A mitochondrial DNA SNP is maternally inherited in hops (*Humulus lupulus*, L.) and segregates varieties of the renowned Brewer's Gold female lineage from the others

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***Abstract*—** *The integration of molecular biology concepts and techniques in cultivated plant breeding programs allows defining more accurately the parents involved in crosses. The analysis of the DNA polymorphism in existing hop cultivars or wild types selected to generate new potent varieties is commonly used. Ribosomal DNA RFLP was the first to be studied in the middle of the 1990's and differentiated at least European from North American cultivated hops. The EcoRI rDNA RFLP has been established here for a selection of 31 H. lupulus plants, 17 of which are cultivars and the remaining 14 include wild types and other genotypes used for breeding. The sequence of the Magnum variety rDNA EcoRI fragment (4,957 bp) covering the intergenic spacer and corresponding to its North American heritage has been determined. As mitochondria are known to play a role in the biosynthesis of the valuable plant secondary metabolites, specially those based on isopentenyl pyrophosphate, the search for mtDNA polymorphism allowing a possible distinction among hops was engaged. After having established the 14,472 bp sequence of mtDNA encompassing the ATP synthase alpha subunit (509 aa), a 560 bp region 5' upstream of this gene allowed to identify a single nucleotide polymorphism. This SNP appeared to be maternally inherited in the lineage of Brewer's Gold, often used in crosses for its bittering properties. This work may stimulate researchers to explore further on the role of mitochondria in the biosynthesis of metabolites, mainly but not only isoprenoids, that make Humulus lupulus so interesting.*

Keywords*— *Cannabaceae, Cannabis sativa, Medicinal plant, Mitochondria, Terpenes.

I. INTRODUCTION

As revised in 2013 by Yang et al. [1], *Humulus* is one of the ten genera of the Cannabaceae family. The three Humulus species, whose cradle is Eastern Asia, are *H. japonicus*, Siebold & Zuccarini (or *H. scandens*, Loureiro), *H. lupulus*, Linné, known as hop, and *H. yunnanensis*, Hu.

H. lupulus, a dioecious diploid perennial plant was distributed primarily in Northern temperate parts of America, Asia and Europe. Its 2.57 Gbp [2] genome is composed of 20 chromosomes (2n=2x=20), of which one pair is recognised as sexual (XX for female and XY for male). Hops also exist as aneuploid, monoecious, triploid or tetraploid individuals [3]. The main use of hops since the Middle Age and up to now is as a "spice" in brewing, bringing bitterness, aroma and flavour to beer [4-6]. Only female plants are cultivated for their inflorescences, called cones (or strobili), which host lupulin glands containing the wanted specific resins. Their principal fraction, the α-acids (humulone), which is the traditional basis of the hop commercial value, contributes, along with β-acids (lupulone), to beer bitterness whereas its aroma and flavour are due to cone essential oils [6]. These remarkable metabolites are mostly synthesized from the isopentenyl pyrophosphate (IPP) by involving nuclear, plastidial and/or mitochondrial pathways [7, 8].

From germplasm collections, hosted in a few places dedicated to *H. lupulus* in producing countries, selected female and male individuals, used for controlled or open pollination, are requested in breeding programs to generate and establish genetically stable new cultivars. Once such a potent new variety is chosen for its properties, it is vegetatively propagated in glasshouses and the resulting developed plantlets are positioned in the prepared gardens for further growth. Two to four-year trials in various places allow to establish its characteristics by both qualitative and quantitative studies, including the ease to cultivate and harvest, the resistance to diseases, the crop yield, sensory evaluations and biochemical analyses. Parental plants used in breeding programs can be already existing varieties (mother plants) and males originating from previous crosses or wild type hops [9-11]. In hop gardens, and for each cultivated plant, up to 4 bines from annual shoots are fixed during Spring on a trellis to allow a fast growth with clockwise climbing. When flowers are harvested early in Autumn, and dependant of the

hop variety, the composition and relative richness of hop resins is determined following a traditional classification in two main groups: aroma and bittering. A third one, dual-purpose (meaning high α-acids content and valuable aroma properties) tends to become generally accepted mainly for commercial reason [12-14]. They rely on the tastes of hop flower teas and of the beer due to hop addition (either as dried cones, pellets or extracts) in the brewing process. This qualitative scoring is completed by quantitative biochemical analyses of a selection of hop secondary metabolites present in the thoroughly dried cones [15, 16].

