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Morphological and Genetic Analysis of Wild Hop (*Humulus lupulus* L.) Germplasm from Calabria Region in South Italy

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Abstract: Hops (*Humulus lupulus* L.) constitute a species that grows spontaneously in the region of Calabria (South Italy), but the species' morphological and genetic characterization have not yet been explored. Thus, we explored some morphological traits related to cones of wild hops from three Calabrian sites: Cosenza (CS), Catanzaro (CZ), and Vibo Valentia (VV). In addition, eight Simple Sequence Repeats (SSR) were adopted to investigate the genetic diversity and population structure of the local germplasm, which were also compared to commercial varieties. Cone length exhibited large variation among the different populations, whereas cone shape was the most discriminant trait according to principal coordinate analysis. Eighty-one alleles were detected with a high mean of alleles per locus (10.12). The SSRs used in the present study were highly informative with a genetic diversity of 0.829 and a PIC value > 0.62, thereby confirming the high genetic variability in Calabria. Finally, genetic structure analysis revealed the existence of two distinct groups regardless of the specimens' sampling sites. Further studies including other wild hops populations from Calabria will be performed in order to detect specific alleles for new breeding programs.

Keywords: hop; molecular markers; population structure; simple sequence repeat; wild population; genetic diversity; polymorphism

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1. Introduction

Three species comprise the *Humulus* genus: *H. lupulus*, *H. japonicus*, and *H. yunnanensis* [1]. Within the species *H. lupulus*, five groups or taxonomic varieties have been identified: *H. lupulus* var. *lupulus* for the European hops, *H. lupulus* var. *cordifolius* for the Japanese hops, and *H. lupulus* var. *neomexicanus*, *pubescens*, and *lupuloides* for the plants native to the American continent [2], for which specific morphological characteristics (the number of lobes on the leaf and hairs on the bine) can distinguish the different varieties [1]. *H. lupulus* is a dioecious perennial plant [3] cultivated for its female inflorescences, commonly known as 'cones', whose commercial value lies mainly in the presence of bitter and aromatic compounds (resins and essential oils). These substances, together with other basic beer ingredients (water, malt, and yeast), contribute to providing a characteristic taste and aroma to the beverage [1]. Nevertheless, the addition of hops improves beer's stability in terms of microbial spoilage, thereby influencing the enhancement of foam as well [4].

Hop cones are utilized during wort boiling to guarantee the isomerization of α and β-acids, i.e., the solubilization of bitter substances, and in the late stage before wort cooling to preserve its aroma in the final beer [5]. Popular in the past as a healing agent in traditional medicine, these cones have recently found novel applications in cosmetic, pharmaceutical, and agricultural industries as a plant-protection product [6]. The species grows wild in various parts of Europe bordering the Mediterranean basin and its presence has also been reported in Calabria (South Italy) [7]. Experimental trials during the 1800s and in the last decades of the 19th century confirmed the high suitability of the Italian pedoclimatic conditions for the cultivation of this climbing plant [8]. Despite these assumptions, its

cultivation never spread throughout Italy in the past due to environmental, historical, and cultural reasons. Indeed, the nation is traditionally devoted to the production and consumption of wine [9]. Wild hops populations with high genetic variability may represent a new resource for breeding. Indeed, hops cultivars and landraces from Europe present less genetic diversity in comparison to Northern American spontaneous hops [10], while the plants distributed in China and Japan are considered as part of a genetically distinct group [11]. Wild hops ecotypes collected in Northern Italy were characterized by major cones yields but possessed fewer aromatic compounds when compared with commercial varieties grown in the same conditions and environment [12]. In addition, beers produced using wild hops from Central Italy have been appreciated for their distinctive herbaceous and flowery aroma [13].

In the last few years, an attempt has been made to enhance hops production, which is confined to a small production area, thereby promoting a focus on local ingredients while trying to meet the new demand [14]. In this context, the main goals are to identify allelic forms from local germplasm conferring adaptability to new cultivation areas and isolate specific aromatic compounds, thereby improving the aromatic and gustative qualities of the final product.

However, although phenotypic selection may represent a starting point from which to individuate useful genotypes for breeding programs, environmental effects reduce the capacity to identify specific genotypes [15]. In contrast, molecular markers are characterized by stability and are not affected by environmental factors. Thus, molecular markers are widely employed to study the genetic diversity and genetic structures of populations. Many studies have focused on the genetic diversity of hops collections based on different types of molecular markers, such as amplified fragment length polymorphisms [16], simple sequence repeats [12,17,18], single-nucleotide polymorphisms [19], and Diversity Arrays Technology [20].

