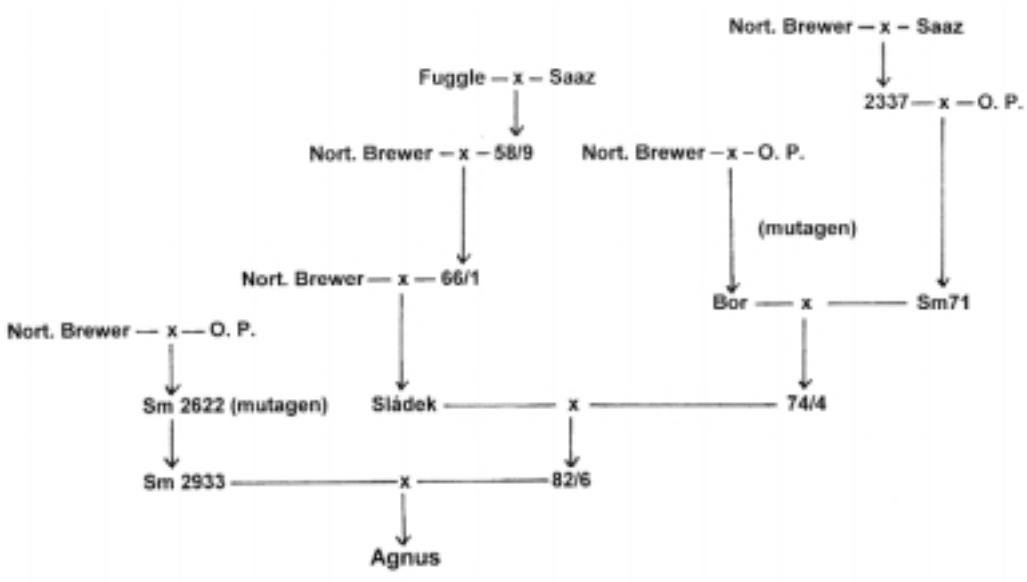

Figure 1. Family tree of Agnus variety

Table 1. Contents and composition of hop resins and bitter acids in variety Agnus in zoning and variety tests in the period of 1998–2001

Locality	Year	Bitter acids* (EBC 7.7)					Hop resins* (EBC 7.5)				
		α	β	α/β	Co-α	Co-β	total	soft	LCV	β-fraction	hard
Stekník+	1998	14.8	7.3	2.03	38.3	59.6	31.5	28.7	15.2	13.5	2.8
Stekník+	1999	11.9	6.7	1.78	37.6	57.3	29.5	26.9	12.4	14.5	2.6
Stekník+	2000	13.4	6.8	1.97	36.3	59.5	–	–	–	–	–
Liběšice	2000	15.1	6.1	2.47	30.3	52.8	–	–	–	–	–
	2001	14.2	7.6	1.87	34.3	54.6	30.0	26.9	14.7	12.2	3.1
Ročov	2000	11.9	5.1	2.33	29.4	51.2	–	–	–	–	–
	2001	14.7	6.3	2.33	31.2	54.4	–	–	–	–	–
Nesuchyně	2000	13.7	5.2	2.63	31.9	54.3	–	–	–	–	–
	2001	13.4	6.8	1.97	34.2	54.8	–	–	–	–	–
H. Počaply	2000	12.1	4.2	2.88	30.3	53.0	27.6	25.5	13.6	11.9	2.1
	2001	12.7	7.0	1.81	35.5	56.8	31.0	28.6	13.8	14.8	2.4
Blšany	2000	15.7	5.5	2.85	29.6	52.6	–	–	–	–	–
	2001	13.7	7.8	1.76	34.8	56.4	–	–	–	–	–
Stekník	2000	13.5	4.8	2.81	29.1	51.3	–	–	–	–	–
	2001	13.6	6.5	2.09	30.3	51.1	–	–	–	–	–
Kolešovice	2000	15.5	6.1	2.54	32.6	55.3	31.8	29.4	16.5	12.9	2.4
	2001	16.1	7.3	2.20	35.4	56.3	32.6	29.8	17.1	12.7	2.7
V. Bystřice	2000	14.7	6.4	2.29	31.5	54.4	–	–	–	–	–
	2001	13.4	6.9	1.94	30.9	51.0	–	–	–	–	–

