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# Genetic Analysis of Tea Gray Blight Resistance in Tea Plants

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## Abstract

Tea gray blight caused by *Pestalotiopsis longiseta* SPEGAZZINI is a severe disease in tea fields in Japan. The resistance of tea plants to the disease was found to be controlled by 2 independent dominant resistance genes $\boldsymbol{Pl_1}$ and $\boldsymbol{Pl_2}$, based on parent-offspring genetic analysis. There were 9 genotypes for resistance to *P. longiseta* in tea plants. Six genotypes $\boldsymbol{Pl_1Pl_1Pl_2Pl_2}$, $\boldsymbol{Pl_1Pl_1Pl_2}pl_2$, $\boldsymbol{Pl_1Pl_1}pl_2pl_2$, $\boldsymbol{Pl_1}pl_1\boldsymbol{Pl_2Pl_2}$, $\boldsymbol{Pl_1}pl_1\boldsymbol{Pl_2}pl_2$, $\boldsymbol{Pl_1}pl_1pl_2pl_2$ were resistant, 2 genotypes $pl_1pl_1\boldsymbol{Pl_2Pl_2}$, $pl_1pl_1\boldsymbol{Pl_2}pl_2$ were moderately resistant and one genotype $pl_1pl_1pl_2pl_2$ was susceptible. Since the cultivars harboring the $\boldsymbol{Pl_1}$ gene with homozygosity always produced resistant plants to the disease in any cross combinations, they could become very important materials for the breeding of cultivars resistant to the disease.

**Discipline:** Plant breeding / Plant disease / Tea industry
**Additional key words:** epistatic effect, genotype, phenotype

## Introduction

Tea gray blight was caused by *Pestalotiopsis longiseta theae* SAWADA up to the early 1970s in Japan. However, since around 1973, outbreaks of the disease associated with severe lesions have been observed mainly in Shizuoka Prefecture[5]. Presently, tea gray blight caused by *P. longiseta* is a very severe disease in the main tea-growing districts of Japan. Hamaya and Horikawa (1982), who revealed that the virulent pathogen causing tea gray blight was not *P. theae* but *P. longiseta* SPEGAZZINI, studied the ecological characteristics of this fungus[2]. As *P. longiseta* attacks leaves as well as the current shoots which eventually wither, both the quality and yield of tea are severely affected[3].

Since it was shown that 'Yabukita', which is presently the leading tea cultivar in Japan, is susceptible to this fungus[1,2,4,6,7], it was deemed essential to develop tea cultivars resistant to the disease. There are considerable differences in the susceptibility to *P. longiseta* among tea cultivars. In addition, a method of testing for resistance to the disease which has been recently developed[2,8] enables to carry out a genetic analysis of the resistance of tea plants to the disease.

The present report deals with the genetic analysis of the resistance of tea plants to *P. longiseta*.

## Materials and methods

Seedlings from reciprocal diallel crosses of 5 tea cultivars, *i.e.* 'Yabukita', 'Fujimidori', 'Sayamakaori', 'Yamatomidori' and 'Z-1' were used as materials. Thirty seedlings for each cross were planted in a field without selection. The resistance of the materials was evaluated after inoculation of the fungus in the field. For the inoculation, a mature leaf on a healthy shoot was wounded 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. Since 5 healthy leaves per plant were inoculated in 2 areas of a leaf, a total of 10 areas were inoculated. The conidia of *P. longiseta* which were used for the experiments were cultured on a medium consisting of autoclaved tea leaves. The number of conidia per inoculation was adjusted to $10^6$ per mL which is the optimum concentration for artificial inoculation. The tests were performed from mid-July to August after the rainy season because high temperatures of 25 to 30ºC enhance the incidence of this disease. The degree of resistance 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; moder-