However, among molecular markers, the use of simple sequence repeats (SSRs) is more frequent in the study of genetic diversity as they provide greater benefits in terms of identifying polymorphisms, multi-allelism, and codominant inheritance modes; accuracy; and reproducibility [15]. Indeed, the genomic mapping of hops and the identification of individuals have mainly been achieved through SSR analysis [21]. In particular, SSRs or microsatellites are molecular markers made of tandemly repeated DNA sequences characterized by high information content. They enable the detection of genetic diversity between individuals within species [22]. Several SSR analyses have been developed for hops plants over the years to assess their genetic variability [21,23–25].

Different studies have been conducted on the genetic and chemical characterization of Italian local genotypes [26,27] without including southern regions such as Calabria, and since wild diversity may constitute an interesting pool of genetic diversity for breeding [28], this work aimed to investigate the genetic diversity of wild hops collected in the South of Italy (Calabria). In addition, our study represents the first step towards determining the diversity of wild hops from Calabria, for which the material collected thus far is unknown.

2. Materials and Methods

2.1. Plant Material

Wild hops plants (*Humulus lupulus* L.) were identified in three different geographical areas in the region of Calabria (southern Italy)—Cosenza (CS), Catanzaro (CZ), and Vibo Valentia (VV)—whereas commercial varieties were grown on farms in the provinces of Reggio Calabria (RC) and VV, as reported in Figure 1 and Table 1.

Specifically, 22 wild plants were sampled, of which 18 were female and 4 males; the plants were labelled and geo-referenced. In addition, 4 commercial varieties were sampled, of which 2 were American (US), 1 was English (GB), and 1 was German (DE). Fresh, young leaves from the apical part of the stems were collected from wild hops plants and commercial varieties grown in different areas of Calabria. Once harvested, the leaves were stored at −80 °C until use.

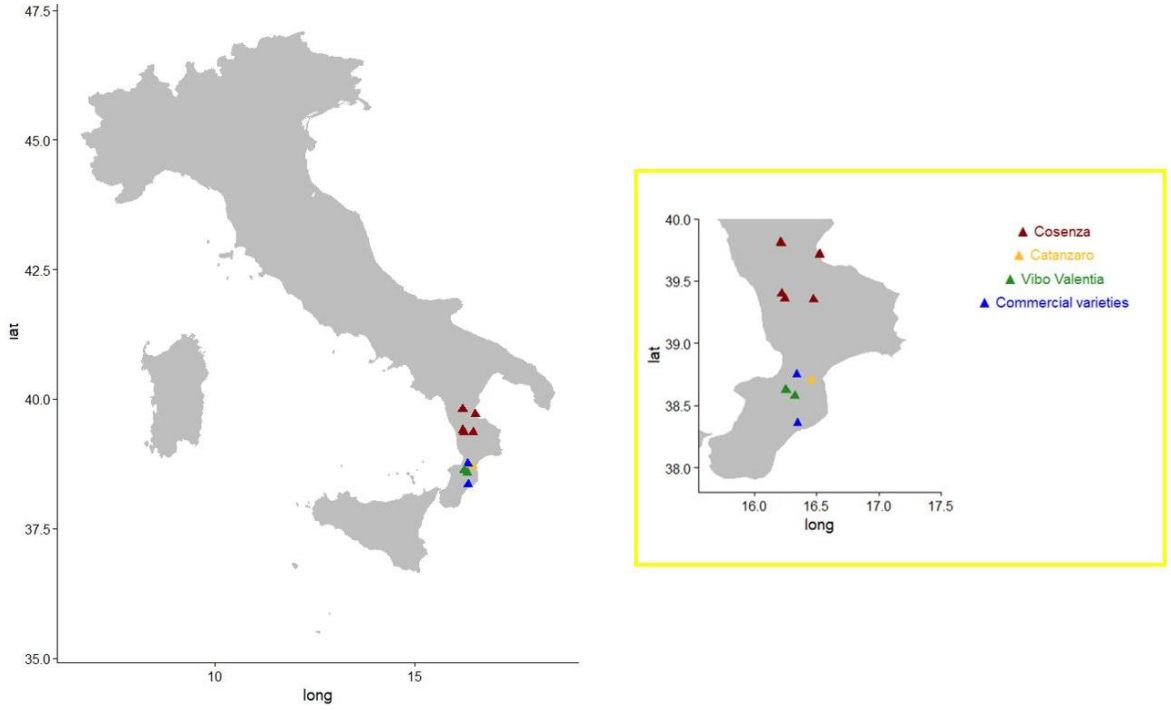

Figure 1. Geographical locations of wild hops (Red—Cosenza; Yellow—Catanzaro; Green—Vibo Valentia; blue—the commercial varieties Comet and Columbia in the province of Reggio Calabria, Fuggle and Hersbrucker in the province of Vibo Valentia).

