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Breeding of flavour hops in the Czech Republic

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Abstract

Hop breeding in the Czech Republic develops a number of flavour hops. The Kazbek hop variety was registered in 2008 and the Mimosa variety in 2019. The best 7 hop genotypes were selected from breeding materials between the years 2014 and 2019. Kazbek, 5512 (Ceres) and 5571 (Eris) expressed a clear citrusy flavour. Mimosa, 5495 (Juno) and 5520 (Saturn) could be described as fruity while 5164 (Uran), 5540 (Jupiter) and 5580 (Pluto) as rather spicy. Genotype 5164 showed the highest aroma intensity, content of alpha acids (11.51% w/w) and alpha/beta ratio (2.20). Whereas Mimosa demonstrated the highest content of beta acids (5.56% w/w) and the lowest alpha/ beta ratio (0.28), 5571 and 5520 had the highest cohumulone amount (49.19% rel. and 47.35% rel., respectively). In contrast, 5580 were distinguished by the lowest cohumulone (21.28% rel.). The highest amount of hop oils was determined in 5164 (1.77 w/w) and 5520 (1.53 w/w). 5164, 5540 and 5495 showed a myrcene level above 40% rel. On the other hand, Kazbek, Mimosa and 5571 indicated a myrcene content below 35%. The highest level of farnesene (12.50% rel.) was detected in 5164; in other samples the values were below 1% rel. Mimosa showed the highest concentration of selinenes (32.75% rel.); other samples demonstrated selinenes below 10% rel. The Kazbek variety is the foundation for the breeding of flavour hops in the Czech Republic, and therefore it is the mother plant of genotypes 5495, 5512, 5520, 5540 and 5571. Harmonie is the mother plant of genotype 5580.

Key words: hop, Humulus lupulus L., flavour hops, hop aroma, hop resins, hop oils

1 Introduction

The associate professor Karel Osvald became a founder of hop breeding in the Czech Republic in as early as the 1930s. Hop breeding was based on a clonal selection in the original Žatec hop vegetations (Fric, 1992). In the 1960s, hop hybridisation was introduced to hop breeding, resulting in the registration of the first Czech hybrid varieties - Sládek and Bor - in the 1990s. In the past, hop breeding mainly focused on aroma hops, which have a lower content of alpha acids and a balanced alpha/beta ratio ranging between 0.8 and 1.5 (Nesvadba et al., 2017a). The first bittering varieties - Agnus, Vital and Rubín - were registered after 2000 (Nesvadba et al., 2013). In terms of beer brewing, hop breeding focuses on hop varieties to be used for lager beers, which are typical for the Czech Republic. Therefore, many additional hop varieties such as Premiant, Harmonie, Saaz Late, Bohemie and Kazbek were used for these beers. As a result of an increasing number of newly-established microbreweries in the Czech Republic, a brewing of top fermented beers started and required a different composition of hop varieties. First, registered Czech hop varieties were tested. Harmonie, Rubín and Vital could be used partly. However, the Kazbek variety became the most widely used. The Kazbek variety is characterised by a citrusy aroma of hop cones (Nesvadba, 2009). This hop variety is becoming established in both Czech and foreign breweries.

From this perspective, the objectives of hop breeding have been expanded to include genotypes of flavour hops (Nesvadba et al., 2016). Currently, many large and small breweries are producing special beers (IPL, IPA, APA, ALE, IBA etc.), using different types of hops. Hops from the USA (Amarillo, Belma, Cascade, Citra, Mosaic

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etc.), Germany (Mandarina Bavaria, Huell Melon, Polaris etc.), Australia (Enigma, Galaxy etc.) and other countries generate interest. Nowadays, there is a total of 284 registered hop varieties from around the world (IHGC hop variety list, 2018). Over the last few years, the interest in flavour hops has grown in Czech breweries as well.