* based on dry matter, + state variety test

w/w and account for a sharp smell of hop cones. Hop oils composition is typical of higher contents of myrcene (40–60% rel.) and low contents of β-farnesene (< 1.0 % rel.). Contents of myrcene, β-caryophyllene and α-humulene are decisive for hop oils composition because usually form 70–85% of its weight. The record of Agnus gas chromatography hop oils analysis is shown in Figure 2.

Up to 1999, the research project EP 9356 Zoning of hop hybrid varieties had been conducted with financial support of the Czech Ministry of Agriculture and Food. The aim of the project is to determine suitable localities for cultivation of all hybrid hops including variety Agnus. Hitherto, results show that hops have higher content of α-bitter acids and give higher yield in localities Blšany, Kolešovice and Liběšice compared with another places.

Table 2. Composition of hop resins in variety Agnus in the period 1998–2001

Component unit		Interval
Total resins*	(% w/w)	27–32
α-Bitter acids*	(% w/w)	11–15
β-Bitter acids*	(% w/w)	5–7.5
Ratio α/β		1.90–2.60
β-Fraction*	(% w/w)	11–14
Hard resins*	(% w/w)	2–3
Cohumulone	(% rel.)	29–38
Colupulone	(% rel.)	51–59

* based on dry matter

These experiences will be utilised in extension of growing area of Agnus in the future.

Agnus variety is cultivated on the area of 3 hectares at an experimental farm of Hop Research Institute in Stekník at the present time (2002). Total crop amount of raw hops is about 5 tons. This amount enabled to process raw hops to hop products, pellets and CO_2-extract for subsequent brewing tests. Since 2000, research project FA-E3/051 Utilisation of high-alpha hops of Czech origin in brewing industry has been funded by the Ministry of Industry and Trade. Many brewing tests in pilot and full scale have been performed in the pilot brewery at Hop Research Institute and several Czech breweries – Pilsner Urquell, King's brewery Krušovice, Bernard Humpolec brewery, Drinks Union and Louny breweries. Fermentation was performed either in classical fermentation cellars or in cylindric-conical tanks. In spite of brewing tests not having been finished, hitherto results show that hop products made from Agnus, have comparable brewing quality to foreign ones. If (partial) substitution of imported hop products by the ones made from Agnus variety were realised, significant stabilisation of Czech hop industry would be achieved.

CONCLUSIONS

Prospective hop variety Agnus has been bred within a framework of the innovation of breeding process in the Czech Republic. Shortening of breeding period significantly makes this process more effective. Final utilisation of new hop varieties in agricultural enterprises and brew-

Table 3. Hop oils composition of variety Agnus in several localities in the year 2000

