Characterization of Czech hop (*Humulus lupulus* L.) genotypes by molecular methods

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ABSTRACT

In the last year, new hybrid hop (*Humulus lupulus* L.) variety Agnus was released for cultivation in the Czech Republic. It has been necessary to prepare the quality system of Agnus identification from other Czech genotypes and characterise the germplasm of this variety by molecular methods. We proved that utilization of five STS primer combinations successfully and completely identified and determined Czech released varieties and new promising breeding materials. The use of STS method was also very effective and sensitive for control of authenticity and purity of variety Agnus in multipropagation cycle. The study of genetic diversity of 61 hop varieties by RAPD, STS, ISSR and AFLP methods confirmed, that germplasm of variety Agnus has ranked among high-alfa varieties. The results can be successfully used for identification, germplasm management, genetic studies and breeding purposes by breeders, multipropagators and hop growers.

Keywords: DNA fingerprinting; RAPD; STS; ISSR; AFLP; genetic diversity

Hop (*Humulus lupulus* L.) is a dioecious perennial climbing plant and only female plants are cultivated for commercial use, mainly in brewing industry. Female inflorescences, referred as cones, contain hop resins, which give beer its bitterness, and essential oils, which give beer its flavour (Neve 1991). Characteristic profile of chemical components in hop cones depends on hop variety. There are about two hundred hop varieties worldwide, which are grown on 80 thousand hectares.

Hop is one of the most important crops in the Czech Republic. Czech hop production is valued on world market and it ranks on fourth place in the world. Cultivation of hop is specially directed by law No. 97/1996 in the Czech Republic. Only Czech released varieties can be planted in hopgarden. In the last year, new hybrid variety Agnus (HML04981) was released for cultivation in the Czech Republic. In this situation, the capability to identify individual Czech hop varieties is critical for hop industry.

Although identification of hop varieties based on the content and composition of volatile compounds have been developed (e.g. Krajl and Zupanec 1991, Peacock and McCarty 1992), the value of analytical parameters can be influenced by environment and harvest. The use of molecular biology methods is more reliable for variety identification, control of variety authenticity and purity. There are many molecular methods, which can be used for DNA fingerprinting (Patzak 2001). Random amplified polymorphism of DNA (RAPD) has mainly been used for identification of hop varieties. Disadvantages of RAPD method were overcome by transformation of specific RAPD markers to sequence tagged sites (STS). These systems have been recently published by Tsuchiya et al. (1997), Araki et al. (1998) and Murakami (1998).

The use of molecular methods provides a possibility to study genetic diversity of hop varieties and their relationships. This knowledge is very important for hop breeders (Murakami 2000a). RAPD method was employed by Šustar-Vozlič and Javornik (1999) in analysis of 65 hop genotypes and by Murakami (2000b) in analysis of 51 hop genotypes. STS and amplified fragment length polymorphism (AFLP) methods were used for analysis of 41 hop genotypes by Jakše et al. (2001). Seefelder et al. (2000) reported about AFLP analysis of 90 hop genotypes. All these methods and inter-simple sequence repeat (ISSR) method have provided very good molecular characterization of hop germplasm and its genetic relationships (Patzak 2001).

In our work, we proved the identification of Czech released hop varieties and new promising breeding materials by STS molecular method. We also proved the possibility to control of authenticity and purity of variety Agnus in multipropagation cycle by STS method. In the next experiments, we analysed 61 hop varieties by RAPD, STS, ISSR and AFLP methods for study of genetic diversity with concentration on new hybrid variety Agnus.

MATERIAL AND METHODS

Plant material and DNA isolation

Five Czech released varieties (Osvald's clone 72, Bor, Sládek, Premiant, Agnus) from maintenance hopgarden and four new promising breeding materials (4382, 4527, 4353, 4715) from breeding hopgarden were used for identification experiments. Sixty-one world hop varieties from the world hop collection of the Hop Research Institute in Žatec were used for genetic diversity experiments. The chemical characteristics and origins of individual hop

Table 1. List of used hop varieties with their origin, morphological and chemical characteristics

