Intraspecific Sequence Variation in the *rbcL-accD* Region of the Chloroplast Genome in Tea (*Camellia sinensis*).

Tomomi Ujihara, Nobuyuki Hayashi and Katsunori Kohata

National Institute of Vegetable and Tea Science (NIVTS),

National Agriculture and Food Research Organization.*

Satoru Matsushita

Houmeikai.**

Sakihito Kitajima

Graduate School of Science and Technology, Kyoto Institute of Technology.***

(Accepted 8 June 2007)

チャ葉緑体*rbcL-accD*領域塩基配列の多様性

独立行政法人 農業・食品産業技術総合研究機構 野菜茶業研究所

氏原ともみ・林 宣之・木幡勝則

財団法人 豊茗会

松下 智

京都工芸繊維大学 大学院工芸科学研究科

北島佐紀人

Summary

In order to reveal intraspecific variation in the tea (*Camellia sinensis*), the nucleotide sequence of the *rbcL-accD* region in the chloroplast genome of 21 Japanese tea cultivars, 22 and 7 tea clones collected in China and Vietnam, respectively, and 6 Chinese cultivars were determined. The nucleotide sequence was well conserved. Eighteen Japanese tea cultivars selected from local varieties were divided into 2 types based on 2 SNPs (Single Nucleotide Polymorphisms). One of these types (Ia) was mainly distributed in Kyoto. Both types were also found in the Asian continent, indicating that they were derived from there to Japan. Twelve additional types were found in tea of the Asian continent. Some of the tea clones from south China had one or two of the three In/Dels (insertion/deletion polymorphism), which are unique in *C. taliensis* and *C. irrawadiensis*. Chloroplast DNA can be used in order to distinguish genetic background of the cultivars of tea leaves on the market.

* 2769 Kanaya, Shimada, Shizuoka 428-8501, Japan

** 3F-A, 4-15-15 Nakamura-ku, Nagoya 450-0002, Japan

*** Sakyo-ku, Kyoto 606-8585, Japan

Key words: Tea, *Camellia sinensis*, chloroplast DNA, sequence variation

キーワード：チャ、*Camellia sinensis*、葉緑体DNA、塩基配列変異

1. Introduction

Consumers in Japan are becoming increasingly interested in the provenance of agricultural products, and techniques to confirm the producing cultivars or place of production in order to discourage inaccurate labeling are in demand. Furthermore, the JAS (Japanese Agricultural Standard) Law requires the country of origin to be labeled on imported tea leaves. This is enforced to avoid the illegal blending of relatively low-priced foreign tea leaves in domestic tea leaves without the consumer's knowledge. Thus, there is a need to develop an analytical method to identify the countries of origin of the tea leaves currently on the market. The cultivars of imported tea leaves are supposed to be local cultivars in producing countries, and they are probably distinguishable from Japanese cultivars by genetic background, except in instances when nursery stock of Japanese cultivars has been exported to a foreign country, where the plants were cultivated and used to produce tea leaves that were then imported to Japan. Therefore, analysis of the genetic background of tea leaves on the market may reveal their origin.

DNA-based techniques have been widely applied to cultivar identification of agricultural products^{1), 2), 3)} and detection of GMOs (Genetically Modified Organisms)⁴⁾. Interspecific and/or intraspecific variations in tea and other *Camellia* species have been reported^{5), 6), 7), 8)}, and they have been applied to cultivar identification of green tea leaves on the market⁹⁾. Thirteen CAPS (Cleaved Amplified Polymorphic

Sequence) markers were developed based on the sequences of 3 nuclear-coded genes and 46 Japanese cultivars were distinguished, and two varieties of tea, var. *assamica* and var. *sinensis*, were differentiated using these CAPS markers^{7), 10)}. However, the geographical distribution of cultivars was not confirmed by these CAPS markers.

