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Genetic difference analysis of *Humulus lupulus* L. (Hop) cultivars grown in Pazaryeri (Bilecik) using the ISSR-PCR method

İsmail POYRAZ[1, 2*], Burcu ÖZMEN[3], Kezban DOĞCA[1]
ORCID: 0000-0003-3651-5885**;** 0000-0003-3667-1597; 0009-0003-0631-1168

[1] Bilecik Seyh Edebali University, Faculty of Science, Molecular Biology and Genetics Depart., 11100, Bilecik, Türkiye
[2] Bilecik Seyh Edebali University, Biotechnology Application and Research Centre, 11100, Bilecik, Türkiye
[3] Bilecik Seyh Edebali University, Graduate School, Biotechnology, 11100, Bilecik, Türkiye

Abstract

Humulus lupulus L. (hop) is an important industrial plant grown only in the Bilecik-Pazaryeri district in Turkey. The most common usage area is the beer industry. It is preferred as a flavour and aroma raw material because it contains secondary metabolites. Different *H. lupulus* varieties are cultivated in the villages in Pazaryeri. Besides the registered varieties used by farmers, it is seen that some farmers also prefer unknown *H. lupulus* seeds in production. This study aimed to determine the genetic diversity of 18 *H. lupulus* varieties and samples cultivated in different locations for the beer industry in Pazaryeri using the ISSR (inter simple sequence repeat)-PCR method. DNA isolations were performed using a commercial kit from plant leaves grounded in liquid nitrogen. 25 ISSR primers were tested for DNA amplifications and 10 primers that produced reproducible DNA fragments were used for genetic diversity analysis. PIC (polymorphism information content) value and PCoA (principal coordinate analysis) were calculated for each primer. DNA bands obtained from PCR products electrophoresed in agarose gel were photographed. Band profiles were compared using the Phoretix1DPro program and binary data analysis was performed as present (1) / absent (0). Jaccard similarity and distance matrixes were created with UPGMA (unweighted pair group method with arithmetic mean) and a dendrogram showing genetic diversity was drawn using the MEGA 11 software. It was determined that different *H. lupulus* varieties grown in the same locations show location-dependent (together) branching in the dendrogram. While it is expected that registered varieties have separate branching from each other in the dendrogram, it was seen that different H. lupulus varieties grown in the same locations show location-dependent (together) branching.

Keywords: hop, genetic diversity, ISSR-PCR method

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Pazaryeri'nde (Bilecik) yetiştirilen *Humulus lupulus* L. (Şerbetçi otu) çeşitlerinin ISSR-PCR Yöntemiyle genetik farklılık analizi

Özet

Humulus lupulus L. (şerbetçi otu), Türkiye'de sadece Bilecik-Pazaryeri ilçesinde yetiştirilen önemli bir endüstriyel bitkidir. En yaygın kullanım alanı bira endüstrisidir. İçerdiği sekonder metabolitler nedeniyle tat ve aroma verici ham madde olarak tercih edilir. Pazaryeri'ndeki köylerde farklı *H. lupulus* çeşitlerinin tarımı yapılmaktadır. Çiftçilerin kullandığı tescilli çeşitlerin yanında bazı adı bilinmeyen *H. lupulus* tohumlarının da üretimde tercih edildiği görülmektedir. Bu çalışmada, Pazaryeri'nde bira endüstrisine yönelik farklı lokasyonlarda tarımı yapılan 18 *H. lupulus* çeşit ve örneğin ISSR (basit dizi tekrarları arası)-PZR yöntemiyle genetik çeşitliliklerinin belirlenmesi amaçlanmıştır. DNA izolasyonları, sıvı azotta öğütülmüş bitki yapraklarından ticari kit kullanılarak gerçekleştirilmiştir. DNA çoğaltımları için 25 ISSR primeri test edilmiş ve tekrarlanabilir DNA fragmanları üreten 10 tane primer genetik çeşitlilik analizi için kullanılmıştır. Her primer için PIC (polymorphism information content) değeri ve PCoA (temel koordinat analizi) hesaplanmıştır. Agaroz jel elektroforezi yapılan PZR ürünlerine ait DNA bantları fotoğraflanmıştır. Bant profilleri Phoretix1DPro program kullanılarak karşılaştırılmış ve var (1) / yok (0) ikili veri analizi yapılmıştır. UPGMA (aritmetik ortalama ile ağırlıksız çift grup yöntemi) ile Jaccard benzerlik ve mesafe matrisi oluşturulmuş ve

[1] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +902282141173; Fax.: +92282141162; E-mail: ismail.poyraz@bilecik.edu.tr
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MEGA 11 yazılımı kullanılarak genetik çeşitliliği gösteren dendrogram çizilmiştir. Tescilli çeşitlerin dendrogramda birbirinden ayrı dallanma göstermesi beklenirken, aynı lokasyonda yetişen farklı *H. lupulus* çeşitlerinin lokasyona bağlı (birlikte) dallanma gösterdiği belirlenmiştir.

