Comparison of Sequence of \textit{rbcL} and Non-coding Regions of Chloroplast DNA and ITS2 Region of \textit{rDNA} in Genus \textit{Humulus}

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The divergence among hop cultivars, as well as wild hops (\textit{Humulus lupulus} var. \textit{lupulus}, \textit{neomexicanus} and \textit{cordifolius}) and related species (\textit{H. japonicus}) was studied using the sequence difference of the partial \textit{rbcL} fragment, the noncoding regions, the \textit{trnL} intron and spacer between the \textit{trnL} 3' exon and \textit{trnF}, of chloroplast DNA and the ITS2, one of the nuclear ribosomal internal transcribed spacer regions. There were no differences within the varieties of \textit{H. lupulus} and within \textit{H. japonicus}. A few nucleotide substitutions were observed between the varieties of \textit{H. lupulus} in the 1260 bp nucleotide sequence of \textit{rbcL} or the 853 bp total of the noncoding regions of chloroplast DNA. There were no nucleotide changes in the 232 bp ITS2, even though this region showed a higher substitution rate when \textit{H. lupulus} and \textit{H. japonicus} were compared. \textit{H. japonicus} showed 13 substitutions in \textit{rbcL}, 9 in the noncoding regions and 24 in the ITS2 region compared with \textit{H. lupulus} var. \textit{lupulus}. Synonymous substitution at the 3rd codon position of \textit{rbcL} enabled to estimate the divergence time of the two species at 3.74 million years ago. This study which revealed a low variability within \textit{H. lupulus} suggests that variation, which could be utilized in hop breeding is limited.

**Key Words:** cpDNA, diversity, evolution, hop, \textit{Humulus}, ITS2, \textit{rbcL}.

\textbf{Introduction}

The hop species, \textit{Humulus lupulus} L., which belongs to the family Cannabaceae, Urticales, is a dioecious and perennial plant. Only the flowers of the female hop are used for brewing beer, to which they impart bitterness and flavor. Wild hops are indigenous to the Northern hemisphere, and records of commercial cultivation date back to the last thousand years (Burgess 1964). Classical European hop cultivars such as Saazer, Tettnangger and Hallertauer seem to be descendants of hops indigenous to each production area, Zatec in the Czech Republic, Tettnang and Hallertau in Germany. The genus \textit{Humulus} consists of \textit{H. lupulus}, \textit{H. japonicus} and \textit{H. yunnanensis} (Neve 1991). \textit{H. yunnanensis} was only reported by Small in 1978. It is generally recognized that the genus \textit{Humulus} (Allaby 1998) consists of only two species, \textit{H. lupulus} and \textit{H. japonicus}. \textit{H. lupulus} includes the European cultivated hop, \textit{H. lupulus} var. \textit{lupulus}, Japanese wild hop, \textit{H. lupulus} var. \textit{cordifolius}, and North American wild hops, \textit{H. lupulus} var. \textit{neomexicanus}, \textit{H. lupulus} var. \textit{pubescens} and \textit{H. lupulus} var. \textit{lupuloides}. However, the distinction between the latter North American hops is unclear (Neve 1991), and in this report only \textit{H. lupulus} var. \textit{neomexicanus} was used to represent North American wild hops. \textit{H. japonicus}, the related annual species, resembles hops morphologically, but it has a different chromosome number, 2n = 17 and 2n = 16 in male and female \textit{H. japonicus}, respectively, compared with 2n = 20 in \textit{H. lupulus} (Neve 1991), resulting in cross incompatibility (Small 1978).

Genetic variation within the genus \textit{Humulus} is poorly documented. Pillay and Kenny (1994, 1996) reported a difference in the restriction digested fragment within \textit{H. lupulus} and between \textit{H. lupulus} and \textit{H. japonicus}. The intergenic spacer region of \textit{rDNA} showed a variation in length among wild and cultivated hops and one chloroplast restriction site with \textit{PstI} was different between \textit{H. lupulus} and \textit{H. japonicus}. Almost no reports are available on the relationship within \textit{H. lupulus} based on DNA sequence differences. In this paper, we described DNA sequence differences within the genus \textit{Humulus}, and analyzed their evolutionary aspect.