Variety	Country	Ploidity	Parentage	Content of			Content of									Yield	Bine
				α-acids	β-acids	α/β ratio	1	2	3	4	5	6	7	8	9	(t.ha^{-1})	color
Bramling Cross	England	2*n*	Bramling × (BB1 × open-pollinated)	6	3	2	27	51	1	37	30	0.8	16	2	2	1.7	green
Brewers Gold	England	2*n*	BB1 × open-pollinated	6.5	3.3	1.9	45	67	2	38.5	25	0	7.5	2	2	2.2	g-r
Fuggle	England	2*n*	landrace	4.8	2.5	1.9	27	51	1.1	26	37	6	12	4	3	1.35	g-r
Golding	England	2*n*	landrace	5.5	2.2	2.1	25	51	0.8	30	41	0	13	1	2	1.3	g-r
Challenger	England	2*n*	17/54/2 × 1/61/57M	7.8	4.2	1.9	23	51	1.3	37	27	1.5	9	5	4	1.8	violet
Northdown	England	2*n*	Northern Brewer × open-pollinated	8.5	5.2	1.6	27	50	2	27	42	0.8	15	4	2	1.7	green
Northern Brewer	England	2*n*	Canterbury Golding × OB21 (male seedling of Brewers Gold)	8.4	4	2.1	30	51	1.7	35	30	0	10	2	1	1.8	violet
Target	England	2*n*	1/61/1 × 27/57/281M	11.1	4.2	2.6	37	61	2	50	20	0	9	1	5	1.7	violet
Yeoman	England	2*n*	43/69/17 × 25/68/173M	10	4	2.5	27	49	1.7	35	26	0	13	3	1	1.7	r-v
Pride of Ringwood	Australia	2*n*	Pride of Kent (derived from Brewers gold) × open-pollinated	8.5	5	1.7	36.5	52	1.5	37	5.5	0.8	7.5	2	2	2.5	r-v
Ringwood Special	Australia	2*n*	Fuggle × open-pollinated	5.5	5.5	1	43	61	1.6	39	19	8	8	2	2	1.6	g-r
Agnus	Czech Republic	2*n*	2933 (Northern Brewer × open-pollinated) × 82/6 [Sládek × 74/4 (Bor × 71)]	14	7.5	1.8	36	57	2.5	50	17	0.2	9	3	3	2.3	green
Bor	Czech Republic	2*n*	Northern Brewer × open-pollinated	9	5	1.8	23.5	46	1.2	42	30	0	9.5	3	3	2	red
Osvald's clone 72	Czech Republic	2*n*	landrace	3.5	5	0.7	25	41	0.5	39	20	16	5.9	3	3	1.1	red
Premiant	Czech Republic	2*n*	Osvald clone 72 × (Osvald clone 72 × american male)	10	4.5	2.2	21	42.5	1.5	42	30	1.8	9	3	3	2.5	green
Sládek	Czech Republic	2*n*	Northern Brewer × [Northern Brewer × (Osvald clone 126 × saazer)]	6.5	7.5	0.9	27	47	1.8	45	25	0	9	3	3	2.5	green
Striesselspalt	France	2*n*	derived from Hersbruck	4.5	4.7	0.9	23	41	0.7	25	20	0.8	9	3	3	1.7	g-r
Golden Star	Japan	2*n*	mutant from Shinshuwase (Saaz × White Bine)	3.7	4.6	0.7	48	69	0.6	35	38	0	12	3	3	1.1	red
Kirin II	Japan	2*n*	clonal selection from Shinshuwase (Saaz × White Bine)	3.5	4.5	0.7	48	70	0.6	30	40	0	13	2	3	0.9	red
Southern Brewer	South Africa	2*n*	Fuggle × seedling of Fuggle	10	4	2.5	39	66	0.8	41	19	4	9	3	3	1.7	r-v
Backa	Yugoslavia	2*n*	derived from Hersbruck, Striesselspalt and Elsaser	3.7	4.8	0.7	24	41	0.7	30	32	0.5	10	2	2	1.6	g-r
Vojvodina	Yugoslavia	2*n*	Northern Brewer × (Savinsky Golding × open-pollinated)	6.5	5.8	1.3	30	58	1.1	32	32	0.5	10	3	3	1.8	violet
Hallertau	Germany	2*n*	landrace	4.5	4.5	1	23	40	0.8	27	48	0	13	3	3	1.2	g-r
Hallertau Tradition	Germany	2*n*	Hallertauer Gold × 75/15/106M	6	4.5	1.5	27.5	48	1.2	23	50	0.5	13	5	3	1.7	g-r
Hersbruck	Germany	2*n*	landrace	3.5	5	0.7	21	38	0.7	20	25	0	11	2	3	1.7	g-r
Magnum	Germany	2*n*	Galena × 75/5/3M	13.5	5	2.4	27	46	2	31	36	0.8	9	4	2	2.2	green
Merkur	Germany	2*n*	Magnum × 81/8/13M	14.5	5.5	2.5	20	43	2.2	32	32	0	9	4	4	2	green
Perle	Germany	2*n*	Northern Brewer × 63/5/27M	7	4	1.6	30	54	1.1	23	35	0	9	4	3	1.9	green