On the other hand, chloroplast DNA has been widely used for phylogenetic analysis and the study of geographic distribution of plants, due to their maternal inheritance and seed-mediated migration properties^{11), 12)}. As the chloroplast genome has a high copy number, it is presumed to be that chloroplast DNA is easily amplified by PCR, even after partial DNA degradation through the green tea manufacturing process, which involves steaming or pan-firing and drying. Tea in Japan is considered to have originated from a small group of tea plants introduced from China, and thus has a lower genetic diversity than tea cultivated in other parts of the world^{13), 14)}. Furthermore, in Japan, 10 cultivars occupy 88% of the total tea cultivating area¹⁵⁾. These leading cultivars were selected from local Japanese varieties. Although some cultivars bred by crossings have become popular in recent years, cultivars bred by selection were used as the maternal parents of many such current cultivars. Therefore, the cytoplasmic genetic diversity of local Japanese varieties is reflected in the current cultivars. Katoh *et al.* reported that genetic diversity was seen in the nucleotide sequences of the *matK* gene in chloroplast DNA in cultivated tea from east to south Asia, in contrast to only 2 types in 30 Japanese cultivars (the names of the

cultivars were not revealed)¹⁶⁾. Thus, chloroplast DNA sequence information will be useful for distinguishing the genetic background of the cultivars of processed tea leaves on the market.

The nucleotide sequence for the *rbcl-accD* region of the chloroplast genome of genus *Camellia* is expected to be variable, as it has been shown to be highly variable in the family *Polygonaceae* at both the intrageneric¹⁷⁾ and intraspecific^{18), 19)} levels, and in family *Moraceae* at the intrageneric level²⁰⁾. In this manuscript, we compared nucleotide sequences in the *rbcl-accD* region in order to study the intraspecific geographic distribution of tea and the intrageneric relationship between tea and other *Camellia* species, because some wild relatives are cross-compatible with tea²¹⁾, and because interspecific hybrids of *C. sinensis* and *C. irrawadiensis* have been cultivated in south Asia^{22), 23), 24)}. We also describe the potential application of the above information in order to distinguish the genetic

background of tea leaf cultivars on the market.

2. Materials and Methods

2.1 Plant materials

Fifty-six tea clones were used in this study. To study types of Japanese tea cultivars, 18 cultivars (var. *sinensis*) and 3 Japanese cultivars (Karabeni, Benihomare and Hatsumomiji) whose maternal parents were Chinese or Indian tea were analyzed. Twenty-two Chinese and 7 Vietnamese samples were collected in tea estates or their neighboring area and morphologically classified as var. *assamica*. Leaves of each clone were collected and herbarium specimens were used for DNA extraction. Six semi-fermented tea leaves made from Chinese cultivars were collection of Dr. Yoshiyuki Takeda. The locality of these clones is shown in Figure 1.

Two wild relatives of cultivated tea, *C. taliensis*, which is native to China, and *C. irrawadiensis*, which is native to Myanmar, and

Fig. 1. Types and localities of *C. sinensis*.

Locality and the number of clones for each type are indicated. For cultivars 'Karabeni', 'Benihomare' and 'Hatsumomiji', the locality of the maternal parent is indicated. For 6 Chinese tea leaves made from cultivars, localities of production are indicated.

11 *Camellia* species: *C. assamensis*, *C. salicifolia*, *C. pitardii*, *C. cuspidata*, *C. saluenensis*, *C. kissi*, *C. sasanqua*, *C. furfurea*, *C. hongkongensis*, *C. japonica* and *C. tsaii* were also analyzed for comparative purposes. Eleven species except for *C. sasanqua* and *C. japonica* are preserved in the Makurazaki tea research station, NIVTS, Kagoshima. *C. sasanqua* and *C. japonica* are preserved in the Kanaya tea research station, NIVTS, Shizuoka.