Component	Liběšice	Ročov	Stekník	Nesuchyně H.	Počaply	Blšany	Kolešovice V.	Bystřice
Total oil (% w/w)	**2.84**	**2.10**	**2.30**	**2.19**	**2.58**	**2.27**	**2.10**	**1.99**
2-Methylbutylacetate	0.05	0.04	0.05	0.05	0.07	0.04	0.03	0.04
Isobutylisobutyrate	0.18	0.14	0.14	0.15	0.18	0.16	0.13	0.14
Methylhexanoate	0.04	0.02	0.03	0.04	0.02	0.03	0.06	0.03
α-Pinene	0.14	0.12	0.12	0.09	0.12	0.14	0.12	0.19
2-Methylbutylpropanoate	0.18	0.10	0.13	0.11	0.14	0.12	0.06	0.11
β-Pinene	1.31	1.20	1.16	0.94	1.21	1.29	1.01	1.56
Myrcene	48.3	46.6	45.6	47.6	48.4	48.3	52.1	48.5
3-Methylbutylisobutyrate	0.16	0.16	0.18	0.18	0.16	0.27	0.23	0.15
2-Methylbutylisobutyrate	1.10	0.85	0.90	1.06	1.12	0.88	0.81	0.99
Methylheptanoate	0.21	0.21	0.18	0.16	0.18	0.19	0.13	0.16
Limonene	0.89	0.81	0.84	0.74	0.81	0.90	0.78	0.92
trans-Ocimene	0.18	0.21	0.27	0.18	0.22	0.23	0.15	0.11
Methyl-6-methylheptanoate	0.80	0.54	0.66	0.71	0.60	0.88	0.85	0.78
2-Nonanone	0.28	0.15	0.16	0.13	0.14	0.19	0.12	0.13
Linalool	0.64	0.59	0.59	0.55	0.61	0.58	0.47	0.66
2-Nonen-1-ol	0.04	0.03	0.02	0.03	0.03	0.06	0.04	0.05
Methyloctanoate	0.31	0.24	0.30	0.26	0.28	0.35	0.22	0.22
2-Decanone	0.11	0.16	0.11	0.11	0.10	0.11	0.09	0.10
Methyl-6-nonenoate	0.04	0.06	0.03	0.04	0.03	0.03	0.05	0.05
Methylnonanoate	0.13	0.13	0.12	0.11	0.12	0.13	0.09	0.10
Geraniol	0.95	0.72	0.72	0.70	1.00	0.63	0.63	0.82
Methyl-8-methylnonanoate	0.35	0.25	0.30	0.35	0.30	0.39	0.38	0.32
2-Undecanone	1.27	1.25	1.45	1.44	1.18	1.60	1.28	1.22
Methyl-4-decenoate	2.02	1.62	1.81	2.03	1.57	2.14	2.06	1.89
Methyl-4,8-decadienoate	0.48	0.56	0.43	0.51	0.44	0.44	0.37	0.45
Methylgeranate	1.85	1.56	1.84	1.74	1.98	1.35	1.40	1.95
Methyldecanoate	0.20	0.21	0.22	0.22	0.20	0.27	0.16	0.18
α-Cubenene	0.08	0.07	0.08	0.07	0.08	0.07	0.07	0.05
α-Ylangene	0.07	0.08	0.08	0.07	0.08	0.08	0.07	0.09
α-Copaene	0.26	0.29	0.31	0.28	0.28	0.29	0.26	0.34
Geranylacetate	0.06	0.06	0.06	0.06	0.07	0.04	0.02	0.06
2-Dodecanone	0.08	0.10	0.10	0.11	0.09	0.10	0.09	0.08
β-Caryophyllene	11.0	10.1	11.6	11.4	10.8	10.2	10.2	10.9
β-Cubenene	0.39	0.41	0.43	0.39	0.43	0.39	0.32	0.37
α-Humulene	16.3	20.0	17.9	16.7	16.3	17.1	16.9	16.2
β-Farnesene	0.20	2.59	0.50	0.39	0.15	0.10	0.05	0.11
γ-Muurolene	0.80	0.87	0.91	0.82	0.89	0.78	0.63	0.84
β-Selinene	0.62	0.67	0.73	0.62	0.68	0.62	0.48	0.67
α-Selinene	0.93	0.98	1.11	0.96	1.04	0.95	0.82	1.04
2-Tridecanone	0.64	0.75	0.46	0.68	0.65	0.73	0.67	0.58
α-Muurolene	0.17	0.19	0.24	0.18	0.21	0.16	0.18	0.16
γ-Cadinene	0.79	0.85	0.85	0.83	0.89	0.68	0.66	0.80
δ-Cadinene	1.26	1.35	1.46	1.37	1.41	1.24	1.14	1.31
Cadina-1,4-diene	0.12	0.14	0.15	0.13	0.15	0.12	0.11	0.13
Caryophyllenepoxide	0.06	0.06	0.07	0.07	0.09	0.07	0.07	0.14
2-Tetradecanone	0.05	0.07	0.07	0.07	0.07	0.06	0.06	0.07
Humulenepoxide II	0.10	0.12	0.12	0.13	0.15	0.10	0.07	0.22
2-Pentadecanone	0.06	0.06	0.08	0.07	0.07	0.07	0.06	0.05