In the last thirty years, several studies on genetic diversity among cultivated hop varieties and wild type hops have been published and used in breeding programs continuously generating numerous new registered varieties. In 2016, the production of 249 commercial cultivars, adapted for various pedoclimatic conditions and bred mostly to fit with market driven product requirements, was reported by the International Hop Growers' Convention (IHGC) [17]. Hop is produced in 30 countries with a total acreage for 2017 of about 55,000 ha. Germany and the USA, the two first producers, represented 2/3rd of the world production. Besides historical cultivation areas of the Northern hemisphere, the development of a hop business in Italy is also going on [18-20] and the Southern hemisphere contribution to the world hop production rely up to now on Argentina, Australia, New-Zeeland and South Africa [17]. Based on their knowledge accumulated over generations, hop breeders take advantages from the availability of fine biochemical analyses and of molecular biology technologies. Genetic distances based on various DNA polymorphisms, expressed sequence tags (ESTs) and other techniques generating vast number of data, allow the clustering of all available and future cultivars along with individuals from wild hop germplasm growing collections [21-26]. One of the very first published study showed that rDNA restriction fragment length polymorphism (RFLP) was able to segregate hops according to their geographical origins [27]. More recently, genomic and plastidial DNA tags identified accurately the continental origins of hops allowing new breeding program strategies [25, 28]. A still current aim of breeding programs is to combine in one variety both aromatic and bitter properties, with high yield, disease resistance and easy crop. Such hunted dual-purpose varieties can preserve aromatic properties without reducing the α-acid contents and the targeted beer quality. Strong efforts have been engaged to find the suitable father plant able to bring bittering, respectively aroma, components in an aroma, respectively bitter, mother variety without altering it first qualification and so to generate suitable F1 plant populations expected to contain future and competitive new variety candidates. Searches for useful quantitative trait loci (QTL) have shown to be highly complex as described in the remarkable work of McAdam et al. published in 2013 [29]. In this reference, unfortunately, no mention about plastid or mitochondrial genome contributions can be read.

With respect to cell energy (ATP production) and metabolism, the fundamental role of mitochondria is recognised [30-33]. The angiosperm mitochondrial genome size and organization differ considerably from one taxa to another and ranges from around 200 to at least 2,500 kbp [34]. The available hop mtDNA assembled sequence of 336,811 bp [2] is positioned at the lower size range. Although maternal inheritance in plants is predominant, biparental mitochondrial transmission have been reported in various taxa [35, 36]. The data published by Clark et al. [8] and the progress in mtDNA sequencing [2] encourage to overcome possible difficulties due to both size and mode of parental transmission to progeny of mitochondrial genome and to dynamize its study in hops which has so far been timorous.

Here, within the 14,472 bp mitochondrial sequence of the Strisselspalt hop variety, a 560 bp region was selected at the 5' end of the gene encoding the α subunit of ATP synthase (EC 3.6.1.14), highly conserved among plants [37-39]. It contains a single nucleotide polymorphism acting as a signature that splits in two groups the 31 selected plants used in a still running hop breeding program and raises the question of maternally inherited mitochondrial genome in hops.

II. MATERIALS AND METHODS

Plant samples, either fresh, dried or lyophilized, were processed upon receipt as follows. Fresh leaves and cones were obtained from growers in various gardens in Alsace (France) within a continuous breeding program established in 2001 and supported by the Cophoudal – Comptoir Agricole (Hochfelden, France). Wild type samples were collected by the authors along the Bruche river near the town of Molsheim (France). Fresh collected samples were placed immediately in a cool box at 4-6 °C and, once in the lab, entered the dedicated DNA extraction procedure or were dropped 2 min in liquid nitrogen and then packaged for storage at – 80°C. Dried or lyophilized plant samples were ground and processed directly with the appropriate protocol depending upon the aim of the experiment. Samples from some of the parental plants used in part of the program were provided, with their code numbers and filiations, by Dr Peter Darby (Wye Hop Ltd, UK). The identity and filiations of all the plants used in this study were gathered from various sources [12-14], checked for consistency and summarized in Table 1.