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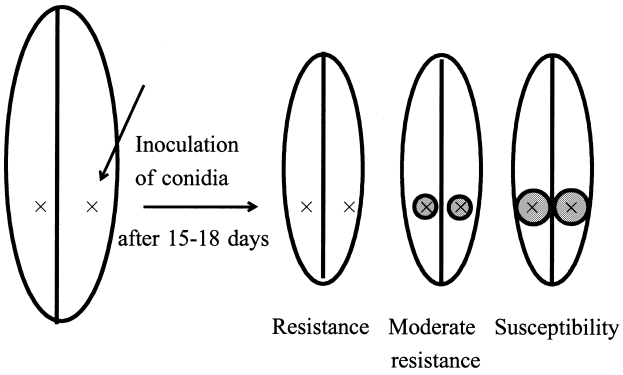


**Fig. 1. Lesions of 3 groups in relation to the resistance to *P. longiseta***
Resistance, lesion size ≦ 5 mm;
Moderate resistance, lesion size 7–10 mm;
Susceptibility, lesion size ≧ 11 mm.

ately resistant, M; and susceptible, S (Fig. 1).

Based on the segregation of each cross combination, genetic analysis of the resistance of tea plants to tea gray blight was carried out and a hypothesis for the resistance of tea plants to the disease was proposed.

Moreover, 16 cross combinations including 14 cultivars were examined to verify the above hypothesis in applying the same method as that previously described.

In addition, the $F_1$ plants between a susceptible cultivar 'Yabukita' and the 3 tea cultivars 'Benihikari', 'Ace 37' and 'Abo 27' which always produced resistant plants to the disease in all the cross combinations were backcrossed again to 'Yabukita' to detect the full genotypes by analyzing the segregation in the $BC_1$ generation.

These experiments were performed from 1989 to 1998 at the Makurazaki Station of the National Institute of Vegetable and Tea Science.

## Results and discussion

### Genetic analysis of the resistance to tea gray blight

The size of the lesions of tea gray blight was measured in the parents of the 5 cultivars whose degree of resistance to the disease had been determined[6]. The size of the lesions exceeded 11 mm in diameter in 'Yabukita', which is a susceptible cultivar (S), while in the case of 'Fujimidori', which is moderately resistant (M), the size of the lesions ranged from 7 to 10 mm. In the case of 'Yamatomidori', 'Sayamakaori and 'Z-1', which were resistant (R), the size of the lesions ranged between 3 and 5 mm in diameter. Thus the criteria for evaluating the resistance to the disease were as follows: resistance, lesion size ≦ 5 mm; moderate resistance, lesion size 7–10 mm; susceptibility, lesion size ≧ 11 mm.

Table 1 indicates the size of the lesions in relation to the degree of resistance to tea gray blight in the parents of the 5 cultivars used for the crosses. Since no significant differences were detected in the segregation for the resistance among the reciprocal crosses (Fig. 2), data on the $F_1$ of the crosses were analyzed together. The segregation for resistance to the disease in the $F_1$ plants from the reciprocal crosses among the 5 cultivars is shown in Table 2. There were 2 or 3 distinct peaks for the segregation of the $F_1$ plants. In the S × R combinations, there were 2 types of segregation patterns, such as segregation ratio 1 : 1 for R and S ('Yabukita' × 'Sayamakaori', 'Yabukita' × 'Yamatomidori') and segregation ratio 1 : 1 for R and M ('Yabukita' × 'Z-1'). It was thus suggested that the resistance gene for tea gray blight did not depend on one pair of alleles but on 2 or more alleles.

In the S×M combinations, the value of the segregation ratio for S and M was approximately identical and there was no segregation for R ('Yabukita' × 'Fujimidori').

In the M×R combinations, 2 patterns of segregation were observed, such as segregation ratio 2 : 1 : 1 for R, M, and S ('Fujimidori' × 'Yamatomidori', 'Fujimidori' ×'Sayamakaori') and segregation ratio 1 : 1 for R and M ('Fujimidori' × 'Z-1').

In the R ×R combinations, the segregation ratio was 3 : 1 for R and S in the cross 'Yamatomidori' × 'Sayamakaori' and 3 : 1 for R and M in the combinations of 'Yamatomidori' × 'Z-1' or 'Sayamakaori' × 'Z-1'.

