Phenotypic and alpha-acid content diversity of wild hop populations in Croatia

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ABSTRACT

An ecogeographical survey of wild hop populations was conducted in the northwest of Croatia in two consecutive years. A total of 121 plants was documented on eight locations. Along with the passport data, the data on three phenotypic traits (no. of leaflets, cone shape, aroma) were gathered during collection, while the content of α-acids in hop cones was determined by lead conductance. Shannon's information index was calculated for each phenotypic trait and was used as a measure of intra- and inter-population diversity. For all analyzed traits, most of the total phenotypic diversity was attributable to differences among plants within populations. The differences in proportions of individuals having a particular trait state between all pairs of populations in the case of leaflet number and cone shapes were non-significant. Four out of 28 pairwise comparisons for aroma were proven significant, indicating the existence of differences in proportions of individuals across populations recorded to have 'typical hoppy', 'fine hop' or 'rough' aroma. No significant differences were observed for α-acids content among populations. Plants exhibiting elongated cone shape had significantly higher α-acids content than those having oval or round cone shapes. Similarly, plants categorized as 'rough' aroma hop cones had the highest content of α-acids compared with those categorized as 'typical hoppy' or 'fine hop aroma' hop cones.

Keywords: *Humulus lupulus* L.; wild hops; hop cones; qualitative traits; quantitative traits; phenotypic diversity; alpha-acid content

It is well-known that first hop cultivars such as English Fuggles and Goldings as well as Czech Saaz hops were selected from wild hop populations (Wagner 1975, Rybáček 1980, Kišgeci et al. 1984, Schattenhofer 1989, Barth et al. 1994, Moir 2000). Studying of hop wild gene pool is of essential need for sustainability of hop production (Nesvadba 2007). Cytogenetic and molecular studies of wild hops have brighten up the phylogeny of hops (Ono 1961, Henning et al. 1997, Hampton et al. 2001, Hampton et al. 2002, Jakše et al. 2004, Murakami et al. 2006, Lutz et al. 2007, Nesvadba 2007, Nesvadba et al. 2007, Patzak et al. 2007, Probasco et al. 2007, Štajner et al. 2008), but a few authors reported on phenotypic variability of wild hop populations (Wormald 1915, Schmidt 1917, Salmon and Wormald 1921, Blattny 1950, Davis 1957, Ono 1961, Wagner 1974 and 1975, Nesvadba 2007). However, phenotypic characterization of hop plants is the very first step that precedes the mentioned molecular analyses.

In the past century, investigations concerning wild hops became more intensive (Wormald 1915, Schmidt 1917, Salmon and Wormald 1921, Blattny 1950, Davis 1957). However, the most important studies of wild hops related to this paper were provided by Wagner (1974, 1975) who described 398 hop habitats in Pannonian, Alpine, Dinaric, Pindo-Rhodopian, Carphato-Balkanian and Mediterranean

macro-regions of former Yugoslavia. He also provided exploration of wide geographical area and determination of phenotypic traits of wild hop plants which are mostly overspread in Subpannonian region of Pannonian macro-region, mainly in the valleys of the rivers Sava and Drava and also on hilly lands of the regions of Slavonia, Zagorje, Posavina, Podravina, Međimurje and Pokuplje. Agriculture is not so intensive in those areas because of the relief and soil so the wild hops were found mostly on the fields near the forest borders. This completely stays in line with research provided by Nesvadba, who organized an expedition in the northern Caucasus to the region of North Osetia in 2006 (Nesvadba 2007).

In 2007 and 2008, we conducted an ecogeographical survey of variability of wild hop populations in Subpannonian micro-region of Croatia. The aim of this research was to determine phenotypic variability of wild hop populations in the northwest and central part of Croatia and to analyze the content of α-acids in hope cones in order to assess its breeding potential.

MATERIALS AND METHODS

Ecogeographical survey. An ecogeographical survey of wild hop populations was conducted in the northwest of Croatia in two consecutive years (2007 and 2008). A total of 360 plants was found on wider areas of Ludbreg, Kalnik, Križevci, Novo Virje, Ozalj, Prelog, Varaždin and Rugvica (Figure 1). According to Wagner (1974, 1975) those areas belong to the Subpannonian micro-region of Pannonian macro-region and partially to Dinaric macro-region. Geographic position of each plant was detected by GPS station and minimal distance between neighboring plants was 250 m. The photographs were taken and after detailed ocular assessments of each of 360 plants we selected 121 plants which were described *in situ* according to the descriptor list for genus *Humulus* L. (Rígr and Faberová 2000, Anonymous 2006). The reason why we selected, described and collected only 121 plants out of 360 was the 250 m of minimal distance between neighboring plants, to avoid the sampling of clones (Rybáček 1980, Kišgeci et al. 1984). The passport data were recorded *in situ* including some phenotypic traits such as: determination of sex, leaf morphology (number of leaflets), cone shape and aroma. Aroma was assessed nasally and defined as: 'typical hoppy', 'fine aroma' and 'rough' or 'bad smell'.

