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Note

Parentage analysis of tea cultivars in Japan based on simple sequence repeat markers

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Tea cultivars have been bred by individual selection of landraces and by crossbreeding, but the validation of the parentage is limited. In this study, we performed parentage analysis of 79 tea cultivars in Japan based on SSR markers to confirm or identify the parent-offspring relationships among them. The effectiveness of nine SSR markers for parentage analysis was validated by comparing them to the existing cleaved amplified polymorphic sequence markers. The former markers were detectable more alleles than the latter. Simulation of parentage analysis of the tea cultivars predicted biparental origins for 12 cultivars ('Houshun', 'Mie ryokuhou no. 1', 'Surugawase', 'Tenmyo', 'Yamanoibuki', 'Harumidori', 'Koushun', 'Minekaori', 'Okumusashi', 'Saemidori', 'Sofu', and 'Toyoka'), in the first five of which candidate parents of yet-to-be-defined pedigree were newly identified. Comparisons of a total of 41 SSR genotypes confirmed the newly-identified parentages of 'Asahi' for 'Tenmyo', 'Rokurou' for 'Houshun', 'Surugawase', and 'Yamanoibuki', and 'Yamatomidori' for 'Mie ryokuhou no. 1'. The maternity of seven cultivars out of the 12 was also confirmed with chloroplast DNA sequences. Uniparental origins were confirmed for 25 cultivars. This parentage analysis has improved our knowledge of tea pedigrees and will aid in the development of new cultivars.

Key Words: DNA marker, parentage analysis, pedigree, simple sequence repeat (SSR), tea (*Camellia sinensis*) cultivar.

Introduction

Tea (*Camellia sinensis* (L.) Kuntze) is a species of evergreen tree that is important for making beverages. This species includes two major varieties, *C. sinensis* var. *sinensis* (L.) Kuntze and *C. sinensis* var. *assamica* (J. W. Mast.) Kitam. (see Chen and Chen 2012 for a review). Its geographic origin is predicted to be southwestern China, and teas are now cultivated in various tropical, sub-tropical, and temperate regions worldwide. Because tea plants undergo outcrossing due to self-incompatibility, seed progenies generally have different allele combinations from parents as well as among siblings. Long-established tea gardens used to be composed of heterogenic, seed-derived populations, known as landraces; in the modern era, individuals with superior traits have been selected and vegetatively propagated as cultivars. To date, hundreds of tea cultivars have been bred in leading tea-producing countries, such as China, India, Sri Lanka, Kenya, and Japan (Chen and Chen 2012). In Japan, tea is an important beverage in tea ceremonies (*Cha-no-yu* or *Sado*) and in daily life. Japanese tea has recently attracted foreign consumers and purchasers. Since the introduction of tea to Japan in the medieval era, tea cultivation has expanded from Kyoto throughout most of Japan. The cultivar 'Yabukita', which is derived from a Shizuoka landrace, is currently the most widely cultivated tea because of its relatively high yield and wide regional adaptability. Many green tea cultivars have been bred by crossbreeding this cultivar and its relatives (Tanaka 2012).

Green tea processed in Kyoto Prefecture is called "*Uji-cha*" and is known as one of the highest-quality teas in the world. It includes *tencha* (tea leaves are steamed and dried without rolling, then ground in a stone mill to a fine powder known as *matcha*) and *gyokuro* (steamed and rolled green tea made from shade-grown leaves) in addition to *sencha*, a popular steamed and rolled green tea. Several cultivars

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have been bred by individual selection of Kyoto landraces or their progenies (e.g. 'Asahi', 'Goko', 'Samidori', 'Ujihikari', and 'Ujimidori'). 'Houshun' and 'Tenmyo' are green tea cultivars suitable for making *gyokuro* and *tencha*, both of which were selected from naturally pollinated seed progenies of 'Samidori' (Kyoto Prefectural Tea Research Institute 2004a, 2004b). However, their paternal parents are unknown.