Female < *Humulus lupulus* > Male

FIGURE 1: PHOTOGRAPHS OF FLOWERING FEMALE AND MALE HOPS.

All molecular biology protocols used unless otherwise indicated were from Ausubel et al. [40]. Enzymes, chemicals, oligonucleotides (primers) and/or materials, depending on public market constraints, were delivered to the laboratory by various companies.

Mitochondrial DNA (mtDNA) was isolated from fresh leaves of the Strisselspalt hop variety following the protocol of Maureen et al. [41]. This mtDNA preparation served for primer walking experiments. For all other experiments, total DNA was purified from hop samples using the Nucleospin Plant kit (Macherey-Nagel, Germany). PCR were performed with high-fidelity polymerases and processed with protocols optimized for each thermal cycler used. PCR products (native and/or after their restriction endonuclease digestion) less than 2 kbp long were analyzed by 2 % w/v agarose gel electrophoresis in Tris-Borate-EDTA buffer and either Gene Ruler Ladder Mix or Range Ruler 50 bp DNA Ladder (Thermo Fisher, USA) was chosen.

DNA sequencing, carried on both strands twice for each purified PCR product, was generated by an ABI Prism sequencer. In some cases, PCR products were inserted into appropriate plasmid vectors. Recombinant DNA from positive clones were purified from 1 mL cultures with NucleoSpin Plasmid QuickPure (Macherey-Nagel, Germany) and standard T3 and T7 primers were then used in sequencing. Sequence analysis and assembly were achieved by using the online software platform at the National Center for Biotechnology Information in the USA [42].

For Southern blot hybridization, probes were generated by random primed DNA labelling with Digoxigenin-dUTP (DIG DNA Labelling Kit) followed by luminescent signal detection (DIG Luminescent Detection Kit). Both kits were from Roche (Germany). The Digoxigenin labelled probe used for the rDNA EcoRI fragment length polymorphism study corresponded to the 819 bp fragment from position 5072 to 5890 of sequence AF223066 (GenBank). The DNA Molecular Weight Marker VII, Digoxigenin-labelled (Roche, Germany) was chosen for 1 or 1.2 % w/v agarose gel electrophoresis. The unlabelled DNA Marker VII (Roche, Germany) or the Gene Ruler Ladder Mix (Thermo Fisher, USA) was used in electrophoresis without subsequent Southern blotting.

III. RESULTS AND DISCUSSION

Linked to the breeding program initiated in 2001 in Alsace (France) and aiming at generating hop varieties with an aroma profile like that of the starting Strisselspalt variety and an elevated content in α-acids, a subset of 31 samples (known cultivars including Strisselspalt as reference, unknown encoded plants and two wild types, one of both sexes) was used in this study (Fig. 1 and Table 1).

TABLE 1

IDENTITIES, ORIGINS, DATES OF RELEASE AND TYPES OF THE *H. LUPULUS* USED.