Laboratory analysis. The content of α-acids in hop cones collected in field was determined by the Lead conductance method for hops, hop powder and pellets (Analytica-EBC 7.4 1998).

Data analysis. Shannon's information index as a measure of phenotypic diversity within populations was calculated according to Lewontin (1972) (Equation 1):

$$H_j = -S\ (p_i \log_2 p_i) \quad (1)$$

Where: p_i is the frequency of each trait state.

Shannon's information index was used to measure the total diversity (H_{total}) as well as the average intra-population diversity (H_{pop}). The percentage of diversity within (H_{pop}/H_{total}) × 100 and among populations [($H_{total} - H_{pop}$)/H_{total}] × 100 was also obtained. Fisher's exact test in Statistical Analysis System – SAS (Anonymous 2004) was used to test for differences in number of individuals having a particular trait state between pairs of populations. The same procedure was employed to test the independence between cone shapes and aroma across all the sampled individuals. The univariate analysis of variance using the PROC GLM in Statistical Analysis System – SAS (Anonymous 2004) was conducted in order to test mean differences among populations and among groups of plants categorized by cone shape and by aroma. Percentages of α-acids content were normalized by arcsine transformation (i.e. *arc. sin.* $\sqrt{x}$; x = % of α-acids/100). Post hoc comparisons of population means were carried out using Tukey's Studentized range test at $P < 0.05$.

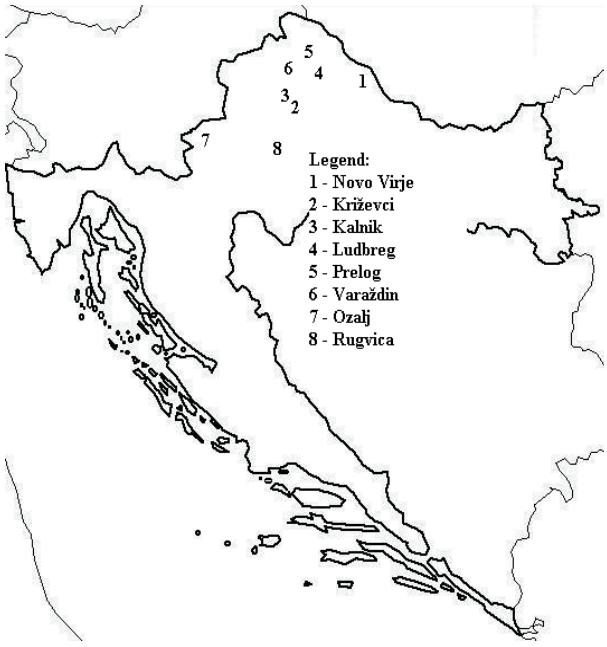

Figure 1. Locations of wild hop populations on the map of Croatia

Table 1. Number of male and female hop plants at eight locations and their ratio (n = 121)

Location	Sample size (n)	Ratio
Kalnik	12	1:2
Križevci	13	1:2.25
Ludbreg	9	1:8
Novo Virje	10	1:2.33
Ozalj	22	1:1.2
Prelog	23	1:1.87
Rugvica	20	1:1.22
Varaždin	12	1:1.4
Overall	121	1:1.75

RESULTS AND DISCUSSION

Number and ratio of male and female plants. The portion of female plants in all eight populations was higher compared to male plants (Table 1) as it was reported in segregating populations. Average sex ratio in eight populations of Subpannonian micro-region between male and female plants was 1:1.75, varying from 1:1.2 for populations of Ozalj and Kalnik to 1:8 for population of Ludbreg (Table 1). However, Fisher's exact test did not show significant differences between populations (Table 2). These results corresponded to those of Wagner (1974, 1975) who found the ratio between male and female plants of 1:1.54 in six macro-regions of former Yugoslavia which varied from 1:1.04 in Pannonian macro-region to 1:5.63 in Mediterranean macro-region. In his ecogeographical survey Wagner collected 398 plants of wild hops found in 398 habitats of six geographical macro-regions whereas, we collected 121 plants found in eight habitats/populations, mostly in Subpannonian micro-region and partially in Dinaric macro-region. Thus, it seems impossible to find the exact number of male and female plants in their habitats regarding the floristic content of plant associations where wild hop usually occurs.