Table 1. List of the studied hops genotypes.

Name	Province	Place	Elevation (m a.s.l.)	E/V	F/M
Coscile1	CS	Castrovillari	224	E	F
Coscile2	CS	Castrovillari	236	E	F
Coscile3	CS	Castrovillari	228	E	F
Coscile4	CS	Castrovillari	221	E	F
Coscile5	CS	Castrovillari	217	E	F
Brullì1	CZ	Petrizzi	328	E	F
Brullì2	CZ	Petrizzi	327	E	M
Brullì3	CZ	Petrizzi	321	E	M
Brullì4	CZ	Petrizzi	322	E	F
Brullì5	CZ	Petrizzi	318	E	F
COMET	RC	Gioiosa I.	198	V	F
COLUMBIA	RC	Gioiosa I.	198	V	F
Quattromiglia1	CS	Rende	173	E	F
Quattromiglia2	CS	Rende	175	E	M
Crati1	CS	Corigliano	3	E	F
Crati2	CS	Corigliano	1	E	F
Garusi	VV	Serra S. Bruno	804	E	M
Caruso	VV	Serra S. Bruno	804	E	F
Siviglia	VV	Serra S. Bruno	802	E	F
HERSBRUCKER	VV	Polia	711	V	F
FUGGLE	VV	Polia	711	V	F
Movigliano1	CS	Montalto Uffugo	184	E	F
Movigliano2	CS	Montalto Uffugo	177	E	F
Molarotta	CS	Spezzano Sila	1204	E	F
Pizzoni1	VV	Pizzoni	325	E	F
Pizzoni2	VV	Pizzoni	277	E	F

CS = Cosenza, CZ = Catanzaro, VV = Vibo Valentia; RC = Reggio Calabria; E = ecotype, and V = commercial variety; F = female and M = male.

2.2. Morphological Measurements

The morphological characteristics of the Calabrian wild hops and the commercial varieties were assessed based on specific cone descriptors using UPOV [29] and the methods of Rígr and Faberová [30]. Morphological measurements were carried out on 20 samples for each genotype. In particular, the following traits were determined: length (cm), diameter (cm), size, and shape of the cones.

2.3. DNA Extraction

Approximately 0.15 g of plant material was ground in liquid nitrogen to a fine powder and the total genomic DNA was extracted and purified by CTAB method [31]. Total DNA was resuspended in 70 µL of water (Nuclease-free water, Merk Millipore Corporation) and quantity and quality were measured using Biophotometer® D30 (Eppendorf) and stored at −20 °C.

2.4. Microsatellite Analysis

Eight SSRs were used in the present study, as reported in Table 2, which had been selected by previous studies based on polymorphisms [21,26,27]. Polymerase chain reactions (PCRs) were performed in 20 µL containing 20 ng of DNA, 1X PCR buffer (20 mM Tris-HCl pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DDT, 50% glycerol, 0.5% Tween® 20, and 0.5% Igepal® CA-630), 0.2 mM of dNTPs (Roche, Basel, Switzerland), 0.32 of µM reverse primer, 0.16 µM of fluorescence-labeled (FAM) universal M13(-21) primer, and 0.16 µM forward primer with M13(-21) tail, as reported by Schuelke [32]. Amplifications were performed using MiniAmpTM Thermal Cycler (Applied Biosystems, Waltham, MA, USA) under different annealing temperatures (Ta), depending on the primer pairs. Amplification was preceded by a denaturing step at 94 °C for 4 min followed by 30 three-step cycles at 94 °C for 45 s, Ta for 45 s, and 72 °C for 30 s. Subsequently, diluted PCR product was added to formamide and ROX standard (Perkin-Elmer, Waltham, MA, USA) and run on ABI PRISM 3500 Genetic Analyzer (Applied Biosystems).

Table 2. List of SSR loci used in the present study.