Thus the pattern of segregation of the $F_1$ plants tended to be identical for 'Sayamakaori' and 'Yamatomidori', suggesting that the genotype for the resistance was identical in the 2 cultivars. Based on the segregation

**Table 1. Reaction of parent cultivars to *P. longiseta* and mean diameter of the lesions**

| Cultivar | Reaction* | No. of plants observed | Mean ± s.d. (mm) |
|---|---|---|---|
| Yabukita | S | 45 | 13.07 ± 1.66 |
| Fujimidori | M | 36 | 8.17 ± 1.38 |
| Sayamakaori | R | 44 | 4.36 ± 0.75 |
| Yamatomidori | R | 45 | 3.22 ± 0.52 |
| Z-1 | R | 46 | 4.17 ± 1.14 |

*R, Resistant; M, Moderately resistant; S, Susceptible.

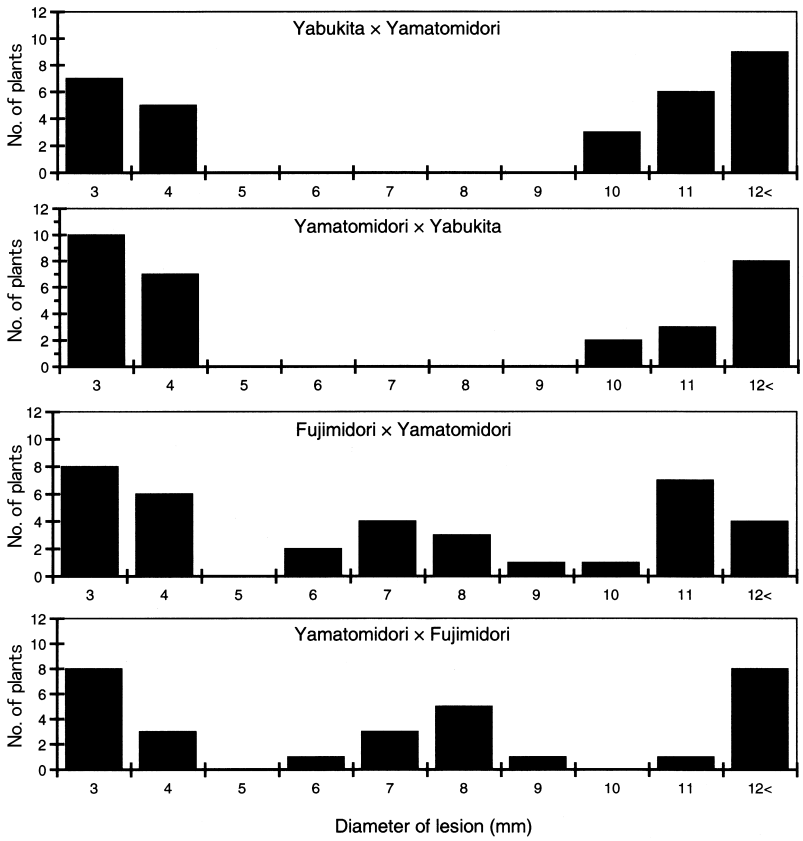


**Fig. 2. Segregation of the lesion size of $F_1$ plants in the reciprocal crosses**

analysis of each cross combination and the degree of resistance of the parents, the genetic pattern of resistance to *P. longiseta* can be interpreted as follows. There are 2 dominant resistance genes acting independently, namely ***Pl₁*** which confers a strong level of resistance and ***Pl₂*** which confers a moderate level of resistance, with ***Pl₁*** showing an epistatic effect in relation to ***Pl₂***.

Table 3 illustrates the proposed genotypes of the parents used for the crosses. For the susceptible cultivar 'Yabukita' which does not harbor the dominant resistance genes ***Pl₁*** and ***Pl₂***, the proposed genotype was $pl_1pl_1pl_2pl_2$. Since half of the plants from the crosses between the moderately resistant cultivar 'Fujimidori' with 'Yabukita' were susceptible and the other half were moderately resistant to the disease, the proposed genotype of 'Fujimidori' was $pl_1pl_1\mathbf{Pl_2}pl_2$ with heterozygosity for the ***Pl₂*** gene which confers a moderate level of resistance.