2.2 DNA extraction, PCR amplification and sequence analysis.

Total DNA was extracted using a modified CTAB method²⁵⁾ from dried or frozen leaves. DNA of Chinese semi-fermented tea leaves were extracted using a modified CTAB method for processed tea leaves⁹⁾. The *accD*-specific primers D1 and D2²⁰⁾ were used to amplify an approx. 200 bp of *accD* gene of *C. sinensis* var. *sinensis* cultivar 'Yabukita'. The obtained PCR product was sequenced directly to design an *accD*-specific primer accDRv1 (5'-CATAACCCCAATT GCTACGG-3'). An *rbcL*-specific primer rbcLFW1 (5'-GAAGGACGTGATCTTGCTCGT-3') was prepared based on the *rbcL* sequence of *C. sinensis* (accession number: AF380037). These two primers accDRv1 and rbcLFW1 were used to amplify a 1.7 kb fragment of *rbcL-accD* from the chloroplast genome of each clone. PCR

conditions were as follows: 100 ng of DNA, 0.25 μ M of each primer, 200 μ M of dNTPs, 2 mM of magnesium chloride, 0.625 U of *Takara ExTaq* DNA polymerase (Takara, Shiga, Japan), 1 x *ExTaq* buffer. Reaction volumes were 25 μ l. PCR reactions were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Tokyo, Japan) by an initial denaturation for 5 min at 94°C followed by 35 cycles of 30 sec at 94°C, 1 min at 60°C, 90 sec at 72°C, and final extension of 10 min at 72°C. PCR products were purified using QIAquick PCR purification kit (QIAGEN, Tokyo, Japan). Nucleotide sequence of purified products were determined using BigDye Terminator v3.0 or v3.1 Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan), and automated DNA sequencer ABI310 genetic analyzer (Applied Biosystems, Tokyo, Japan).

3. Results and Discussion

3.1 Sequence variation in *rbcL-accD* region of tea.

In order to analyze intraspecific geographic distribution within the tea, the *rbcL-accD* region of the chloroplast genome was amplified by PCR and their nucleotide sequences were determined. Amplified fragments contained approximately 110 bp of the 3' portion of the *rbcL* gene, 530 bp of the intergenic spacer region, and 1030 bp of the 5' portion of *accD* gene. Nucleotide position

Table 1 Interspecific sequence variation of *rbcL-accD* region among *C. sinensis*, *C. irrawadiensis* and *C. taliensis*

sample	polymorphic site									Accession No.
	528 ^a	703	831	954	1038	1218	1236	1328	1422	
<i>C. sinensis</i>	(T) ₁₁	G	A	G	A	sin	sin	sin	G	AB207869
<i>C. irrawadiensis</i>	• ^b	A	•	A	C	ta/ir	ta/ir	ta/ir	T	AB207874
<i>C. taliensis</i>	(T) ₁₂	A	C	A	C	ta/ir	ta/ir	ta/ir	T	AB207881

^a Numbers correspond to the position of nucleotide in the sequence of *C. sinensis* cultivar 'Yabukita'.

^b Dot means the nucleotide is the same as that of *C. sinensis* cultivar 'Yabukita'.

sin or ta/ir means *sinensis*- or *taliensis/irrawadiensis*-type In/Del, respectively.

number 1 in this study corresponds to position 1302 of the published sequence of the *rbcL* gene of *C. sinensis* (accession number: AF380037). The 1.7 kb fragments were easily amplified from DNA of tea leaves on the market and herbarium specimen as well as DNA of fresh leaves. The sequences between the position 52 and 1566 were used to compare samples.

The sequence variation among tea, *C. taliensis* and *C. irrawadiensis* is shown in Table 1. These species belong to section *Thea*. When compared with tea cultivar 'Yabukita', 5 and 4 SNPs were found in *C. taliensis* and *C. irrawadiensis*, respectively. *C. taliensis* showed different repeat number of SSR at position 528. The repeat number of *C. taliensis* was 12 while the repeat number of *C. irrawadiensis* was 11 as tea cultivar 'Yabukita'. In addition, these two species had 3 In/Dels in the *accD* coding region and thus were easily distinguishable from tea. Compared to *C. taliensis* and *C. irrawadiensis*, In/Dels in tea at position 1218 and 1236 were insertion of 6 nucleotides (CAATTA and TTTATA, respectively) and In/Del at position 1328 was deletion of 6 nucleotides (TATAAA). These In/Dels were not found in other 11 *Camellia* species: *C. assamensis* (accession number AB207870), *C. salicifolia* (AB207878), *C. pitardii* (AB207877), *C. cuspidata* (AB207871), *C. saluenensis* (AB207879), *C. kissi* (AB207876), *C. sasanqua* (AB207880), *C. furfuracea* (AB207872), *C. hongkongensis* (AB207873), *C. japonica* (AB207875) and *C. tsaii* (AB207882), except for tea collected at Yunnan Province, China as mentioned below.