Anahtar kelimeler: şerbetçi otu, genetik çeşitlilik, ISSR-PCR yöntemi

1. Introduction

Humulus lupulus L. (hop) is a dioecious (2n=20) climbing plant belonging to the order Urticales (Nettles), family Cannabaceae family. It is widely distributed in the northern [1]. *H. lupulus* also has names such as Maya grass, Beer grass, Ömer grass, Beer flower, and Sarısarmışık in Turkey [2]. There are three main types of hops: *H. lupulus* (beer hop), *H. jabonicus* (Japanese hop) and *H. yunnanensis* (Chinese hop). *H. jabonicus* and *H. yunnanensis* are only grown only in the country where they are located and local [3-4]. *H. lupulus* only among Humulus species is grown for medical and industrial purposes [3-5].

In *H. lupulus* agriculture, only the female plants are grown to use the flower clusters, the so-called hop cones. [6]. At the basis of the female cones are glandular trichomes in lupulin glands where some secondary metabolites are biosynthesized, including terpenoids, phenolic compounds and prenylated flavonoids. These compounds accumulate throughout their ripening, except for β-acids [7]. The essential oil obtained from hops is rich in volatile compounds such as terpenes and sesquiterpenes. These compounds give beer its aromatic properties. While alpha acids obtained by boiling the beer wort with iso-alpha acids give bitterness of beer [8], β-acids have antimicrobial properties [7]. β-acids are also effective for use as an insecticide in cases where chemical pesticides cannot be used [9].

Hop cultivation started in the 16th century in all European countries and the world, especially in England. Hops were first produced in our country in 1955 by the Ministry of Agriculture. Trials were carried out in 21 provinces, especially in Edirne, Bursa, Bolu, Bilecik and Kütahya. According to the results obtained, hop cultivation was started in Bilecik province in 1965 by the Ministry of Agriculture due to the high level of alpha acids in the culture variety tested in Bilecik [10]. Bilecik province has been declared a pilot region in hop production [10]. The first use of hops was in Europe because of their antimicrobial properties. Then, it was found that hop has a calming effect, and their milk yield increased in animals. It is assumed that the increase in milk yield is because of the calming and estrogenic effect of hops. However, it has been preferred more in the drink industry due to the rapid increase in beer consumption [11]. In studies carried out in recent years, it has been found that hop seeds are a potent source of antioxidant activity and have cytotoxic effects against various types of cancer cells in vitro [12].

Studies for the assessment of genetic diversity in the hop use some molecular markers and methods such as ISSR, SSR (simple sequence repeat), RAPD (random amplified polymorphic DNA), STS (sequence-tagged sites), and AFLP (amplified fragment length polymorphism) [13-14]. Among molecular markers, the use of ISSR markers is more frequent in the study of genetic diversity as they provide greater practicality and economic benefits [15-16].

In this study, we have aimed at H. lupulus (hop) cultivars produced in the Bilecik-Pazaryeri using the ISSR markers to uncover their spatial genetic profiles besides genetic differences. The obtained data is useful for the parent selection of future hop breeding programs towards using potentially superior hop varieties and providing industrial production with a competitive advantage in producing high-quality raw materials.

2. Materials and methods

2.1 Plant materials

Hop female samples were collected during fieldwork in the Bilecik-Pazaryeri (Table 1). The obtained plant samples were ground with liquid nitrogen and made ready for DNA isolation. DNA samples were stored at -20° C until use.

2.2 DNA isolation

H. lupulus DNA samples were obtained from bean leaves powdered with liquid nitrogen using the GeneMATRIX Plant DNA isolation kit. DNA concentration and quality were measured using a UV-enabled Nanodrop spectrophotometer (Shimadzu, Japan). DNA samples were diluted to 2 ng.

2.3 ISSR-PCR analysis

ISSR-PCR analysis was performed according to the sequences obtained from the University of British Columbia (Canada). After screening, an initial primary screening was performed using 25 primers (Table 2). Amplification of ISSR fragments from *H. lupulus* genomic DNA was performed in a total reaction volume of 25 µL containing 2 ng template DNA, 1× Taq polymerase reaction buffer, 2 mM $MgCl_2$, 0.1 mm each of dNTPs, 0.2 mM

primer and 1 U Taq DNA polymerase (Fermentas, USA). Amplifications were performed in the Thermo Arctic Thermal Cycler (Thermo Scientific, USA) programmed as follows: 4 min. of denaturation at 95 °C and 40 cycles of 45 seconds of denaturation at 95 °C, 45 seconds of annealing at 48–65 °C for ISSR amplification and a 1.5 min. extension at 72 °C followed by a final extension at 72 °C for 7 min.