Spalt	Germany	2*n*	landrace	5	4.9	1	26	43	0.7	42	25	12	9	3	3	1	red
Spalter Select	Germany	2*n*	76/18/80 × 71/16/7M	5	4	1.2	23	43	0.7	20	20	20	9	4	3	1.95	red
Taurus	Germany	2*n*	82/39/37 × 85/54/15M	15.5	5	3	24	47	1.4	30	23	0	8	3	2	2.2	g-r
Tetnang	Germany	2*n*	landrace	4.6	4.6	1	27	45	0.8	23	24	15	9	3	3	1.4	red
Calicross	New Zealand	2*n*	Late Cluster × seedling of Fuggle	10.5	7	1.5	41	57	1.1	52	19	0	6.5	1	3	1.2	r-v
Smooth Cone	New Zealand	2*n*	Late Cluster × seedling of Fuggle	11	6.8	1.8	39	55	1.4	48	21	0	6.5	2	3	1.3	r-v
Estera	Poland	2*n*	Savinsky Golding × Nadwislavsky	4	6.8	0.6	29	42	1.2	34	39	0	12	3	3	1.8	green
Izabela	Poland	2*n*	Lubelski × Yugoslavian male	8.5	9.9	0.8	29	55	2	42	29	0	10	3	3	2.8	g-r
Lubelski	Poland	2*n*	landrace (Saazer)	4.7	9.9	0.5	28	47	1.1	23	42	1.5	10	3	3	1.7	g-r
Marynka	Poland	2*n*	Brewers Gold × Yugoslavian male	10	11.7	0.85	26	54	2	31	33	2	11	2	2	2.2	green
Nadwislavsky	Poland	2*n*	landrace	3.8	8.5	0.45	31	43	1.3	34	37	1	9	3	3	1.5	g-r
Serebrjanka	Russland	2*n*	selection from landrace of Siberian hop	3.5	5.5	0.6	23	42	0.7	40	27	14	9	3	3	1	red
Atlas	Slovenia	2*n*	Brewers Gold × 3/3	8.5	3.7	2.3	37	62	2	38	16	15	6	2	2	2.1	red
Aurora	Slovenia	2*n*	Northern Brewer × TG	9.5	3.8	2.5	24	52	1.2	45	24	7.5	6	4	4	2.4	red
Blisk	Slovenia	3*n*	Atlas (4*n*) × 1/9	6	3	2	37.5	61	2.2	45	12	18	6	2	3	2.2	green
Bobek	Slovenia	2*n*	Northern Brewer × TG	5.3	5.1	1.1	30	51	2	52	16	5.5	5	3	4	2	g-r
Buket	Slovenia	2*n*	Northern Brewer × 2/137	8	3.5	2.3	24	54	2.1	44	22	5.5	7.5	3	4	1.9	g-r
Celeia	Slovenia	3*n*	Savinsky Golding (4*n*) × 105/58	6	2.8	2.1	28	55	2.1	34	20.5	5	8.5	4	4	1.9	g-r
Cicero	Slovenia	3*n*	Aurora × 3/3 (4*n*)	8	3	2.6	27	50	1.7	33	26	5	9	3	2	2.1	red
Savinsky Golding	Slovenia	2*n*	derived from Fuggle	4.5	2.7	1.7	30	50	1	32	33	7	10.5	3	4	1.7	g-r
Cascade	USA	2*n*	[Fuggle × (Serebjanka × seedling of Fuggle)] × open-pollinated	6	6	1	36	58	1.2	52	13	6	5	4	3	1.8	g-r
Centennial	USA	2*n*	OR6619-04 × USDA63015M	10.5	4	2.6	30	52	1.8	50	15	0.5	6.5	4	3	1.85	g-r
Columbus	USA	2*n*	unknown	16	5.2	3	32	54	2.5	33	18	0.5	9.5	1	3	2.8	green
Comet	USA	2*n*	Sunshine (derived from *H. aeureus*) × utah wild male	7	3	2	43	68	0.8	58	1	0	6	2	2	1.8	g-r
Crystal	USA	3*n*	Hallertauer Mtf. (4*n*) × 21381M	3.5	5.5	0.6	23	44	1.2	52	21	0.5	6	2	2	1.6	g-r
Eroica	USA	2*n*	Brewers Gold × open-pollinated	12	4.7	2.5	40	64	1.1	60	1	0	10	3	2	2.5	g-r
Galena	USA	2*n*	Brewers Gold × open-pollinated	13	8	1.6	40	65	1.1	57	12	0	5	2	2	2.2	g-r
Chinook	USA	2*n*	Petham Golding × USDA63012M (Brewers Gold × utah wild hop)	13	3.5	3.6	32	54	2	37	22	0	10	4	2	2.3	red
Late Cluster	USA	2*n*	landrace	7	5	1.5	40	64	0.6	50	16.5	0	6.5	1	2	2	r-v
Liberty	USA	3*n*	Hallertauer Mtf. (4*n*) × 64035M	4	3.5	1.1	27	57	0.9	37	37	0.5	10.5	4	3	1.5	g-r
Mt. Hood	USA	3*n*	Hallertauer Mtf. (4*n*) × USDA19058M (Early Green open-pollinated)	6	6	1	22	46	1.2	50	25	0.5	10	4	3	1.8	g-r
Nugget	USA	2*n*	USDA65009 (Brewers Gold × Early Green Golding) × USDA63015M (Brewers Gold × East Kent Golding)	13	5	2.6	26	56	2	56	17	0	8.5	3	4	2.1	g-r
Wilamette	USA	3*n*	Fuggle (4*n*) × seedling of Fuggle	5	3.5	1.5	32	63	1.2	50	25	5.5	7.5	4	3	1.6	green

