

Article

Characterization of Wild Corsican Hops and Assessment of the Performances of German Hops in Corsican Environmental Conditions through a Multidisciplinary Approach

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Featured Application: This work could lay the foundation for future hop breeding research; moreover, the results of this research answers to the brewers' question of how territory, in this case the Corsican territory, influences aromatic and morphological characters of cultivars.



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Abstract: Hops (*Humulus lupulus* L.) is a species that grows spontaneously in Corsica, but the characterization of this species in this territory has not yet been investigated. The main objectives of this study are to explore the features of wild hops from Corsica and to determine the effect of the island terroir on some cultivars in the first year of growth. A multidisciplinary approach consisting of the genetic analysis, morphological comparison and chemical characterization of essential oils was carried out on four wild Corsican hops and three hop cultivars grown in Tettngang, Germany and Corsica, France. The morphological and GC-MS analysis of Corsican wild hops, set cluster coastal samples apart from the one far from the coast. This dissimilarity is supported by the SSR analysis by two of the three coastal accessions. The genetics demonstrate a proximity between the European noble cultivar Tettnganger and the mountain Corsican wild hop from Corte. The morphological comparison between German hops cultivated in Tettngang and in Corsican soil, and the GC-MS characterization of their essential oils' chemical profiles, show different features between year 0 and year +1 for each sample. This multidisciplinary approach highlights an acclimatization of hop cultivars to the Corsican terroir one year after planting.

Keywords: *Humulus lupulus* L.; GC-MS; essential oil characterization; plant morphological analysis; plant SSR fingerprints; plant adaptability

1. Introduction

Hops (*Humulus lupulus* L.) have been used in beer brewing since the 13th century for their aroma, bitterness, and preservative properties. The volatile compounds of hops and the chemical variability of essential oils have generated interest in the brewery industry. Several studies have focused on their chemical characterization by GC-MS analysis. Usually, the main constituents are myrcene, humulene and caryophyllene [1–6]. To our knowledge there are five botanical varieties of hops (var. *lupulus*, -*cordifolius*, -*neomexicanus*, -*pubescens* and -*lupuloides*) [7]. So far, only var. *lupulus* grows spontaneously in Europe. These botanical varieties differ in their chemical composition, genetics, and morphology. For plants, and specifically for hop genotypes differentiation, it is possible to use morphological, chemical and or genetic markers [8–13]. The use of morphological or chemical markers alone make the differentiation among the studied genotypes not simple because of

the small differences often present within the species, and because morphological and chemical characters are often dependent on biotic and abiotic factors and on plant age [14–19]. Several studies differentiate hop varieties by observing the ratio between antioxidants and aromatic compounds; Kralj and coworkers [4] observed that the α humulene/ β caryophyllene ratio is a varietal trait used as a marker because it is independent from ripeness and storage, and it is characteristic of each variety. Instead, the genetic study allows the differentiation among genotypes that could present the same morphological or chemical characters, and allows the recognition and discrimination of genotypes grown in different conditions or plant age; DNA analyses are able to reveal variations in genomic sequence with frequencies over 1% among individuals of the same species [20]. Among the molecular markers, SSRs are relevant and effective tools to determine genetic differences within plant genotypes [20–28]. Patzak and collaborators [23] used nine SSR makers and three STS to genetically characterize 136 hop genotypes from Czech Republic, France, Switzerland, the Caucasus Region, Canada, the U.S.A. and five world cultivars, highlighting the genetic differences between American and European genotypes. In the study of Rodolfi and coworkers [27], nine SSR markers were successfully used to obtain the genetic structure of a population of 123 Italian wild hop samples and commercial cultivars, highlighting, in particular, the marked genetic differences between wild and cultivated hops. In Corsica, the characterization of the wild hop biodiversity was never studied before, and it is a priority for a sector, such as brewing in the continuous expansion and interest in peculiar new varieties. The most widely cultivated varieties of the current crops were selected using conventional methods [29], and the level of biodiversity of the starting material is the base for a successful plant selection. In hops, wild material was usually used for the most important varieties cultivated today, providing resistance to diseases or high percentages of alpha acids and peculiar aromas [30]. Hops, around the globe, is distributed between the 35 and 55° parallel [31], and in Corsica (43° parallel North), wild hop is naturally present in riverbeds, such as the Restonica, in river mouths near Ajaccio and Bastia, and in northern sandy clay soils. Corsica could be a new place for hop cultivation. All the environmental and territory effects on crop or the final product, biotic and abiotic factors, including climate, soil composition, living environment ecosystem, cultivar, but also human factors, such as agronomic practices, constitute the terroir concept [32]. A study on the influence of territory on hop field performances, made by Rodolfi and collaborators [27], evaluated the different performances of Cascade cultivar growth in 13 growing areas in Italy, Slovenia, Germany, Michigan and Ohio, with particular attention given to the Italian territory; the results showed important differences in the quality of this cultivar, concerning bitter acids, antioxidants and aroma. Differences in bitter acids and essential oil content were reported for hops grown very far from each other, but also (in particular in Italy) for hops grown at relatively small distances, due to the peculiar conformation of the territory. Moreover, Van Holle and collaborators [17] studied the differences between Amarillo hops grown in Washington State and Idaho (U.S.A.), highlighting differences, especially in the quality and quantity of essential oils; the monovarietal beers produced with the studied hops showed differences in flavors associated with the provenience of hops.

In this study, two aims were addressed: (i) to evaluate the morphological, genetic and aromatic characteristics of wild Corsican hops, in order to characterize new sources of genetic material for breeding purposes and (ii) to evaluate the terroir adaptability/influence of/on Cascade, Smaragd, and Tettninger cultivars by measuring aromatic and morphological differences when grown in a native territory (Tettninger) and in a new growing area (Corsica).

2. Materials and Methods

2.1. Plant Material

Corsican wild hops were found in different Corsican riverbeds and planted in an experimental field in Patrimonio (Corsica, France). Leaves sample were collected for the DNA analysis. Rizhomes of Cascade, Tettninger and Smaragd cultivars were obtained

from German grower (Locher-Hopfen-Tettngang, Missenhard, Germany), and planted in the experimental field. The hop cones of all the studied hops were picked two times: three months after planting and the following picking year.