Table 1. *H. lupulus* sample names, locations, and altitudes

Sample Number	Location	Altitude (m)	Variety
1	Pazaryeri, Merkez Mah.	787	Braves
2	Pazaryeri, Merkez Mah.	788	Aroma, Hallertan Hüller
3	Pazaryeri, Merkez Mah.	818	9 Number
4	Pazaryeri, Merkez Mah.	818	Magnum
5	Pazaryeri, Merkez Mah.	818	Nugget
6	Pazaryeri, Merkez Mah.	818	Wye Target
7	Pazaryeri, Avşar Mevki	758	9 Number
8	Pazaryeri, Arapdede Köyü	832	Hallertan Hüller
9	Pazaryeri, Arapdede Köyü	832	Hallertan Hüller
10	Pazaryeri, Dereköy	877	Braves
11	Pazaryeri, Dereköy	877	Braves
12	Pazaryeri, Dereköy	790	Hallertan Hüller
13	Pazaryeri, Dereköy	790	Hallertan Hüller
14	Pazaryeri, Dereköy	779	Unknown
15	Pazaryeri, Küçükelmalı Köyü	810	Unknown
16	Pazaryeri, Kınık köyü	811	Unknown
17	Pazaryeri, Kınık köyü	811	Unknown
18	Pazaryeri, Kınık köyü	811	Unknown

Table 2. ISSR primer sequences, polymorphism rates and PIC values

Primer Names	Sequence (5'-3')	Total Band Number	Polymorphic Band Number	Polymorphism Rate (%)	PIC value
ISSR-01	AGAGAGAGAGAGAGAGG	17	16	94,1	0.574
ISSR-02	GAGAGAGAGAGAGAGAT	27	27	100	0.344
ISSR-03	GTGTGTGTGTGTGTGTC	-	-	-	-
ISSR-04	ACACACACACACACACC	-	-	-	-
ISSR-05	GGGTGGGTTGGGGTG	20	20	100	0.525
ISSR-06	TCTCTCTCTCTCTCTCG	28	27	96,4	0.280
ISSR-07	CTCTCTCTCTCTCTCTA	-	-	-	-
ISSR-08	CACACACACACACACAG	40	38	95	0.302
ISSR-09	GGATGGATGGATGGAT	-	-	-	-
ISSR-10	GGGTGGGTTGGGGTG	30	29	96,7	0.510
ISSR-11	TGTGTGTGTGTGTGTGA	-	-	-	-
ISSR-12	GTGCGTGCGTGCGTGC	-	-	-	-
ISSR-13	CGATGGATGGATGGAT	-	-	-	-
ISSR-14	GAGAGAGAGAGAGAGAC	45	44	97,8	2.708
ISSR-15	CTCTCTCTCTCTCTCTG	-	-	-	-
ISSR-309	GAGAGAGAGAGAGAGAA	30	30	100	0.478
ISSR-312	ACACACACACACACAC	-	-	-	-
ISSR-324	AGAGAGAGAGAGAGAGYT	-	-	-	-
ISSR-829	TCTCTCTCTCTCTCTCG	31	31	100	0.458
ISSR-835	AGAGAGAGAGAGAGAGYC	-	-	-	-
ISSR-847	CACACACACACACACARC	36	36	100	0.383
ISSR-849	GTGTGTGTGTGTGTGTYA	-	-	-	-
ISSR-880	GGAGAGGAGAGGAGA	-	-	-	-
ISSR-886	VDVCTCTCTCTCTCTCT	-	-	-	-
ISSR-890	CCGCCGCCGCCGCCGCCG	-	-	-	-
ISSR-891	AGAGAGAGAGAGAGAGC	-	-	-	-
Total		305	299	98	0.656

The PCR-amplified fragments were separated on a 1.3% agarose gel containing ethidium bromide (0.5 µg/mL). The gels were visualized under UV light and photographed using the Gel Logic 212Pro Imaging System (Carestream, USA). The molecular weights of the ISSR-PCR products were estimated using a 100 bp Plus DNA ladder (Fermentas).

2.4 Data analysis

The observed DNA bands on the jele photographs were scored as present (1) or absent (0) binary data using Phoretix1DPro software (TotalLab, UK). The polymorphism information content (PIC) value for each primer was calculated using the formula PIC = $2f(1-f)$ (f: allele frequency) [17]. Principal coordinate analyses (PCoA) on the genetic distance matrix were computed using GenAlExV6.51b2 software [18]. The genetic similarity between *H. lupulus* samples was calculated using Jaccard's genetic similarity coefficient and a dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA) The data obtained were drawn as a phylogenetic tree using the MEGA 11.0.3 software.