The nucleotide substitution rate in many genes and noncoding DNA regions is known to behave like a molecular clock which is correlated with the length of the evolutionary time and shows an approximately constant rate defined by the kind of genes and regions analyzed, among a wide range of species (Kimura 1986, Nei 1990). The substitution of the 3rd codon position in the coding region is more useful than that of the 1st and 2nd positions in the construction of a phylogenetic tree (Manhart 1994). \textit{rbcL} also shows the same clock-like behavior, although the rate is not necessarily constant among species, especially between perennial and annual taxa (Clegg et al. 1994, Daughjberg and Anderson 1997, Frascaria et al. 1993, Manen et al. 1998, Wilson et al. 1990). Furthermore, the conservative property of the \textit{rbcL} fragment which prevents nucleotide substitution makes it difficult to analyze closely related species (Gielly...
and Taberlet et al. (1994). Taberlet et al. (1991) reported that the trnL intron and spacer between the trnL 3’ exon and trnF of cpDNA accumulate substitutions at a higher rate than that of rbcL, and that these noncoding regions may enable to resolve the conservation problem when using rbcL. Nuclear ribosomal internal transcribed spacer (ITS) regions display high rates of substitution compared to cpDNA and they are useful in the generic and species level relationship in plants (Baldwin et al. 1995).

The objectives of this report were to determine the diversity within the genus *Humulus* at the molecular level using the sequence of *rbcL* and noncoding region on cpDNA and ITS2, one of the ITS regions between 5.8 s and 26 s rDNA. The species differentiation within the genus *Humulus* and their evolutionary divergence were elucidated.

**Materials and Methods**

**Plant materials and DNA extraction**

The hop cultivars (*H. lupulus* var. *lupulus* and *neomexicanus*), the wild hop species (*H. lupulus* var. *neomexicanus* and *cordifolius*), and their related species (*H. japonicus*), used in the experiments are listed in Table 1. Some hop cultivars were used for commercial brewing at Kirin, while others were obtained from hop dealers. The North American wild hop, USDA 60016, which was imported from the USA in 1972 is being preserved in the Kirin hop breeding experimental field. Japanese wild hops No. 1 and No. 2 were provided by courtesy of Dr. Sano of Hiroasaki University. *H. japonicus* No. 1 through No. 3 were collected in Yokohama city in 1998.

Total DNA was isolated from hop pellets by the cetyltrimethylammonium bromide (CTAB) method or from fresh leaves using the Isoplant DNA extraction kit (Nippon Gene, Japan). The procedure was described in detail previously (Murakami 1998).

**Sequencing and analysis of the partial rbcL fragment, trnL intron and spacer between trnL 3’ exon and trnF and ITS2 region**

Primers for amplification and sequencing were designed by Hasebe et al. (1994) for rbcL, by Taberlet et al. (1991) for the trnL intron and spacer between the trnL 3’ exon and trnF and by Gigliano (1998) for ITS2. A partial rbcL fragment was amplified by PCR (Murakami 1998) with primers rbcL1 and L4 (Table 2). A single product was obtained by PCR using a total volume of 100 μL, a temperature of 65°C for annealing and 30 cycles for amplification. The PCR products were purified using a High Pure PCR Products Purification kit (Boehringer Mannheim), followed by dRhodamine Terminator Cycle Sequencing Kit (PE Applied Biosystems) with primers rbcL1 through L4, respectively. The 1260 bp sequence, which encompasses position 31 from the start codon to position 1290, was analyzed using the ABI PRISM™ 310 Genetic Analyzer (PE Applied Biosystems). The DNA fragment including the trnL intron and spacer between the trnL 3’ exon and trnF was amplified with primers INT1 and SP2 (Table 2). PCR and sequencing were performed under the same conditions as those for rbcL, except that annealing was carried out at 55°C. The 541 bp of the trnL intron sequence and the 312 bp of the spacer sequence between the trnL 3’ exon and trnF were determined using primers, INT1 and INT2, and SP1 and SP2, respectively. Table 2 also shows the primers ITS2F and ITS2R for the ITS 2 region (Gigliano 1998). Amplification and the sequencing were performed by the same procedures as those used for rbcL.