To have the evidence of the varieties and to be sure to compare the same varieties, leaves samples of the German hops cultivated in Patrimonio and in Tettngang region were genetically analyzed. The climatic conditions of the Tettngang valley and the four studied Corsican areas have been gathered from the open database “climate-data.org” for which the results shown are averaged from 1999 to 2019, and from the database “me-teoblue” for which the data are averaged from 2010 to 2020. The “Patrimonio” data can be considered common to “Oletta” due to the geographical proximity. During the growth period of hop from May to September, the Tettngang valley is characterized by its very high rain content (90–130 mm/month; av: 114 mm/month), low temperatures (13–18 °C; av: 15.8 °C), strong south-west to north-east winds (5–50 km/h) and a low solar exposition (4–7 days/month; av: 5.6 days/month). For the same period, the Corsican planting site (Patrimonio) and Oletta are characterized by their low rain content (10–54 mm/month; av: 30 mm/month), high temperatures (16–24 °C; av: 21.2 °C), strong south-east to north-west winds (5–50 km/h) and a very high solar exposition (10–20 days/month; av: 14.2 days/month). Ajaccio is characterized by its low rain content (15–55 mm/month; av: 30 mm/month), high temperatures (17–24 °C; av: 21.4 °C), medium west-south-west to east-north-east winds (5–19 km/h) and a high solar exposition (5–14 days/month; av: 9 days/month). Corte is characterized by its medium rain content (15–60 mm/month; av: 38 mm/month), medium temperatures (15–21 °C; av: 18.6 °C), weak north to south winds (0–38 km/h) and a high solar exposition (7–16 days/month; av: 11.4 days/month).

In the first part of this study, wild hops (Table 1), located in Ajaccio, Corte, Patrimonio and Oletta, were characterized by a morphological, genetic and chemical approach.

Table 1. List of the four wild hops and characteristics of their provenance.

Accessions/Cultivars	Geographic Coordinates	Soil Type	Altitude	Sea Proximity	Salinity
Ajaccio Wild	41.915528 N, 8.819222 E	Clay-sand	5 m	Very close	High
Oletta Wild	42.666066 N, 9.308305 E	Clay-sand	20 m	Very close	High
Patrimonio Wild	42.697306 N, 9.361750 E	Silt-sand	10 m	Very close	High
Corte Wild	42.303591 N, 9.153470 E	Clay-sand	410 m	Away	Weak

In the second part of the study were compared three hops cultivars: Cascade, Smaragd and Tettnganger. In this case, the hops have been identified with the commercial name of the cultivar followed by the geographical origin, for example, Cascade Tettngang, Cascade Corsica, etc. The geographical characteristics of the two growing sites (Tettngang valley, Tettngang, Germany; Patrimonio, Corsica, France) are shown in Table 2.

Table 2. Geographical indication and characteristics of the two growing sites.

Growing Sites	Geographic Coordinates	Soil Type	Altitude	Sea Proximity	Salinity
Corsica	42.695583 N, 9.344222 E	Silt-sand	10 m	Very close	High
Tettngang	47.668861 N, 9.604528 E	Clay-sand	466 m	Far away	None

2.2. SSR Analysis

Genomic DNA of the 10 samples (Tables 1 and 2) was extracted from (i) young leaves (L) collected from young Corsican wild hops and from (ii) Cascade, Tettnganger and Smaragd cvs grown in the Tettngang valley and in Corsica in order to highlight the terroir modification or retention of their genome. The samples, after immersion in liquid nitrogen, were stored at −80 °C and then lyophilized until DNA extraction. Genomic DNA was extracted following the CTAB protocol [33].

For DNA amplification, six SSR primer sets were used, which had shown a high discriminating power [34] (Table S1).

The PCR amplification was performed in a 25 μ L volume containing: 1x Reaction Buffer (Biotools, B&M Labs, S.A., Madrid, Spain), 1.5 mM MgCl₂ (Biotools, B&M Labs, S.A., Madrid, Spain), 0.2 mM dNTPs (Amersham Biosciences, Piscataway, NJ, USA), 0.2 μ M primer (MWG Biotech, Ebersberg, Germany), 20 ng genomic DNA and 0.6 U of Taq polymerase (Biotools, B&M Labs, S.A., Madrid, Spain). For primer HIACA3 and HIGA23, the MgCl₂ concentration was 2.5 mM to obtain a better quality of amplification.

The PCR amplifications were performed in thermal cycler MJ PCT 100 Research (Watertown, MA, USA), using the following amplification protocol: programming a first step at 95 °C for 5 min followed by 30 cycles of 45" at 94 °C, 30" at the specific annealing temperature for each couple of primers, and 90" at 72 °C, for denaturation, annealing and primer extension, respectively; then a final extension at 72 °C for 8 min.

The amplification products were separated with a CEQ 2000 Genetic Analysis System (Beckman Coulter, Inc., Brea, CA, USA) sequencer on acrylamide gel CEQ Separation Gel LPA-1 (Beckman Coulter, Inc., Brea, CA, USA). A marker CEQ DNA Size Standard kit 400 (Beckman Coulter, Inc., Brea, CA, USA) was used to estimate the approximate molecular weight of the amplified products. One of the two PCR primers in each reaction was end-labelled with a fluorescent dye (Cy5, MWG-Biotech, Ebersberg, Germany). The analysis was performed in triplicate.

2.3. Morphological Comparison

Many parameters were observed and measured at different times of maturity, following the UPOV (International Union for the Protection Of new Varieties of plants) recommendations (Table S2). These observations were made directly on field or in riverbeds on thirty plants for each sampled hop. The hop plants were observed and compared to literature standards (UPOV) during growing from May to August in two growing seasons (2019 and 2020). The first growth season was named "year 0" and the second one, "year +1".

2.4. Essential Oils Chemical Characterization

Three hydrodistillations for each accession were performed on hop cones in a Clevenger apparatus for four hours, as recommended by the European Pharmacopoeia [35]. The essential oils were collected and then analyzed by GC-FID and GC-MS.

These analyses were carried out using a PerkinElmer Autosystem GC apparatus equipped with a dual flame ionization detection (FID) and fused-silica capillary columns (60 m \times 0.22 mm i.d., film thickness 0.25 μ m) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). The oven temperature program was from 60 to 230 °C at 2 °C/min and then was held isothermally (30 min). The carrier gas was helium (1 mL/min). Injector and detector temperatures were held at 280 °C. Split injection was performed with a fractional ratio of 1:80. The injected oil volume was 0.1 μ L. The relative percentage of the oil constituents was calculated from the GC peak areas without using correction factors.

