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Phenotypes and Genotypes Related to Tea Gray Blight Disease Resistance in the Genetic Resources of Tea in Japan

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Abstract

The phenotypes related to tea gray blight resistance were evaluated by the artificial inoculation method in the accessions of tea germplasm preserved at the National Institute of Vegetable and Tea Science in Makurazaki, Kagoshima Prefecture. The genotypes of 453 plants including 89 main tea cultivars in Japan were also determined by using the parent-offspring genetic analysis of many cross combinations. A wide variation in the resistance of tea plants to tea gray blight was observed both in terms of phenotypes and genotypes. The majority of the Assam plants (*Camellia sinensis* var. *assamica*) showed a high level of resistance and very few variations, both in genotypes and phenotypes. The Japanese native plants (*C. sinensis* var. *sinensis)* showed a wider genetic diversity than any other groups of plants in the resistance to tea gray blight. Since many of the tea plants derived from other countries were highly resistant to the disease and harbored 2 ***Pl1*** genes which confer a high level of resistance, they are very important materials for the breeding of cultivars that are resistant to the disease. The phenotype and genotype analysis was found to be very useful to identify doubtful cultivars.

Discipline: Plant breeding / Plant disease
Additional key words: *Camellia sinensis*, genetic analysis, genetic diversity

Introduction

Tea gray blight caused by *Pestalotiopsis longiseta* and tea anthracnose caused by *Colletotrichum teae-sinensis,* are serious tea diseases in Japan. Since it was shown that 'Yabukita', which is presently the leading tea cultivar in Japan, is susceptible to the fungus[1–4,8,9], it was deemed essential to develop tea cultivars resistant to the disease in Japan.

There are considerable differences in the susceptibility to *P. longiseta* among the tea cultivars[5]. A method of detecting the resistance to the disease has been developed[2,8,9] that enables a genetic analysis of the resistance to the disease. Genetic analysis revealed that the resistance of tea plants to the disease is controlled by 2 independent dominant resistance genes, ***Pl1*** and ***Pl2***. The ***Pl1*** gene, which confers a high level of resistance, is genetically epistatic in relation to the ***Pl2*** gene, which confers a moderate level of resistance[8,9].

The present report deals both with phenotypes and genotypes related to the resistance of tea plants to *P. longiseta* in the tea germplasm accessions preserved at the Makurazaki Station, which is the largest gene bank station of tea in Japan.

Materials and methods

The resistance of the materials was evaluated after inoculation of the fungus in the field. To evaluate the resistance, 5 healthy mature leaves per plant were wounded in 2 areas of a leaf by the sharpened tip of a 3 mm wide (+) screw driver and infected with a water suspension of conidia placed on the tip of the instrument.

The conidia of *P. longiseta* were cultured on a medium consisting of autoclaved tea leaves. The number of conidia per inoculation was adjusted at the optimum concentration to 10^6 per mL. Phenotypes were determined by the degree of resistance which was evaluated 15–18 days after the inoculation by measuring the diameter of the lesions, and the plants were divided into 3 groups: resistant, R; moderately resistant, M; and susceptible, S (Fig. 1).

Based on the segregation of cross combinations

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between the susceptible cultivar, 'Yabukita' and tested tea plants preserved as genetic resources, their genotypes were determined (Fig. 2).

Nine genotypes of tea plants related to the resistance to tea gray blight could be analyzed by this method because the resistance is controlled by 2 independent dominant resistance genes, ***Pl1*** and ***Pl2***. The ***Pl1*** gene which confers a high level of resistance, is genetically epistatic in relation to the ***Pl2*** gene, which confers a moderate level of resistance[8,9]. However, 3 genotypes harboring the ***Pl1*** gene with homozygosity could not be analyzed fully because all the F_1 plants exhibited the resistance without separation of the phenotype. In this case, as the F_1 plants harbored 1 or 2 ***Pl1*** genes regardless of cross combinations, all of the F_1 plants were resistant, and the ***Pl2***-*pl2* gene reaction could not be analyzed in the F_1 generation. Thus the incomplete genotype ***Pl1Pl1*** ? ? comprised 3 genotypes, ***Pl1Pl1Pl2Pl2***, ***Pl1Pl1Pl2pl2*** and ***Pl1Pl1pl2pl2***.