The origin of the genus *Camellia* has been supposed to be a region of south China to north Myanmar²⁶⁾. It is well known that crops were highly variable at the neighboring area of its origin, *C. sinensis* of this region was expected to

have high genetic diversity. The nucleotide sequence of 21 Japanese cultivars (18 cultivars selected from Japanese local varieties and 3 cultivars whose parents were Chinese or Indian tea, Figure 1) and 22 Chinese clones (21 were collected in Yunnan Province and 1 in Guangdong Province), 6 Chinese cultivars (5 were cultivated in Fujian Province and 1 was in Guangdong Province) and 7 Vietnamese clones (5 were collected in Tuyen Quang and Bac Thai Province and 2 were collected in Lam Dong Province) were determined. The sequence variation is shown in Table 2. Though this nucleotide sequence was highly conserved among the Japanese cultivars, 2 SNPs were found at positions 692 and 764. Based on these SNPs, Japanese cultivars were divided into 2 types: Ia and II. Type Ia included 'Yabukita', which occupies over 75 % of the total tea cultivation area¹⁵⁾, type II included 'Asatsuyu', maternal parent of 'Yutakamidori' which occupies the second largest area¹⁵⁾. Type Ia was distributed in Kyoto, while type II was distributed mainly in Shizuoka, and Nara, Fukuoka and Miyazaki (Figure 1). Two Japanese cultivars ('Hatsumomiji' and 'Benihomare') selected from seeds of var. *assamica* introduced from India, were also divided into these types. Chinese cultivars produced in Fujian Province ('Baxian', 'Huangdun', 'Huangjingui', 'Wuyi Rougui' and 'Maoxie') divided into 2 types. These results indicated that the two types were not raised in Japan but originated from genetic diversity in the Asian continent, and, furthermore, the 2 types did not correspond to the two varieties, var. *sinensis* and var. *assamica*. Tanaka and Takeda reported that most Japanese cultivars were divided into 2 types using maternal-inherited RAPD (Random Amplified Polymorphic DNA) markers²⁷⁾. One was 'Yabukita'-type and the other was 'Asatsuyu'-

Table 2 Intraspecific sequence variation of *rbcL-accD* region in *C. sinensis*

type	polymorphic site															Accession No
	280 ^a	349	495	503	528	645	692	703	764	954	1038	1107	1218	1328	1375	
I a	C	C	C	T	(T) ₁₁	T	G	G	G	G	A	G	sin	sin	G	AB207869
b	.	^b	.	.	(T) ₁₄	.	.	.	.	.	.	.	.	.	.	AB231630
II	.	.	.	.	.	.	A	.	A	.	.	.	.	.	.	AB231628
III	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	AB231629
IV a	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	AB231631
b	.	.	.	.	(T) ₁₃	.	.	.	.	.	.	A	.	.	.	AB231632
c	.	.	.	.	(T) ₁₄	.	.	.	.	.	.	A	.	.	.	AB231633
V a	.	.	.	.	(T) ₁₃	C	.	.	.	.	.	A	.	.	.	AB231634
b	.	.	.	.	(T) ₁₄	C	.	.	.	.	.	A	.	.	.	AB231635
VI a	.	.	.	.	(T) ₁₂	.	.	A	.	A	.	A	ta/ir	.	T	AB231636
b	.	.	.	.	(T) ₁₃	.	.	A	.	A	.	A	ta/ir	.	T	AB231637
c	.	.	.	.	(T) ₁₅	.	.	A	.	A	.	A	ta/ir	.	T	AB231638
VII	T	T	T	.	(T) ₁₇	.	.	A	.	A	.	.	ta/ir	ta/ir	T	AB231639
VIII	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	AB306664

^a Numbers correspond to the position of nucleotide in the sequence of *C. sinensis* cultivar 'Yabukita'.