The oils were analyzed with a PerkinElmer TurboMass detector (quadrupole), coupled in line to a PerkinElmer Autosystem XL equipped with two fused-silica capillary columns (60 m \times 0.22 mm i.d., film thickness 0.25 μ m), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). The other GC conditions were the same as described above. The ion source temperature was 150 °C, the energy ionization was 70 eV and the electron ionization (EI) mass spectra were acquired over the mass range of 35–350 Da. The volume of oil injected was 0.1 μ L. The main volatile compounds were identified on the basis of their mass spectra compared with the reference mass spectra libraries (WILEY275, NBS75K, Adams 2001) and of their calculated retention indexes (RIs) through the application of the Kovats index (KI) formula compared with those reported in the literature and linear retention index (LRI). When it was not possible to find the KI in the literature, a tentative identification was obtained by matching with mass spectra libraries data: a match quality

of 98% minimum was used as a criterion. The gas-chromatographic signals were manually integrated, and the resulting peak areas were compared with the total sum of area and expressed in percentage.

2.5. Data Analysis

The nuclear DNA fragments, resulted by the DNA amplification with the SSR markers, were sized by using a conservative binning approach [36] through the statistical R software (R Development Core Team 2005), which takes into account the type of replicate and compensates for the limits of the fragment resolution. The differences among the examined hops were evaluated through the genetic similarity matrix using Euclidean distance [25,37,38]. The cluster analysis and construction of the dendrogram relative to genetic distances were obtained by using the unweighted pair-group method with arithmetic mean (UPGMA) algorithm, with Genetix [39] and XLSTAT 2009 software (Addinsoft™ 1995–2009). The univariate clustering analysis was performed using XLSTAT 2009 software (Addinsoft™ 1995–2009) to discriminate the genotypes.

A statistical analysis of the chemical composition of essential oils was performed using the opensource R studio software (version 1.2.5001, Factoextra package). The normalized data were put in a dissimilarity matrix using Euclidean distance. The hierarchical tree resulting of the matrix linkage was then graphically represented into a dendrogram.

3. Results and Discussion

3.1. SSR Analysis

From the genetic analysis, the four wild accessions were separated; the cultivars are recognized to be the same in Tettang and Corsica and the genetic profile of the analyzed hops are reported in Table 3. The dissimilarity in the studied samples were highlighted and analyzed in the dendrogram resulted by the Cluster analysis (UPGMA) at Euclidean distance (Figure 1). The relationship between all the accessions is shown with a dissimilarity index from 0 to 76. The examined population is divided in two main clusters (76% of dissimilarity); the first cluster is composed of the accessions Patrimonio, Ajaccio, Cascade and Smaragd; the other two Corsican accessions, Corte and Oletta, fall in the second cluster with the cultivar Tettanger (Figure 1). Corte accession is separated from those of Patrimonio and Ajaccio; this could be explained by their very different growing conditions (Table 1), such as distance from the coast (respectively away, very close, and very close), temperatures (respectively av: 18.6 °C, av: 21.2 °C, and av: 21.4 °C), salinity (respectively weak, high, and high), altitude (respectively 410 m, 10 m, and 5 m), winds (respectively weak from north to south, strong from south-east to north-west, and medium from west-south-west to east-north-east), and precipitations (respectively av: 38 mm/month, 30 mm/month, and 30 mm/month) (“climate-data.org” and “meteoBLUE” data). However, these peculiar growing conditions do not apply to Oletta. As no hop traceability data are available in Corsica, it is difficult to estimate when or how the plant was established on the island, nor if it was implanted from different cultivars. Currently, no origin or environmental condition has been found explaining the dissimilarity among the coastal hops’ genotypes. Another possibility is the autochthonous origin of Corsican germplasm; no data, however, are available to support this hypothesis.

Tettanger cultivar resulted closer to Corte and Oletta wild Corsican accessions (Figure 1). This result indicates the affinity of wild hops with some European cultivar selected from landraces. Other authors confirm this sentence [25,40]. Rodolfi and collaborators [25], in a population study on 123 hop genotypes, observed that Tettanger and other European cultivars (Galena, East Kent Golding, Goldings, Challenger, H. Mittelfrüh) were clustered together with Italian wild accessions. This sentence is also in accord with Patzak and coworkers [38]; the authors assert that Tettanger cultivar, selected by German landrace, have a close relationship with the wild European ancestors [40]. Smaragd and Cascade cultivar resulted in a separated cluster together with Patrimonio and Ajaccio accessions (Figure 1). The genetic similarity to the Corsican accessions could be due to

Smaragd and Cascade origins: Smaragd was developed from the Hallertau variety that belongs to the wild German hop population, but Cascade possesses a complex genetic structure [25] and it has among its ancestors, Fuggle, an English hop variety selected from wild [21,40]. This similarity could also demonstrate the correlations between European ancestors and Corsican wild hops.

Table 3. Genetic profiles of ten hop samples (six cultivars and four wild hops).

Accessions/Cultivars	H1ACA3	H1AGA7	H1GA23									
SMARAGD Corsica	211	223	187	212	243	249	165	167	233	233	195	195
SMARAGD Tett nang	211	223	187	212	243	249	165	167	233	233	195	195
CASCADE Corsica	211	211	163	187	241	267	157	169	233	233	177	187
CASCADE Tett nang	211	211	163	187	241	267	157	169	233	233	177	187
TETTNANGER Corsica	211	239	183	209	287	301	167	167	209	231	179	195
TETTNANGER Tett nang	211	239	183	209	287	301	167	167	209	231	179	195
Ajaccio Wild	230	236	167	187	243	265	161	167	229	233	179	187
Oletta Wild	211	233	187	187	295	295	165	165	229	229	179	179
Patrimonio Wild	211	239	159	181	243	277	165	167	215	234	187	187
Corte Wild	209	231	187	187	295	295	165	165	215	234	179	179

Columns list the allele sizes for the six investigated loci [34].