Locus	Repeat Motif	5′-Forward-3′	5′-Reverse-3′
HLGA23	$(CT)_{24}$	AAGCACGAAAACTGACTTG	GTTGCCCAAAATCACTGTT
HLGA48	$(TC)_{17}$	CTCTCCCTTACCTTATATACAG	AAGCTTCCAGCCTAAAATTC
HLGA42	$(CT)_{17}$	TGTCTTCAGGAACCCTTAACT	CCACCTTTGCTGATCCTTTCTA
HLGA27	$(AG)_{23}$	ATGCAAACGAATGAGCCTT	CCATAACCCATAATCAAACCA
HLGT17	$(GT)_{15}$	GGTCCTTAGTCACTTGCCAAT	GACTGTTCGAAGCACAATCAA
HLGA53	$(CT)_{16}$	GGACCGGGTTACTACCAGTG	AGCCTTCAACCTCAAAGCAC
HLAGA1	$(CTT)_{19}$	TCAAGAGCACAAATCCAAA	AAGGGAGATACACGTAAAG
HLGA31	$(GA)_{17}$	CAAACTTGGTGCTCTAAGATGAA	CGTTTTCCCAACACCTAGTTC

2.5. Statistical Analysis

2.5.1. Morphological Data Analysis

The map and its detail shown indicating sampling areas in Figure 1 were created using *maps* and *cowplot* based on *ggplot2* [33]. Four morphological traits of Calabrian hops' germplasm were evaluated: cone size, cone shape, cone diameter (cm), and length (cm). Diameter and length data were analyzed by one-way ANOVA, whereas an χ^2 analysis was performed to analyze the shape and size of the cone. In addition, the Coefficient of Variation (CV) was also calculated as the ratio between standard deviation and mean. Moreover, Principal Coordinate Analysis (PCA) was performed by using *factoextra* package based on *ggplot2* package [33]. Both data analyses were performed in R software v.3.4.3 [34].

2.5.2. Molecular Data Analysis

For molecular analysis via SSR markers, the allele number (N), effective allele number (Ne), expected Heterozygosity (H), observed Heterozygosity (Ho), Shannon diversity index (I), and inbreeding coefficient (F) were determined using GenAlex software version 6.5 [35]. Cervus v. 3.0.7 software was used to estimate the polymorphic information content (PIC) for each SSR locus (Copyright Tristan Marshal, Field Genetic, Ltd., London, UK). A dendrogram was created using MEGA software version X [36] using the pair-wise distances matrix developed by Nei and Li [37] and the unweighted pair group method of the arithmetic clustering algorithm (UPGMA) [38] to identify differences among populations. Principal Coordinates Analysis (PCoA) was conducted based on Fst genetic distances by using GenAlEx6 software version 6 [35]. Finally, to evaluate the genetic relationships among individuals and populations, model-based (Bayesian) clustering was performed using STRUCTURE software [39], assigning an admixture coefficient (Q) $\geq$ 0.9 as the assignment probability of each group. The program was set as reported by Aci et al. [40] and a criterion (ΔK) was adopted to determine the probable K value [41].

3. Results

3.1. Morphological Data

The ANOVA and χ^2 test for analyzing the four morphological features (Figure S1) established among the Calabrian hops population showed significant differences, and the highest and lowest CV (coefficient of variation) were observed in cone length (28.24%) and cone shape (13.91%), respectively (Table 3). Cone size ranged from 3 to 7, whereas cone shape was from 3 to 4 (Table 4).

Table 3. Analysis of variance and χ2 test of the studied morphological variables in hops plants.

Quantitative Traits			
Source	***df***	**Diameter**	**Length**
Population	22	0.623 ***	6.63 ***
Error	437	0.0451	0.222
CV%		17.72	28.24
Qualitative traits			
		Shape	***Size***
df		3	4
χ^2		23.78 ***	18.08 ***
CV%		13.91	28.16

df = degree of freedom; CV, coefficient of variation; *** = $p < 0.001$.

Table 4. The statistical characteristics of the studied traits in hops plants.

	Size	**Shape**	**Diameter (cm)**	**Length (cm)**
Min	3	3	0.9	1.3
Max	7	4	2.5	5.0
Mean	5.261	3.565	1.523	2.574
St. Dev.	1.482	0.496	0.270	0.727
Skewness	−0.213	−0.264	0.340	0.509
Kurtosis	−1.156	−1.939	−0.0158	−0.495

Moreover, the diameter and length of the cones ranged from 0.9 to 2.5 cm and from 1.3 to 5.0 cm, respectively (Table 4). Based on different Calabrian sampling areas (CS, CZ, and VV), the morphological data displayed a higher diameter in the VV populations followed by CS and CZ, as well as when considering cone length (Table S1). In addition, the PCoA based on morphological traits displayed two principal coordinates that contributed to 91.31% of the overall variability among the Calabrian hops populations (Figure 2). In

particular, PC1 and PC2 explained 69.1% and 22.2% of the total variation, respectively (Figure 2). In PC1, the size, diameter, and length of the cone were mainly involved, whereas PC2 included cone shape. These two components were able to distinguish two groups in the region of Calabria regardless of the plants' geographic origins and highlight the difference between Hersbrucker and other commercial varieties (Figure 2).