3. Results

In this study, to determine genetic relationships among 18 *H. lupulus* samples, 10 ISSR primers out of pre-tested 25 ISSR primers were used in PCR amplifications (Figures 1 and 2). The PIC value average was found to be 0-1.0 range for ISSR markers (Table 2). PCoA graphic on the genetic distance matrix was obtained (Figure 3). The similarity and distance matrixes were calculated with the Jaccard coefficients. Using the information obtained with the Jaccard matrix in Newick format, a dendrogram showing genetic diversity was created with the MEGA 11 software (Figure 4).

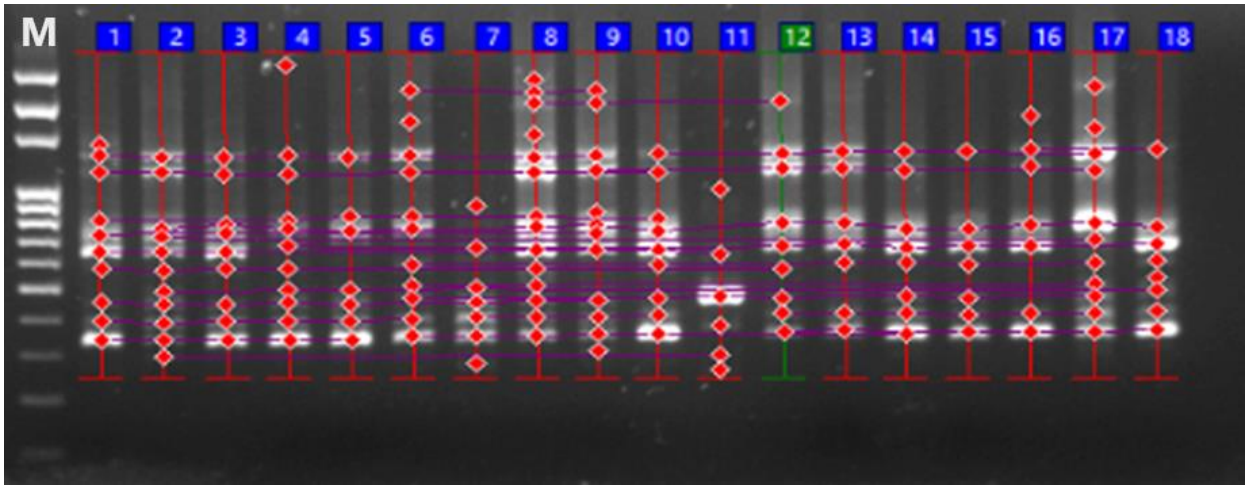

Figure 1. PCR band profiles amplified with the ISSR-14 primer. M: Marker GeneRuler 100 bp Plus DNA ladder, 1-18: *H. lupulus* samples. ♦: DNA bands

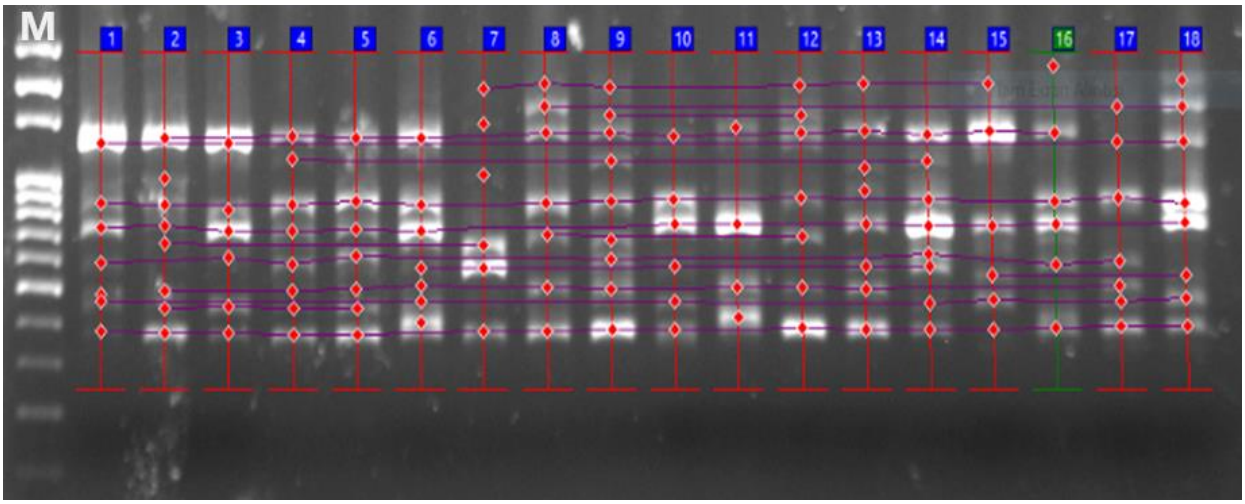

Figure 2. PCR band profiles amplified with the ISSR-10 primer. M: Marker GeneRuler 100 bp Plus DNA ladder, 1-18: *H. lupulus* samples. ♦: DNA bands