Since in the $F_1$ plants from crosses between the

**Table 2. Segregation for the resistance to *P. longiseta* in $F_1$ plants obtained from crosses among 5 cultivars**

| Cross combination | No. of observed plants / groups | | | | Expected ratio | | | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|---|---|
| | R | M | S | Total | R | M | S | | |
| Yabukita(S) × Yamatomidori(R) | 29 | | 31 | 60 | 1 | | 1 | 0.067 | 0.9<P |
| Yabukita(S) × Sayamakaori(R) | 32 | | 28 | 60 | 1 | | 1 | 0.267 | 0.8<P<0.9 |
| Yabukita(S) × Z-1(R) | 33 | 27 | | 60 | 1 | 1 | | 0.600 | 0.7<P<0.8 |
| Yabukita(S) × Fujimidori(M) | | 35 | 25 | 60 | | 1 | 1 | 1.667 | 0.4<P<0.5 |
| Fujimidori(M) × Yamatomidori(R) | 25 | 20 | 15 | 60 | 2 | 1 | 1 | 2.500 | 0.2<P<0.3 |
| Fujimidori(M) × Sayamakaori(R) | 30 | 15 | 15 | 60 | 2 | 1 | 1 | 0.000 | – |
| Fujimidori(M) × Z-1(R) | 34 | 26 | | 60 | 1 | 1 | | 1.067 | 0.5<P<0.6 |
| Yamatomidori(R) × Z-1(R) | 46 | 14 | | 60 | 3 | 1 | | 0.088 | 0.9<P |
| Sayamakaori(R) × Z-1(R) | 43 | 17 | | 60 | 3 | 1 | | 0.356 | 0.8<P<0.9 |
| Yamatomidori(R) × Sayamakaori(R) | 50 | | 10 | 60 | 3 | | 1 | 1.112 | 0.5<P<0.6 |

**Table 3. Reaction of parent cultivars for the resistance to *P. longiseta* and the proposed genotype**

| Cultivar | Reaction | Proposed genotype |
|---|---|---|
| Yabukita | S | $pl_1pl_1pl_2pl_2$ |
| Fujimidori | M | $pl_1pl_1\mathbf{Pl_2}pl_2$ |
| Sayamakaori | R | $\mathbf{Pl_1}pl_1pl_2pl_2$ |
| Yamatomidori | R | $\mathbf{Pl_1}pl_1pl_2pl_{2s}$ |
| Z-1 | R | $\mathbf{Pl_1}pl_1\mathbf{Pl_2Pl_2}$ |

highly resistant cultivars 'Sayamakaori' or 'Yamatomidori' and the susceptible cultivar 'Yabukita' the segregation pattern for the resistance and the susceptibility was 1 : 1, the genotype $\mathbf{Pl_1}pl_1pl_2pl_2$ was proposed for 'Sayamakaori' and 'Yamatomidori' .

In the case of 'Z-1', no plants were susceptible and only highly resistant plants or plants with a moderate level of resistance were produced regardless of the cross combinations. Therefore, the $\mathbf{Pl_1}pl_1\mathbf{Pl_2Pl_2}$ genotype was proposed, with homozygosity for $\mathbf{Pl_2}$ which confers a moderate level of resistance and heterozygosity for $\mathbf{Pl_1}$ which confers a high level of resistance. Based on the genotype of the parents which had been tentatively proposed, chi-square test of the segregation pattern of each cross combination was performed and the value obtained fitted well to the theoretical values (Table 2).