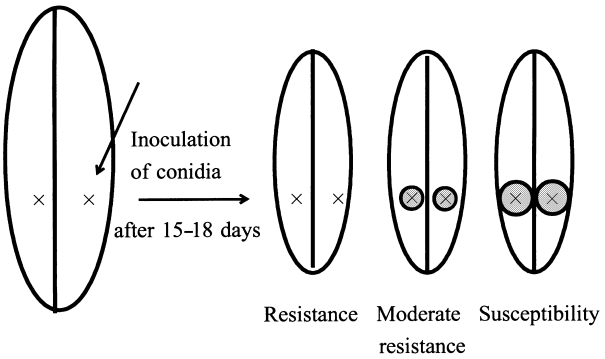

Fig. 1. Lesions of 3 groups of tea plants in relation to the resistance to *P. longiseta*
Resistance: Lesion size ≦ 5 mm.
Moderate resistance: Lesion size 6–10 mm.
Susceptibility: Lesion size ≧ 11 mm.

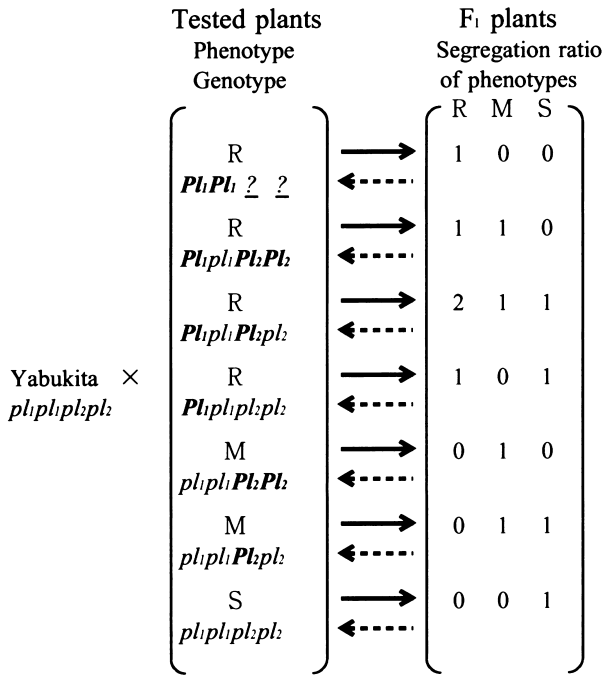

Fig. 2. Genotypes and phenotypes of tea plants in relation to the resistance to *P. longiseta*
Phenotypes: R, resistance; M, moderate resistance; S, susceptibility.
→ : Theoretical segregation ratio of phenotype in F_1.
⇠ : Genotypes estimated from the theoretical segregation ratio of phenotypes in F_1.

These experiments were performed from mid-July to August after the rainy season in 1989–2000 at the Makurazaki Station of the National Institute of Vegetable and Tea Science.

Results and discussion

1. Evaluation of the resistance to tea gray blight in the accessions of tea germplasm

Screening tests for the resistance to tea gray blight were carried out for 2,480 plants collected worldwide and maintained at the Makurazaki Station. The results are shown in Fig. 3.

The Japanese native plants collected from all over Japan showed considerable variations in the resistance. Susceptible plants accounted for 16.5% of the total number of plants, the same rate as that for the plants with moderate resistance to the disease. Of the 1,178 plants, 774 were resistant. In the Chinese variety (var. *sinensis*), there were large differences between the Japanese native plants and introduced Chinese and Indian plants which had been collected from Zhejiang, Jiangxi, and Anhui Provinces in China and Darjeeling in India, respectively. These foreign materials belonging to the var. *sinensis* were generally resistant phenotypically, although some of the materials were moderately resistant and susceptible. In 723 plants belonging to the Assam variety (var. *assamica*) which had been collected from India, Sri Lanka, Myanmar, Vietnam, Bangladesh and Taiwan, all of the materials showed a high level of resistance except for 8 plants that showed only a moderate level of resistance. There were no susceptible plants in the Assam variety.