Information about hop varieties were obtained from official materials of Hop Growers of America, Germany, England, Czech Republic, Poland, New Zealand, South Africa and Slovenia
1 – cohumulone (% α-acids), 2 – colupulone (% β-acids), 3 – total oils (% DW), 4 – myrcene (% oils), 5 – humulene (% oils), 6 – farnesene (% oils), 7 – caryophylene (% oils), 8 – resistance to downy mildew, 9 – resistance to powdery mildew

varieties are shown in Table 1. DNA was isolated from young leaves according to Saghai-Maroof et al. (1984) modified by Patzak et al. (1999). DNeasy Plant Mini kit (Qiagen, Hilden, FRG) was used for DNA isolation from multipropagated plants of Agnus in glasshouse.

Molecular methods

STS analyses were performed according to Brady et al. (1996) – alleles 11a59, 3a88 and 5-2, Tsuchiya et al. (1997) – locus B72WF2/R2, Araki et al. (1998) – primer combination 1, and Murakami (1998) – primer combinations no. 1 and 2. New specific primer Nug2 (TCTTATGTGAGCCTCAGCAAG) was also used in STS reactions. RAPD analyses were performed according to Patzak et al. (1999). Eight decamer oligonucleotide primers OPA-11, OPB-08, OPC-08, OPC-09, OPV-17, $(GACA)_2GA$, $(AGC)_3A$ and M13 were used in RAPD analyses. ISSR analyses were performed according to Patzak (2001). ISSR reactions were based on six microsatellite sequences: $(GACA)_4G$, $(TGTC)_4T$, $(AGC)_5A$, $(GCT)_5G$, $(TCG)_5T$ and $(CGT)_5C$,