Thanks to hop breeding, it is possible to gain new promising hop genotypes with different aromas. Numerous hybridisations have been aimed at developing new hop genotypes with a high intensity of specific aromas (Nesvadba, 2018). The basis for hop breeding is a collection of genetic resources of hops, which is part of the "National Programme on Conservation and Utilization of Plant Genetic Resources and Agro-biodiversity" (Charvátová et al., 2017). The collection includes all of the world's hop varieties and wild hops that are used for the breeding of flavour hops. Flavour hop varieties and wild hops are the most used. Promising genotypes are tested in terms of their content and composition of hop resins and oils, aroma of hop cones and utilisation in beer brewing. Agro-technological requirements are among the crucial aspects. The new varieties need to be successful in hop growing as well (Wirowskij, 1980).

2 Material and Methods

The new genotypes were developed as a selection of descendants resulting from hybridization. Subsequently, the best genotypes are propagated and planted in breeding nurseries (2 plants from each of selected genotype). After an evaluation over a period of at least 3 years, the best genotypes are once again propagated and planted (50 to 100 plants from each of reselected genotype). Based on partial results, 26 hop genotypes showing specific hop aromas were closely watched and evaluated in the years 2015 to 2019. Kazbek and Mimosa were selected as benchmark

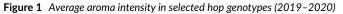
varieties. The evaluation focused on hop yields, growth characteristics (plant shape, length of stems, density of hop cones etc.), the intensity and the character of hop cone aroma. Chemical analyses of hop cones were crucial as well. The content and composition of hop resins were determined on the basis of liquid chromatography (EBC 7.7, 1998). The content and composition of hop oils were determined from dry hop cones by using liquid chromatography (Krofta, 2008).

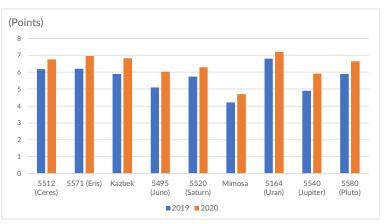
The evaluated genotypes originated from the same locations. Data were based on evaluations performed between the years 2015 and 2019. A Wolf picking machine was used for harvesting. Hop cones were dried at the temperature of 55°C. An organoleptic test was applied to evaluate the aromas in the years 2019 (37 evaluators) and 2020 (28 evaluators). The aroma intensity was evaluated on a scale from 1 (the lowest intensity) to 10 (the highest intensity). The aroma character was divided into the following groups: hoppy, citrusy, floral, fruity, grassy and woody. The procedure included a detailed description of the identified aroma. Basic statistical methods were used for the full set of clones: average and variability are expressed in % (centuple of the variation coefficient).

3 Results and discussion

26 promising hop genotypes with specific hop aromas were evaluated between the years 2014 and 2019. Preference was given to a strong aroma intensity and aroma character. Promising genotypes were also named after planets to have a better overview of the samples. The names were selected because of the non-hoppy aromas (aromas from a different planet).

Figure 1 shows that Uran has the highest intensity – 7.2 points in 2020 and 6.8 points in 2019. In 2020, a high intensity was determined in Eris (7.0 points), Kazbek (6.8 points), Ceres (6.8 points) and Pluto (6.6 points). In 2019, these genotypes exceeded the level of 6 points. Mimosa has the lowest intensity – 4.7 points in 2020 and 4.2 points in 2019. The results show that aroma intensity was higher in 2020 than in 2019. Some genotypes show lower year-on-year differences, e.g. Uran 0.4 points and Ceres 0.5 points. In contrast, the aroma intensity of Jupiter in 2019 was lower by 1.03 points compared to 2020. Juno shows a year-on-year difference of 0.9 points. These genotypes are likely to have a higher variability of aroma intensity, depending on the year.