The sex distortion ratio is evident in hop breeding programs worldwide (Polley et al. 1997, Jakše et al. 2008); also monoecious plants are detected in different crossing families (Neve 1991, Čerenak et al. 2006).

According to the number of leaflets per leaf the hop leaves are divided into three types: unifoliar, three foliar and five foliar (Rybáček 1980, Kišgeci et al. 1984).

The most frequent type of leaf in eight habitats of Croatian Subpannonian micro-region was three foliar leaf followed by unifoliar and five foliar types. Shannon's information index for number of leaflets per leaf varied from 0.00 for populations of Kalnik and Križevci to 0.92 for population of Novo Virje (Table 3). Values of Shannon's information index showed smaller phenotypic diversity for leaf morphology among populations compared to diversity within populations (Table 3). Also in this case, the results of Fisher's exact test were not significant (Table 2). In two (Kalnik and Križevci) out of eight populations this trait was found monomorphic as all the analyzed plants showed three foliar leaf. For that reason populations of Kalnik and Križevci were not applicable for testing the differences in number of leaflets by using Fisher's exact test.

Cone shape, aroma and the content of α-acids in hop cones. Cone shape is one of the most important quality traits in hop breeding (Wormald 1915, Schmidt 1917, Salmon and Wormald 1921, Blattny 1950, Davis 1957, Ono 1961, Wagner 1974, 1975, Rybáček 1980, Kišgeci et al. 1984, Schattenhofer 1989, Barth et al. 1994, Henning et al. 1997, Moir 2000, Rígr and Faberová 2000,

Table 2. Significance of Fisher's exact test for differences in number of individuals having a particular trait state between pairs of populations

	Trait: Aroma	Kalnik	Križevci	Ludbreg	Novo Virje	Ozalj	Prelog	Rugvica
1	Kalnik							
2	Križevci	0.0209						
3	Ludbreg	0.7824	0.2507					
4	Novo Virje	0.3691	0.0691	0.5493				
5	Ozalj	0.8166	0.0464	1.0000	0.2862			
6	Prelog	0.0207	1.0000	0.2150	0.1364	0.0345		
7	Rugvica	0.4165	0.2112	0.7166	0.8350	0.4129	0.2887	
8	Varaždin	0.1841	0.7577	0.7650	0.3881	0.4074	1.0000	0.5475

Table 3. Shannon's information index (H_j) of three morphological traits in eight hop populations in Croatia

Population	Sample size	Trait		
		no. of leaflets	cone shape	aroma
Kalnik	12	0.00	0.81	1.30
Križevci	13	0.00	0.76	0.76
Ludbreg	9	0.86	0.54	1.41
Novo Virje	10	0.92	1.38	1.56
Ozalj	22	0.06	1.25	1.33
Prelog	23	0.57	1.51	1.05
Rugvica	20	0.07	1.54	1.54
Varaždin	12	0.12	1.45	1.15
H_t	121	0.37	1.35	1.46
H_p		0.32	1.16	1.26
Among (%)[a]		11.77	14.52	13.31
Within (%)[b]		88.23	85.48	86.69

[a]of total diversity attributable to differences among populations; [b]of total diversity attributable to differences within populations

Hampton et al. 2001, Hampton et al. 2002, Srečec 2004, Nesvadba 2007).

In spite of precise descriptors for genus *Humulus* sp. (Rígr and Faberová 2000, Anonymous 2006) it was impossible to divide the hop cones *in situ* to more than three morphological types, primarily because of the different conditions of their habitat (shadowing, competition among vegetation, soil conditions etc.). For that reason the hop cones were divided into three morphological types: elongated, oval and round which are also the most representative shapes of cones at well known varieties. Comparing 77 female plants found in eight populations (Table 4) the oval one was the most dominant morphological type of hop cones (45 individuals), followed by elongated (22 individuals) and round type (10 individuals). Similarly to cone morphology the plants were categorized into three basic types of aroma: 'typical hoppy', 'fine hop' (in brewing terminology known also as 'grassy' or 'fruity') and 'rough' (unpleasant aroma; sometimes could smell of garlic; term also known in brewing terminology). Before analyses of α-acids share, the sensory (nasal) assessment of hop cones was provided by three assessors. 'Typical hoppy' and/or 'fine hop' aroma were found in most of plants having the oval hop cones while 'rough' aroma was present in most of the plants with elongated hop cones (Table 4). The association between shape of cones and quality of hop aroma was not observed in known varieties since both types of cone shapes are presented in both types of hop cultivars.