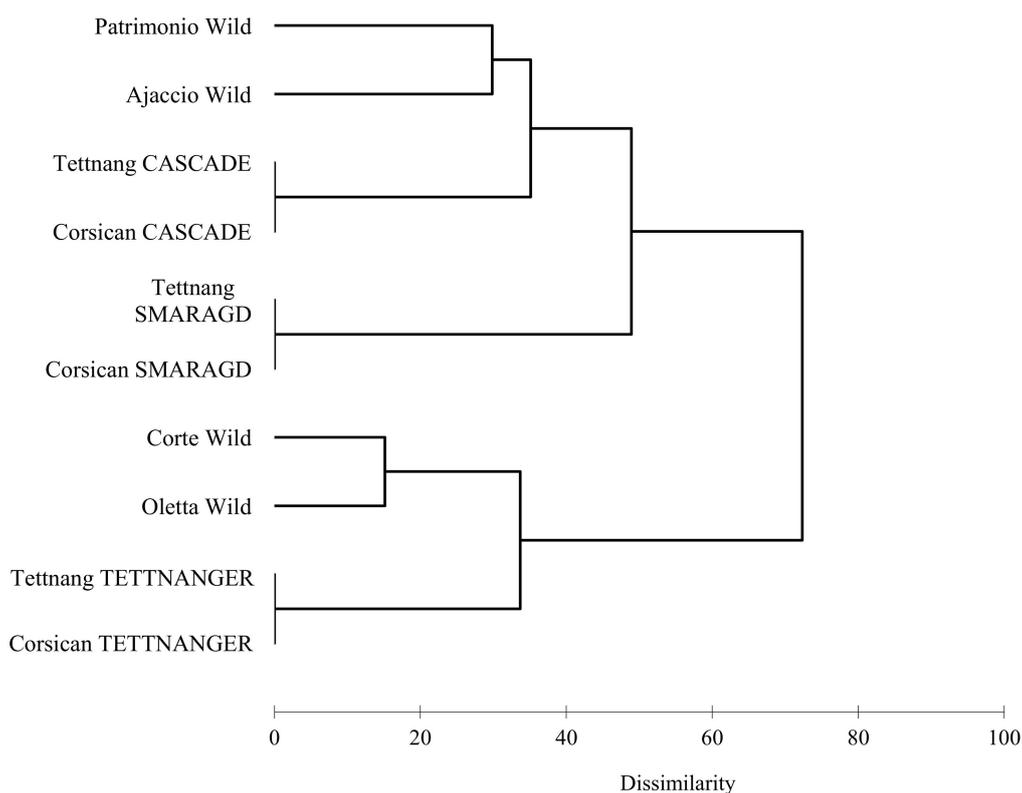


Figure 1. UPGMA dendrogram based on Euclidean distance of cultivated and Corsican wild hops.

3.2. Morphological Comparison

Morphological characters of the four wild Corsican hops are described in Table 4.

Table 4. Morphological description of wild hops from Corsica. In bold, the differences are highlighted.

Morphological Characteristics	Corte	Oletta	Patrimonio	Ajaccio
Main shoot: anthocyanin coloration	Strong	Absent	Absent	Absent
Leaf: size of blade	Medium	Large	Medium	Large
Leaf: blistering of upper side of blade	Absent	Absent	Absent	Absent
Leaf: color of upper side of blade	Green	Green	Green	Green
Leaf: if green, intensity of green color of the upper side of blade	Dark	Light	Dark	Dark
Time of flowering	Late	Late	Medium	Late
Plant: Growth type	Normal	Normal	Normal	Normal
Side shoot from the middle third of the plants: length	Long	Short	Short	Short
Side shoot from the upper third of the plants: Length	Long	Short	Short	Short
Side shoot from the middle third of the plants: density of foliage	Dense	Dense	Medium	Medium
Side shoot from the middle third of the plants: number of cones per node	Many	Few	Medium	Few
Side shoot from the middle third of the plants: total number of cones	Many	Few	Few	Few
Side shoot from the upper third of the plants: total number of cones	Many	Medium	Medium	Few
Time of picking maturity of cones	Medium	Late	Medium	Late
Cone: size	Large	Large	Medium	Medium
Cone: shape	Broad ovate	Medium ovate	Cylindric	Cylindric
Cone: degree of opening of bracts	Clearly open	Just open	Just open	Closed
Cone: intensity of green color	Light	Medium	Light	Light
Bract: size	Large	Small	Large	Medium
Bract: ratio length/width	Medium	Small	Medium	Medium
Bract: length of tip	Short	Short	Medium	Short

From Table 4 it is possible to observe the absence of anthocyanin coloration in three wild hops from coastal areas of Corsica (Oletta, Patrimonio and Ajaccio). As Treutter et al. [41] explained, the absence of plant pathogens, such as *Tetranychus urticae* Koch, downy mildew, and powdery mildew, makes the biosynthesis of protective anthocyanins unnecessary. Therefore, the absence of these biotic stresses in the studied areas could explain the absence of anthocyanin coloration of the main shoot of these wild accessions. The side shoots of the coastal plants Oletta, Patrimonio, and Ajaccio are short, and the number of cones is low (Table 4). As exposed previously, these three Corsican areas are characterized by high temperatures, high solar exposition, medium to strong winds and low precipitations, leading to a severe hydric stress, especially during the hop growing period. Therefore, this observation supports the correlation between the size of the plant, the number of cones, and water stress exposed by Lisar and collaborators [42]. Moreover, even if salt sensitivity of hop plants has not yet been studied, Oletta, Patrimonio and Ajaccio accessions grow in the coastal area, and salt stress could influence the plants' morphological traits [43].

Corte accession, instead, comes from a particular area of Corsica characterized by a humid microclimate caused by its weak winds, its medium precipitations and temperatures and the presence of two big rivers [44]. This leads to the presence, in Corte accessions,

of a strong anthocyanin coloration of the main shoot (Table 4). This humid microclimate is perfect for the growth of powdery and downy mildew. Anthocyanins are supposed to have a role in the plant resistance to pathogens and herbivores, but their biosynthesis requires energy [42] at the expense of the biosynthesis of prenylated compounds, such as xanthohumol [45]; this theory is supported by the anthocyanin coloration of the main shoots of wild hops from Corte. In the same way, the major length of the side shoots and the higher number of cones in wild hops from Corte compared to the other wild hops (Table 4) prove that these characteristics can be influenced by water stress (less present in Corte area), as a response to preserve the vital parts of the plant [43]. These observations seem to corroborate the previously enounced fact that the peculiar growing conditions of the Corte accessions lead to differentiations, both genetic and morphological.