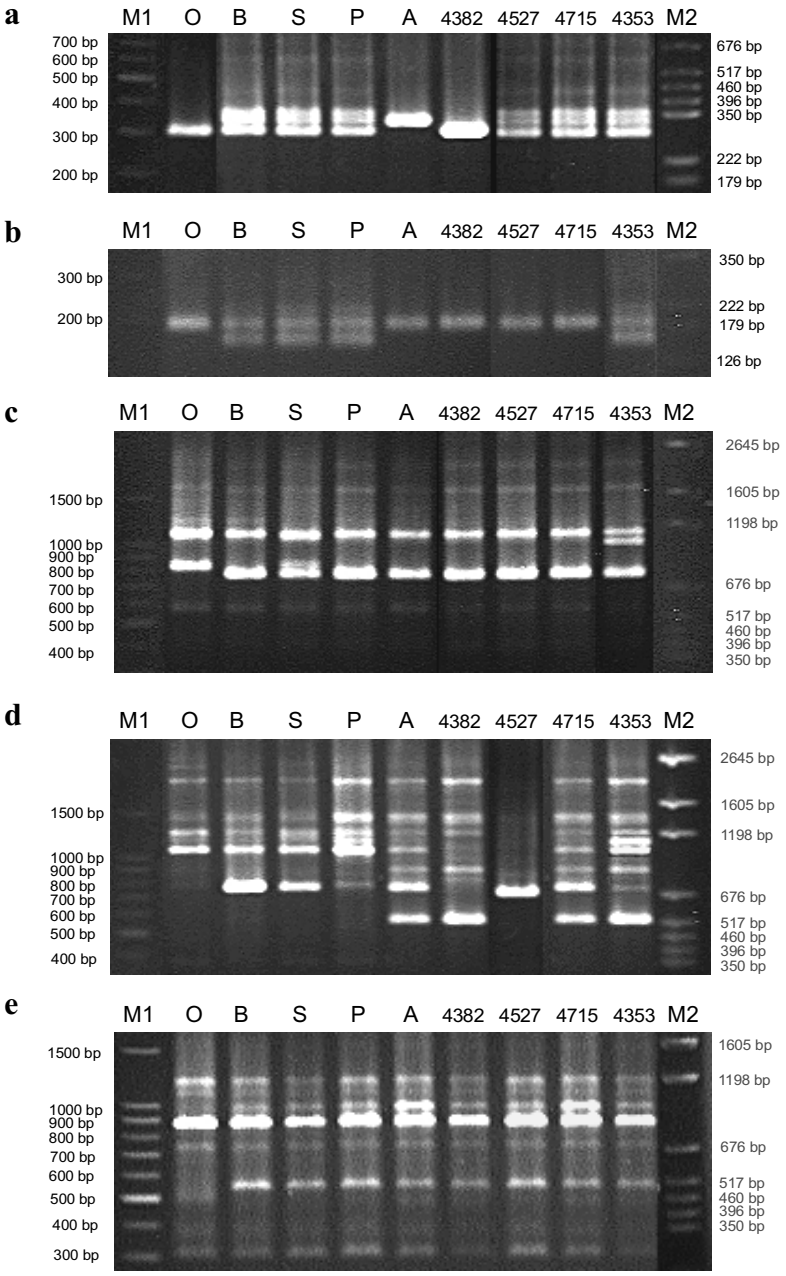

Figure 1. Analyses of amplified products in STS reactions of Czech released varieties and new promising breeding materials with primer combinations B72WF2/R2 (Tsuchiya et al. 1997) (a), I. (Araki et al. 1998) (b), 1 (c) and 2 (d) (Murakami 1998) and Nug2 (e) in 2% agarose gels; O – Osvald's clone 72, B – Bor, S – Sládek, P – Premiant, A – Agnus, M1 – 100 bp Ladder, M2 – pGEM DNA marker (Promega, Madison, Wisconsin, USA)

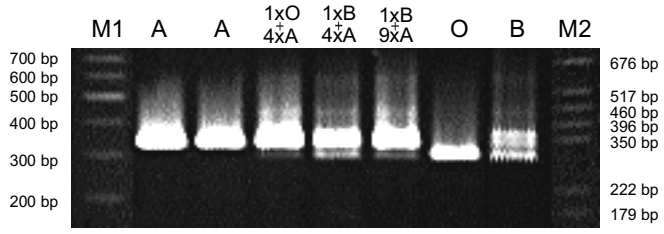

Figure 2. Analysis of amplified products in STS reactions of variety Agnus and contaminated DNAs with primer combinations B72WF2/R2 (Tsuchiya et al. 1997) in 2% agarose gel; O – Osvald's clone 72, B – Bor, A – Agnus, M1 – 100 bp Ladder, M2 – pGEM DNA marker (Promega, Madison, Wisconsin, USA)

which were used as PCR primers and their three combinations: $(GACA)_4G + (TGTC)_4T$, $(TGTC)_4T + (GCT)_5G$, $(AGC)_5A + (CGT)_5C$. AFLP analyses were performed according to Patzak (2001). Six primer combinations, with three selective bases on the 3'end, E-ACG + M-CAT, E-ACG + M-CTA, E-ACG + M-CTG, E-ACG + M-CTC, E-ACT + M-CTC, E-AAC + M-CTG.