Based on the hypothesis put forward previously, 9 genotypes of tea plants for the resistance to tea gray blight were proposed. The relation between the genotype and phenotype is displayed in Table 4. The 6 genotypes showing high levels of the resistance gene $\mathbf{Pl_1}$ with homozygosity or heterozygosity showed a resistance in the phenotype. The 2 genotypes which harbored the $\mathbf{Pl_2}$ gene with homozygosity or heterozygosity without the $\mathbf{Pl_1}$ gene showed a moderate resistance in the phenotype. One genotype which did not harbor the 2 types of dominant resistance genes $\mathbf{Pl_1}$ and $\mathbf{Pl_2}$ showed a susceptibility

**Table 4. Proposed genotype and relationship between the genotype and phenotype**

| Genotype | Phenotype |
|---|---|
| $\mathbf{Pl_1Pl_1Pl_2Pl_2}$ | Resistant |
| $\mathbf{Pl_1Pl_1Pl_2}pl_2$ | Resistant |
| $\mathbf{Pl_1Pl_1}pl_2pl_2$ | Resistant |
| $\mathbf{Pl_1}pl_1\mathbf{Pl_2Pl_2}$ | Resistant |
| $\mathbf{Pl_1}pl_1\mathbf{Pl_2}pl_2$ | Resistant |
| $\mathbf{Pl_1}pl_1pl_2pl_2$ | Resistant |
| $pl_1pl_1\mathbf{Pl_2Pl_2}$ | Moderately resistant |
| $pl_1pl_1\mathbf{Pl_2}pl_2$ | Moderately resistant |
| $pl_1pl_1pl_2pl_2$ | Susceptible |

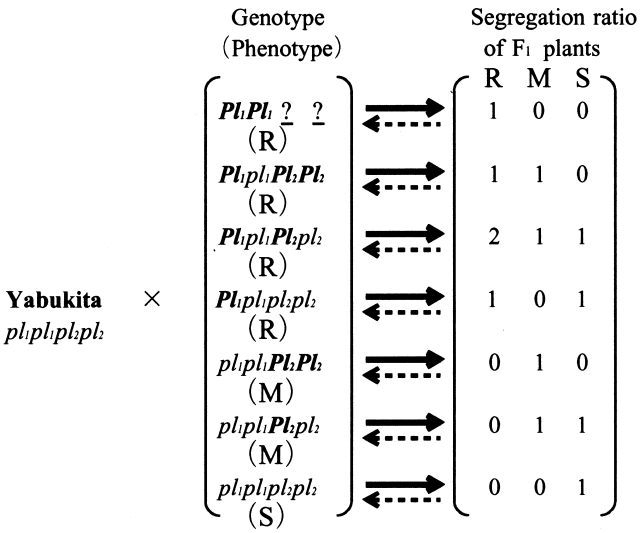


**Fig. 3. Estimation of the genotypes based on the segregation of the $F_1$ plants for the resistance to *P. longiseta***

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 $F_1$.

in the phenotype.

According to the hypothesis put forward previously, we were able to analyze the genotype of the tea plants for resistance to tea gray blight in the $F_1$ generation. The model for analyzing the genotype of the tested plants is shown in Fig. 3.

Initially, the tested plants were crossed with susceptible cultivars such as 'Yabukita'. Thus, their genotypes could be estimated based on the model illustrated in Fig. 3. In this method, 3 genotypes harboring the $\mathbf{Pl_1}$ gene with homozygosity could not be analyzed fully because all the $F_1$ plants exhibited the resistance without separation in the phenotype. In this case, as the $F_1$ plants harbored one or two $\mathbf{Pl_1}$ genes regardless of cross combinations, all the $F_1$ plants were resistant, and the $\mathbf{Pl_2}$-$pl_2$ gene reaction could not be analyzed in the $F_1$ generation. Thus the unidentified genotype $\mathbf{Pl_1Pl_1}$ ? ? may include 3 genotypes, $\mathbf{Pl_1Pl_1Pl_2Pl_2}$, $\mathbf{Pl_1Pl_1Pl_2}pl_2$ and $\mathbf{Pl_1Pl_1}pl_2pl_2$.