Multiple alignment and homology of the sequences were analyzed using DNASIS-Mac (Hitachi Software Engineering).

For the estimation of nucleotide substitution, Kimura’s two-parameter method (Kimura 1986) was used to estimate the average nucleotide substitution per site (K) and the synonymous fraction of the third codon position of the rbcL fragment was calculated as Ks with a standard error.

Nucleotide diversity, measurement of DNA polymorphism (Li 1997), were also calculated within *H. lupulus*.

**Results**

Sequences of the partial rbcL fragment, and the noncoding region, the trnL intron and the spacer between the trnL 3’ exon and trnF of cpDNA and the ITS2 region of the hop cultivars, wild hops and *H. japonicus* were registered in DDBJ as accesses No. AB033761 and AB033889 through AB033898, as shown in Table 1.

**Variation within Humulus lupulus**

Hop cultivars were classified into two groups by nucleotide differences based on the taxonomic classification. One group comprised two varieties, Brewers gold and Nugget in the USA, and the other group other cultivars listed in Table 1. Only two nucleotide substitutions were found between the two groups in the partial rbcL sequence, and they were located at the 1st and 3rd codon positions of the rbcL gene. The noncoding and the ITS2 regions were not different in any of the cultivars. North American wild hop USDA6001, *H. lupulus* var. *neomexicanus*, also had the same sequence as that of Brewers gold and Nugget.

Within the Japanese wild hops, *H. lupulus* var. *cordifolius*, no nucleotide substitution was detected in the regions studied. *H. lupulus* var. *lupulus* differed from *H. lupulus* var. *cordifolius* by two nucleotides, one in the rbcL sequence and the other in the noncoding region (Table 3). In the comparison of *H. lupulus* var. *neomexicanus* and *cordifolius*, one nucleotide was modified in the rbcL sequence and one in the noncoding region (Table 3). *H. lupulus* var. *neomexicanus* and *cordifolius* had the same nucleotide on the 1st codon of rbcL which differed from that of *H. lupulus* var. *lupulus*.