The morphological comparison between the Tettang cultivars of hops and their cuttings planted in Corsica is described in Table 5. It is possible to observe that in the first year after planting, the morphological characters of the cultivars remain the same in Corsica and in Tettang. It is instead very interesting to observe that morphological modifications occurred from the year +1 after planting. The first important change occurred to the main shoots of all cultivars (Table 5), which lost their anthocyanin coloration. The presence of *Tetranychus urticae* Koch, downy mildew, and powdery mildew in the Tettang area [46] could explain the coloration of the main shoot in Tettang samples as a result of the synthesis of anthocyanins responding to stress (Table 5) [47]. Therefore, the absence of these attacks on the coastal areas of Corsica could explain the anthocyanins disappearing a year after transplantation. Salt stress is often related to an increase in anthocyanin content, but Akula and Ravishankar [43] reported that salt stress in salt sensitive species could decrease anthocyanins level, as may have happened in the studied plants. Another observable change in the cultivars occurred after one year in the Corsican territory: the shortening of side shoots (Table 5). Moreover, in Table 5, it is highlighted the reduced number of cones in the side shoot in hops cultivated in Corsica. These last two modifications are probably symptoms of plant stress, especially water, wind and salt stress [43], that affects most plants in the northwest of Corsica. Evidence of the modification of plant character caused by the zone of cultivation is also the picking time. Not all the cultivars react in the same way, but Cascade shows anticipation in picking maturity (Table 5). It is well known that the maturity of cones depends on their percentage of water loss. This anticipation could therefore be explained by the dry climate of the new growing site, leading to a faster loss of water. Moreover, the early production of cones could be a response to plant stress; plants often react to stress by shortening flowering and maturation of fruit, in order to produce seeds for the survival of the species [48]. These data are very important for growers to let them know how hop cultivars react to a new growing condition.

Table 5. Morphological description of hop cultivars (Tettnanger, Smaragd and Cascade) grown in Tettnang and in Corsica. In bold are highlighted the morphological modifications.

Morphological Characteristics	TETTNANGER				SMARAGD				CASCADE			
	Tettnang		Corsica		Tettnang		Corsica		Tettnang		Corsica	
	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1
Main shoot: anthocyanin coloration	Weak	Weak	Weak	Absent	Strong	Strong	Strong	Absent	Medium	Medium	Medium	Absent
Leaf: size of blade	Small	Small	Small	Small	Small	Small	Small	Small	Medium	Medium	Medium	Medium
Leaf: blistering of upper side of blade	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Leaf: color of upper side of blade	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Leaf: if green, intensity of green color of the upper side of blade	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark
Time of flowering	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Early	Early	Early	Early
Plant: growth type	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Side shoot from the middle third of the plants: length	Long	Long	Long	Short	Medium	Medium	Medium	Short	Long	Long	Long	Medium
Side shoot from the upper third of the plants: Length	Medium	Medium	Medium	Short	Medium	Medium	Medium	Short	Long	Long	Long	Medium
Side shoot from the middle third of the plants: density of foliage	Dense	Dense	Dense	Dense	Sparse	Sparse	Sparse	Sparse	Medium	Medium	Medium	Medium
Side shoot from the middle third of the plants: number of cones per node	Few	Few	Few	Few	Few	Few	Few	Few	Medium	Medium	Medium	Medium
Side shoot from the middle third of the plants: total number of cones	Medium	Medium	Medium	Few	Medium	Medium	Medium	Few	Many	Many	Many	Medium
Side shoot from the upper third of the plants: total number of cones	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Many	Many	Many	Many

Table 5. Cont.

Morphological Characteristics	TETTNANGER				SMARAGD				CASCADE			
	Tettngang		Corsica		Tettngang		Corsica		Tettngang		Corsica	
	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1
Time of picking maturity of cones	Early	Early	Early	Early	Late	Late	Late	Late	Medium	Medium	Medium	Early
Cone: size	Small	Small	Small	Small	Small	Small	Small	Small	Large	Large	Large	Large
Cone: shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Med. ovate	Med. ovate	Med. ovate	Med. ovate
Cone: degree of opening of bracts	Open	Open	Open	Open	Open	Open	Open	Open	Closed	Closed	Closed	Closed
Cone: intensity of green color	Light	Light	Light	Light	Dark	Dark	Dark	Dark	Medium	Medium	Medium	Medium
Bract: size	Small	Small	Small	Small	Small	Small	Small	Small	Large	Large	Large	Large
Ratio length/width	Small	Small	Small	Small	Small	Small	Small	Small	Medium	Medium	Medium	Medium
Length of tip	Short	Short	Short	Short	Short	Short	Short	Short	Long	Long	Long	Long

Year 0 = first growing season; Year +1 = second growing season.

3.3. Essential Oils Chemical Characterizations

The essential oils chemical profiles of the German and Corsican hops were characterized. The GC/MS analysis shows a similarity between the wild hops from Corsica (Table 6), but the abundance of the four compounds shows variation, dependent on the location: myrcene, (E)- β -farnesene, α -humulene, and α -selinene.

Table 6. Essential oils composition of the wild Corsican hops by GC/MS.

Compounds	LRI ^a	RI ^a	RI ^P	Ajaccio	Oletta	Patrimonio	Corte
Hydrocarbonates							
β -Pinene	964	970	1110	0.3	0.4	0.1	0.3
Myrcene	981	976	1159	20.1	19.0	19.1	6.7
p-Cymene	1010	1011	1268	0.1	0.1	-	0.1
Limonene	1029	1020	1199	0.1	0.2	0.2	0.1
α -Ylangene	1373	1375	1476	0.3	0.1	0.8	-
α -Copaene	1381	1379	1488	-	0.3	0.1	0.1
Isocaryophyllene	1402	1407	1571	0.2	0.1	0.1	0.5
(E)- β -Caryophyllene	1414	1424	1591	8.2	10.0	13.0	8.8
β -Copaene	1432	1431	1581	1.1	0.1	0.4	0.1
(E)- α -Bergamotene	1432	1432	1580	1.4	0.1	0.2	0.2
(E)-β-Farnesene	1445	1448	1661	2.0	2.5	2.8	0.1
α-Humulene	1455	1456	1665	18.3	15.2	24.9	5.4
4.5-Di- <i>epi</i> -aristolochene	1469	1467	1665	1.0	0.5	0.5	1.2
γ -Muurolene	1477	1471	1681	1.7	-	2.9	-
γ -Himachalene	1479	1479	1693	0.2	3.6	-	1.4
β -Selinene	1485	1483	1712	6.7	10.0	2.6	26.8
Zingiberene	1493	1489	1717	11.7	11.6	5.8	1.6
Valencene	1495	1497	1719	3.1	-	1.5	-
α-Selinene	1505	1494	-	9.3	10.1	7.7	25.9
(E,E) α -Farnesene	1506	1498	1744	0.3	-	0.1	0.1
γ -Cadinene	1513	1507	1752	2.1	0.4	1.2	0.1
δ -Cadinene	1524	1516	1752	0.1	1.1	0.1	0.1
γ -Bisabolene	1529			1.9	2.3	0.2	0.1
α -Cadinene	1538	1535	1743	0.8	0.1	0.7	0.1
Alcohols							
Linalool	1087	1081	1544	0.3	0.1	0.4	0.6
α -Terpineol	1179	1179	1700	0.1	-	0.5	0.1
8-caryolanol	1562	1559	2044	0.1	0.1	0.2	0.2
Viridiflorol	1593	1591	2089	0.1	-	1.7	-
Humulol	1601	1588	2165	0.5	-	0.5	0.8
Zingiberenol 1	1614	1599	2109	0.3	0.1	0.3	0.1
Zingiberenol 2	1620	1613	2190	0.3	0.1	0.2	0.1
α -Cadinol	1642	1641	2231	0.7	1.2	1.2	2.2
Eudesm-11-en-4 α -ol	1651	1642	2241	0.3	2.3	1.1	3.1
α -Bisabolol	1680	1672	2217	0.6	-	0.3	0.1
Ketones							
2-nonanone	1091	1070	1388	0.4	0.4	-	0.1
2-decanone	1172	1176	1495	0.3	0.5	-	-
2-undecanone	1291	1273	1592	1.1	2.0	0.8	0.3
2-dodecanone	1371	1385	1711	0.1	0.3	0.1	0.2
2-tetradecanone	1576	1580	1909	0.4	0.1	0.2	0.3
(Z) 2-pentadec-6-enone	1652	1647		0.1	0.8	-	0.8
Aldehydes							
Nonanal	1084	1083	1394	0.2	0.9	0.7	0.1
Decanal	1184	1185	1498	0.1	0.1	0.1	-
Geranial	1242	1244	1731	0.2	0.1	0.3	0.1