^b Dot means the nucleotide is the same as that of *C. sinensis* cultivar 'Yabukita'.

sin or ta/ir means *sinensis*- or *taliensis/irrawadiensis*-type In/Del, respectively.

type, and according to listed cultivars, they correspond to Ia and II in this manuscript, respectively. Katoh *et al.* reported that, by comparison of the nucleotide sequence of the chloroplast *matK* gene, Japanese tea cultivars and tea clones of east China were divided into two types although the names of the cultivars were not described. In addition, one of the types was found in China, Laos, Vietnam, Myanmar, India and Bangladesh¹⁶⁾. 'Karabeni', a progeny of var. *sinensis* introduced from Hubei Province, China, had another type (III).

In contrast to Japanese cultivars, tea clones were more divergent in south Asia, as expected. In Vietnamese and Yunnan clones, 3 and 8, respectively, types were found in addition to Ia (Table 2, Figure 1). Although morphologically classified as *C. sinensis* var. *assamica*, type VIa, VIb, VIc and VII, collected in Yunnan, had one or two of the three In/Dels found in *C. taliensis* and *C. irrawadiensis*. Type VII had unique SNPs at positions 280, 349 and 495, which were not observed in *C. taliensis* and *C. irrawadiensis* (Table 2). In these types, SNPs at positions 703,

954 and 1375 were associated with the presence of the In/Del at position 1218. A Chinese cultivar, namely 'Fenghuang Dancong', cultivated in Guangdong Province was another haplotype (VIII).

These In/Dels seem to have occurred recently because other *Camellia* species did not have such In/Dels. Because no tea having all of the three *taliensis/irrawadiensis*-type In/Dels was found, it is possible that *C. taliensis* and *C. irrawadiensis* evolved from a population of tea in south Asia after these indels had occurred and then the 2 species were differentiated. Detailed analysis of section *Thea* in south China using larger numbers of samples will reveal the phylogenetic relationships among these species.

Based on the RFLP analysis using the phenylalanine ammonia lyase (PAL) gene^{14), 28)},²⁹⁾ and the fact that the tea plant has never been found in regions far from tea fields³⁰⁾, Japanese tea is believed to be those introduced from China and their progenies, even if this is a debatable point³¹⁾. Lower genetic variation in chloroplast genome of Japanese cultivars selected from local

varieties suggests that Japanese tea originated from limited population, as previous studies on nuclear genome^{14), 28), 29)}.

In conclusion, by comparison of the nucleotide sequence of the *rbcL-accD* region of the chloroplast genome, in contrast to Japanese cultivars which had only two types, Chinese and Vietnamese tea was rather divergent. The information on the In/Dels, SNPs and SSRs should be helpful in distinguishing the genetic background of the cultivars of tea leaves on the market. Identifying difference of chloroplast genome from Japanese cultivars is effective in the illegal blending of foreign green tea leaves into domestic ones.

Acknowledgement

We thank Dr. Yoshiyuki Takeda for tea leaves of Chinese cultivars, Dr. Jun-Ichi Tanaka of Agriculture, Forestry and Fisheries Research Council for plant materials and information on *Camellia* species, Mr. Atsushi Nesumi of NIVTS for informative advice of Japanese cultivars. We thank Dr. Satoru Matsumoto of NIVTS for constructive comments during preparation of this manuscript. This work was supported by a Research project for utilizing advanced technologies in agriculture, forestry and fisheries, The Ministry of Agriculture, Forestry and Fisheries of Japan.