Because of the highly outcrossed nature, information on tea pedigrees would be helpful to avoid inbreeding depression in breeding programs. To date, identification and parentage analyses of tea cultivars have been conducted at the DNA level (see Ni *et al.* 2012 for a review). Such analyses have also been performed in Chinese and Japanese cultivars based on flower morphology (Takeda and Toyao 1980), disease resistance (Takeda 2003), and DNA markers (Katoh *et al.* 2011, Kaundun and Matsumoto 2003, 2004, Matsumoto 2006, Matsumoto *et al.* 1994, Tanaka and Yamaguchi 1996, Tanaka *et al.* 2001, Ujihara *et al.* 2009, 2011, Zhang *et al.* 2020). Analyses of DNA markers have shown that parentages were in some cases misunderstood, and the pedigrees of some cultivars have been revised in light of this new information (Matsumoto 2006, Tanaka and Yamaguchi 1996). Among DNA markers, simple sequence repeats (SSRs), which are repeat sequences composed of 1–6 nucleotide motifs, are frequently used as they present several advantages over other DNA markers (Merritt *et al.* 2015). More recently, next-generation sequencing (NGS)-based approaches have also been applied (e.g. Zhang *et al.* 2020), though there could be limitations of such approaches related to highly repetitive and heterozygous tea genomes (Xia *et al.* 2020). Actually, we initially tried an analysis using single nucleotide polymorphism data obtained from our previous NGS-based approach in 44 cultivars (Kubo *et al.* 2019), but failed in accurate parentage prediction (data not shown) due to the above limitations. Classification of tea landraces and cultivars has been performed based on SSR markers (e.g. Kubo *et al.* 2019, Liu *et al.* 2017, Meegahakumbura *et al.* 2016, 2018, Ohsako *et al.* 2008, Tamaki *et al.* 2016, Taniguchi *et al.* 2014, Wambulwa *et al.* 2016, 2017). These analyses have revealed relationships among tea varieties, but the pedigrees of these cultivars are yet to be examined. To date, only a limited number of pedigrees have been validated. 'Meiryoku' seems to be derived from a cross between 'Yabukita' and 'Z1', whereas 'Yutakamidori' is more likely to be derived from an outcrossing of 'Asatsuyu' instead of selfing (Matsumoto 2006, Tanaka and Yamaguchi 1996). The paternal parental line of 'Okumidori' does not seem to be 'Shizu zai 16' (Matsumoto 2006). Twenty-nine and four cultivars are suggested to be offspring of 'Dabaicha' and 'Tieguanyin', respectively. The parentages of 'Echa 5', 'Foxiang 1', 'Foxiang 2', and 'Huangguanyin' have also been confirmed (Tan *et al.* 2015), and eight individuals were identified as selfing from 'Ziyan' (Tan *et al.* 2019).

In the present study, we conducted parentage analysis of selected tea cultivars in Japan, including 'Houshun' and 'Tenmyo', based on SSR markers in order to confirm or newly identify parent-offspring relationships.

Materials and Methods

Plant materials and DNA extraction

A total of 79 cultivars, representative of those grown in Japan, were used for analysis (**Table 1**). For easy identification, each cultivar name is indicated with the sample number in square brackets (e.g. 'AN5' [1]) hereafter. Of these, 36 cultivars were newly included for genotyping in this study (**Table 1**, asterisks). Fresh leaves of four cultivars ('Kurasawa' [21], 'Sofu' [50], 'Toyoka' [56], and 'Yamatomidori' [66]) were kindly supplied by Tea Industry Research Center, Shizuoka Prefectural Research Institute of Agriculture and Forestry (Kikugawa, Japan), Division of Tea Research, Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization (NARO) (Shimada, Japan), Saitama Tea Research Institute (Iruma, Japan), and Yamato Tea Research Center, Nara Prefecture Agricultural Research and Development Center (Nara, Japan), respectively. Fresh leaves of the remaining 32 cultivars were collected from plants grown under natural conditions in tea genetic resource gardens at the Tea Industry Research Division, Agriculture and Forestry Technology Department, Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Center (Uji, Japan), and the University Farm, Faculty of Life and Environmental Sciences, Kyoto Prefectural University (Soraku-gun, Japan).

DNA was extracted with a DNeasy Plant Mini Kit (Qiagen, Valencia, USA) or crude DNA lysate was prepared from small pieces of leaves according to the manufacturer's instructions (KOD FX Neo, Toyobo, Osaka, Japan).