Based on the above differences, since the average nucleotide substitution per site (K) was calculated and all
<table>
<thead>
<tr>
<th>Classification</th>
<th>Name</th>
<th>Country</th>
<th>cpDNA origin</th>
<th>Sequence accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivated hop</td>
<td>Saazer</td>
<td>Czech Republic</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td>AB033761</td>
</tr>
<tr>
<td></td>
<td>Hallertauer</td>
<td>Germany</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td>AB03389</td>
</tr>
<tr>
<td></td>
<td>Hallertauer tradition</td>
<td>Germany</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td>AB03390</td>
</tr>
<tr>
<td></td>
<td>Hersbrucker</td>
<td>Germany</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td>AB033891</td>
</tr>
<tr>
<td></td>
<td>Northern brewer</td>
<td>Germany</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spalt select</td>
<td>Germany</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spalter</td>
<td>Germany</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tettnanger</td>
<td>Germany</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kirin No. 2</td>
<td>Japan</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green bullet</td>
<td>New Zealand</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Super styrian</td>
<td>New Zealand</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Styrian golding</td>
<td>Slovenia</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bramling cross</td>
<td>UK</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>East Kent goldings</td>
<td>UK</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WGV</td>
<td>UK</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brewers gold</td>
<td>USA</td>
<td><em>H. lupulus</em> var. <em>neomexicanus</em></td>
<td>AB033892</td>
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<tr>
<td></td>
<td>Cluster</td>
<td>USA</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td>AB033893</td>
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<tr>
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<td>Fuggle</td>
<td>USA</td>
<td><em>H. lupulus</em> var. <em>lupulus</em></td>
<td>AB033894</td>
</tr>
<tr>
<td></td>
<td>Nugget</td>
<td>USA</td>
<td><em>H. lupulus</em> var. <em>neomexicanus</em></td>
<td>AB033895, AB033896, AB033897, AB033898</td>
</tr>
<tr>
<td>Wild hop</td>
<td>Japanese wild No. 1</td>
<td>Japan</td>
<td><em>H. lupulus</em> var. <em>cordifolius</em></td>
<td>AB033892</td>
</tr>
<tr>
<td></td>
<td>Japanese wild No. 2</td>
<td>Japan</td>
<td><em>H. lupulus</em> var. <em>cordifolius</em></td>
<td>AB033893</td>
</tr>
<tr>
<td></td>
<td>North American Wild (USDA60016)</td>
<td>Japan</td>
<td><em>H. lupulus</em> var. <em>neomexicanus</em></td>
<td>AB033894</td>
</tr>
<tr>
<td>Related species</td>
<td>H. japonicus No. 1</td>
<td>Japan</td>
<td><em>H. japonicus</em></td>
<td>AB033895</td>
</tr>
<tr>
<td></td>
<td>H. japonicus No. 2</td>
<td>Japan</td>
<td><em>H. japonicus</em></td>
<td>AB033896</td>
</tr>
<tr>
<td></td>
<td>H. japonicus No. 3</td>
<td>Japan</td>
<td><em>H. japonicus</em></td>
<td>AB033897</td>
</tr>
</tbody>
</table>

1) sequenced for partial rbcL only.
2) Brewers gold contains cpDNA derived from North American wild hop (*H. lupulus* var. *neomexicanus*), since it was developed from the crossing of the wild hop female designated as Manitoba B11. Nugget’s female ancestor is Brewers gold, and both hops have an identical cpDNA, as confirmed by this study.
3) Japanese wild hops No.1 and No.2 were provided by courtesy of Dr. Sano, Hiroasaki University.
4) North American wild hop was imported from the USA. It is being cultivated in Kirin’s experimental field. Register number is USDA No. 60016.
5) H. japonicus No. 1 through No. 3 were indigenous species, collected in Yokohama city, Japan, 1998.
6) trnL intron of cpDNA
7) Spacer between *trnL* and *trnF* of cpDNA
Table 2. Primers used for target region amplification and sequencing

<table>
<thead>
<tr>
<th>Target region</th>
<th>Primer name</th>
<th>Sequence (5'→3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>rbcl</td>
<td>rbcl1</td>
<td>ATGTCACCCAAACAGAGATAAAGC</td>
</tr>
<tr>
<td></td>
<td>rbcl2</td>
<td>TGAAAAACGTGAAATTCACAGTGTCAG</td>
</tr>
<tr>
<td></td>
<td>rbcl3</td>
<td>CTTCTGCTACAAATAGAATCGATCTCCTCA</td>
</tr>
<tr>
<td></td>
<td>rbcl4</td>
<td>GACGAGCCGCTCCGCCCTC</td>
</tr>
<tr>
<td>trnL. intron</td>
<td>INT1</td>
<td>CGAAATCCTGTAAGCCTAG</td>
</tr>
<tr>
<td></td>
<td>INT2</td>
<td>GGGGATAGGGACTTGAGAC</td>
</tr>
<tr>
<td>trnL-trnF spacer</td>
<td>SP1</td>
<td>GCTTTAACGCTCCTCCTATCCC</td>
</tr>
<tr>
<td></td>
<td>SP2</td>
<td>ATTTGAATCGTGACGAG</td>
</tr>
<tr>
<td>ITS2</td>
<td>ITS2F</td>
<td>TGCGAGATCCGCTGAACCATCG</td>
</tr>
<tr>
<td></td>
<td>ITS2R</td>
<td>CCAAAACACCCCTCGTACGACAGC</td>
</tr>
</tbody>
</table>