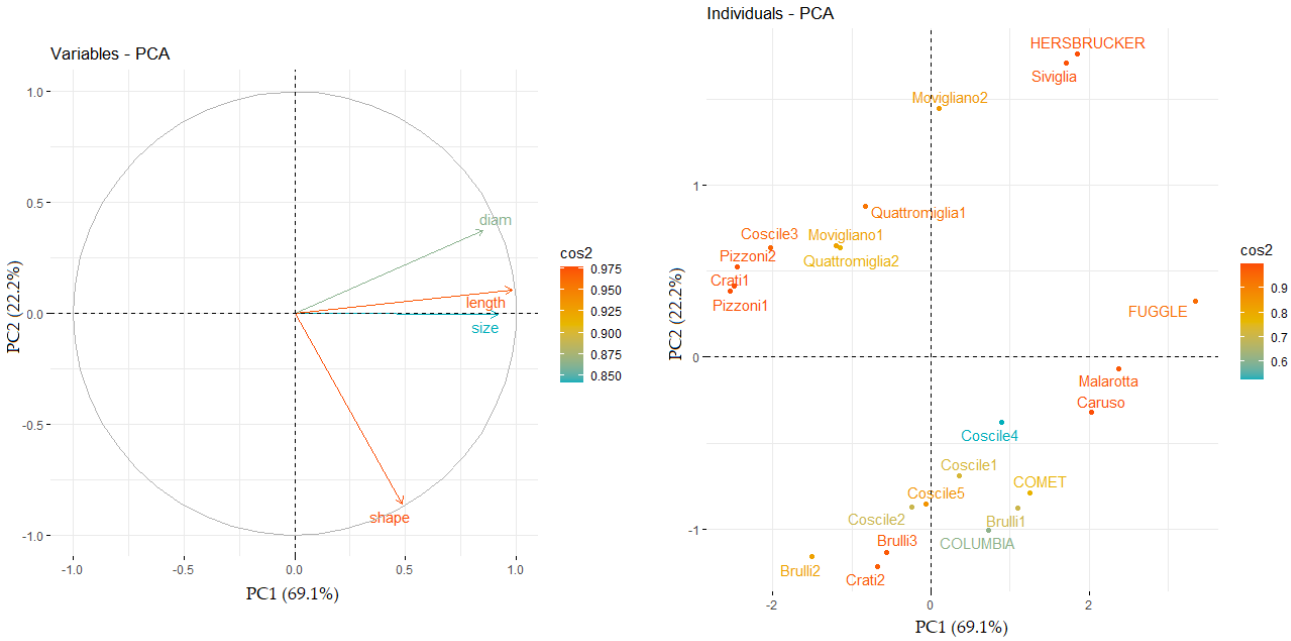

Figure 2. Principal coordinates analysis (PCoA) of 23 Calabrian hops populations based on agro-morphological traits (length = cone length; diam = cone diameter; size = cone size; shape = cone shape).

3.2. Molecular Data

Eight SSR markers were applied to evaluate the genetic variability among the twenty-six *H. lupulus* genotypes, and the data for genetic and statistical analysis are presented in Table 5.

Table 5. Genetic diversity parameters among hops populations based on SSR markers.

Locus SSR	N	Ne	He	Ho	PIC	I	F
HlGA23	14	8.167	0.899	0.619	0.866	2.320	0.295
HlGA48	7	4.630	0.799	0.385	0.755	1.704	0.509
HlGA42	7	5.869	0.847	0.760	0.806	1.822	0.084
HlGA27	17	9.941	0.917	0.615	0.891	2.521	0.316
HlGT17	7	4.036	0.767	0.808	0.716	1.599	−0.074
HlGA53	7	2.895	0.667	0.769	0.621	1.399	−0.175
HlAGA1	16	10.331	0.922	0.760	0.895	2.520	0.159
HlGA31	6	4.934	0.813	0.654	0.767	1.672	0.180
Mean	10.125	*6.350*	*0.829*	*0.671*	*0.790*	*1.945*	*0.162*

N, number of alleles; Ne, number of effective alleles; PIC, Polymorphic Information Content; He, expected Heterozygosity or gene diversity; Ho, observed Heterozygosity; I, Shannon's information index; F, fixation index.