Numerous genotypes were provided to microbreweries for brewing tests. The breeding efforts spreading over a period of five years resulted in the development of the best 9 genotypes, including Kazbek and Mimosa, which are already registered. Based on their aroma character, the genotypes are divided into 3 groups:

Citrusy aroma – Ceres, Eris and Kazbek;
 Fruity aroma – Juno, Saturn and Mimosa;
 Spicy aroma – Uran, Jupiter and Pluto.

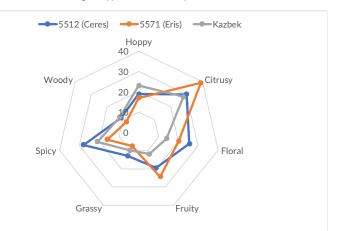
1. Citrusy aroma

The genotypes of Ceres, Eris and Kazbek have the strongest citrusy aroma. Ceres

and Eris resulted from the hybridization of a maternal plant of Kazbek. The male plant of Ceres has European hop varieties in its origin and the genotype of Eris originated from the Figure 2 variety. The Kazbek variety was developed from the hybridization of the Bor variety and a male plant with Russian wild hops in its origin. Figure 2 shows that Ceres is characterised by a high share of citrusy (lemon, lime, grapefruit, and orange), spicy (star anise) and floral (mint) aromas. Eris has the strongest citrusy (lime, grapefruit, tangerine and orange) and fruity (tropical fruits) aromas. Kazbek is characterised by a citrusy aroma and a lower share of spicy and hoppy aromas.

Table 1 shows the average content and composition of hop resins. Eris has the highest average content of alpha acids – 7.31% w/w. The average content of beta acids is 4.94% w/w. The alpha/beta ratio ranges between 1.31 (in 2018) and 1.68 (in 2017). Eris is characterised by a very high cohumulone content of

Figure 2 Aroma character in genotypes with a citrusy aroma (2019–2020)



49.19% rel. A similar content of alpha acids was determined in Ceres (5.95% w/w) and Kazbek (5.35% w/w) but Kazbek has a higher content of beta acids (4.42%w/w) than Ceres (3.30% w/w). Therefore, Kazbek has a lower alpha/beta ratio (1.24) than Ceres (1.81). Ceres (36.21% rel.) and Kazbek (35.57% rel.) have a similar average cohumulone content.

Table 2 shows that Eris has a higher content of hop oils than other genotypes. It is rather interesting that average compositions of hop oils in these genotypes are very similar. Genotype Ceres has a slightly higher content of myrcene and humulene and Kazbek a slightly higher content of selinenes. However, the differences are negligible.

The results demonstrate that the genotypes are similar in terms of their content and composition of hop resins and oils but their aroma characters are very different. Even though they have citrusy aromas, the compositions are not identical.

Table 1	Average content and	composition of he	op resins in gel	notypes with a	citrusy aroma	a (2015-2019)

Genotype	Alpha acids (% w/w)	Beta acids (% w/w)	Alpha/beta ratio	Cohumulone (% rel.)
5512 (Ceres)	5.95	3.30	1.81	36.21
5571 (Eris)	7.31	4.94	1.47	49.19
Kazbek	5.35	4.42	1.24	35.57

 Table 2
 Average content and composition of hop oils in genotypes with a citrusy aroma (2015-2019)

Genotype	Weight (w/w)	Myrcene (% rel.)	Caryophyllene (% rel.)	Farnesene (% rel.)	Humulene (% rel.)	Selinenes (% rel.)
5512 (Ceres)	1.06	38.21	10.56	0.18	28.30	1.40
5571 (Eris)	1.15	32.21	11.56	0.26	24.90	2.66
Kazbek	1.02	32.75	11.76	0.42	21.00	3.83

2. Fruity aroma

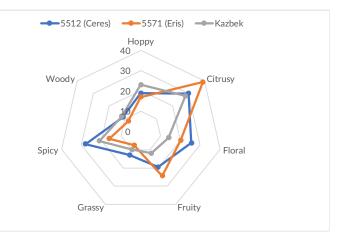
Among the evaluated genotypes, the strongest fruity aroma was found in the genotypes of Juno and Saturn as well as in the Mimosa variety. Juno was developed from the hybridization of Kazbek and a wild hop from Canada. The genotype of Saturn resulted from the hybridization of Kazbek and a male plant that is a multiple cross of European hops. Mimosa was developed from the hybridization of Czech and South African breeding materials.