Hop identity §	Pedigree/ Origin	Date of release	Type#
Admiral	Derived from Northdown and Challenger breeding lines	1996	B
Columbus	Unknown.	1970's	B
Elsaesser	An old landrace (Alsace, France) distinct from Strisselspalt.	Na	A
Glacier	Progeny of Elsaesser (mother lineage), Northern Brewer and Brewer's Gold (Washington State University, USA).	2000	A
Saphir	Cross of 83/17/20 x 80/56/6 (Hüll, Hallertau, Germany).	2002	A
Select	76/18/80 x 71/16/7 (derived from Hallertauer Mittelfrüh and Spalt, one of the world oldest hop varieties, Hüll, Hallertau, Germany).	1993	A
Strisselspalt	First reported in 1885 and probably issued from a cross involving a Spalt mother (Alsace, France).	Na	A
Strisselspalt 4X	Generated for the breeding program in 2001/2002.	Na	Na
Target	Lineage of Northern Brewer and Eastwell Golding.	1972	B
Tradition	Cross between Hallertauer Gold and male 75/15/106 (Hüll, Hallertau, Germany).	1993	A
Whitbread Golding variety	Bates Brewer – open pollination in 1911; WGV name given in 1920 (England).	1953	A
Wild type F	Wild female ecotype (Alsace, France).	Na	Na
Wild type M	Wild male ecotype (Alsace, France).		
TB104	Progeny of Pilgrim used as mother crossed with a male having Challenger as grandmother.	*	Na
TC61	Progeny of the mother 25/91/12 having Yeoman in its mother lineage and Olympic in its father one (3/4 of Brewer's Gold) as grandmother and crossed with a male having Bramling Cross as one of its grandmother.		
TC132	Same mother as for TC61 but crossed with a male having as grandmothers Yeoman and the same grandmother as Pilgrim and Admiral as mother.		
TC169			
TD161	Progeny of a male having Yeoman and Olympic (3/4 of Brewer's Gold) as grandmothers and crossed with a female having the same grandmother as Pilgrim.		
2/82/12	Progeny of Challenger.		
46/01/02	Progeny of Pilgrim as a mother in an open pollination.		
48/02/09	Progeny of Hallertau INT32.		
48/02/14			
54/97/01	From an open pollination of Early Bird, a Goldings aroma hop, discovered in 1887 at Wye (Kent, England).		
318/01/10	Male issued form crosses using Strisselspalt as mother plant with a male issued from Mathon x wild type male.		
Brewer's Gold	Wild female named BB1 isolated in Morden (Manitoba, Canada) x wild male (Kent, England) in 1919.	1934	B
Horizon	Half Sister of Nugget (lineage of Brewer's Gold and Early Green, bred at Oregon State University (USA).	1997	B
Magnum	Galena x male 75/5/3 (Hüll, Hallertau, Germany) (Galena released in 1978 is a daughter of Brewer's Gold in an open pollination).	1993	B
Merkur	Magnum x male 81/8/13 (Hüll, Hallertau, Germany).	2001**	B
Millenium 47	Daughter of a tetraploid Nugget (a granddaughter of Brewer's Gold) (Yakima, WA USA). Two isolates used (47 & 52)	2000	B
Millenium 52			
Nugget	Daughter of Brewer's Gold (Oregon State University, USA).	1983	B

§: Female plants are in bold; #: A = aroma, B = bittering; Na: not applicable;

****: From various breeding programs (Peter Darby personal communication); **: registered***

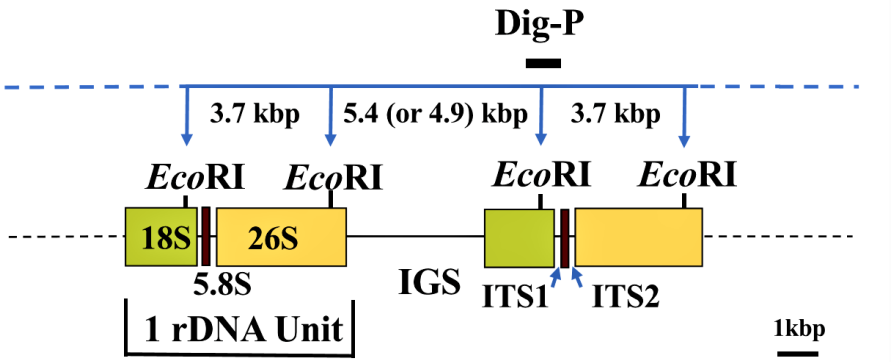

FIGURE 2: MAP OF THE HOP rDNA TANDEM REPEAT ORGANIZATION SHOWING TWO rDNA UNITS, THE ECORI SITES AND CORRESPONDING FRAGMENT SIZES (IGS: INTERGENIC SPACER, ITS: INTERNAL TRANSCRIBED SPACER). Note the position and extent of the Digoxigenin-labelled probe (Dig-P).