To verify the hypothesis, the resistance to tea gray blight was evaluated in 9 cross combinations between the susceptible cultivars ('Yabukita', 'Asatsuyu' and 'Saemifori') and tested cultivars. The segregation analysis of the $F_1$ plants in each cross combination is shown in Table 5. The method depicted in Fig. 3 was applied to each segregation ratio for resistance (R), moderate resistance (M) and susceptibility in the $F_1$ of 9 cross combinations and fitness to the expected ratio was verified by using the

**Table 5. Genotypes estimated from the segregation of $F_1$ plants between the susceptible cultivar and tested cultivar**

| Cultivar (estimated genotype) | Tested cross combination | No. of observations | | | Expected ratio | | | Probability ($\chi^2$ value) |
|---|---|---|---|---|---|---|---|---|
| | | R | M | S | R | M | S | |
| Benitachiwase (***Pl*$_1$*pl*$_1$*Pl*$_2$*Pl*$_2$**) | Yabukita×Benitachiwase (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | 43 | 30 | | 1 | 1 | | 0.3<P<0.4 (2.315) |
| Saemidori (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | Yabukita×Saemidori (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | | | 71 | | | 1 | – (0) |
| $F_1$ 10115 (***Pl*$_1$*pl*$_1$*Pl*$_2$*pl*$_2$**) | $F_1$10115×Asatsuyu (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | 28 | 7 | 7 | 2 | 1 | 1 | 0.05<P<0.1 (4.667) |
| Shizu-Zai-16 (***Pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$**) | Yabukita×Shizu-Zai-16 (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | 14 | | 13 | 1 | | 1 | 0.95<P (0.037) |
| Makurazaki-13 (***Pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$**) | Yabukita×Makurazaki-13 (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | 62 | | 85 | 1 | | 1 | 0.1<P<0.2 (3.598) |
| Asatsuyu (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | Yabukita×Asatsuyu (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | | | 57 | | | 1 | – (0) |
| Mak-Kei-29-5 (***Pl*$_1$*pl*$_1$*Pl*$_2$*pl*$_2$**) | Yabukita×Mak-Kei-29-5 (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | 27 | 11 | 13 | 2 | 1 | 1 | 0.7<P<0.8 (0.568) |
| Meiryoku (*pl*$_1$*pl*$_1$***Pl*$_2$***pl*$_2$) | Yabukita×Meiryoku (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | | 15 | 13 | | 1 | 1 | 0.4<P<0.5 (0.143) |
| Yutakamidori (*pl*$_1$*pl*$_1$***Pl*$_2$***pl*$_2$) | Yutakamidori×Saemidori (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | | 27 | 28 | | 1 | 1 | 0.95<P (0.143) |

chi-square test. All the 9 cross combinations fitted well to the expected values deduced from Fig. 3, with probabilities of the chi-square values exceeding 0.05. Twelve cultivars whose genotypes had been identified as shown in Table 3 and Table 5 were used for 7 cross combinations to verify the hypothesis (Table 6). The segregation ratio for resistance, moderate resistance and susceptibility in the $F_1$ plants fitted well to the expected values deduced from the genotype of the parents. It is thus concluded that the resistance to tea gray blight is controlled

**Table 6. Chi-square analysis of the segregation ratio of $F_1$ plants among the cultivars with identified genotypes**

| Cross combination (Genotype) | | | Observation No. | | | Expected ratio | | | Probability ($\chi^2$ value) |
|---|---|---|---|---|---|---|---|---|---|
| | | | R | M | S | R | M | S | |
| Benitachiwase (***Pl*$_1$*pl*$_1$*Pl*$_2$*Pl*$_2$**) | × | Sayamakaori (***Pl*$_1$***pl*$_1$*pl*$_2$*pl*$_2$) | 27 | 13 | | 3 | 1 | | 0.5<P<0.6 (1.200) |
| Z-1 (***Pl*$_1$*pl*$_1$*Pl*$_2$*Pl*$_2$**) | × | Saemidori (*pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$) | 77 | 75 | | 1 | 1 | | 0.95<P (0.026) |
| Kanayamidori (***Pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$**) | × | $F_1$ 10115 (***Pl*$_1$*pl*$_1$*Pl*$_2$*pl*$_2$**) | 40 | 7 | 7 | 6 | 1 | 1 | 0.95<P (0.025) |
| Yutakamidori (*pl*$_1$*pl*$_1$***Pl*$_2$***pl*$_2$) | × | Mak-Kei-29-5 (***Pl*$_1$*pl*$_1$*Pl*$_2$*pl*$_2$**) | 31 | 19 | 12 | 4 | 3 | 1 | 0.2<P<0.3 (3.108) |
| Yamatomidori (***Pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$**) | × | Meiryoku (*pl*$_1$*pl*$_1$***Pl*$_2$***pl*$_2$) | 21 | 10 | 12 | 2 | 1 | 1 | 0.9<P<0.95 (0.209) |
| Shizu-Zai-16 (***Pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$**) | × | Makurazaki-13 (***Pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$**) | 69 | | 21 | 3 | | 1 | 0.9<P<0.95 (0.133) |
| Sayamakaori (***Pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$**) | × | Makurazaki-13 (***Pl*$_1$*pl*$_1$*pl*$_2$*pl*$_2$**) | 79 | | 24 | 3 | | 1 | 0.9<P<0.95 (0.133) |