Genotyping of DNA markers

For validation of the DNA markers in the parentage analysis, three tea cultivars with known pedigrees ('Harumidori' [10], 'Minekaori' [29], and 'Saemidori' [44]; Takeda *et al.* 1991, 2002, Ueno and Furuno 1989) were examined based on eight cleaved amplified polymorphic sequence (CAPS) markers (**Supplemental Table 1**; Ujihara *et al.* 2011). The allele data of the CAPS markers were confirmed to be identical to those in the previous reports (Kitajima-Ujihara 2013, Ujihara *et al.* 2011), whereas those of 'Unkai' [60] were newly analyzed in this study. Genotypes of the three cultivars were also analyzed based on nine SSR markers (MSG0403, MSG0421, MSG0572, MSG0609, MSG0699, MSG0703, MSG0795, MSG0800, and MSG0811; Taniguchi *et al.* 2012) (**Supplemental Table 1**) using fluorescent-labeled primers (Sigma-Aldrich, St. Louis, USA), localized to independent linkage groups (**Supplemental Table 3**).

The genotypes of 79 cultivars were used to examine the pedigrees based on the nine SSR markers. Genotype data for 43 cultivars were retrieved from our previous study

Table 1. List of 79 tea cultivars examined in this study

Type	No.[a]	Cultivar[b]	Pedigree[c]		Type	No.[a]	Cultivar[b]	Pedigree[c]	
Green tea cultivar						41	Okuyutaka	**Yutakamidori** × F_1NN8	
	1	AN5*	A2 × S24			42	Rokurou*	(Selection of a landrace of unknown origin)	
	2	Asagiri*	(Selection of Uji landrace)			43	Ryofu	Houryoku × **Yabukita**	
	3	Asahi	(Selection of Uji landrace)			44	Saemidori	**Yabukita** × **Asatsuyu**	☆
	4	Asanoka	**Yabukita** × Cp1			45	Sakimidori	F_1NN27 × ME52	
	5	Asatsuyu	(Selection of Uji landrace)			46	Samidori	(Selection of Uji landrace)	
	6	Fujimidori*				47	Sayamakaori	**Yabukita** × ?	
	7	Fukumidori*	**Yabukita** × 23F_1-107			48	Sayamamidori*	(Selection of Uji landrace)	
	8	Fushun	Z1 × **Kanayamidori**			49	Shunmei	**Yutakamidori** × F_1NN8	
	9	Goko	(Selection of Uji landrace)			50	Sofu*	**Yabukita** × **Shizu-inzatsu 131**	☆
	10	Harumidori*	**Kanayamidori** × **Yabukita**	☆		51	Surugawase*	**Yabukita** × ? **[Rokurou]**	★
	11	Harumoegi*	F_1NN27 × ME52			52	Takachiho	(Selection of Miyazaki landrace)	
	12	Hatsumidori*	(Selection of Mie landrace), triploid			53	Tamamidori*	(Selection of Uji landrace)	
	13	Himemidori*	(Selection of Fukuoka landrace)			54	Tenmyo	**Samidori** × ? **[Asahi]**	★
	14	Hokumei	**Sayamamidori** × 5507			55	Terakawawase	(Selection of Uji landrace)	
	15	Hoshinomidori*	(Selection of Fukuoka landrace)			56	Toyoka*	**Sayamamidori** × **Yabukita**	☆
	16	Houshun	**Samidori** × ? **[Rokurou]**	★		57	Tsuyuhikari*	Shizu 7132 × **Asatsuyu**	
	17	Izumi*	**Benihomare** × ?			58	Ujihikari	(Selection of Uji landrace)	
	18	Kanayamidori	S6 × **Yabukita**			59	Ujimidori	(Selection of Uji landrace)	
	19	Komakage	(Selection of Uji landrace)			60	Unkai	**Takachiho** × F_1 9-4-48	
	20	Koushun	**Kurasawa** × **Kanayamidori**	☆		61	Yabukita	(Selection of Shizuoka landrace)	
	21	Kurasawa*	**Yabukita** × ?			62	Yaeho	(Selection of Shizuoka landrace)	
	22	Kuritawase	(Selection of Shizuoka landrace)			63	Yamakai	**Yabukita** × ?	
	23	Kyomidori*	(Selection of Uji landrace)			64	Yamanami*	Seedling introduced from Hubei Province, China	
	24	Makinoharawase*	(Selection of Shizuoka landrace), triploid			65	Yamanoibuki*	**Yabukita** × ? **[Rokurou]**	★
	25	Meiryoku	**Yabukita** × Z1[d]			66	Yamatomidori*	?[d]	
	26	Mie ryokuhou no. 1*	**Yabukita** × ? **[Yamatomidori]**	★		67	Yumekaori	**Sayamakaori** × Miyazaki no. 8	
	27	Minamikaori	**Yabukita** × Miya A11			68	Yumewakaba*	**Yabukita** × Saitama no. 9	
	28	Minamisayaka	MiyA-6 × F_1NN27			69	Yutakamidori*	**Asatsuyu** × ?[d]	
	29	Minekaori	**Yabukita** × **Unkai**	☆	Black and oolong tea cultivar				
	30	Miyamakaori*	Kyoken 283 × Saitama no. 1			70	Becchan (Betjan)		
	31	Miyoshi*	(Selection of Uji landrace)			71	Benifuji*	**Benihomare** × C19	
	32	Musashikaori	**Yabukita** × 27F_1-73			72	Benifuuki	**Benihomare** × Mak Cd 86	
	33	Narino	(Selection of Uji landrace)			73	Benihikari*	Benikaori × Cn1	
	34	Natsumidori*	(Selection of Shizuoka landrace)			74	Benihomare	(Selection of Indian hybrids, Tada series)	
	35	NN38*	**Yabukita** × Shizu zai 16			75	Hatsumomiji*	Ai2 × NKa05	
	36	Oguramidori*	(Selection of Uji landrace)			76	Indo	(Selection of Indian hybrids, Tada series)	
	37	Okuhikari*	**Yabukita** × Shizuoka Cy225			77	Ooba oolong		
	38	Okumidori	**Yabukita** × ?[d]			78	Seishin oolong	(Derived from Taiwan)	
	39	Okumusashi	**Sayamamidori** × **Yamatomidori**	☆		79	Shizu-inzatsu 131	(Selection of a natural cross population of Manipur no. 5)	
	40	Okunoyama*	(Selection of Uji landrace)						