References

- 1) Ohtsubo, K., T. Fuji, Y. Hashino, H. Toyoshima, H. Okadome, S. Nakamura, T. Fuse and S. Kawasaki (1999): Identification of domestic rice cultivars by RAPD method using milled rice grains as samples (in Japanese). J. Jpn. Food Sci. Tech. 46, 117-122.
- 2) Wünsch, A. and I. Hormaza (2002): Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA markers. Euphytica, 125, 59-67.
- 3) Wakao, T., Y. Hikida, T. Tsuneyoshi, S. Kaji, H. Kubota and T. Kubota (1999): A simple DNA analysis for identifying eel species by using polymerase chain reaction-restriction fragment length polymorphism method (in Japanese). Nippon Suisan Gakkaishi, 65, 391-399.
- 4) Ahmed, F. E. (2002): Detection of genetically modified organisms in foods. Trends Biotechnol. 20, 215-223.
- 5) Magoma, G. N., F. N. Wachira, M. O. Imbuga and S. G. Agong (2003): Biochemical differentiation in *Camellia sinensis* and its wild relatives as revealed by isozyme and catechin patterns. Biochem. Syst. Ecol. 31, 995-1010.
- 6) Chen L. and S. Yamaguchi (2002): Genetic diversity and phylogeny of tea plant (*Camellia sinensis*) and its related species and varieties in the section *Thea* genus *Camellia* determined by randomly amplified polymorphic DNA analysis. J. Hortic. Sci. Biotech. 77, 729-732.
- 7) Kaundun, S. S. and S. Matsumoto (2003): Development of CAPS markers based on three key genes of the phenylpropanoid pathway in tea, *Camellia sinensis* (L.) O. Kuntze, and differentiation between *assamica* and *sinensis* varieties. Theor. Appl. Genet. 106, 375-383.
- 8) Wachira, F., J. Tanaka, Y. Takeda (2001): Genetic variation and differentiation in tea (*Camellia sinensis*) germplasm revealed by RAPD and AFLP variation. J. Hortic. Sci. Biotech. 76, 557-563.
- 9) Ujihara, T., S. Matsumoto, N. Hayashi and K. Kohata (2005): Cultivar identification and

- analysis of the blended ratio of green tea production on the market using DNA markers. Food Sci. Technol. Res. 11, 43-45.
- 10) Kaundun, S. S. and S. Matsumoto (2003): Identification of processed Japanese green tea based on polymorphisms generated by STS-RFLP analysis. J. Agric. Food Chem. 51, 1765-1770.
- 11) Bremer, B., K. Bremer, N. Heidari, P. Erixon, R. G. Olmstead, A. A. Anderberg, M. Källersjö, E. Barkhordarian (2002): Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. Mol. Phylogenet. Evol. 24, 274-301.
- 12) Kanno, M., J. Yokoyama, Y. Suyama, M. Ohyama, T. Itoh and M. Suzuki (2004): Geographical distribution of two haplotypes of chloroplast DNA in four oak species (*Quercus*) in Japan. J. Plant Res. 117, 311-317.
- 13) Yamaguchi, S., S. Matsumoto and J. Tanaka (1999): Genetic dispersal of tea plant. Global Advantage in Tea Science, Aravali Books International (P) LTD, India, pp. 413-426
- 14) Matsumoto, S., Y. Kiriwa and Y. Takeda (2002): Differentiation of Japanese green tea cultivars as revealed by RFLP analysis of phenylalanine ammonia-lyase DNA. Theor. Appl. Genet. 104, 998-1002
- 15) Cha Kankei Shiryō (2006), (in Japanese), Nihon Chagyo Chuoh Kai, Japan, pp. 25-26
- 16) Katoh, Y., M. Katoh, Y. Takeda and M. Omori (2003): Genetic diversity within cultivated tea based on nucleotide sequence comparison of ribosomal RNA maturase in chloroplast DNA. Euphytica, 134, 287-295.
- 17) Yasui, Y. and O. Ohnishi (1998): Interspecific relationships in *Fagopyrum* (*Polygonaceae*) related by the nucleotide sequences of the *rbcL* and *accD* genes and their intergenic region. Am. J. Bot. 85, 1134-1142.
- 18) Inamura, A., Y. Ohashi, E. Sato, Y. Yoda, T. Masuzawa, M. Ito and K. Yoshinaga (2000): Intraspecific sequence variation of chloroplast DNA reflecting variety and geographical distribution of *Polygonum cuspidatum* (*Polygonaceae*) in Japan. J. Plant Res. 113, 419-426.
- 19) Yamane, K., Y. Yasui and O. Ohnishi (2003): Intraspecific cpDNA variations of diploid and tetraploid perennial buckwheat, *Fagopyrum cymosum* (*Polygonaceae*). Am. J. Bot. 90, 339-346.
- 20) Matsuda, Y., H. Yoshimura, H. Kanamoto, T. Ujihara, K-I. Tomizawa, Y. Sugimura and S. Kitajima (2005): Sequence variation in *rbcL-accD* region in the chloroplast genome of *Moraceae*. Plant Biotechnology, 22, 231-233.
- 21) Takeda, Y. (1990): Cross compatibility of tea (*Camellia sinensis*) and its allied species in the genus *Camellia*. JARQ, 24, 111-116.
- 22) Wight, W. and P. K. Barua (1957): What is tea? Nature, 179, 506-507.
- 23) Wood, D. J. and P. K. Barua (1958): Species hybrid of tea. Nature, 181, 1674-1675.
- 24) Wachira, F. N., W. Powell and R. Waugh (1997): An assessment of genetic diversity among *Camellia sinensis* L. (cultivated tea) and its wild relatives based on randomly amplified polymorphic DNA and organelle-specific STS. Heredity, 78, 603-611.
- 25) Kaundun, S. S. and Y. G. Park (2002): Genetic structure of six Korean tea plantations as revealed by RAPD-PCR markers. Crop Sci. 42, 594-601.
- 26) Chu, D. C. (1997): Green tea-its cultivation,