Figure 3 shows that Juno and Saturn genotypes have a similar composition of aroma character. However, evaluations

of a hop aroma point to major differences in aroma descriptions. The aroma of Juno includes apple and tropical fruits (banana, mango and honeydew melon) as well as citrusy (lemon, lime zest and tangerine) and floral aromas. Saturn has a fruity (apricot, peach, watermelon and mango), citrusy (lemon, lime zest, tangerine and red orange), spicy (black pepper, chilli and anise) and a slightly woody (pine, needles) aroma. Fruity and citrusy aromas are dominant in the Mimosa variety. The aroma of Mimosa includes tropical fruits (banana, mango, papaya and passion fruit) and a citrusy (lemon, lime and grapefruit) aroma; other aromas – grassy (hemp and nettle) and floral (mint and rosemary) – are weaker.

Table 3 shows that contents and compositions of hop resins differ greatly in the genotypes with a fruity aroma. The highest average content of alpha acids was determined in Saturn (7.5% w/w), followed by Juno (4.78% w/w), and the lowest in Mimosa (1.58% w/w). Juno and Saturn have nearly identical beta acid con-

Figure 3 Aroma character in genotypes with a fruity aroma (2019–2020)



tents but they have different ratios of alpha/beta acids. Saturn is characterised by the alpha/beta ratio of about 2 and Juno has the ratio of 1.28. A high content of beta acids, and thus a very low alpha/beta ratio (0.28) is typical of Mimosa. The genotypes also differ in terms of cohumulone content. The highest cohumulone content was found in Saturn (47.35% rel.), followed by 5495 (38.53% rel.), and the lowest in Mimosa (29.44 % rel.).

Table 4 shows that the genotypes have very different contents and compositions of hop oils once again. Saturn has the highest average content of hop oils (1.53 w/w). In the years 2017 and 2018, the content of hop oils exceeded 2.00 w/w in some locations. Juno has the average content of hop oils of 1.04 w/w and Mimosa only 0.75 w/w. Juno has the highest content of myrcene (44.66% rel.) and caryophyllene (11.20% rel.). Saturn has the highest humulene content (27.49% rel.). Mimosa has the highest content of selinenes (32.75% rel.).

Table 3	Average content and	composition of ho	p resins in genotypes	s with a fruity arom	a (2015-2019)
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Genotype	Alpha acids (% w/w)	Beta acids (% w/w)	Alpha/beta ratio	Cohumulone (% rel.)
5495 (Juno)	4.78	3.76	1.28	38.53
5520 (Saturn)	7.50	3.60	2.07	47.35
Mimosa	1.58	5.56	0.28	29.44

Table 4 Average content and composition of hop oils in genotypes with a fruity aroma (2015-2019)

Genotype	Weight (w/w)	Myrcene (% rel.)	Caryophyllene (% rel.)	Farnesene (% rel.)	Humulene (% rel.)	Selinenes (% rel.)
5495 (Juno)	1.04	44.66	11.20	0.27	4.33	5.30
5520 (Saturn)	1.53	36.37	10.66	0.24	27.49	1.90
Mimosa	0.75	32.07	5.79	0.96	2.25	32.75

3. Spicy aroma

Uran resulted from the hybridization of the Columbus variety and semi-finished breeding materials. Jupiter was developed as a result of the hybridization of the Kazbek variety and international breeding materials. Pluto resulted from the hybridization of the Harmonie variety and an inbreeding line of Saaz hops.