[a] Sample number is indicated in each cultivar for easy identification hereafter.
[b] Cultivars whose SSR allele data were fully obtained in this study are indicated with asterisks.
[c] Origins and parentages (♀ × ♂) of cultivars are shown if described (full references are listed in **Supplemental Text 1**). Question marks mean unknown parentages. Parentages confirmed in this study are indicated with larger bold text, in which candidate parents newly predicted in this study are indicated with square brackets and filled stars (★) on the right. Parentages, in which both of the parents were confirmed, are indicated with open stars (☆) on the right. Cultivars directly selected from landraces or populations are shown in parentheses.
[d] Cultivars whose pedigrees have previously been suspected and corrected.

(Kubo *et al.* 2019). Allele data were treated as missing data (**Supplemental Table 2**) as three alleles were detected in triploid cultivars ('Hatsumidori' [12] and 'Makinoharawase' [24]; **Table 1**), and all allele data of these two cultivars were omitted from the estimation of genetic polymorphic parameters to avoid potential errors. The 32 SSR markers (MSG0083-MSE0335; Taniguchi *et al.* 2012) (**Supplemental Table 5**) were further used for genotyping 'Asahi' [3], 'Houshun' [16], 'Mie ryokuhou no. 1' [26], 'Rokurou' [42], 'Samidori' [46], 'Surugawase' [51], 'Tenmyo' [54], 'Yabukita' [61], 'Yamanoibuki' [65], and 'Yamatomidori' [66] using fluorescent-labeled primers or a post-labeling method (Shimizu and Yano 2011). Estimation of genetic polymorphic parameters and simulation of parentage analysis were conducted with Cervus 3.03 (Kalinowski *et al.* 2007) as reported previously (Kubo *et al.* 2009). LOD (natural log of the likelihood ratio) scores for tests, in which the most likely candidate parent is the true parent or not, were calculated with Cervus 3.03. In each of the 12 cultivars whose biparental pedigrees were newly identified or confirmed (**Supplemental Table 4**, **Supplemental Fig. 1**), only a single parent pair without mismatch in the SSR locus as well as the pedigree was identified, although more than one parent pair were predicted for some cultivars. In such cases, the identified parents mostly showed the highest LOD score (data not shown).

Chloroplast DNA analysis for confirmation of maternity

PCR amplification was performed for three chloroplast intergenic spacer regions (*ndhF-rpl32*, *trnSGG-trnSr*, and *trnSf1-trnGGG*; Wambulwa *et al.* 2016) in 23 cultivars (**Supplemental Table 6**). PCR products were directly sequenced after purification. The nucleotide sequences determined in this study are available in the DDBJ/EMBL/GenBank databases under accession numbers LC630487-LC630555.