Table 6. Cont.

Compounds	LRI ^a	RI ^a	RI ^P	Ajaccio	Oletta	Patrimonio	Corte
Esters							
2-methylbutyl isobutyrate	989	1004	1176	0.7	0.2	-	0.1
Methyl heptanoate	1006	1010		-	0.5	0.4	0.1
Methyl 6-methylheptanoate	1070	1068	1338	0.3	0.5	0.6	-
Methyl octanoate	1210	1205		-	0.1	0.2	0.1
Methyl nonanoate	1290	1290	1611	0.6	0.4	0.8	0.3
Methyl 4-decenoate				-	-	-	-
Methyl geraniate	1301	1301	1680	0.1	0.1	0.1	0.1
Other							
Caryophyllene oxide	1571	1576	1980	0.1	0.8	1.2	0.1
Humulene epoxide II	1602	1601	2044	0.1	1.0	0.1	0.8
Humulene epoxide III	1626	1626		0.2	0.1	0.9	0.1
Aromadendrene oxide	1650	1617	2002	-	0.8	1.5	0.1
Hexadecanoic acid	1961			0.2	0.3	0.2	0.1
Essential oil rate %				0.3	0.1	0.1	0.2

RI^a and RI^P: retention index on RTX-1 apolar column and RT-Wax polar column, respectively. LRI^a: retention indices reported from literature [49]. In bold, the compounds that show variation are highlighted.

The essential oil chemical profiles of Ajaccio, Oletta and Patrimonio are very similar. They contain respectively 20.1, 19.0, 19.1% of myrcene (characterized by spicy and balsamic herbal notes), 2.0, 2.5, 2.8% of (E)- β -farnesene (citrus herbal notes), 18.3, 15.2, 24.9% of α -humulene (woody notes), and 9.3, 10.1, 7.7% of α -selinene (herbal notes), showing especially a spicy citrus and woody character (Table 6). Instead, the essential oil of the Corte hop contains 6.7% of myrcene, 0.1% of (E)- β -farnesene, 5.4% of α -humulene, and 25.9% of α -selinene, thus it is characterized prevalently by herbal notes. This clustering of the Ajaccio, Oletta, and Patrimonio essential oils (Figure 2) could be explained by the specific environment conditions inherent to the coastal areas of Corsica, especially high temperatures, low rain content, medium to strong winds, and high salinity of soils (Table 1). The differentiation of the essential oil of Corte hops, grown in altitude and far from the coast, corroborates this view. The high presence of selinene in Corte genotypes, characterized by a humid environment, is in accord with the study of Patzak and coworkers [50], where European hops were found to be characterized by higher amounts of selinene compared to American hops. Moreover, this result is also in accord with the study of Mongelli and collaborators [51] on Italian wild and cultivated hops, where a correlation was found between high selinene presence and wild Italian hops.

The GC/MS analysis of the Tettngang cultivars and those transplanted in Corsica is shown in Appendix A. It reveals an evolution of the abundance, especially of four compounds from year 0 to year +1 after transplantation: β -pinene, α -humulene, zingiberene, and linalool.

The amount of these four compounds evolved in the same way for the three German hops grown in Corsica. The abundance of β -pinene (resinous and piney aroma) and linalool (floral, citrus, sweet notes) decrease after planting, respectively from 2.0% to 0.9%, and 1.0% to 0.3% of the essential oils for the Cascade hops; from 1.5% to 1.2%, and 1.5% to 0.5% for the Smaragd hops; and from 1.3% to 0.7%, and 1.5% to 0.4% for the Tettnganger hops. The abundance of α -humulene (woody notes), and zingiberene (spicy notes) increase after planting, respectively from 9.2% to 13.7%, and <0.1% to 5.1% of the essential oils for the Cascade hops; from 16.2% to 18.2%, and <0.1% to 7.1% for the Smaragd hops; and from 8.2% to 10.2%, and <0.1% to 7.9% for the Tettnganger hops. Similar results were found in a study [52] on the evaluation of the quality parameters of Cascade to the Sardinian environment; in the chemical composition of Sardinian Cascade oil was observed a low content in β -pinene and linalool, and a high percentage of α -humulene, especially in

the samples coming from the two coastal areas (Alghero and Orosei). As Sardinia is an island characterized by geographic and pedoclimatic conditions similar to Corsica, it is possible to assume a comparable oil composition and an analogous performance of the cultivars. This trend in the aromatic compound development highlights a modulation of the German hops to the Corsican coastal terroir and its environmental conditions. The increase in zingiberene, characterized by strong organoleptic properties and efficiency in hop aggressor repellence [53], is a beneficial consequence of the German hops' acclimatization. Moreover, the comparison of Table 6 and Appendix A shows that the abundance of these four compounds tends toward that of the hops growing wild in coastal areas of Corsica (on average 0.3% for the β -pinene, 19.5% for the α -humulene, 9.7% for the zingiberene, and 0.3% for the linalool). These results are supported by the dendrogram of the essential oil characterizations (Figure 2).