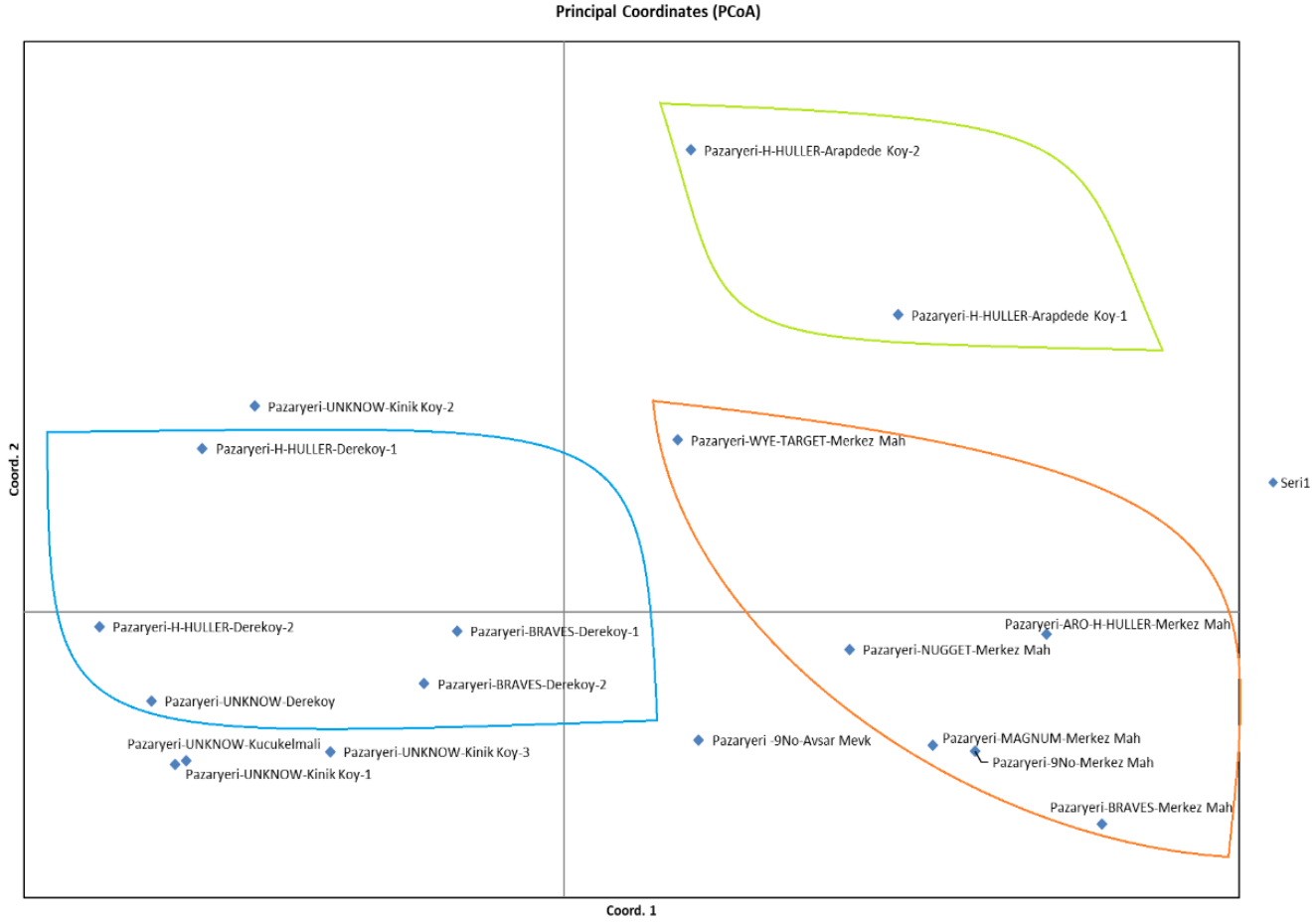

Figure 3. PCoA analysis results for *H. lupulus* samples amplified by ISSR primers. Shape colours; Orange: Merkez Mahallesi, Blue: Dereköy, Green: Arapdede Köyü locations

4. Conclusions and discussion

Hop is an important crop that has commercial value. Its production can limit due to its lack of tolerance to different climatic conditions and the associated limited acreage. The increasing demand caused to ensure more sales of hops [19]. Hundreds of varieties of the hop plant are grown. At the same time, the production of new varieties is performed and tested. Most of the hops in Europe are old and are already known. For this reason, hop varieties in Europe are not very different from each other genetically, morphologically, and ecologically [20]. In contrast to Europe, great genetic diversity has been discovered in many wild hop varieties in America and Asia [20-21]. The American subspecies of wild hops, ssp. neomexicanus and ssp. lupuloides, have been found to have greater genetic diversity than the European variety ssp.lupulus. This subspecies has a high alpha acid content and resistance to Verticillum wilt (a fungal disease [21]. For producers, it is critical to determine the originality and origin of the hops purchased. The desire to make this determination has led to the development of new DNA-based analytical methods [22].