2. Analysis of the genotypes of tea plants in relation to the resistance to tea gray blight

The genotypes related to the resistance to tea gray blight caused by *P. longiseta* were analyzed in 453 plants based on the segregation ratio of the F_1 progenies between the susceptible cultivar 'Yabukita' and tested plants (Fig. 2 and Table 1).

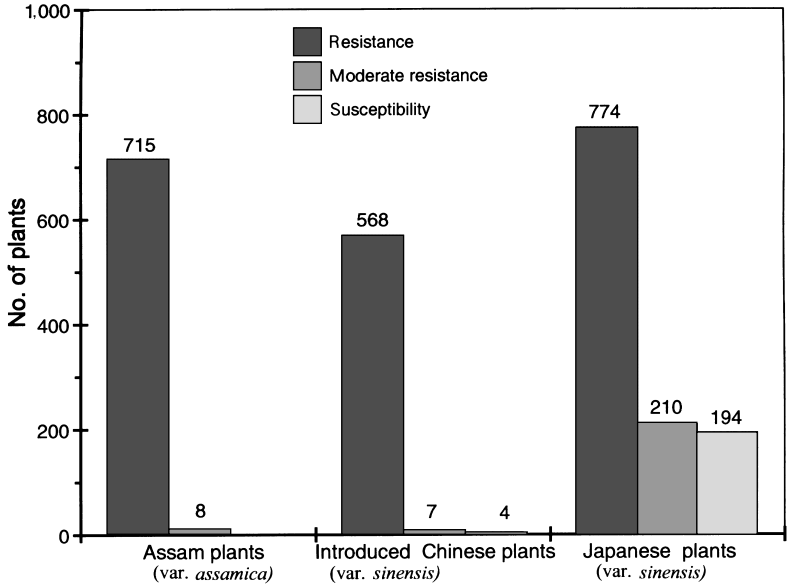

Fig. 3. Phenotypes of the tea plants in relation to the resistance to *P. longiseta*
Collection sites were as follows.
Assam plants (var. *assamica*): India, Sri Lanka, Myanmar, Bangladesh, Vietnam, Malaysia, Taiwan.
Chinese plants (var. *sinensis*): China, Korea, India (Darjeeling).
Japanese plants (var. *sinensis*): Japan.

More than 98% of the introduced Chinese plants (var. *sinensis*) had a phenotype with a high level of resistance, as in the case of the Assam variety (Fig. 3). However, the genotypes related to the resistance to tea gray blight were markedly different between the Assam variety and the Chinese variety, and clear differences were also observed between the introduced Chinese plants and Japanese native plants in the same Chinese variety (Table 1). It was found that the genotypes of the Chinese variety displayed wider variations than those of the Assam variety. In the Chinese variety, the Japanese native plants showed a wider genetic polymorphism than the introduced Chinese plants, including the small-leaf plants collected from China and Darjeeling in India.

The genotypes of the Assam variety were very simple and 76.4% of the plants showed homozygosity for the ***Pl*$_1$** gene which confers a high level of resistance to tea gray blight. Although the phenotypes of the introduced Chinese plants were very similar to those of the Assam variety in the resistance to the disease, the genotypes displayed wider variations than those of the Assam variety and all the 7 genotypes shown in Fig. 2 were identified. In the Japanese native plants of the Chinese variety, 80.6% of the 62 plants showing the highest level of resistance phenotypically harbored only one ***Pl*$_1$** gene. The variations in the genotype of the Darjeeling plants were as narrow as those of the Assam variety. It was assumed that the small-leaf plants of Darjeeling had been affected