PCR chemicals and electrophoresis

A *Taq* PCR master mix kit (Qiagen, Hilden, FRG) was used for STS, RAPD and ISSR reactions, and an AmpliTaq Gold DNA polymerase (Applera Corporation, Applied Biosystems, Foster City, California, USA) was used for AFLP reactions. All PCR amplifications were performed using a Genius thermocycler (Techne, Cambridge, UK). A minimum of two amplifications was performed in order to check consistency.

Horizontal agarose gel electrophoresis was performed according to Patzak (2001) for the separation of RAPD (1.5% gel) and STS products (2% gel). Sequencing vertical polyacrylamide gel electrophoresis was performed according to Patzak (2001) for separating AFLP, STS (5% gel, 8M urea) and ISSR products (4% gel, 8M urea) at 45W.

Genetic diversity analysis

The cluster analysis was revealed by NTSYS-pc v. 2.02 for WINDOWS (Exeter Software, New York, New York, USA). Only sharp, strong and reproducible PCR products on gels for individual varieties were used for the analyses. Similarity was estimated using Jaccard's (1908) similarity coefficient (JCS), which ranges from 0 (all products between evaluated varieties were different) to 1 (all products between evaluated varieties were identical). The dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA) clustering procedure.

RESULTS

The use of STS was the most reliable method for identification of Czech released varieties and new promising breeding materials. The utilization of four STS primer combinations by Tsuchiya et al. (1997), locus B72WF2/R2 (Figure 1a), Araki et al. (1998), primer combination I. (Figure 1b), Murakami (1998), primer combinations no.1 (Figure 1c) and no.2 (Figure 1d), and STS primer Nug2 (Figure 1e) successfully and completely identified and determined all Czech tested genotypes. STS patterns contained several discrete polymorphic products among varieties, however they were not different for each variety. Number and set of primer combinations for identification of variety were influenced by each variety. Specific STS patterns of primer combination no. 2 (Murakami 1998) and locus B72WF2/R2 (Tsuchiya et al. 1997) were obtained for variety Agnus. Unfortunately, STS pattern of primer combination no. 2 (Figure 1d) contained all polymorphic products, which were amplified in Czech released varieties and new promising breeding materials and this primer combination were not suitable for control of authenticity and purity of variety Agnus in multipropagation cycle. In opposite, the specific polymorphic product of locus B72WF2/R2 in STS reactions was very suitable for this purpose. Alternative contaminations of variety Agnus with other genotypes can be successfully identified by STS analysis in mixture DNA samples. For example, one plant of Osvald's clone 72 was detected in mixture DNA sample of five Agnus plants and one plant of Bor was detected in mixture DNA sample of five or ten Agnus plants (Figure 2). The use of STS locus B72WF2/R2 has been very effective and sensitive for control of authenticity and purity of variety Agnus in multipropagation cycle.

The use of molecular methods for measuring of genetic diversity plays an important role in evaluating hop genetic resources. Variety Agnus belongs to high-alfa varieties (Table 1) and we first finely characterised its genetic germplasm by molecular methods. For study of genetic diversity, we analysed 61 hop varieties by RAPD, STS, ISSR and AFLP methods. Cluster analysis confirmed, that variety Agnus has ranked among high-alfa varieties (Figure 3). Variety Agnus, as separately cluster 5, was closely related to cluster 4, which contained the most of high-alfa varieties (Figure 3). Other high-alfa varieties, with influence of wild American gene pool, were grouped in cluster 6 and 7 (Figure 3). Old European and new aroma varieties were mostly grouped in cluster 1 (Figure 3).