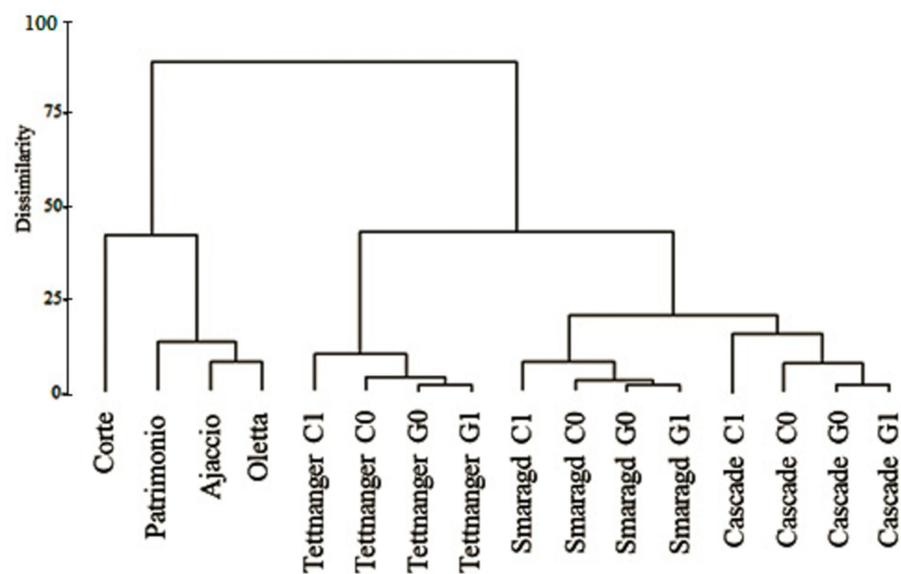


Figure 2. Dendrogram of German (G) and Corsican (C) hops essential oils. The numbers indicate the year of the plant age (0 = first growing season; 1 = second growing season).

Data of essential oils of German and Corsican (cultivars and wild) hops were used in the cluster analysis to characterize the hop samples (Figure 2). The resulted dendrogram indicates the presence of two clusters with 85% dissimilarity. The first cluster is characterized by the sole presence of wild Corsican hops, while the second cluster contains the studied cultivars. These data, according to a previous study on wild hops [24], demonstrated the discrimination power of the aromatic profile of hops. At the same time, these results are in partial disagreement with another study [13] where, among 75 Portuguese native hops studied, similarities were found between the aromatic profiles of 11 wild hops and 4 cultivars. As our results suggest, the essential oils of the accessions located in coastal areas of the island (Patrimonio, Ajaccio, Oletta) are similar (13% dissimilarity), unlike the one located in Corte far from the coast (40% dissimilarity). This discrimination of the Corte essential oil supports the genetic and the morphological analyses. As explained before, it is apparent that this isolation is caused by the peculiar environmental conditions of the area of Corte. In Figure 2, the cluster analysis shows one homogeneous cluster for each cultivar. While the essential oils of Smaragd and Cascade are close (20% dissimilarity), Tettmanger's oil presents more than 40% of dissimilarity with the other German hops. This difference corroborates the observations from the genetic analysis and can be explained by the peculiar aromatic profile of Tettmanger, which is considered one of the few noble aromatic cultivars [54]. An interesting result can be observed on the clustering of the cultivars grown in Corsica compared with those grown in Germany. The results indicate that after one year of cultivation in Corsica, all three analyzed cultivars differ markedly

(10% dissimilarity for Tettanager, 8% for Smaragd, 15% for Cascade); the same cultivars grown in Germany show almost identical essential oil contents (3% dissimilarity). These observations show that the terroir and the climatic conditions play a fundamental role on the quality performance of these cultivars.

4. Conclusions

The analysis of wild Corsican hops highlights the presence of interesting genotypes both from the genetic and aromatic point of view, as they possess a unique genetic profile and peculiar aromatic bouquet. It is possible to observe the apparent epigenetic influence of the territory on wild hops, as they show differences in morphological characters and, in particular, the anthocyanin coloration of the main shoot, presumably linked to the pedoclimatic conditions. The observation of the influence of the Corsican territory on three hop cultivars, from the morphological and aromatic point of view, allow us to confirm the adaptability of the German hops to the Corsican terroir after planting. In this study, we can recognize that the acclimatization to the new environment of hop cultivars is not immediate, but it is observable almost one year after planting. The variation among the profiles of the studied hop varieties concerns the increase of zingiberene and modifications in the content of α -humulene, β -pinene and linalool. Moreover, we can observe that German cultivars established in Patrimonio present some common features in morphological characters with the wild hops from coastal areas of Corsica (Oletta, Patrimonio, Ajaccio). The obtained results are important for future Corsican hop growers and for brewers. The investigation demonstrates the key role of the origin of the hop cultivars that they use and the importance of knowing the characteristics that the territory gives to the hop in order to produce beers with the desired aromatic character.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/app11093756/s1>.

Author Contributions: A.D.-W. contributed to the project development, performed the chemical and morphological analysis, and interpreted the results. M.R. contribute to the project development, performed the genetic analysis and interpreted the results. T.G. planned the project concept and contributed to the genetic analysis, statistical analysis and results interpretation. J.P. and J.C. contributed to the project development. A.D.-W., M.R., T.G., were involved in the first draft writing and final manuscript editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Essential oils composition of the German hops by GC/MS (Year 0 = first growing season; Year +1 = second growing season).