by 2 independent dominant genes ***Pl₁*** and ***Pl₂***, with ***Pl₁*** exhibiting an epistatic effect in relation to ***Pl₂***.

The current genetic analysis, which indicated for the first time that an important trait related to the resistance to the disease in the tea plant is controlled by major genes, should contribute to the promotion of breeding work for the development of varieties resistant to *P. longiseta*.

**Genotype analysis of the cultivars harboring *Pl₁* with homozygosity**

Three genotypes harboring ***Pl₁*** with homozygosity could not be analyzed directly in the $F_1$ generation by the method indicated in Fig. 3. However, when the $F_1$ plants between a susceptible cultivar 'Yabukita' and tested cultivar were backcrossed to 'Yabukita' (the susceptible cultivar), $BC_1$ plants obtained from each cross combination always segregated for resistance, moderate resistance and susceptibility to the disease in the phenotype. The genotype analysis in this case is illustrated in Fig. 4.

When the $BC_1$ plants of each cross combination between the susceptible cultivar 'Yabukita' and $F_1$ plants showed the segregation ratio 2 : 1 : 1 for resistance, moderate resistance and susceptibility, the genotypes of all the $F_1$ plants were identical and the phenotype was estimated to be ***Pl₁pl₁Pl₂pl₂*** based on the model shown in Fig. 3. Therefore, the genotype of the tested cultivar used as the first parent was estimated to be ***Pl₁Pl₁Pl₂Pl₂*** based on the model shown in Fig. 4.

When the segregation ratio of every cross combination in $BC_1$ was 1 : 0 : 1 for resistance, moderate resistance and susceptibility, the $F_1$ plants were estimated to show the same genotype ***Pl₁pl₁pl₂pl₂***. The genotype of the cultivar used as the first parent was assumed to be ***Pl₁Pl₁pl₂pl₂***.

When the segregation ratio of $BC_1$ was separated into 2 types, such as 2 : 1 : 1 and 1 : 0 : 1 for resistance, moderate resistance and susceptibility, the genotype of the former was ***Pl₁pl₁Pl₂pl₂***, and that of the latter was ***Pl₁pl₁pl₂pl₂***. And when the number of $F_1$ plants showing the genotype ***Pl₁pl₁Pl₂pl₂*** and that of the $F_1$ plants showing the genotype ***Pl₁pl₁pl₂pl₂*** were equal statistically, the genotype of the parent of $F_1$ was estimated to be ***Pl₁Pl₁Pl₂pl₂***.

To verify the existence of the 3 genotypes harboring the ***Pl₁*** gene with homozygosity, the resistant cultivars 'Benihikari', 'Ace 37' and 'Abo 27' were used and their genotypes were analyzed based on the model illustrated in Fig. 4.