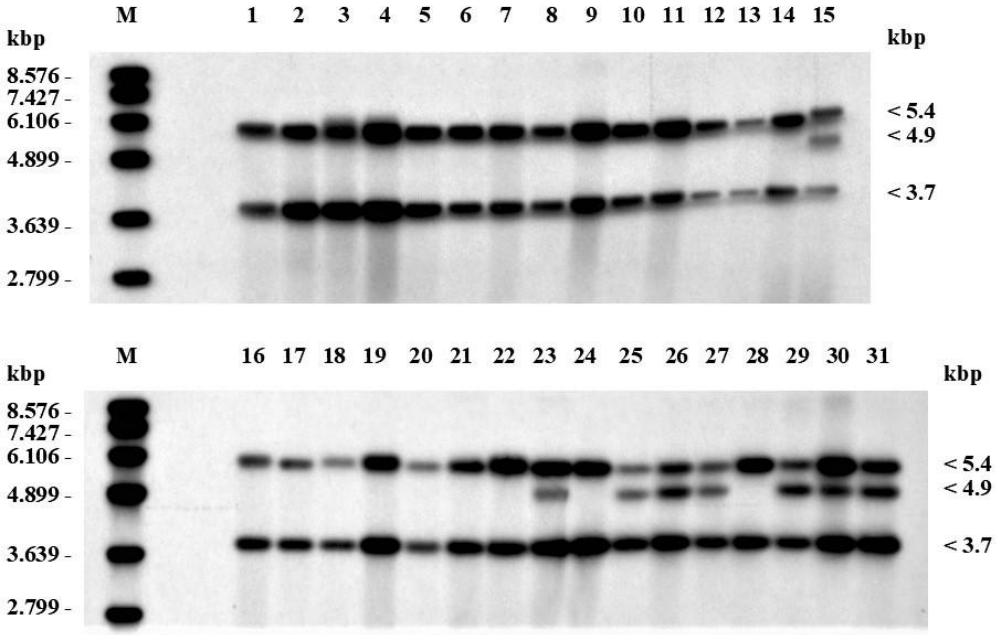

FIGURE 3: RESULTS OF SOUTHERN BLOT HYBRIDIZATION OF ECORI-DIGESTED HOP TOTAL DNA.

Samples of about 10 µg of digested DNA corresponding to the different varieties (lanes 1-15 upper panel and 16-31 lower panel) and the Dig-labelled DNA marker VII (lane M, sizes in kbp on the left) were loaded on 1% agarose gel. After electrophoresis, the Southern blot hybridization and detection procedure was followed using a Dig-labelled rDNA probe. The specific sizes of the hybridized rDNA fragments are indicated (in kbp on the right). The samples were (lane-name): 1-Admiral, 2-Columbus, 3-Elsaesser, 4-Glacier, 5-Saphir, 6-Select, 7-Strisselspalt, 8-Strisselspalt tetraploid, 9-Target, 10-Tradition, 11-WGV, 12-Wild type female, 13-Wild type male, 14-TB104, 15-TC61, 16-TC132, 17-TC169, 18-TD161, 19-2/82/12, 20-46/01/02, 21-48/02/09, 22-48/02/14, 23-54/97/01, 24-318/01/10, 25-Brewer's Gold, 26-Horizon, 27-Magnum, 28-Merkur, 29-Millenium 47, 30-Millenium 52, 31-Nugget.

A first view of the distribution of this selection in terms of rDNA restriction polymorphism profiles was obtained. A Southern blot hybridization of EcoRI total DNA digestions of the 31 plant samples with the Dig-labelled Strisselspalt rDNA probe (819 bp) covering the EcoRI site of the 18S sequence gave typical results. The presence of two types of rDNA RFLP was revealed with either two hybridized fragments (profile R1: 5.4 and 3.7 kbp) for European and Asian type hops or three (profile R2: 5.4, 4.9 and 3.7 kbp) in the case of varieties containing rDNA inherited from North American cultivated hop (Fig. 2 & 3 and Table 2). The sequence length divergence occurs in the IGS part of the rDNA repeat organisation.

The sequence of the specific 4.9 kbp EcoRI rDNA fragment was determined for Magnum (GenBank accession number MH205730). It may serve in the understanding of the various IGS sequence rearrangements in the *Humulus* genera and completes previous contributions [27, 44]. The results highlight the relevant case of Merkur (see lane 28 in Fig. 3, and Table 2). This bittering hop variety inherited via its Magnum mother plant some characteristics of Brewer's Gold, but the rDNA

polymorphism indicates that the component of North American cultivated hops disappeared as a consequence of the cross. This is in accordance with the conclusion of Pillay & Kenny [27] indicating that "Ribosomal DNA length phenotypes showed simple Mendelian inheritance in hop".