The eight loci generated a total of 81 alleles with a mean of 10.12 alleles per locus. For each locus, the allele numbers varied from 6 (HlGA31) to 17 (HlGA27). The polymorphism information content (PIC) for the eight SSR primers extended from 0.621 (HlGA53) to 0.895 (HlAGA1) with a mean of 0.79. Expected heterozygosity (He) was between 0.667 (HlGA53) and 0.922 (HlAGA1) with an average of 0.829, whereas Ho ranged from

0.615 (HlGA27) to 0.808 (HlGT17) with a mean of 0.671. The fixation index (F), which estimates the degree of allelic fixation, was 0.162 and ranged from −0.175 in HlGA53 to 0.509 in HlGA48. Shannon's information index ranged from 1.399 to 2.52 with a mean of 1.945. In addition, when grouping the genotypes analyzed by geographical area, high genetic diversity was observed in CS, while VV and CZ showed similar characteristics (Table S2). Moreover, based on SSR marker data, a cluster analysis was performed using Nei and Li distances and an UPGMA dendrogram. The twenty-six genotypes were classified into three clusters (Figure 3). Cluster I grouped 16 genotypes, including Coscile (1-5), Pizzoni (1-2), Quattromiglia (1-2), Crati (1-2), Movigliano 2, Brulli 3, Garusi, and 2 commercial varieties (Comet and Fuggle); cluster II grouped 7 genotypes, including Brulli (1-2-4-5), Movigliano 1, and 2 commercial varieties (Columbia and Hersbrucker); and cluster III included Molarotta, Caruso, and Siviglia (Figure 3).

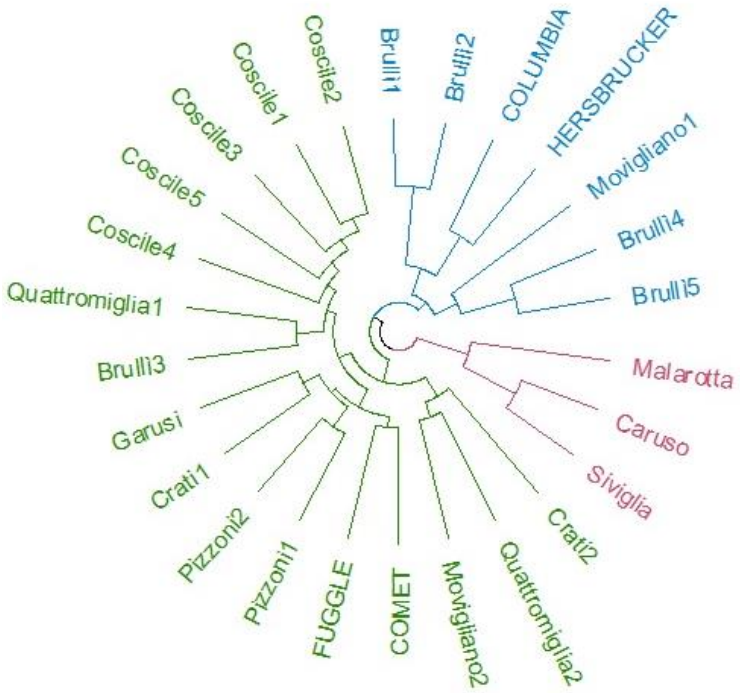

Figure 3. Unrooted dendrogram of the 22 Calabrian hops populations from three geographical areas and of four commercial varieties (Comet, Fuggle, Columbia, and Hersbrucker) based on eight SSR data points according to UPGMA method. The genotypes in red, blue, and green indicate three different clusters.

In addition, genotypes were clustered based on their genetic similarity using a Principal Coordinates Analysis (PCoA) (Figure 4). The first and second components were responsible for 29.05% of the overall variation, of which each component explained 17.25% and 11.80%, respectively. The PCoA was not able enough to distinguish genotypes according to their geographical origin and thus confirmed the results observed in the cluster analysis. Moreover, the correlation coefficient obtained by Mantel's test did not a show significant correlation between morphological and SSR information (r = −0.108, p = 0.0531) as well as geographical and molecular data (r = 0.0166, p = 0.8053), including altitude as well.

Moreover, when grouping the genotypes by geographical area (CS, CZ, and VV), AMOVA demonstrated that most of the total genetic diversity was explained within individuals (76%) followed by among individuals (16%) and populations (8%) (Table 6). High genetic distance was observed between CS and CZ, and pairwise Fst revealed a high degree of differentiation between CZ and VV (Table 7).