Compounds	Cascade				Smaragd				Tettninger			
	Tettngang		Corsica		Tettngang		Corsica		Tettngang		Corsica ²	
	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1
Hydrocarbonates												
β-Pinene	2.0	2.0	1.2	0.9	1.5	1.4	1.3	1.2	1.3	1.0	1.0	0.7
Myrcene	50.3	49.1	45.6	58.1	46.1	46.3	45.8	47.3	40.6	40.9	39.9	42.5
p-Cymene	0.3	0.1	0.2	0.3	1.1	1.1	1.1	1.1	0.7	0.7	0.8	0.7
Limonene	0.9	1.1	1.1	0.9	0.1	0.1	0.5	0.3	0.6	0.6	0.6	0.6
α-Ylangene	0.2	0.1	0.4	0.2	0.2	0.1	0.1	0.2	0.5	0.3	0.8	0.6
α-Copaene	-	-	-	-	-	-	-	-	-	-	-	-
Isocaryophyllene	-	-	0.1	-	-	-	0.4	0.2	-	-	-	-
(E)-β-Caryophyllene	4.8	4.3	7.7	6.4	6.1	6.0	7.2	6.5	5.7	4.7	6.1	5.5
β-Copaene	-	-	0.1	0.2	0.2	0.2	0.3	0.4	0.2	0.2	-	0.1
(E)-α-Bergamotene	0.4	0.7	0.3	-	0.3	0.2	0.4	-	0.8	0.9	0.9	0.7
(E)-β-Farnesene	6.8	6.6	8.2	3.9	4.1	4.5	3.9	4.0	20.2	20.8	21.2	19.3
α-Humulene	9.2	8.9	9.8	13.7	16.2	16.1	16.9	18.2	8.2	8.1	8.6	10.2
4.5-Di-epi-aristolochene	0.1	0.5	0.1	0.6	0.1	0.1	0.2	0.4	-	-	-	-
γ-Muurolene	0.2	0.1	0.8	0.1	0.2	0.2	0.5	0.3	0.9	0.7	1.2	0.7
γ-Himachalene	-	-	0.1	0.1	0.2	0.4	0.1	0.1	0.1	0.1	0.1	0.1
β-Selinene	1.2	1.3	1.5	1.2	5.1	5.9	4.9	5.1	0.6	0.7	0.7	0.5
Zingiberene	-	-	1.1	5.1	-	-	0.2	7.1	-	-	2.1	7.9
Valencene	1.1	0.8	2.1	1.2	0.5	0.3	0.7	0.5	0.1	0.1	-	0.1
α-Selinene	0.4	0.4	0.1	0.4	0.5	0.5	0.6	0.5	0.5	0.4	0.6	0.3
(E,E) α-Farnesene	0.5	0.4	0.1	0.1	0.1	0.3	0.1	-	-	-	-	-
γ-Cadinene	0.7	0.4	0.6	0.5	1.3	1.6	1.5	1.3	0.1	0.1	0.2	0.1
δ-Cadinene	-	-	1.2	0.9	0.1	0.1	0.1	0.9	0.1	0.1	-	0.1
γ-Bisabolene	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1
α-Cadinene	0.3	0.1	0.4	0.2	-	-	-	-	0.2	0.2	0.4	0.2
Alcohols												
Linalool	1.0	1.4	0.4	0.3	1.5	1.4	1.0	0.5	1.5	1.2	1.1	0.4
α-Terpineol	0.1	-	0.1	-	-	-	-	-	0.1	0.1	-	-
8-caryolanol	0.3	0.3	0.1	0.2	0.1	0.1	-	0.1	0.1	0.1	0.2	0.1
Viridiflorol	-	-	0.2	-	1.0	1.2	1.2	1.0	1.3	1.7	0.9	0.9
Humulol	-	-	0.1	0.2	-	-	-	0.1	-	-	-	-
Zingiberenol 1	-	-	0.2	0.5	-	-	0.2	0.9	-	-	0.5	1.2
Zingiberenol 2	0.1	0.1	0.1	-	0.1	0.1	0.2	0.1	-	-	-	-
α-Cadinol	0.4	0.3	1.7	0.7	0.1	0.2	0.1	0.2	0.2	0.2	0.3	0.1
Eudesm-11-en-4α-ol	0.1	0.2	1.1	0.1	-	-	-	-	0.2	-	0.5	0.1
α-Bisabolol	-	-	0.1	-	-	-	-	-	0.1	0.1	-	-
Ketones												
2-nonanone	0.1	0.2	0.1	-	0.1	0.1	0.2	0.1	0.1	0.2	-	-
2-decanone	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.5	0.4	0.2	0.1
2-undecanone	0.6	0.8	0.4	0.2	1.0	1.1	0.8	0.4	1.7	1.4	1.6	0.9
2-dodecanone	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
2-tetradecanone	0.2	0.1	0.1	-	0.3	0.3	0.5	-	0.1	0.1	0.2	0.1
(Z) 2-pentadec-6-enone	-	-	-	-	0.1	0.4	-	-	0.1	0.1	-	-

Table A1. Cont.

Compounds	Cascade				Smaragd				Tettninger			
	Tett nang		Corsica		Tett nang		Corsica		Tett nang		Corsica ²	
	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1	Year 0	Year +1
Aldehydes												
Nonanal	0.4	0.6	0.8	0.7	0.2	0.2	0.3	0.4	-	-	-	-
Decanal	-	-	0.1	0.1	-	-	-	-	0.1	-	0.1	0.1
Geranial	0.1	0.1	0.2	0.1	0.1	0.1	0.4	0.3	0.1	0.2	0.1	0.1
Esters												
2-methylbutyl isobutyrate	0.3	0.1	0.2	0.1	0.1	0.1	-	0.1	0.1	0.1	-	0.1
Methyl heptanoate	1.8	1.5	0.1	0.1	0.3	0.1	0.4	0.1	0.1	0.1	0.2	0.2
Methyl 6-methylheptanoate	0.2	0.5	0.2	0.1	0.5	0.6	0.8	0.5	0.6	0.4	0.7	0.7
Methyl octanoate	0.1	0.3	0.2	0.1	0.7	0.7	0.5	0.7	0.1	-	0.1	0.1
Methyl nonanoate	0.1	0.1	0.1	0.1	-	-	-	-	0.2	0.1	0.5	0.5
Methyl 4-decenoate	0.6	0.8	0.7	0.6	1.7	1.9	0.8	1.4	0.7	0.9	0.4	0.7
Methyl geraniate	0.3	0.1	0.4	0.6	0.5	0.4	0.4	0.7	0.2	0.2	-	0.1
Other												
Caryophyllene oxide	-	-	0.1	0.1	-	-	-	-	0.3	0.5	-	0.3
Humulene epoxide II	-	-	0.1	0.1	-	-	-	-	-	-	-	-
Humulene epoxide III	0.8	1.0	0.4	0.1	-	-	-	-	0.1	0.1	0.4	0.5
Aromadendrene oxide	0.1	0.1	0.1	0.1	-	-	-	-	-	-	-	-
Hexadecanoic acid	0.2	0.1	0.2	0.4	0.1	0.1	0.1	0.3	0.2	0.1	0.3	0.3
Essential oil rate %	1.0	1.0	1.0	1.0	1.2	1.2	1.9	1.2	0.3	0.3	0.3	0.4

Bold highlights the four components for which the contents seem to be influenced by transplantation.

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