Extreme climatic conditions such as long periods of drought and high temperatures have caused negative impacts on yields and quality in recent decades. Therefore, breeding studies with high tolerance, resilience to environmental conditions, productivity, and disease resistance are needed [9]. As a result of the phylogenetic analyses to carry out among the different hop cultivars, breeding studies can carry out with suitable parents to selected among the hop plants whose genetic diversity and profiles have been determined. Molecular marker-assisted analyses can be done in a shorter time and offers significant economic benefits [23].

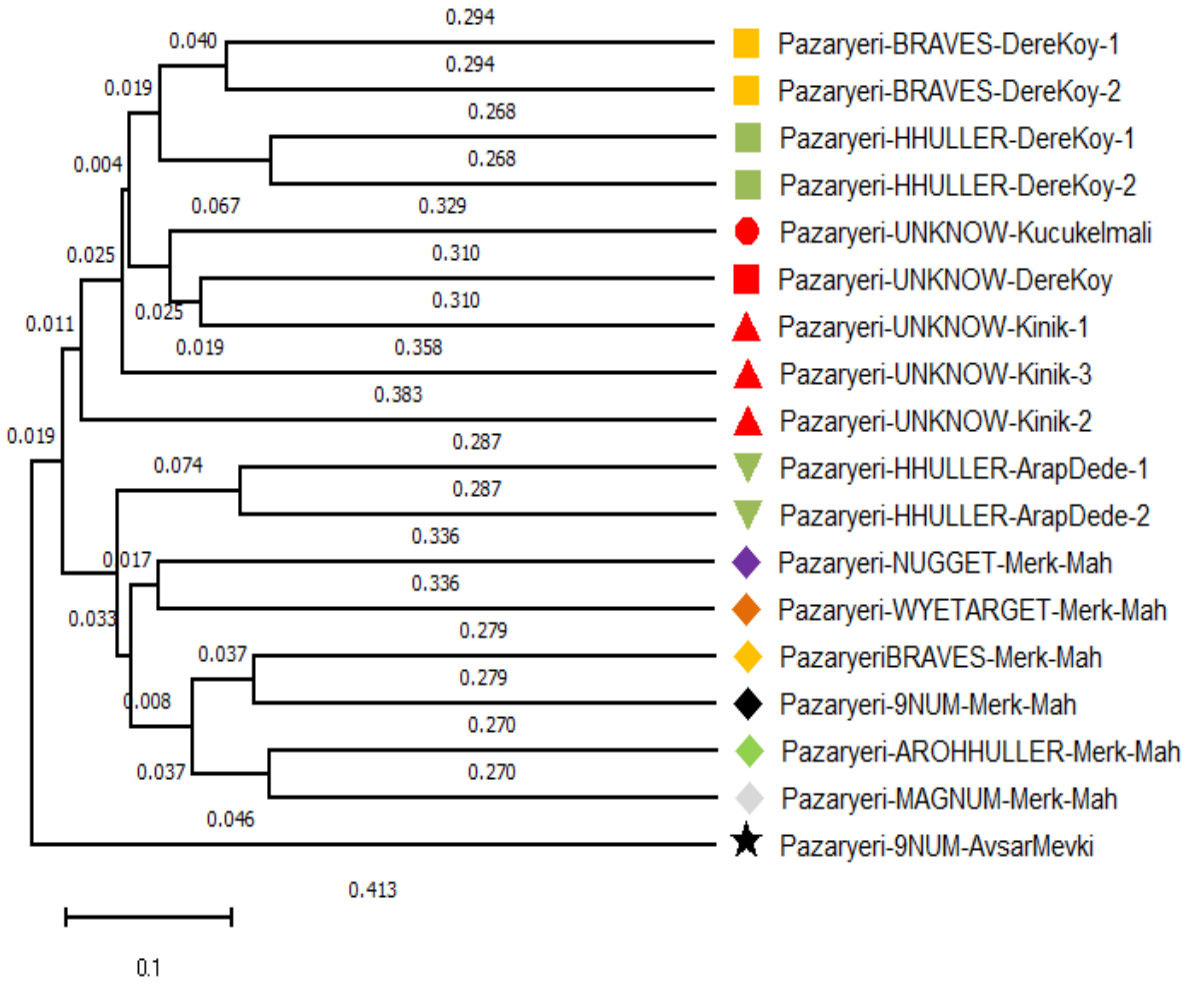

Figure 4. Dendrogram of different *H. lupulus* samples. Locations; ■: Dereköy, ●: Küçükelmalı Köyü, ▲: Kınık Köyü, ▼: Arapdede Köyü; ♦: Merkez Mahallesi, *: Avşar Mevki. Sample colours; Orange: Braves, Dark green: Hallertan Hüller, Light green, Red: Unknown samples, Purple: Nugget, Brown: Wye target, Black: 9 number, Gray: Magnum