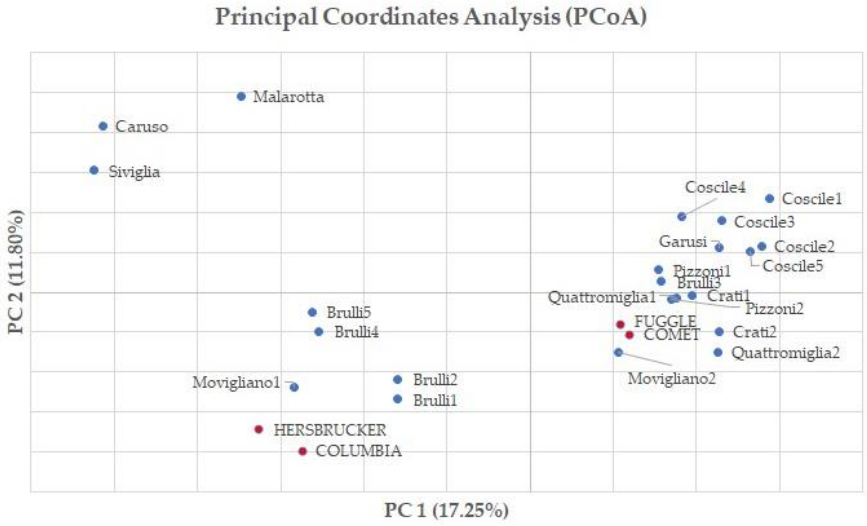

Figure 4. A two-dimensional plot of Principal Coordinate Analysis (PCoA) based on Fst genetic distance showing the clustering of hops genotypes (in red: commercial varieties).

Table 6. Analysis of molecular variation (AMOVA) among and within three Calabrian hops populations.

Source	df	SS	MS	Est. Var.	%
Among Pops.	2	14.873	7.437	0.286	8%
Among Indiv.	19	69.558	3.661	0.535	16%
Within Indiv.	22	57.000	2.591	2.591	76%

Table 7. Nei and Li (1979) [37] genetic distance (below diagonal) and Pair-wise estimates Fst (above diagonal) based on SSR markers among Calabrian hops populations.

	CS	***CZ***	***VV***
CS	0	0.095	0.08
CZ	0.686	0	0.109
VV	0.51	0.55	0

Finally, the genetic structures of the 26 hops genotypes were determined using Structure software (Figure 5). The optimum number of the genetic groups (K) of our collection was K = 2 based on ΔK peaks (Figure 5). Based on admixture coefficient (Q) of ≥0.9 as an assignment probability of each population to a group, it was possible to assign 16 and 9 to groups 1 (green) and 2 (red), respectively; one genotype (Malarotta) exhibited Q values of 0.673 and 0.327, thereby showing an admixed genetic structure (Figure 5).

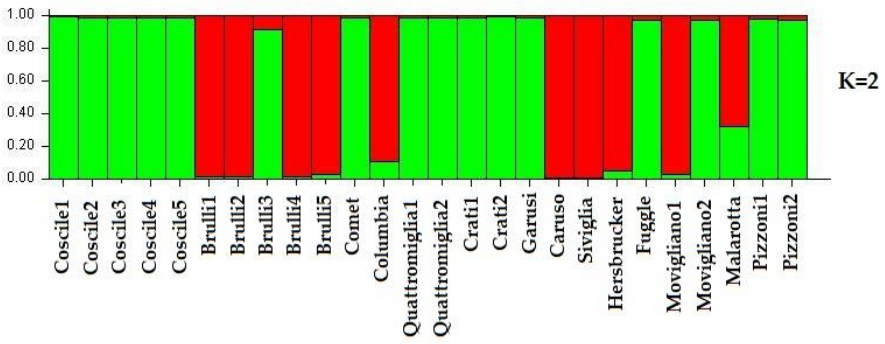

Figure 5. Bayesian individual clustering based on 8 SSR datasets of 22 hops populations from 3 different areas and of 4 commercial varieties (Comet, Comet, Fuggle, Columbia, and Hersbrucker) as inferred by STRUCTURE (Group 1 = green; Group 2 = red).

4. Discussion

To exploit the genetic resources in plants and highlight new insights for their use and conservation, the study of genetic diversity and population structures is necessary. Thus, in this study, we analyzed, for the first time, the genetic diversity of *H. lupulus* L. wild germplasm in Calabria (South Italy). Morphological and molecular analyses were adopted, as these techniques are frequently employed for hops [26,42,43]. Although morphological analysis presents many limitations with respect to the influence of the environment and low polymorphism in phenotypic expression, these traits were helpful in the preliminary evaluation of genetic diversity [44]. On the other hand, molecular markers have been used successfully for genetic diversity and population structure studies on hops [44–46]. In this study, high variation was detected in the yield components, such as the size and length of the cones, thereby confirming the samples' potential with respect to breeding. In addition, based on a PCA of the morphological traits, it was possible to surmise that cone shape was a highly-discriminant trait among Calabrian hops populations, as previously observed using genotypes from Northern Italy [26]. In the present study, the morphological traits obtained for the wild hops were not compared to commercial varieties due to the different management strategies for cultivation, for example, in terms of growth in the natural environment vs. agro-soil.