Although Merkur is a bittering hop, it can be seen on web sites from various companies commercializing hops, that there is an increased tendency to requalify some varieties from bittering to dual-purpose type, which is the case for Merkur, probably because the race aiming only at higher α-acids cone content shifted towards other goals. Among these, already targeted since decades, are the selection of disease resistant hops, improvement of aroma and flavour, drought tolerance with an easier cultivation and post-harvest production processes. As well as for the hop resin synthesis [8], many properties rely on the plant metabolism which involves mitochondrial activity [45].

To start the search for hop mitochondrial genome polymorphism information, the hop mtATPase was used to anchor the study. A 166 bp sequence was identified in 1999 by sequencing a mtDNA fragment generated by PCR using degenerated primers deduced from alignment of the 17 sequences (mostly partial) of plant mitochondrial ATPase α subunit available at that time (our unpublished report). From this sequence a primer walking strategy in both direction was developed. A contig of 14,472 bp hop mtDNA was obtained for the Strisselspalt variety (Genbank access number: MH016574). It encompasses the mtATPase α subunit gene (position 9090 – 10619). The deduced 509 aa protein sequence (calculated MW of 55,337 Da) is identical except one amino acid (position 485: I versus V) to that of *Cannabis sativa* (GenBank KU363807.1).

This sequence will contribute to the accuracy of the available hop mitochondrial sequences in public databases because all of them showed unproperly assembled sequences in this specific region. This is the reason why, on the contrary of all plant ATPAse α subunits that gave at least 95 % identities using a BlastX search, no high-quality match with *H. lupulus* sequence data in either hopbase.org or GenBank [42] was obtained prior to our present contribution.

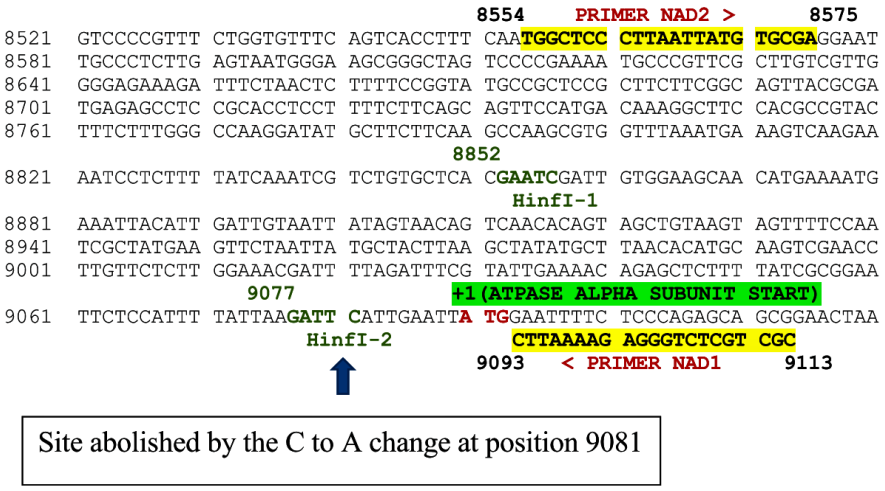

FIGURE 4: PARTIAL STRISSELSPALT HOP MTDNA SEQUENCE FROM THE 14,472 BP REGION (GENBANK MH016574). The primers NAD1 and NAD2 used for PCR, the translation start (+1, ATG) of the ATPase α subunit coding gene and the HinfI sites are positioned.

The sequence 5' upstream of the mtATPase α subunit gene diverged rapidly from that of *C. sativa*. So, it was interesting to look if any variability occurred within a maximum 600 bp region ahead of the highly conserved gene sequence among the 31 selected hop genotypes used in this study. This limited short size fitted to practical conditions for further easy analysis, which could be achieved in a one-work day experiment starting with a minimum sample size (about 1 g) thus preserving the plant and therefore acting as a non-destructive assay. This size was also chosen for convenience in sequencing. The search for an informative potent polymorphic restriction site resulted in the choice of HinfI, recognizing the sequence 5' – G/ANTC – 3', present twice in the Strisselspalt mtDNA explored region (HinfI-1 and HinfI-2, Fig. 4). Two primers NAD 1 and NAD2 were designed (Fig.4). Used in PCR assays the expected fragment of 560 bp was generated. The size was identical for all genotypes tested during this study. The comparison and analysis of the sequences from 4 hop varieties (Brewer's Gold, Nugget, Select and Strisselspalt) revealed an HinfI restriction polymorphism to be further investigated (Fig.4). The electropherogram of the two typical HinfI profiles is presented with a slight sample overload to see the 36 bp fragment (Fig.5).

