Genetic variability of wild hops (*Humulus lupulus L.*) in Caucasus region.

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Figure 1: Caucasus mountains nearby the biggest mount Elbrus (5642 m).

Figure 2: Map of individual localities of wild hops in Caucasus region.

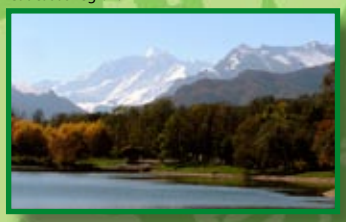


Figure 3: Vladikavkaz locality.

Figure 4: Wild hops on Terek locality.

Figure 5: Wild hops on Komsomolskoje locality.

Figure 6: Wild hop on Craj locality.

Introduction

Hop (*Humulus lupulus* L.) is a dioecious perennial climbing plant and only female plants are cultivated for commercial use, mainly in brewing industry and to a smaller extent for pharmaceutical purposes. Female inflorescences, referred to as cones, contain hop bitter resins, essential oils, polyphenols and tannins. The origin of the genus is considered to be China (Neve 1991). Wild hops are distributed throughout the Northern Hemisphere, and have been classified into a number of taxonomic varieties based on their morphology. Historically, hop improvement has been based on European landraces because they provide the flavour qualities preferred by brewers. Wild germplasm provides new genetic resources for breeding to overcome the limited genetic variation present in modern hop breeding programmes. One of the interesting wild hop regions is Caucasus (Figure 1). It is supposed that this region is a half way of cultivated hop from Asia to Europe. In our experiments, we studied the genetic variability of wild hops from this region.

Materials and methods

In 2005 and 2006, we realized successful expeditions for wild hops in Caucasus region. Wild hops were collected from nine localities: 1 – Vladikavkaz, 2 – river Terek, 3 – Zmenskaja, 4 – Sunža, 5 – Gizel, 6 – Komsomolskoje, 7 – Ursdon, 8 – Craj and 9 – Kabardino-Balkar Republic (Figure 2, 3, 4, 5, 6). Samples were transferred as rootstock (Table 1) or dry cones (Tables 2). Dry cones were used for chemical analyses of hop resins. Hop resins were determined according to EBC 7.7. method by HPLC on SHIMADZU LC 10A (Shimadzu, Japan). Wild hop rootstocks were multipropagated in glasshouse and transferred to field condition. DNAs were isolated from young leaves according to Patzak (2001). For molecular analyses, we used nine SSR (Hadonou et al., 2004; Jakše et al., 2002) and three STS (Patzak et al., 2007) loci. PCR reactions were performed in TGradient thermocycler (Biometra, FRG). The genetic diversity analysis was evaluated by cluster analysis, which was revealed by NTSYS-pc v. 2.11V for WINDOWS (Exeter Software, USA).

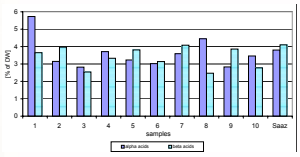
Table 1: Localities of samples used for molecular analyses.

Label	Locality
Caucasus 1	Sunža
Caucasus 2	Komsomolskoje
Caucasus 4	Vladikavkaz
Caucasus 5	Sunža
Caucasus 6	Ursdon
Caucasus 7	Craj
Caucasus 8	Craj
Caucasus 9	Ursdon
Caucasus 17	river Terek

Table 2: Analyses of hop resins of samples from different localities

No.	Locality	alfa acids [% of DW]	beta acids [% of DW]	ratio alfa/beta	cohumulone [% of alfa]	colupulone [% of beta]
1	Zmenskaja	5,73	3,65	1,57	20,3	42,1
2	Ursdon	3,15	3,97	0,79	23,7	51,5
3	river Terek	2,82	2,54	1,11	31,7	39,8
4	Gizel	3,71	3,33	1,11	34,1	54,5
5	Komsomolskoje	3,23	3,81	0,85	19,1	41,5
6	Zmenskaja	3,02	3,14	0,96	25,2	48,7
7	Vladikavkaz	3,59	4,08	0,88	22,4	45,6
8	river Terek	4,45	2,47	1,80	19,4	41,5
9	Vladikavkaz	2,83	3,86	0,73	20,8	42,1
10	Zmenskaja	3,46	2,78	1,24	22,8	41,8
	Saaz	3,0 - 6,0	4,5 - 8,0	0,6 - 0,9	23 - 26	39 - 43
	Sládek	4,0 - 8,0	3,5 - 8,0	0,7 - 1,3	25 - 31	45 - 51
	Premiant	7,0 - 11,0	3,5 - 6,0	1,7 - 2,3	18 - 23	39 - 44
	Agnus	11,0 - 15,0	5,0 - 8,0	1,9 - 2,6	29 - 38	51 - 59

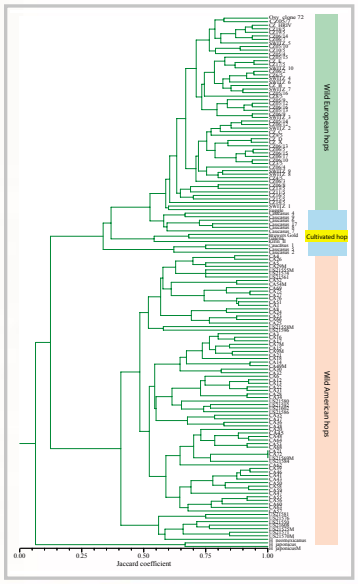
Figure 7: Alpha and beta acid contents in wild hops from Caucasus



Results

Chemical taxonomy is commercially used for evaluation of cultivated hops and control of variety purity and identification. In spite of fact that natural conditions significantly influence the content of hop resins, the contents of cohumulone and colupulone are stable and belong to reliable genetic determined features. We analysed ten different samples of wild hops from Caucasus region and results are shown in Table 2 and Figure 7. We found that some samples (2,5,6,7,9) were very similar to old European varieties. In the other hand, samples with higher content of cohumulone and colupulone (3,4) evidence for origin from another germplasm like American. The molecular DNA technology is a useful method for the study of genetic diversity, individual genotyping, population structure and phylogeny. Therefore, we used it for these purposes. In our experiment, we tested nine Caucasus wild genotypes in comparison to Czech, European and American wild hops (totally 150 genotypes). We found that Caucasus wild hops were clustered to European hop germplasm (Figure 8), which is evidently separated from wild American germplasm and *H. japonicus*. Caucasus wild hops were divided to two groups by submerged group of cultivated hops with mixed Euro-American germplasm. From this analysis is evident that Caucasus wild hops include wide genetic diversity with Euro-Asian germplasm and can be promising sources for hop breeding programmes.

Figure 8: Dendrogram of individual wild hops revealed by UPGMA cluster analysis based on Jaccard's similarity coefficient determined using 22 STS and 46 SSR markers. CZ – Czech, CA – Canada



Acknowledgements

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