

**POLYMORPHISM OF CHALCONE SYNTHASE ENCODING
GENES AND THEIR RELATION WITH BITTER SUBSTANCES LEVEL
IN HOP'S CONES**

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Analysis of polymorphism of encoding chalcone synthase genes of Ukrainian hop varieties was conducted. The dependence between gene polymorphism and the level of bitter substances in hop cones was shown. The possibility of usage of molecular markers that show the polymorphisms of encoding chalcone synthase genes for determination of hop varieties type (aroma of bitter) was shown.

Key words: *hop, chalcone synthase, polymorphism, bitter and aromatic varieties, cluster analysis.*

Hop (*Humulus lupulus* L.) is a dioecious perennial climbing plant from *Cannabaceae* family. The primary commercial application of the hop plant has historically been in the beer brewing industry. Also, there is potential to develop new hop products, such as phytoceuticals, due to various biological activities (antibacterial, antifungal, anticancer, sedative, soporific, estrogenic) of unique secondary metabolites. Secondary metabolites (some of which impart the bitter taste and aroma to beer), such as bitter acids (alpha-acids humulone and cohumulone, beta-acids lupulone and colupulone) and prenylflavonoids (xanthohumol) accumulate in the lupulin glands. Enzymes, which catalyze biosynthesis of unique secondary metabolites in hop, are chalcone synthases. There are five chalcone synthases in hop: chalcone synthase 1 (CHS_H1), chalcone synthase 2 (CHS2), chalcone synthase 3 (CHS3), chalcone synthase 4 (CHS4) and valerophenone synthase (VPS), which are encoded by genes *chs_H1*, *chs2*, *chs3*, *chs4* and *vps*, respectively.

Functions of CHS2, CHS3 and CHS4 *in vivo* are not detected. There was described catalytic activity of CHS2 and CHS4 *in vitro* in syntheses of 6-isobutyl-4-hydroxy-2-pyrone. Functions of CHS3 are not detected.

Depending on cohumulone in content of alpha-acids hop varieties are derived in aroma (till 28 % of cohumulone in content of alpha-acids) and bitter (30-50 % of cohumulone in content of alpha-acids) samples.

Determination of α - and β -acids level is carried out by gas and high performance liquid chromatography, which are expensive and long-time procedures. Despite the considerable amount of molecular genetic and genomic research of hop literature review showed no works devoted to the development of molecular markers to identify the type of variety. Therefore, the purpose of this study was to determine the type of hop varieties using molecular markers of *chs_H1*, *chs2*, *chs3*, *chs4* and *vps* genes.

MATERIAL AND METHODS. Hop varieties are Al'ta, Zmina, Ksanta, Kumyr, Nadiya, Nazaryi, Obolons'kyi, Poles'kyi, Promin', Chaklun (bitter varieties); Vydybor, Haidamats'kyi, Zhytomyrs'kyi 75, Zahrava, Klon 18, Oskar, Pyvovar, Polisianka, Slavianka, Khmeleslav (aroma varieties) from Institute of Polesie Agriculture hop collection (Ukraine). The results of molecular genetic analysis of gene polymorphism *chs_H1*, *chs2*, *chs3*, *chs4* and *vps* (length amplification products in bp) in the samples of above mentioned varieties were obtained in our previous studies. Polymorphic regions of chalcone synthase genes are: 3'-untranslatable region, intron and exon 2 of *chs_H1*; intron of *chs2*; intron of *chs3*; intron of *chs4*; promoter and region, which include part of exon 1, intron and exon 2 of *vps*. Cluster analysis of 20 hop's varieties based on polymorphism of *chs_H1*, *chs2*, *chs3*, *chs4* and *vps* genes was conducted with TREES 4.0 program by unweighted pair group method with arithmetic mean. The reliability was tested using Spearman's rank correlation coefficient for small number of samples.

RESULTS. In accordance with results of cluster analysis of summary data on polymorphism of chalcone synthase encoding genes there was obtained grouping of varieties according to their type. In dendrogram there were identified two clusters: cluster I included all the bitter varieties and cluster II - all aromatic. All obtained results are found to be reliable according to Spearman's rank correlation coefficient. Thus, the type of clustering is fulfilled only by using summary data on polymorphism of all studied regions of genes. This means that for the synthesis of the cohumulone, colupulone and getting of some ratio of β - and α -acids that leads to the formation of "type of variety" index, allelic form of all chalcone synthase genes is found to be of great importance.

CONCLUSIONS

Dependence of the type of hop varieties from gene polymorphism *chs_H1*, *chs2*, *chs3*, *chs4* and *vps* was obtained. Statistically significant approach was developed for the evaluation of hop varieties type by molecular markers, which can reduce the time and material costs during the identification of hop genotypes.

REFERENCES

1. Assessment of the genetic diversity of wild hops (*Humulus lupulus* L.) in Europe using chemical and molecular analyses / [J. Patzak, V. Nesvadba, A. Henychova, K. Krofta] // Biochemical Systematics and Ecology. 2010. №38-P. 136–145.
2. Cloning and characterisation of chs-specific DNA and cDNA sequences from hop (*Humulus lupulus* L.) / [J. Matousek, P. Novak, J. Patzak, H. Niedermeierova] // Plant Science. 2002. №162-P. 1007–1018.
3. DNA Sequence and Expression Variation of Hop (*Humulus lupulus*) Valerophenone Synthase (*VPS*), a Key Gene in Bitter Acid Biosynthesis / [C. Castro, L. Whittcock, S. Whittcock, G. Leggett] // Ann Bot. 2008. № 102 (2).- P. 265–273.
4. Genbank Derived microsatellite markers in hop [N. Bassil, B. Gilmore, J. Oliphant, J. Henning] // Acta hort. 2005. № 668.- P. 47– 52.

5. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods / [K. Tamura, D. Peterson, N. Peterson, G. Stecher et all] // Molecular Biology and Evolution. 2011. № 28 (10).- P. 2731-2739.
6. Venger A. Molecular-genetic polymorphism of *vps* gene in Ukrainian hop varieties / A. Venger, N. Volkova // Modern science. 2014. № 1.- P. 28-34.
7. Patzak J. New STS molecular markers for assessment of genetic diversity and DNA fingerprinting in hop (*Humulus lupulus* L.) / J. Patzak, I. Vrba, J. Matousek // Genome. 2007. № 50.-P. 15– 25.
8. Schröder J. Stilbene and chalcone synthases: related enzymes with key functions in plant-specific pathways / J. Schröder, G. Schröder // Journal of biosciences. 1990. №45.- P. 5– 8.
9. Chadwick L. R. The pharmacognosy of *Humulus lupulus* L. (hops) with an emphasis on estrogenic properties / L. R. Chadwick, G. F. Pauli, N. R. Farnsworth // Phytomedicine. 2006. №13.-P. 119–131.
10. Spearman C. The proof and measurement of association between two things / C. Spearman // Amer. J. Psychol. 1987. № 100.- P. 441–471.
11. Ляшенко Н. И. Физиология и биохимия хмеля /, Н. Г. Михайлов, Р. И. Рудык / Житомир: Полісся, 2004. – 405 с.
12. Венгер А. М. Молекулярно-генетичний поліморфізм генів *chs2*, *chs3*, *chs4* у сортів хмеля звичайного української селекції / А. М. Венгер, Н. Е. Волкова // Вісник Запорізького національного університету. – 2014. – Т. 1. – 13-21 с.