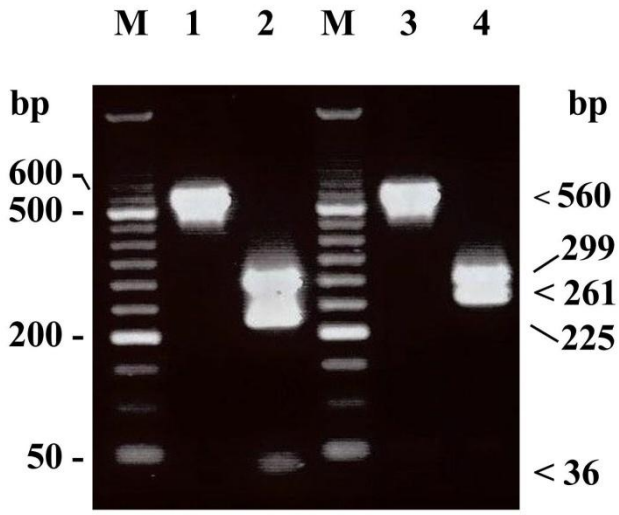

FIGURE 5: ELECTROPHEROGRAM (2% AGAROSE GEL) OF THE FRAGMENT PRODUCED BY PCR USING PRIMERS NAD1 AND NAD2 ON HOP MTDNA FOR STRISSELSPALT (LANE 1 UNCUT & LANE 2 HINFI CUT) AND FOR MAGNUM (LANE 3 UNCUT & LANE 4 HINFI CUT). The Range Ruler 50 bp DNA Ladder was used as marker (lane M, sizes in bp). The 560 bp amplified fragment is either cut twice generating three bands (299 + 225 + 36 bp, lane 2) or once, yielding two bands (299 + 261 bp, lane 4).

The 560 bp PCR product for each of the remaining 27 plants used in this study was sequenced and the HinfI polymorphism checked by agarose gel electrophoresis (data not shown). The summarized results (Table 2) indicate clearly that this polymorphism of the chosen 5' upstream sequence of the hop mitochondrial ATPase α subunit gene split the 31 genotypes used here in two groups (profiles H1 and H2).

Coming back to the filiation of the plants used (Table1), the prevalence of Brewer's Gold came as an evidence. The question was then to give sense to the potent maternal transmission of this mitochondrial single nucleotide polymorphism (C/A at position 9081, see Fig. 4) as a signature of the original mother plant which originated from North America, as confirmed by its rDNA profile and by the known history of the English hop varieties parentage [10, 43]. Ernest Stanley Salmon at Wye College (Kent, UK) used a wild hop collected at Morden (Manitoba, Canada) as a mother plant (designed BB1) in an open pollination (with English wild type males) to generate, in 1919, the successful Brewer's Gold variety [10, 43]. One sister is Bullion and one of its brother was used to generate the Bramling Cross in 1927. The BB1 genetic background, seen as a founder, spread through various breeding programs in an abundant and rich progeny although its genome part was therefore diluted cross after cross. This parentage including many of the varieties issued from Wye (Table 1) until mid-1980s, was broader and famous names can be cited such as Nugget, Galena, Northern Brewers, Magnum, Horizon, Millenium, Target, Challenger, Northdown, Saxon, Viking, Alliance as well as Glacier [10, 43].

Maternal inheritance of mtDNA is probably the common way in *H. lupulus*. The single nucleotide polymorphism revealed in this study showed to be strictly conserved over years and through all breeding programs in which the female plant is the daughter of a mother plant harbouring this mtDNA signature. In crosses involving female plants showing a H1 mtDNA profile with H2 mtDNA males, their progeny showed H1 profile (see Glacier as an example for cultivars and TC61, TC132, TC169 and TD161 for other breeding plants, Table 1 and Table 2). Considering all the information presented in the 2 tables, Merkur shows the interesting case of the inherited mother H2 mtDNA profile of Brewer's Gold (and most probably BB1) brought by its daughter Magnum, and the rDNA R1 profile of European hops resulting from the exclusion of the specific rDNA sequences from North American cultivated hops still present in Magnum.