Table 1. Genotypes of tea plants in relation to the resistance to *P. longiseta*

Varieties	Phenotype	Resistance				Moderate resistance		Susceptibility	Total
	Genotype	***Pl*$_1$*Pl*$_1$ *?* *?***	***Pl*$_1$*pl*$_1$*Pl*$_2$*Pl*$_2$**	***Pl*$_1$*pl*$_1$*Pl*$_2$*pl*$_2$**	***Pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$**	***pl*$_1$*pl*$_1$*Pl*$_2$*Pl*$_2$**	***pl*$_1$*pl*$_1$*Pl*$_2$*pl*$_2$**	***pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$**	
var. *assamica*									
Assam plants		96	28	1	4				129
Taiwan wild tea		11							11
var. *sinensis*									
Introduced Chinese plants									
Darjeeling		23	6						29
China		42	30	2	9	1	5	1	90
Japanese native plants		12	29	17	4	8	8	1	79
Breeding cultivars		3	11	8	16	3	10	13	64
Hybrid		6	18	3	9	4	6	5	51

Genotype ***Pl*$_1$*Pl*$_1$** *?* *?* consists of 3 genotypes: ***Pl*$_1$*Pl*$_1$*Pl*$_2$*Pl*$_2$**, ***Pl*$_1$*Pl*$_1$*Pl*$_2$*pl*$_2$**, ***Pl*$_1$*Pl*$_1$*pl*$_2$*pl*$_2$**.
Assam plants were collected in India, Sri Lanka, Myanmar, Vietnam, Bangladesh and Malaysia.
Taiwan wild tea refers to Taiwan mountain wild tea collected in Taiwan.
Breeding cultivars were bred in Japan by crossing or selection in the tea field.

by the Assam plants (var. *assamica*) over the long period of cultivation in India. These facts were reflected in the characters, such as color of mature leaf[8], distribution and density of pubescence on young leaves[6], cold hardiness in winter[11] and caffeine and tannin contents in young leaves[7].

3. Analysis of the genotypes and phenotypes of major tea cultivars in Japan

The phenotypes and genotypes related to the resistance to tea gray blight of 89 main tea cultivars in Japan were identified (Table 2). Of these, 65 cultivars were derived from Japanese native plants, 9 cultivars originated from mainland China and Taiwan and 15 cultivars were hybrids between the Assam variety and the Chinese variety. As for the 89 cultivars of Japan, the ***Pl*$_1$** gene conferring the highest level of resistance to the disease was mainly derived from plants introduced from foreign countries that had contributed to the breeding for resistance to tea gray blight in Japan. The cultivars for Sencha, a kind of Japanese green tea, included a high rate of susceptible cultivars to the disease since the susceptible cultivar 'Yabukita' had been used many times as a breeding material. The susceptible cultivars derived from 'Yabukita' included 'Saemidori', 'Toyoka', 'Fukumidori', 'Hokumei', 'Harumidori', 'NN-27' and 'Kanaya-No.5'. Of the total 50,146 ha area planted with tea in Japan, 38,738 ha are planted with 'Yabukita'.

4. Identification of doubtful cultivars based on phenotypes and genotypes

The parents of the 3 main breeding cultivars, i.e. 'Meiryoku', 'Yutakamidori' and 'Shunmei' had remained doubtful in terms of transmission of the resistance. The genotypes and phenotypes of these 3 cultivars were examined in relation to the resistance to the disease based on parent-offspring genetic analysis.

'Meiryoku' was moderately resistant phenotypically and the genotype was pl_1pl_1***Pl*$_2$**pl_2, as shown in Table 2. As the parents of this cultivar were assumed to be 'Yabukita' (seed parent) and 'Yamatomidori' (pollen parent), the genotype of 'Meiryoku' could not be produced in this cross combination, because the genotype of 'Yabukita' is $pl_1pl_1pl_2pl_2$ and that of 'Yamatomidori' is ***Pl*$_1$**$pl_1pl_2pl_2$ and these 2 cultivars do not harbor the ***Pl*$_2$** gene. This error was also indicated by the analysis of DNA markers of the 2 cultivars[10].