The genetic diversity dendrogram of 18 different hop varieties and samples cultivated in various locations in Pazaryeri district has two main groups and one outgroup. It has been seen that the samples are grouped according to locations instead of their registration names. Especially, while samples of the same registered variety are expected to branch together, it is seen that they are positioned separately depending on the location. All hop samples that have unknown registration status are branched together in the 1st major group with plant samples from different registrations located in the Dereköy location. The samples in the Merkez Mah and Arapdede village locations, which are close to each other, are branched into clusters belonging to the 2nd major group. The example of 9-Number-Avşar village, which is different from the others in terms of location, acted as an out-group (Figure 4).

Genetic diversity dendrogram data also appears to be confirmed with the PCoA data graphic. Coordinates based on distance and similarity using the PCoA method show that hop samples are grouped locationally into major clusters compatible with the dendrogram (Figure 3).

In 2023, Somalraju et al. evaluated the genetic structure and diversity among 25 wild-collected hops from three Canadian Maritime provinces using microsatellite (SSR) markers. Dissection of the genetic structure among hop samples using phylogenetic tree and PCoA analyses has revealed the four distinct groups. They reported that four distinct subgroups were found, with a low molecular variance between subgroups and a high variance within subgroups, and only seven of the 18 SSR markers also differentiated two clones from the same site from one another [24]. In another study performed in the same year (2023), Calvi et al. analysed the genetic diversity of 22 wild hops from Italy and four commercially registered from the USA using 8 SSR markers. Through dendrogram and PCoA analysis, they reported that an increase in the level of differentiation could be correlated with distance [14].

An example of studies conducted at the genome level on the genetic diversity of hops is the SNP-NGS Genotyping conducted by Tegopoulos et al. in 2023. The results of the study performed with wild hop germplasm in Greece have revealed low differentiation among populations, with the spatial genetic patterns observed relating mainly to topographical elements rather than geographic distance [25].

According to the dendrogram derived from the data obtained by ISSR-PCR experiments, there is a pronounced genetic polymorphism (or genetic diversity) in hop samples grown in different locations of Pazaryeri. The fact that all registered and unregistered commercial hop samples clustered according to their grown locations, suggests that they have undergone genetic mixing over time due to close cultivation. We report that genetically mixed hop samples that occur without conscious hybridization-based breeding efforts may be risky and impractical for future breeding studies. These results can be useful to identify specific genotypes to employ in breeding efforts to develop more productive hop varieties.

References

[1] Paguet, A. S., Siah, A., Lefèvre, G., Moureu, S., Cadalen, T., Samaillie, J., ... & Rivière, C. (2023). Multivariate analysis of chemical and genetic diversity of wild *Humulus lupulus* L.(hop) collected in situ in northern France. *Phytochemistry*, *205*, *113508*. https://doi.org/10.1016/j.phytochem.2022.113508

[2] Baytop, T. (1999). *Türkiye'de bitkiler ile tedavi: geçmişte ve bugün.* Nobel Tıp Kitabevleri.

[3] Koday, S. (2000). Türkiye'de kivi üretimi/yield of kiwi in Turkey. *Doğu Coğrafya Dergisi*, 6(3), 103-122.

[4] Carbone, K., & Gervasi, F. (2022). An updated review of the genus Humulus: A valuable source of bioactive compounds for health and disease prevention. *Plants*, 11(24), 3434. doi: 10.3390/plants11243434.

[5] Zugravu, C. A., Bohiltea, R. E., Salmen, T., Pogurschi, E., & Otelea, M. R. (2022). Antioxidants in hops: Bioavailability, health effects and perspectives for new products. *Antioxidants*, 11(2), 241. doi: 10.3390/antiox11020241.

[6] Rossini, F., Virga, G., Loreti, P., Iacuzzi, N., Ruggeri, R., & Provenzano, M. E. (2021). Hops (*Humulus lupulus* L.) as a novel multipurpose crop for the Mediterranean region of Europe: Challenges and opportunities of their cultivation. *Agriculture*, 11(6), 484. https://doi.org/10.3390/agriculture11060484

[7] Bocquet, L., Sahpaz, S., Hilbert, J. L., Rambaud, C., & Rivière, C. (2018). *Humulus lupulus* L., a very popular beer ingredient and medicinal plant: Overview of its phytochemistry, its bioactivity, and its biotechnology. *Phytochemistry reviews*, 17(5), 1047-1090.

[8] Kishimoto, T., Teramoto, S., Fujita, A., & Yamada, O. (2022). Evaluation of components contributing to the international bitterness unit of wort and beer. *Journal of the American Society of Brewing Chemists*, 80(1), 53-61. https://doi.org/10.1080/03610470.2021.1878684

[9] Korpelainen, H., & Pietiläinen, M. (2021). Hop (*Humulus lupulus* L.): Traditional and present use, and future potential. *Economic botany*, 75(3-4), 302-322.