- processing of the leaves for drinking materials, and kinds of green tea. In: Chemistry and applications of green tea, T. Yamamoto, L. R. Juneja, D. C. Chu and M. Kim, CRC Press, New York, pp. 1-11.
- 27) Tanaka, J. and Y. Takeda (2000): Saiboushitsu iden suru DNA marker ni yoru nihon no cha hinsyu no ruikeika. (in Japanese), http://www.affrc.go.jp/ja/db/seika/data_nivot/h12/nivot00037.html
- 28) Matsumoto, S., A. Takeuchi, M. Hayatsu and S. Kondo (1994): Molecular cloning of phenylalanine ammonia-lyase cDNA and classification of varieties and cultivars of tea plants (*Camellia sinensis*) using the PAL cDNA probe. Theor. Appl. Genet. 89, 671-675.
- 29) Matsumoto, S., Y. Kiriwa and S. Yamaguchi (2004): The Korean tea plant (*Camellia sinensis*): RFLP analysis of genetic diversity and relationship to Japanese tea. Breeding Sci. 54, 231-237.
- 30) Matsushita, S. (1978): Nippon cha no denrai. (in Japanese), Yasaka-shobo, Japan, pp. 94-100.
- 31) Oishi, S. (2004): Nihon cha jisei ron. (in Japanese), Nihon Chagyou Hattatsu-shi, Nounsangyoson bunka kyokai, Japan, pp. 183-203.

摘 要

チャの遺伝的差異を明らかにするため、21の日本品種、22個体および7個体の中国およびベトナムで採集されたチャ、6つの中国品種について、葉緑体*rbcL-accD*領域の塩基配列を決定した。この領域はよく保存されていた。日本品種および5つの中国品種は、一塩基多型に基づき2つのタイプに分類された。両タイプとも日本固有ではなく、アジア大陸に起源し日本に導入されたと考えられた。中国南部で収集されたチャでは、近縁種のタリエンシスおよびイラワジエンシスに特異的な3つのIn/Del (insertion/deletion)のうち1つまたは2つを有するものが複数見出された。葉緑体の塩基配列の多様性を用いることにより、市販茶の原葉として使用された品種の遺伝的背景を判別することができる。