Results

Validation of SSR markers for the parentage analysis of three tea cultivars with known pedigrees

To assess the effectiveness of the DNA markers in the given samples, eight CAPS and nine SSR markers were compared to analyze the known pedigrees of 'Harumidori' [10], 'Minekaori' [29], and 'Saemidori' [44]. There was no incongruence in their pedigrees based on the eight CAPS markers, as the offspring always shared alleles with their maternal or paternal parent (**Supplemental Table 1**, upper panel, red, blue, and gray boxes), confirming their pedigrees. Similar results were obtained using the nine SSR markers (**Supplemental Table 1**, lower panel), but the average number of alleles was higher in the SSRs (3.6) than in the CAPSs (2.1). Therefore, we used the SSR markers to detect the alleles more accurately and examine the pedigrees more clearly.

Polymorphisms of the SSR markers

In the genotypes of 77 tea cultivars with the nine SSR markers (**Supplemental Table 2**) excluding two triploid cultivars, the mean number of alleles (N_A) was 14.22 per locus (**Supplemental Table 3**). This value was higher than that reported previously in 44 cultivars (8.61; Kubo *et al.* 2019) probably because of the increased number of cultivars examined in the present study. Mean values of the observed (H_O) and expected heterozygosities (H_E), and the polymorphic information content (*PIC*) in this study were 0.7922, 0.7910, and 0.7624, respectively; the H_O and H_E values were similar to but slightly lower than those in the previous study (Kubo *et al.* 2019). The null allele frequency estimate (F (Null)) of the marker MSG0699 was the highest (0.0282) among the nine SSR markers. There was no significant deviation from the Hardy-Weinberg equilibrium in any of the nine markers (data not shown).

Parentage analysis of the tea cultivars

Parentage analysis was performed for 79 cultivars based on the nine SSR markers. Biparental origins were identified or confirmed in nine cultivars, 'Houshun' [16], 'Mie ryokuhou no. 1' [26], 'Surugawase' [51], 'Tenmyo' [54], 'Yamanoibuki' [65], 'Koushun' [20], 'Okumusashi' [39], 'Sofu' [50], and 'Toyoka' [56] (**Supplemental Table 4**, **Supplemental Fig. 1**). Including the three cultivars, 'Harumidori' [10], 'Minekaori' [29], and 'Saemidori' [44], whose parentages were already confirmed (**Supplemental Table 1**), biparental origins were predicted for 12 cultivars (**Table 1**, filled and open stars). Notably, the candidate parents of 'Houshun' [16], 'Mie ryokuhou no. 1' [26], 'Surugawase' [51], 'Tenmyo' [54], and 'Yamanoibuki' [65] were newly identified in the present parentage analysis (**Table 1**, brackets and filled stars, **Supplemental Table 4** and **Supplemental Fig. 1**, larger bold text). In the 12 parent-offspring relationships, all showed positive LOD scores and there was no SSR locus mismatch (**Supplemental Table 4**). For example, a 300-bp allele of the marker MSG0403 in 'Samidori' [46] was shared by 'Houshun' [16] and Tenmyo [54] (**Table 2**, red and gray boxes). 'Rokurou' [42] and 'Asahi' [3] were most likely the other parents of 'Houshun' [16] and 'Tenmyo' [54], respectively, as evidenced by the fact that there was no incongruence in the SSR markers between each of them (**Table 2**, blue and gray boxes). To confirm the five newly identified parentage results ('Houshun' [16], 'Mie ryokuhou no. 1' [26], 'Surugawase' [51], 'Tenmyo' [54], and 'Yamanoibuki' [65]), the number of examined SSR markers was increased to 41 in total. Again, this comparison showed no discrepancy in the five cultivars and their predicted parental cultivars (**Supplemental Table 5**, red, blue, and gray boxes). Uniparental origins were also confirmed for 25 cultivars (**Supplemental Table 4**, 'Asanoka' [4]-'Tsuyuhikari' [57]), but the remaining parent for each was unassigned.