[10] Başkaya, Z. (2011). The Geographical Foundations of Hops Production in Bilecik, Eas. *Geogr. Rev*, 25(16), 209-230.

[11] Luo, J., Pan, Q., Chen, Y., Huang, W., Chen, Q., Zhao, T., ... & Lu, B. (2024). Storage stability and degradation mechanism of xanthohumol in *Humulus lupulus* L. and beer. *Food Chemistry*, 437, 137778. https://doi.org/10.1016/j.foodchem.2023.137778

[12] Alonso-Esteban, J. I., Pinela, J., Barros, L., Ćirić, A., Soković, M., Calhelha, R. C., ... & Ferreira, I. C. (2019). Phenolic composition and antioxidant, antimicrobial and cytotoxic properties of hop (*Humulus lupulus* L.) Seeds. *Industrial Crops and Products*, 134, 154-159.

[13] Sözen, E., & Yücel, E. (2015). Determination of genetic relationships between some endemic Salvia species using RAPD markers. *Biyolojik Çeşitlilik ve Koruma*, 8(3), 248-253.

[14] Calvi, A., Aci, M. M., Lupini, A., & Preiti, G. (2023). Morphological and genetic analysis of wild hop (*Humulus lupulus* L.) germplasm from Calabria Region in South Italy. *Agronomy*, 13(1), 252. https://doi.org/10.3390/agronomy13010252

[15] Hilooğlu, M., Poyraz, İ., Poyraz, İ., Ataşlar Göymen, E., & Sözen, E. (2016). Genetic relationships among some Turkish Petrorhagia (Ser.) Link (Caryophyllaceae) taxa using ISSR markers. *Phytotaxa*, 272(2). https://doi.org/10.11646/phytotaxa.272.2

[16] Dağ., E. Yıldırım, N., & Korkmaz, M. (2023). Erzincan ve çevresinde yayılış gösteren A*stragalus* L. türlerinin genetik çeşitliliğinin ISSR yöntemi ile belirlenmesi. *Biyolojik Çeşitlilik ve Koruma*, 16(3), 203-209. https://doi.org/10.46309/biodicon.2023.1255384

[17] De Riek, J., Calsyn, E., Everaert, I., Van Bockstaele, E., & De Loose, M. (2001). AFLP based alternatives for the assessment of distinctness, uniformity and stability of sugar beet varieties. *Theoretical and applied genetics*, 103, 1254-1265. https://doi.org/10.1007/s001220100710.

[18] Peakall, R. O. D., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular ecology notes*, 6(1), 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

[19] Şahin, G., & Erbilen, S. Ü. (2012). Türkiye'de Yetiştirilen Keyf Bitkiler İçerisinde Özel Bir Tür: Şerbetçiotu (*Humulus lupulus* L.). *Zeitschrift für die Welt der Türken*, 4(3), 339.

[20] Van Holle, A., Muylle, H., Ruttink, T., Van Landschoot, A., Haesaert, G., Naudts, D., ... & Roldán-Ruiz, I. (2019). Single nucleotide polymorphisms and biochemical markers as complementary tools to characterize hops (*Humulus lupulus* L.) in brewing practice. *Journal of agricultural and food chemistry*, 67(13), 3761-3771.

[21] Patzak, J., Nesvadba, V., Henychová, A., & Krofta, K. (2010). Assessment of the genetic diversity of wild hops (*Humulus lupulus* L.) in Europe using chemical and molecular analyses. *Biochemical Systematics and Ecology*, 38(2), 136-145.

[22] Ocvirk, M., Nečemer, M., & Košir, I. J. (2019). The determination of the geographic origins of hops (*Humulus lupulus* L.) by multi-elemental fingerprinting. *Food chemistry*, 277, 32-37.

[23] Š Uštar-Vozlič, J., & Javornik, B. (1999). Genetic relationships in cultivars of hop, *Humulus lupulus* L., determined by RAPD analysis. *Plant breeding*, 118(2), 175-181.

[24] Somalraju, A., Soto-Cerda, B., Ghose, K., McCallum, J., Knox, R., & Fofana, B. (2023). Structure and genetic diversity of Canadian Maritimes wild hops. *Genome*, 67(1), 24-30. http://dx.doi.org/10.1139/gen-2023-0045

[25] Tegopoulos, K., Fountas, D. V., Andronidou, E. M., Bagos, P. G., Kolovos, P., Skavdis, G., ... & Grigoriou, M. E. (2023). Assessing Genetic Diversity and Population Differentiation in Wild Hop (*Humulus lupulus*) from the Region of Central Greece via SNP-NGS Genotyping. *Diversity*, 15(12), 1171. https://doi.org/10.3390/d15121171