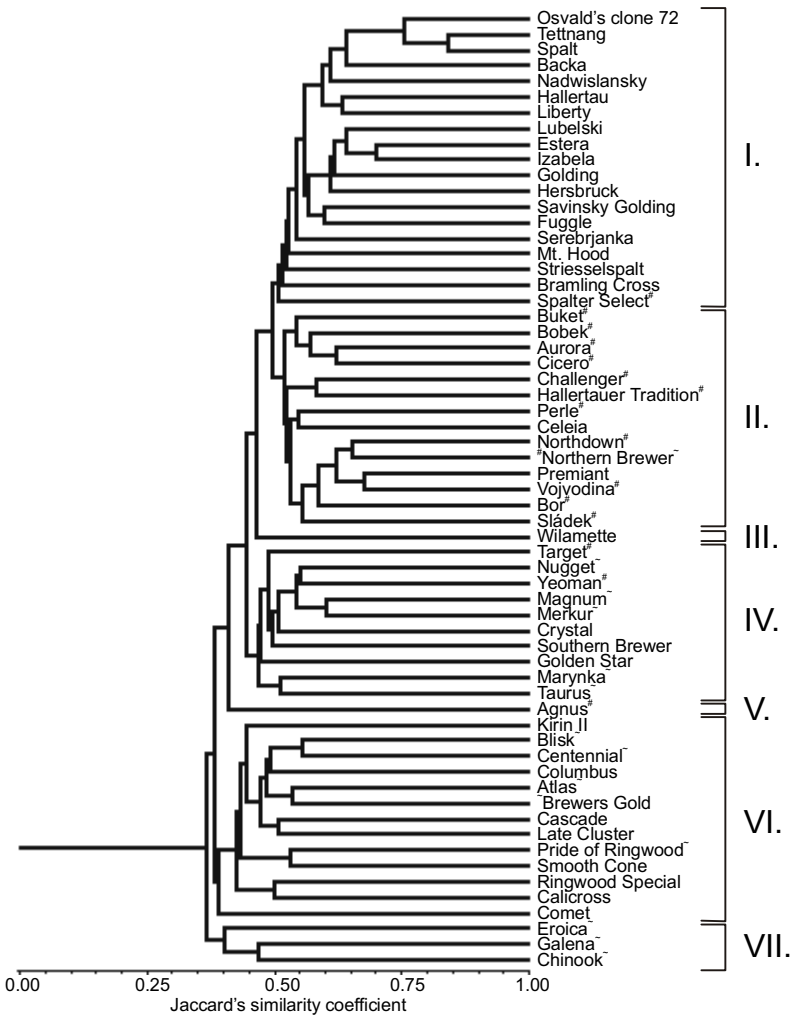

Figure 3. Cluster analysis of individual hop varieties based on 38 RAPD, 55 STS, 112 ISSR and 321 AFLP polymorphic markers revealed by NTSYS-pc v.2.01 (Exeter software, New York, New York, USA)

varieties derived or related to Northern Brewer
~ varieties derived or related to Brewers Gold

Genotypes, clustering in cluster 2, were derived or related to variety Northern Brewer (Figure 3). Clustering was generally consistent with existing knowledge of the origin of varieties. The results confirmed existing genetic diversity of hop varieties and assessed germplasm of variety Agnus to high-alfa hop varieties.

DISCUSSION

A utilization of five STS primer combinations in characterization of Czech released varieties and new promising breeding material revealed a sufficient polymorphism for their identification. The use of STS method was more reliable than use of RAPD method, which Patzak et al. (1999) used for identification of Czech released varieties without Agnus. Using the original RAPD method, it would be difficult to distinguish the difference in the mobility of each RAPD products (Tsuchyia et al. 1997). Although, we tried to convert polymorphic RAPD products to STS marker system (data not shown), the STS primers produced unsatisfactory results, because they amplified common products for most of the varieties, similar to results reported by Brady et al. (1996) and Murakami (1998). The results show that STS marker systems can be used as a method to identify variety Agnus from all Czech released varieties and new promising breeding material. STS method was also very effective and sensitive for control of authenticity and purity of variety Agnus in multipropagation cycle. In our experiments, we successfully detected 20% of contamination with another variety in Agnus rootstocks. It was also possible to detect 10% of contamination. The sensitivity of STS analysis could detect as little as 5% contamination of Northern Brewer in Hersbruck (Tsuchyia et al. 1997, Araki et al. 1998) and of Brewers Gold in Hersbruck (Murakami 1998). Jakše and Javornik (1999) reported that it was possible to detect 5% of contamination by RAPD method and detection limit was a minimum 15% for STS method. STS method is very simple method of DNA fingerprinting with good reproducibility and reliability (Araki et al. 1998). Its utilization for identification of variety Agnus and control of authenticity and purity of this variety can be very effective tool for breeders, multipropagators and hop growers.