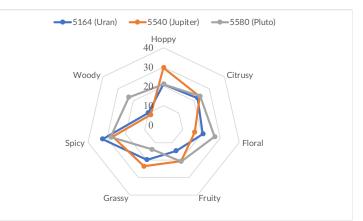
Figure 4 shows that Uran and Jupiter are very different from Pluto. Uran is characterized by a spicy aroma (black pepper, curry and star anise) and a mixture of aromas of garlic, onion, olive and needles (pine). Jupiter has a spicy aroma

(star anise and bay leaf) as well as aromas of herbs (mint, lemon balm), flowers (jasmine, rose) and resin. Pluto has a broad range of aromas. There is a distinct woody aroma, spicy (needles, chamomile and lemon balm), sweet (yoghurt, almond and vanilla) and fruity (green fruits, lemon and pineapple) aromas.

The highest average content of alpha acids (11.51% hm.) was determined in Uran (Table 5). With an average beta acid content of 5.26% w/w, it has a high alpha/beta ratio (2.20). Pluto has the average content of alpha acids of 4.33% w/w and beta acids of 3.29% w/w. Pluto shows the average alpha acid content of 6.43% and an average beta acid content of 5.42% w/w, resulting into a low alpha/beta ratio of 1.19. The content of cohumulone in these genotypes ranges between 21.28% rel. (Pluto) and 28.31% rel. (Jupiter).

Table 6 shows that the highest content of hop oils was (% rel.) found in Uran (1.77 w/w), followed by Pluto (1.35 w/w) and the lowest in Jupiter (0.71 w/w). Uran has the highest content of myrcene (49.42% rel.) and farnesene

Figure 4 Aroma character in genotypes with a spicy aroma (2019–2020)



(12.50% rel.). Jupiter has the highest content of caryophyllene (12.46% rel.) and selinenes (7.50% rel.), while Uran has the highest humulene content (22.22% rel.).

Data collected over a period of five years was used to determine the variability of content and composition of hop resins (Table 7). The centuple of the variation coefficient shows in percentage to what extent a characteristic was influenced by the environment in evaluations over a period of five years. The highest variability of the alpha acid content was determined in Jupiter (30.85%) and Mimosa (30.13%). In contrast, the most stable content of alpha acids was found in Juno, Pluto and Uran, with the variability of less than 15%. Jupiter has the highest variability of other characteristics as well - content of beta acids (18.35%), alpha/beta ratio (39.13%) and cohumulone content (12.27%). The characteristics of this genotype are likely to be more influenced by the environment than those of other genotypes. For example, genotypes Pluto, Juno and Uran as well as Kazbek have a low variability. Overall, it can be stated that all genotypes have an appro-

Table 5	Average content and com	position of hop res	ins in genotypes wi	ith a spicy aroma	(2015-2019)

Genotype	Alpha acids (% w/w)	Beta acids (% w/w)	Alpha/beta ratio	Cohumulone (% rel.)
5164 (Uran)	11.51	5.26	2.20	24.98
5540 (Jupiter)	4.33	3.29	1.37	28.31
5580 (Pluto)	6.43	5.42	1.19	21.28

 Table 6
 Average content and composition of hop oils in genotypes with a spicy aroma (2015-2019)

Genotype	Weight (w/w)	Myrcene (% rel.)	Caryophyllene (% rel.)	Farnesene (% rel.)	Humulene (% rel.)	Selinenes (% rel.)
5164 (Uran)	1.77	49.42	4.97	12.50	6.87	1.51
5540 (Jupiter)	0.71	44.94	12.46	0.38	4.41	7.50
5580 (Pluto)	1.35	35.59	8.26	0.35	22.22	1.98

priate variability of the alpha acid content. Between the years 2010 and 2019, the variability of the alpha acid content was evaluated in selected hop varieties (Nesvadba and Charvátová, 2020). The variability of Czech hop varieties ranged from 8.89% (Premiant) to 26.30% (Sládek). Foreign hop varieties had a higher variability – between 16.03% (in the Target variety from England) and 33.81% (in the Bobek variety from Slovenia).