This mtDNA SNP may shed a new light on strategic breeding approaches opening yet unexplored routes for introducing more cellular work, allowing cytoplasmic hybrid (cybrid) formation followed by plant regeneration [46,47].

TABLE 2
POLYMORPHIC PROFILES SUMMARIZED FOR rDNA AND mtDNA

<table>
<tr><th rowspan="2">Hop identity §</th><th rowspan="2">Type #</th><th>rDNA as target</th><th>mtDNA as target</th></tr>
<tr><th>Genomic DNA/ EcoRI digestion & probe hybridization*</th><th>PCR 5'of ATPase α-Subunit / HinfI digestion**</th></tr>
<tr><td>Admiral</td><td>B</td><td rowspan="11">R1</td><td rowspan="23">H1</td></tr>
<tr><td>Columbus</td><td>B</td></tr>
<tr><td>Elsaesser</td><td>A</td></tr>
<tr><td>Glacier</td><td>A</td></tr>
<tr><td>Saphir</td><td>A</td></tr>
<tr><td>Select</td><td>A</td></tr>
<tr><td>Strisselspalt</td><td>A</td></tr>
<tr><td>Strisselspalt 4X</td><td>Na</td></tr>
<tr><td>Target</td><td>A</td></tr>
<tr><td>Tradition</td><td>A</td></tr>
<tr><td>WGV</td><td>A</td></tr>
<tr><td>Wild type F</td><td rowspan="12">Na</td></tr>
<tr><td>Wild type M</td></tr>
<tr><td>TB104</td></tr>
<tr><td>TC61</td><td>R2</td></tr>
<tr><td>TC132</td><td rowspan="6">R1</td></tr>
<tr><td>TC169</td></tr>
<tr><td>TD161</td></tr>
<tr><td>2/82/12</td></tr>
<tr><td>46/01/02</td></tr>
<tr><td>48/02/09</td></tr>
<tr><td>48/02/14</td><td rowspan="3">R2</td></tr>
<tr><td>54/97/01</td></tr>
<tr><td>318/01/10</td><td>R1</td></tr>
<tr><td>Brewers Gold</td><td>B</td><td rowspan="3">R2</td><td rowspan="6">H2</td></tr>
<tr><td>Horizon</td><td>B</td></tr>
<tr><td>Magnum</td><td>B</td></tr>
<tr><td>Merkur</td><td>B</td><td>R1</td></tr>
<tr><td>Millenium 47</td><td rowspan="2">B</td><td rowspan="3">R2</td></tr>
<tr><td>Millenium 52</td></tr>
<tr><td>Nugget</td><td>B</td></tr>
</table>

§: Female are in bold; #: A= aroma, B = bittering and Na = not applicable;
**** Number of EcoRI hybridized fragments: two for R1 (5.4 + 3.7 kbp) and three for R2 (5.4 + 4.9 + 3.7 kbp).***
***** Number of HinfI fragments: three for H1 (299 + 225 +36 bp) and two for H2 (299 + 261 bp).***

Many attempts to focus on the key genes in the synthesis pathway of the valuable secondary metabolism to set up breeding programs seem to have only partly succeeded up to now [48]. In the search for QTL, McAdam et al. made great progress in the understanding of the pleiotropic nature of hop secondary metabolite synthesis [29] and mentioned the possible action of still unknown regulatory elements. As seen for other regulated gene expression, allostery [49] is probably involved in the crosstalk between nuclei, plastid and/or mitochondria in the case of the synthesis flow based on IPP [8, 50-52]. The mitochondria contribution must be further investigated considering the importance of the chloroplast too. Understanding how gene expression and protein activities are regulated in hop specific pathways, either by biotic or abiotic factors, will allow enhancement of expected secondary metabolite biosynthesis. This point of view is reinforced by the fact that *H. lupulus* laborious cultivation is balanced by its richness in substances of remarkable properties from which only a few have been extensively studied yet [53, 54]. Knowing also that the amount of hop products used in beer making dropped drastically since the 1970's, there might be an opportunity for developing *H. lupulus* as a medicinal plant and re-boosting its production with new cultivation areas [18-20, 55, 56]. This possibility, extended to the other species of the *Humulus* genus, will reinforce the research interests in Cannabaceae almost solely driven by *Cannabis sativa.*

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