Confirmation of maternity for the determined parentages

Chloroplast DNAs were analyzed to confirm the maternity of the present parentages. Three and one nucleotide polymorphisms were detected in the *ndhF-rpl32* and *trnSf1-trnGGG* loci, respectively (**Supplemental Table 6**). Seven cultivars ('Mie ryokuhou no. 1' [26], 'Harumidori' [10], 'Koushun' [20], 'Minekaori' [29], 'Saemidori' [44], 'Sofu' [50], and 'Toyoka' [56]) shared polymorphisms with their predicted maternal parents (**Supplemental Table 6**, red box), confirming their maternal parentages. Maternity was not confirmed for the other five cultivars, as there was no polymorphism in their tested chloroplast DNA.

Discussion

In this study, we performed parentage analyses of selected tea cultivars in Japan based on SSR markers, as to date, only a limited number of these analyses have previously been reported. The present study involved 79 cultivars, representing finer and larger scales of parentage analysis of tea cultivars in Japan than the previous reports. The 37 pedigrees were successfully confirmed or predicted from the 79 cultivars (**Table 1**, larger bold text). Notably, candidate parents ('Asahi' [3], 'Rokurou' [42], and 'Yamatomidori' [66]) were newly identified for the cultivars 'Houshun' [16], 'Mie ryokuhou no. 1' [26], 'Surugawase' [51], 'Tenmyo' [54], and 'Yamanoibuki' [65] (**Table 1**, brackets and filled stars, **Supplemental Table 4** and **Supplemental Fig. 1**, larger bold text). 'Asahi' [3] is one of the best *tencha* cultivars, whereas 'Samidori' [46] is suitable for *tencha* and *gyokuro*; both of these cultivars were selected from Kyoto landraces by breeders in the private sector. Both were planted next to each other as they were expected to introduce their superior traits for *tencha* to their offspring ('Tenmyo' [54]) during its breeding process (our unpublished information).

The present study reveals that 'Rokurou' [42] is a

Table 2. Genotypes of nine SSR markers in 'Tenmyo', 'Houshun', 'Surugawase', 'Yamanoibuki', 'Mie ryokuhou no. 1', and their predicted parental cultivars

No.	Cultivar[a]	Marker[b]	MSG0403			MSG0421			MSG0572			MSG0609		
♀ 46	Samidori				300			280	152		158			166
54	Tenmyo		270		300		278	280			158		106	166
♂ 3	Asahi		270	285		276	278		150		158	104	106	
♀ 46	Samidori				300			280	152		158			166
16	Houshun			281	300	276		280			158	98		166
♂ 42	Rokurou		275	281		276		300	156		158	98	104	
♀ 61	Yabukita		275		300			282		158	166		106	156
51	Surugawase			281	300	276		282		158	166	98	106	
♂ 42	Rokurou		275	281		276		300	156	158		98	104	
♀ 61	Yabukita		275		300			282		158	166		106	156
65	Yamanoibuki		275	281				282	300	158	166	98		156
♂ 42	Rokurou		275	281		276		300	156	158		98	104	
♀ 61	Yabukita		275		300			282		158	166		106	156
26	Mie ryokuhou no. 1		275				277	282			166	177		156	166
♂ 66	Yamatomidori		275			276	277		150			177		156	166

No.	Cultivar[a]	Marker[b]	MSG0699			MSG0703			MSG0795				MSG0800			MSG0811			
♀ 46	Samidori			255	267	135		169			155	168		219	223	137	139		
54	Tenmyo		253	255		135		169			155	168	200	219		137		145	
♂ 3	Asahi		253	255		135		169	139			168	200					145	
♀ 46	Samidori			255	267	135		169			155	168		219	223	137	139		
16	Houshun			255	259		163	169		147	155				223		139	145	
♂ 42	Rokurou		253		259		163		173	147	153		200		223			145	147
♀ 61	Yabukita			255		135				145		157	200				139	145	
51	Surugawase			255	259	135	163			145	153		200		223			145	147
♂ 42	Rokurou		253		259		163		173	147	153		200		223			145	147
♀ 61	Yabukita			255		135				145		157	200				139	145	
65	Yamanoibuki			255	259	135			173	145	153		200		223		139	145	
♂ 42	Rokurou		253		259		163		173	147	153		200		223			145	147
♀ 61	Yabukita			255		135				145		157	200				139	145	
26	Mie ryokuhou no. 1			255	267	135			139	145			200		223		139	141	
♂ 66	Yamatomidori			255	267	135			139		155			205	223		139	141	

[a] The maternity and paternity (♀ and ♂, respectively) of cultivars were assigned based on the described pedigrees and the prediction in this study.