Shannon's information index for cone shape varied from 0.54 for population of Ludbreg to 1.54 for population of Rugvica. Total diversity for hop cones shape within population was higher in comparison with total diversity among different populations (Table 2). The results of Fisher's exact test were not significant for cone shape indicating similar frequency of this trait in different populations (Table 4). Shannon's information index for hop cones aroma varied from 0.76 for population of Križevci to 1.56 for population of

Table 4. Contingency table of cone shape and aroma for 77 female hop plants Fisher's exact test of significance ($P < 0.0001$)

No. of individuals		Aroma			Total
		(1) typical hoppy	(2) fine hop aroma	(3) rough	
Cone shape	(1) elongated	7	1	14	22
	(2) oval	26	18	1	45
	(3) round	8	2	0	10
Total		41	21	15	77

Table 5. Population means, standard deviations, minimum and maximum values for α-acid content among eight wild hop populations in Croatia

Population	*n*	α-acid content			
		mean	S.D.	min	max
Kalnik	7	1.971	0.472	1.400	2.700
Križevci	8	2.363	0.835	1.600	4.000
Ludbreg	8	2.088	0.948	1.100	3.800
Novo Virje	6	2.750	1.340	1.200	4.200
Ozalj	11	2.236	0.584	1.800	3.800
Prelog	14	2.557	0.723	2.000	4.400
Rugvica	11	2.400	0.898	1.400	3.800
Varaždin	5	2.260	0.488	1.700	3.000
Total	70	2.343	0.799	1.100	4.400
P-value*		0.643			

**P*-value of the analysis of variance

Novo Virje (Table 2) but in this case the results of Fisher's exact test were significant ($P < 0.05$) in four comparisons: Križevci vs. Kalnik, Kalnik vs. Ozalj, Kalnik vs. Prelog and Ozalj vs. Prelog. No significant differences ($P = 0.643$) were found for α-acids content across populations (Table 5).

The differences among groups (Table 4) categorized by cone shape and aroma were highly significant ($P < 0.0001$). According to Tukey's Studentized range test the plants exhibiting elongated cone shape had significantly higher share of α-acids ($P < 0.05$) than those having oval or round cone shapes (Table 6). Similarly, plants categorized by aroma as 'rough' had the highest share of α-acids ($P < 0.05$) compared to those categorized by 'typical hoppy' or 'fine hop aroma'. The same objections are found in hop breeding since high alpha varieties rarely have good aroma properties.

Average share of α-acids in hop cones of 77 collected samples varied from 1.8 to 3.6 % (w/w) and these results were in agreement with the results of Wagner's research (Wagner 1974). In 1971 he collected 15 samples of hop cones from different macro-regions of former Yugoslavia and analyzed α-acids using conductometric method. In his study the samples also contained low share of α-acids varying from 1.7 to 5.18% (w/w). In Wagner's as well as in our studies the high share of seeds (in some cases more than 15% of weight of hop cones) obviously caused a decrease of α-acids share in hop cones. In general it is established that seeded cones of different varieties have approximately by 10% lower content of α-acids. At the Slovenian Institute for Hop Research and Brewing in Žalec, Slovenia, the gene bank of 60 female and 50 wild female hops was established. All wild samples analyzed in previous years showed the same lower content of α-acids compared to the breeding goals as it is evident in report survey.

Based on the obtained results it is possible to conclude that diversity of all investigated phenotypic traits was higher within than among populations of wild hops in the northwest part of Croatia. At the same time there was a very strong linkage between cone shape and aromatic traits which could be analyzed during next season in the gene bank of 140 different hop varieties planted in Žalec. The performed analyses represent a first step in the characterization of Croatian wild hop populations. A further analysis of their genetic diversity is a subject of ongoing research, including genotyping of wild hop plants using microsatellite markers.

Table 6. Mean comparisons for α-acid contents among groups of hop plants categorized by cone shape (elongated, oval, round) and by aroma (typical hoppy, fine hop aroma, rough)

Trait	State	*n*	α-acid content	
			mean	S.D.
Cone shape	elongated	19	3.232[a]	0.800
	oval	44	2.016[b]	0.520
	round	7	1.986[b]	0.204
Aroma	typical hoppy	35	2.126[b]	0.380
	fine hop aroma	20	1.765[c]	0.394
	rough	15	3.620[a]	0.490

Means identified by different letters denote significant ($P < 0.05$) post hoc comparisons of means carried out by Tukey's Studentized Range test

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