Employing RAPD, STS, ISSR and AFLP methods, we analysed germplasm of variety Agnus and genetic diver-

sity among other 60 hop varieties. Clustering studies of hop varieties matched well with those based on morphological features, geobotanical and analytical data. Cluster analysis confirmed, that variety Agnus, as separately cluster 5, has ranked among high-alfa varieties grouped in cluster 4, 6 and 7. Division of high-alfa varieties to three clusters was caused by European and American gene pools within varieties. The influence of these two major groups of hop varieties on genetic diversity studies was reported by Šustar-Vozlič and Javornik (1999) and Murakami (2000b) for RAPD analysis and by Seefelder et al. (2000) and Jakše et al. (2001) for AFLP analysis. The most of high-alfa varieties has been based on variety Brewers Gold, with wild American gene pool, and its daughter variety Northern Brewer, with European gene pool (Neve 1991). Genotypes derived or related to variety Northern Brewer were mainly grouped in cluster 2 and in cluster 4. Genotypes derived or related to variety Bre-wers Gold were grouped in cluster 4, 6 and 7. The other varieties in these three clusters, which have not been related to Northern Brewer or Brewers Gold, inherited the quality of high-alfa varieties from another source of wild American germplasm. For example, New Zealand varieties Smooth Cone and Callicross were derived from variety Late Cluster (Šustar-Vozlič and Javornik 1999). American gene pool was suggested also in Japanese varieties Kirin 2 and Golden Star (Murakami 2000b). Clustering of varieties in dendrogram reflected a greater or lesser extent of wild American germplasm infiltration to European gene pool (Seefelder et al. 2000). European aroma varieties were grouped in cluster 1. Murakami (2000b) reported, that two original strains existed in European hops and English old varieties originated from both strains. Seefelder et al. (2000) reported about three groups: Hallertauer, Fuggle/Golding and Saazer, which were similar to our results. In addition, American aroma varieties Mt. Hood and Liberty, which originated from Hallertauer Mfr., were clustered to European aroma hops. Cluster analysis significantly correlated with published studies of genetic diversity (Jakše et al. 2001, Šustar-Vozlič and Javornik 1999, Murakami 2000b, Seefelder et al. 2000), except microsatellite analysis by Jakše et al. (2001). Microsatellite analysis or STS analysis of genetic diversity can be slightly different (Patzak 2001); therefore, it is better to use several different molecular methods together for study of genetic diversity of hop varieties. The results of genetic diversity studies can be suitable for predicting phenotypic values of chemical components of hop varieties (Murakami 2000a). The results of genetic diversity can be successfully used for germplasm management, genetic studies and breeding purposes.

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ABSTRAKT

Charakteristika českých genotypů chmele (*Humulus lupulus* L.) pomocí molekulárních metod

Nová hybridní odrůda chmele (*Humulus lupulus* L.) Agnus byla v minulém roce povolena pro pěstování v ČR. Bylo nezbytné připravit kvalitní systém identifikace odrůdy Agnus mezi ostatními českými genotypy a charakterizovat zárodečnou plazmu této odrůdy pomocí molekulárních metod. Ověřili jsme, že při použití pěti STS primerových kombinací lze úspěšně a kompletně identifikovat a determinovat české povolené odrůdy a nové nadějné šlechtitelské materiály. Použití STS metody bylo též velice efektivní a citlivé pro kontrolu pravosti a čistoty odrůdy Agnus v množitelském cyklu. Studium genetické diverzity 61 chmelových odrůd pomocí RAPD, STS, ISSR a AFLP metod potvrdilo, že odrůda Agnus patří mezi vysokoobsažné odrůdy. Výsledky mohou být úspěšně využity pro identifikaci, spravování genových zdrojů, genetické studie a šlechtitelské záměry šlechtiteli, množiteli i pěstiteli chmele.

Klíčová slova: DNA fingerprinting; RAPD; STS; ISSR; AFLP; genetická diverzita

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