Table 3. Comparison of sequences within genus *Humulus*

<table>
<thead>
<tr>
<th>Compared species region</th>
<th>Number of sites compared</th>
<th>Number of substituted nucleotides</th>
<th>Substitution rate(^{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Codon position</td>
<td>Substitution type(^{1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>total 1st 2nd 3rd Transition Transversion</td>
<td></td>
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<tr>
<td><em>H. lupulus var. lupulus vs. H. lupulus var. neomexicanus</em> rbcl</td>
<td>1260</td>
<td>2 1 0 1 0 1</td>
<td>0 1</td>
</tr>
<tr>
<td></td>
<td>noncoding(^{3})</td>
<td>853</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>ITS2</td>
<td>232</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td><em>H. lupulus var. lupulus vs. H. lupulus var. cordifolius</em> rbcl</td>
<td>1260</td>
<td>1 1 0 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td></td>
<td>noncoding</td>
<td>853</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>ITS2</td>
<td>232</td>
<td>0 0 0</td>
</tr>
<tr>
<td><em>H. lupulus var. cordifolius vs. H. lupulus var. neomexicanus</em> rbcl</td>
<td>1260</td>
<td>1 0 0 1</td>
<td>0 1</td>
</tr>
<tr>
<td></td>
<td>noncoding</td>
<td>853</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>ITS2</td>
<td>232</td>
<td>0</td>
</tr>
<tr>
<td><em>H. japonicus vs. H. lupulus</em> rbcl</td>
<td>1260</td>
<td>13 3 1 9 4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>noncoding</td>
<td>853</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>ITS2</td>
<td>231</td>
<td>24</td>
</tr>
</tbody>
</table>

\(^{1}\) Type of substitution (Kimura 1986). In the case of *rbcl*, the number of substituted nucleotides was counted at the 3rd codon position.

\(^{2}\) Calculated from the number of nucleotides substituted of the 3rd codon position in *rbcl* and total number in the noncoding region by using the two-parameter method (Kimura 1986).

\(^{3}\) *trnL* intron and *trnL-trnF* spacer regions. Figures counted based on sum of both regions.

\(^{4}\) SE: standard error (Kimura 1986).

\(^{5}\) NA: not available.

The values were equal to the standard error, they were not significant (Table 3). Nucleotide diversity among varieties of *H. lupulus* in the studied regions, *rbcl* and noncoding regions, of cpDNA which was estimated to be π=0.0009, was not considered to be significant because of the standard deviation, 0.0009, equivalent to π.

**Differences between the two species**

*H. japonicus* differed from *H. lupulus var. lupulus* in 13 nucleotides in the partial *rbcl* sequence, 9 nucleotides in the noncoding region and one gap and 24 nucleotides in the ITS2 region, respectively. Similar results were obtained when both *H. lupulus var. cordifolius* and *H. lupulus var. neomexicanus* were compared. Three of the nucleotide changes in the partial *rbcl* sequence occurred at the 1st, one at the 2nd and nine at the 3rd codon positions, respectively (Table 3). The K value for the averaged substitution at the 3rd position was calculated to be 0.0217±0.0073, Ks' was calculated to be 0.0157±0.0056. The noncoding regions showed a K value of 0.0106±0.0036, and the ITS2 region showed the highest K value of 0.1137±0.0241.

**Discussion**

This study showed that the North American wild hop (USDA 60016) and the two cultivated hops, Brewers gold and Nugget, had the same sequences. These findings imply that no difference was found within *H. lupulus var. neomexicanus*, since Brewers gold was recorded to have developed from the crossing of the North American wild female hop, designated as Manitoba BBI (Burgess 1964), and cpDNA must have been derived from *H. lupulus var.*
neomexicanus. Nugget also had the same cpDNA because Brewers gold was its female ancestor (Haunold et al. 1984a, b), which was confirmed in the results.