Moreover, the molecular diversity of the hops populations was also analyzed using eight SSR markers, and the number of alleles and their frequencies at each locus were analyzed as polymorphism indicators. Although the number of genotypes analyzed was lower than that of other works, in total, 81 alleles were detected among 26 hops genotypes, with an average of 10.12 alleles per locus. The high number of alleles per locus found in this study was also confirmed by the higher genetic diversity of the investigated germplasm. Indeed, similar values were detected by Stajner et al. [47], who found 10.88 alleles per locus; Rodolfi et al. [48], who detected 11.87 alleles per locus after genotyping 60 hops genotypes from Northern and Central Italy using 8 SSR markers; and Riccioni et al. [27], who reported 12.5 alleles per locus when analyzing 12 populations (165 samples) from Central Italy. In contrast, lower values of alleles per locus were detected when considering European (5.38) and North American (8.88) hops populations using 8 SSRs [10], and by Mafakheri et al. [44] using hops populations from Northern Iran (4.57). These differences in the number of alleles among the studies could be explained by considering the methodologies used, the size of the collection under study, and mainly the SSR panel adopted [27]. However, the selected loci used in the present study were sufficiently informative, which was also confirmed by the high values of genotypic diversity. In particular, different levels of polymorphism (PIC) were detected among the loci, for which HlGA23 (0.866), HlAGA1 (0.895), and HlGA27 (0.891) exhibited higher values, which is in agreement with Stajner et al. [47]; in addition, according to Rodolfi et al. [48], HlGA23 was also most informative with respect to differentiating the Calabrian wild genotypes. The genotypes analyzed in the present study showed a high level of heterozygosis (from 0.667 to 0.922), and this could be explained by the outcrossing of a dioecious species or due to long-distance pollen dispersal [4,27]. The differences observed among the different areas (CS, CZ, and VV) could also be due to the different success of clonal propagation conditioned by the environment, and, therefore, to a fixation of heterozygosity, which was confirmed by AMOVA, highlighting a high variation among individuals, as reported for other species with clonal propagation [49]. In contrast, despite the data discussed above, our results, in some cases, highlight genetic differences even within the same sampling area, confirming an absence of clonality as already reported [28]. This could be justified by the distance among the sampling areas, but further analysis is needed to confirm our hypothesis.

Moreover, our results confirmed that an increase in the level of differentiation could be correlated with distance. Indeed, VV and CZ exhibited similar genetic structures, which was also confirmed by PCoA and Bayesian analysis, in which CS was different from the CZ and VV sites. Yet, STRUCTURE analysis showed two different clusters of hops plants in Calabria, CS, and CZ-VV. Moreover, based on the abundance of private alleles in these wild

genotypes, the population from CS exhibited a higher number. This result can be useful when discriminating between the populations to identify specific genotypes to employ in new breeding programs [50,51].

5. Conclusions

Our results confirmed how the use of microsatellites in hops, due to their high degree of polymorphism, can be considered an excellent marker system for identification. In addition, although previous studies [51] showed that five polymorphic microsatellites can differentiate genotypes, in our study, eight microsatellites were assayed, thereby permitting us to discriminate wild populations within the Calabrian sampling area. In addition, although eight SSRs were used in the present study, we can affirm that Calabrian wild populations were characterized by a high degree of genetic variability, as demonstrated by the high level of heterozygosis.

Moreover, given that the craft beer sector in Italy is constantly developing, it would be desirable to stimulate and incentivize the cultivation of cereals and hops suitable for beer production. The creation of a local supply chain could allow farms to diversify their production and microbreweries in order to characterize and link their beers to a given territory. Therefore, further studies will focus on the selection of representative wild hops germplasms and compare them with aromatic commercial varieties grown under the same conditions both from a bio-agronomic and, above all, a chemical point of view to establish whether the local germplasm can offer a solid basis for the selection of new varieties suitable for developing hops cultivation in Southern Italy.

However, to consolidate this investigation, an *ex situ* analysis should be performed along with the analysis of metabolomic profiles to identify genotypes potentially suitable for use in breeding programs, while also highlighting hops' adaptability to specific environments.

In conclusion, considering the obtained results and comparing them to others, preserving the Calabrian wild hops population should represent a primary target whose achievement will avoid the loss of specific allelic forms that could be useful in both the agronomic and agri-food fields.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13010252/s1, Figure S1: Plant and cones of Calabrian wild hops. (a) Wild hops plant; (b) fresh cones from Coscile area; (c) fresh cones from Corigliano area; Table S1: Morphological traits of the Calabrian hops populations; Table S2: Genetic diversity parameters among hops populations of the three Calabrian areas (CS, CZ and VV) based on SSR markers.

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