4 Conclusion

Two flavour hop varieties are registered in the Czech Republic – Kazbek (2008) and Mimosa (2019). Thanks to extensive breeding efforts, additional promising hop genotypes were developed. Ceres and Eris are remarkable for their intensive and pleasant citrusy aroma. These genotypes rank with the Kazbek variety because

Genotype	Alpha acids (%)	Beta acids (%)	Alpha/beta ratio (%)	Cohumulone (%)
5512 (Ceres)	24.17	12.45	21.90	10.30
5571 (Eris)	20.18	12.60	12.23	4.88
Kazbek	17.40	12.83	17.37	6.85
5495 (Juno)	12.42	10.40	10.58	7.42
5520 (Saturn)	25.52	13.89	19.22	8.80
Mimosa	30.13	14.76	24.23	5.24
5164 (Uran)	14.73	12.79	8.33	4.29
5540 (Jupiter)	30.85	18.35	39.13	12.27
5580 (Pluto)	14.05	6.91	13.94	4.34

Table 7	Variability of content and composition of hop resins (2014-2019)
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As shown above, the selected genotypes differ greatly in terms of aroma intensity, aroma character, content and composition of hop resins and oils. Some of their characteristics may be similar to those of already registered hop varieties. As to the alpha acid content, Uran is similar to the Gaia and Boomerang varieties, which also show their alpha acid content above 10% w/w. (Nesvadba et al., 2017b). The Gaia variety has a high content of selinenes (25-27% rel.) and Boomerang a high content of humulene (17-27% rel.). Out of all the registered Czech hop varieties, Mimosa has the lowest content of alpha acids. Therefore, it is used by brewers for dry hopping. Thanks to its low alpha acid content, Mimosa does not have any impact on the bitterness intensity - unlike hop varieties with a higher content of alpha acids. The hop variety is currently being used for the breeding of flavour hops (Nesvadba et al., 2017c). Mimosa is the maternal plant of genotypes Juno, Ceres, Saturn and Jupiter. It is worth mentioning that Juno has a male wild hop from Canada in its origin. Pluto resulted from the inbreeding of the Harmonie variety. Genotypes Juno, Ceres, Saturn, Jupiter and Pluto belong to the group of aroma hops, together with Sládek, Premiant, Harmonie, Bohemie etc. However, they have a very different aroma character (Nesvadba et al., 2012). Other genotypes have a hop from North America with a high cohumulone content in their origin (Hampton et al., 2002). Therefore, they have a high cohumulone content as well.

of their citrusy aroma. However, their aroma character is different. Two genotypes – Juno and Saturn – can be assigned to Mimosa, which has a fruity aroma. But the aroma intensity of these two genotypes is higher. The third group is comprised of genotypes with a spicy aroma. Pluto is also different because of its woody aroma. Uran has the highest intensity of spicy aroma, which can be even garlicky. Jupiter is characterized by a balanced mixture of spicy, fruity, citrusy, hoppy and grassy aromas. It must be stated that aromas in beer may differ for several reasons. Aroma character is influenced by beer style, combination of hops and quantity. Quantity and maceration period are important for dry hopping.

Currently, the Kazbek variety is being grown on 26.4 hectares (UKZUZ, 2020). The Mimosa variety is being grown in pilot conditions on 0.30 hectares. Other genotypes are being grown at the total number of 100 to 300 plants each. The Kazbek variety is used in many large and small breweries. The Mimosa variety and other genotypes are tested in large and small breweries as well as by homebrewers. Partial results point to good qualities in terms of hop growing and beer brewing. A registration of the new flavour hops is expected in 2022. Currently, additional new hop genotypes are being developed in hop breeding. They have a different aroma character from the aforementioned varieties and genotypes.

5 Acknowledgement

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