A high rate of nucleotide substitution was observed between H. lupulus and H. japonicus in the ITS2 region. However, the difference within H. lupulus was found only in cpDNA. These discrepancies may be due to the following reasons. Firstly, the conservation property of the ITS2 region may result in evolutionary constraint. This region is necessary for appropriate RNA-splicing, which suggests the presence of secondary structural constraints on ITS2 (Baldwin et al. 1995). Secondly, ITS2 does not necessarily show a rapid evolution. Baldwin et al. (1995) noted that a low rate was found in some ancient woody plants, as in the case of cpDNA, for unknown reasons. Thirdly, since ITS2 is a part of nuclear rDNA, which occurs in large arrays of tandem repeats and is subjected to concerted evolution (Edward and Holtsford 1996), a slight difference may be eliminated quickly. As long as the degree of divergence between mutated and original sequences is not large, the diverged copy may be deleted by unequal crossing-over or converted to the conserved copy by gene conversion (Li 1997). Thus, H. lupulus may require a short period of time for rapid concerted evolution.

One nucleotide substitution in rbcL, when H. lupulus var. neomexicanus and H. lupulus var. cordifolius, occurred at the first codon position, which corresponds to a change in amino-acids. Although it remains to be determined how the substitution affects the enzyme activity, it may have been used by natural selection for adaptation to climatic differences in the growing areas between Europe and USA or Japan. However, the nucleotide substitution rates in rbcL and the non-coding region of cpDNA (Table 3) as well as the nucleotide diversity of studied cpDNA region, \( \pi = 0.0009 \pm 0.0009 \), were not significant within H. lupulus. Since nucleotide diversity is related to effective population size and mutation rate (Edward and Holtsford 1996, Li 1997), hops in Europe, North America and Japan may have been recently derived from small size populations or they may show a decreased mutation rate in cpDNA. Pillay and Kenny (1996) assumed the existence of a bottleneck because of the lack of genetic variability in the nuclear rDNA of cultivated and wild hops. Wolfe et al. (1987) estimated the rate of nucleotide substitution in cpDNA using the divergence time, 100-140 million years, between monocots and dicots, and a value of 2.1-2.9 x 10^{-9} per site per year was obtained. This substitution rate of rbcL (Ks') suggests that the average divergence time between H. lupulus and H. japonicus occurred 3.74 million years ago when the rate of 2.1 x 10^{-9} per site per year was used. However, the divergence time within H. lupulus could not be calculated because of the lack of the significant nucleotide substitution rate obtained in this study. We need to use a region with a higher substitution rate to study the diversity within H. lupulus.

Pillay and Kenny (1996) reported length variants in the intergenic spacer region between 26 s and 18 s rDNA (IGS), including the external transcribed spacer (ETS) and non-transcribed spacer (Hershkovits et al. 1999), among cultivated and wild hops, and European and native North American hops, corresponding to H. lupulus var. lupulus and neomexicanus, respectively, and differing by approximately 1-kb. They concluded that there were at least two populations in H. lupulus. Baldwin et al. (1998) and Bena et al. (1998) reported a high substitution rate in the ETS region. Since more information on the divergence within H. lupulus could be obtained from this region, DNA sequence of entire IGS as well as ETS is being studied in an increasing number of wild hop samples.

Wild hop has already been found to be a source of valuable characteristics such as high alpha acid content, from which the bitter taste of beer is derived, resistance to Verticillium albo-atrum and downy mildew (Neve 1991). However, this study suggests that variation, which could be utilized in hop breeding is limited, although further studies should be carried out. For further promotion of the use of genetic resources in breeding programs, the centre of origin should be specified. Alternatively, interspecific hybrids between H. lupulus and H. japonicus need to be developed as new breeding materials.

It is possible that many related species have not yet been discovered. Although the centre of origin of hops is unclear, Neve (1991) suggested that hops may have originated in China and migrated to Europe and North America. If this assumption is valid, an increasing number of wild hops should be collected in China, for the detection of a wide range of diversity.

Acknowledgment

The author thanks Dr. Sano, Hiroasaki University, for providing samples of Japanese wild hops.

Literature Cited