[b] Allele sizes (bp) are indicated below marker names. For each combination of three cultivars, alleles shared with the predicted maternal and paternal parents are colored in red and blue, respectively. Alleles of unclear parental origins are colored in gray. See online article for color version of this table.

candidate parent of 'Houshun' [16], 'Surugawase' [51], and 'Yamanoibuki' [65] (**Table 1**, **Supplemental Table 4**, **Supplemental Fig. 1**). 'Surugawase' [51] and 'Yamanoibuki' [65] are the offsprings of 'Yabukita' [61], bred by Shizuoka Prefecture (Kuranuki *et al.* 1997, Oishi and Hitaka 1966). 'Rokurou' [42] was selected from a landrace of unknown origin (Agriculture, Forestry and Fisheries Research Council 1968). Part of such pedigrees was in a good agreement with the inheritance of the disease resistance. 'Surugawase' [51] and 'Rokurou' [42] have strong resistance to gray blight disease, whereas 'Yabukita' [61] is susceptible to it (Takeda 2003). The genotypes of these cultivars were predicted to be $Pl_1pl_1pl_2pl_2$ ('Surugawase' [51]), $Pl_1pl_1Pl_2pl_2$ ('Rokurou' [42]), and $pl_1pl_1pl_2pl_2$ ('Yabukita' [61]), where Pl_1 and Pl_2 confer strong and moderate levels of resistance, respectively (**Supplemental Fig. 2**; Takeda 2003). It is reasonable to infer that the haplotype Pl_1pl_2, derived from 'Rokurou' [42], combined with pl_1pl_2 from 'Yabukita' [61] to generate the $Pl_1pl_1pl_2pl_2$ genotype of 'Surugawase' [51] (**Supplemental Fig. 2A**). 'Houshun' [16] also has strong resistance to gray blight disease, whereas the resistance of its parent 'Samidori' [46] is slightly low (Kyoto Prefectural Tea Research Institute 2004a), suggesting that its strong resistance (Pl_1) is derived from 'Rokurou' [42] (**Supplemental Fig. 2B**). Hybridization with 'Rokurou' [42] could have provided the disease resistance trait in 'Houshun' [16] and 'Surugawase' [51]. 'Mie ryokuhou no. 1' [26] is the offspring of 'Yabukita' [61] (Ikeda *et al.* 1996). Its paternal parent was successfully predicted to be 'Yamatomidori' [66] in this study. 'Mie ryokuhou no. 1' [26] and 'Yamatomidori' [66] are late maturing cultivars, whereas 'Yabukita' [61] is a medium maturing one (Agriculture, Forestry and Fisheries Research Council 1968, Ikeda *et al.* 1996). The late maturing trait of 'Mie ryokuhou no. 1' [26] may be derived from 'Yamatomidori' [66].

The maternity for the pedigrees of the 12 cultivars was tested based on the maternally inherited chloroplast DNA.

This approach was successful in seven of the 12 cultivars (**Supplemental Table 6**). The sequences were monomorphic in the other five cultivars despite the testing of several other chloroplast markers (data not shown). This was probably because of the lower mutation rate in the chloroplast DNA than in the nuclear DNA (Wolfe *et al.* 1987) as previously reported (e.g. Wambulwa *et al.* 2016). Irrespective of such limitations, we assumed that the probability of misidentifying maternal plants is low as mistakes in the crossbreeding of tea can occur more frequently during the process of pollination than seed harvesting, and therefore more frequently in paternal than maternal parents (Tanaka *et al.* 2001).

In conclusion, the present parentage analysis based on SSR markers has confirmed tens of the described pedigrees in the tea cultivars, and especially have unlabeled the parents of 'Houshun' [16], 'Mie ryokuhou no. 1' [26], 'Surugawase' [51], 'Tenmyo' [54], and 'Yamanoibuki' [65]. This parentage analysis improves our knowledge of tea pedigrees and will aid in the development of new cultivars with superior traits in breeding programs.

Author Contribution Statement

NK, TM, YM and MK designed this study. TM and YH maintained tea samples and helped in sampling. NK and CY performed the genotyping, genetic diversity and parentage analyses. NK, TM, YM and MK performed interpretation of results. NK drafted the manuscript. All authors read and approved the final manuscript.

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