'Yutakamidori' was moderately resistant phenotypically and the genotype was pl_1pl_1***Pl*$_2$**pl_2. 'Yutakamidori' was derived from the self-fertilization of 'Asatsuyu'. The genotype of 'Asatsuyu' which was $pl_1pl_1pl_2pl_2$ without the ***Pl*$_2$** gene is shown in Table 2. Therefore, it was

Table 2. Phenotypes and genotypes of 89 main tea cultivars in Japan

Phenotype	Genotype	Cultivars
Resistance	***Pl*$_1$*Pl*$_1$** ? ?	**Benihomare Benihikari Benifuuki Indo Benifuji Inzatsu131 Tadanishiki** Fushun Kuritawase Minamisayaka Karabeni Chin-Shin-Oolong San-Cha-Tsi-Lan Chin-Shin-Da-Pan Huang-Gan
	Pl*$_1$pl*$_1$***Pl*$_2$***Pl*$_2$**	**Benitachiwase Akane Houryoku Satsumabeni Benikaori** Yaeho Miyoshi Izumi Takachiho Himemidori Tamamidori Unkai Hoshinomidori Yamanami Asagiri Komakage Z-1 Okuhikari Kanaya-No.15 **Makurazaki-No.4 Makurazaki-No.5**
	Pl*$_1$pl*$_1$***Pl*$_2$***pl*$_2$**	**Hatsumomiji** Rokuro Koyanishi Shunmei ME-52 Nka-O3 Makurazaki-No.7 Makurazaki-No.8 S-6
	Pl*pl*$_1$*pl*$_2$*pl*$_2$**	Sayamakaori Yamatomidori Surugawase Okumidori Kanayamidori Minamikaori Satouwase Asanoka Ooiwase NN-27 Shizu-zai-16 Saitama-No.9 Kanaya-No.7 Miyakei-No.2 Makurazaki-No.11 Makurazaki-No.13 Makurazaki-No.16
Moderate resistance	*pl*$_1$*pl*$_1$***Pl*$_2$***Pl*$_2$**	Makizono-dai-chaju Makurazaki-No.1 Nagasaki-No.2
	pl$_1$*pl*$_1$***Pl*$_2$***pl*$_2$**	Yutakamidori Meiryoku Fujimidori Minekaori Kurasawa Yamakai Nka-O-278 Unryuu-cha Makurazaki-No.18 Makurazaki-No.23
Susceptibility	*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$*	Yabukita Asatsuyu Saemidori Toyoka Fukumidori Natsumidori Sayamamidori Okumusashi Okuyutaka Hokumei Harumidori NN-12 Mie-No.260 Kanaya-No.5

***Pl*$_1$** gene confers a high level of resistance.
***Pl*$_2$** gene confers a moderate level of resistance.
Cultivars written in bold letters are Assam hybrids between var. *assamica* and var. *sinensis*.
Underlined cultivars are of Chinese origin (mainland China and Taiwan).
Cultivars without marks are suitable for Sencha, a kind of Japanese green tea.

assumed that the pollen parent was a cultivar with either the ***Pl*$_1$*pl*$_1$***Pl*$_2$***Pl*$_2$, ***Pl*$_1$*pl*$_1$***Pl*$_2$*pl*$_2$, *pl*$_1$*pl*$_1$***Pl*$_2$***Pl*$_2$ or *pl*$_1$*pl*$_1$***Pl*$_2$*pl*$_2$ genotypes.

The cultivar 'Shunmei' (***Pl*$_1$*pl*$_1$***Pl*$_2$*pl*$_2$) was derived from a cross combination between 'Yutakamidori' and 'Kana NN 8'. As shown in Table 2, the genotype of 'Yutakamidori' was *pl*$_1$*pl*$_1$***Pl*$_2$*pl*$_2$ and the cultivar was moderately resistant phenotypically. On the other hand, both the genotype and phenotype of 'Kana NN 8' had been found to be the same as those of 'Yutakamidori' in another experiment[8]. Therefore, the genotype of the plants obtained from this cross combination did not harbor the ***Pl*$_1$** gene and the plants were resistant phenotypically. It was thus demonstrated that the assumed parent of this cultivar was incorrect.

The analysis of the genotypes and phenotypes related to the resistance to tea gray blight also enabled to discriminate similar cultivars, such as 'Himemidori' and 'S6', 'Kanayamidori' and 'NN 12' because of the differences in the genotypes or phenotypes, as shown in Table 2. However, 'Rokuro' and 'Koyanishi' could not be distinguished because their phenotypes and genotypes were identical.

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