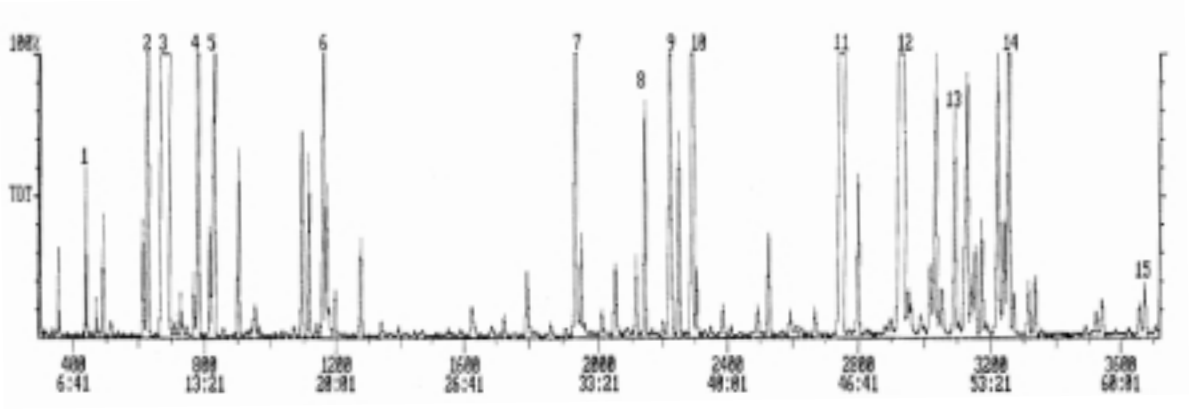

Figure 2. Chromatogram of essential oils of Agnus variety (locality Steknik, crop 2001); column DB 5-MS, 30 m × 0.25 mm × 0.25 μm; temperature programmed 60–250°C; helium carried gas, flowrate 1 ml.min^{-1}; split injection 1:50; gas chromatograph Varian 3400, mass detector Finnigan ITD 800

1. isobutylisobutyrate, 2. β-pinene, 3. myrcene, 4. 2-methylbutylisobutyrate, 5. limonene, 6. linalool, 7. geraniol, 8. 2-undecanone, 9. methyl-4-decenoate, 10. methylgeranate, 11. β-caryophyllene, 12. α-humulene, 13. β-selinene, 14. δ-cadinene, 15. humulenepoxide II

eries can be accelerated by zoning and brewing tests performed with sufficient time in advance. Some new lines of hybrids with very good qualitative and quantitative parameters are available at Hop Research Institute at the present time.

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ABSTRAKT

Nová chmelová odrůda Agnus jako výsledek inovace šlechtitelského procesu v ČR

Metodika šlechtění byla v ČR v průběhu 90. let uplynulého století inovována. Výsledkem této inovace je registrace nové odrůdy chmele Agnus v roce 2001. Odrůda Agnus představuje první vysoce obsažnou odrůdu chmele v sortimentu českých chmelů. Obsahuje v sušině 11 až 15 % hmotn. α-hořkých kyselin a 5 až 7,5 % hmotn. β-hořkých kyselin. Výnos obvykle přesahuje 2 t.ha^{-1}. Pěstování nové odrůdy je testováno v různých lokalitách pěstitelských oblastí v rámci rajonizačních pokusů. Současně jsou prováděny pivovarské testy ve čtvrtprovozním i provozním měřítku. Provozní varní testy jsou realizovány v pěti českých pivovarech.

Klíčová slova: chmel; šlechtění chmele; chmelové pryskyřice; chmelové silice; rajonizace; pivo

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