In the case of 'Benihikari', 21 $F_1$ plants were obtained from the cross of 'Yabukita' and they were all resistant to the disease. Each of the 21 plants of the $F_1$ generation was crossed again with 'Yabukita' and 20 to 30 seedlings were obtained from each cross combination

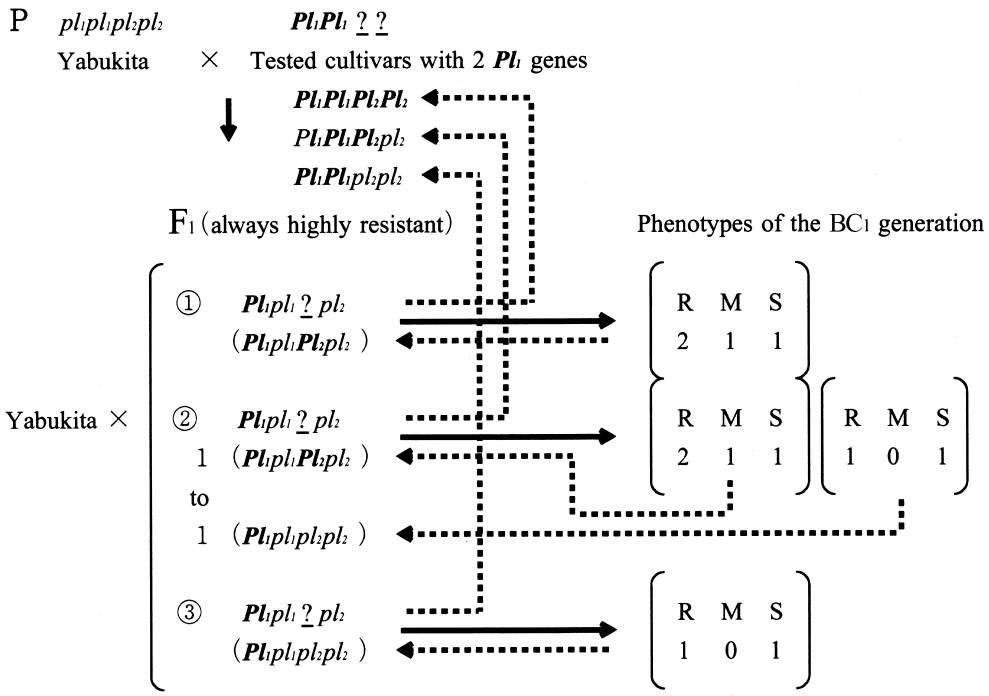


**Fig. 4. Genotype analysis model for cultivars harboring 2 *Pl₁* genes which confer a high level of resistance**

→ Results of the observation, - - - → Results of the estimation.

in $BC_1$. These $BC_1$ seedlings were evaluated for their resistance to the disease and $BC_1$ plants obtained from each of the 21 cross combinations between $F_1$ plants and 'Yabukita' showed the segregation ratio 2 : 1 : 1 for resistance, moderate resistance and susceptibility. Therefore, the genotype of the 21 plants in the $F_1$ generation was assumed to be $\boldsymbol{Pl_1pl_1Pl_2pl_2}$, and the genotype of 'Benihikari' was estimated to be $\boldsymbol{Pl_1Pl_1Pl_2Pl_2}$ (Fig. 5).

Sixteen $F_1$ plants were obtained from the cross combination between 'Yabukita' and 'Ace 37' and they were all resistant to the disease. The $F_1$ plants were crossed again with 'Yabukita' and many $BC_1$ seedlings in each cross combination were obtained. These $BC_1$ plants were evaluated for their resistance to tea gray blight. As a result of the evaluation, 7 cross combinations showed the segregation ratio 2 : 1 : 1 and 9 cross combinations showed the segregation ratio 1 : 0 : 1 for resistance, moderate resistance and susceptibility. The segregation ratio of the genotypes in the $F_1$ plants was analyzed by the chi-square test. The segregation ratio 7 : 9 for $\boldsymbol{Pl_1pl_1Pl_2pl_2}$ and $\boldsymbol{Pl_1pl_1pl_2pl_2}$ fitted well to the expected ratio 1 : 1 because the probability value was more than 0.6. Therefore, the genotype of 'Ace 37' was estimated to be $\boldsymbol{Pl_1Pl_1Pl_2pl_2}$ (Fig. 5).

The cultivar 'Abo 27' was crossed with 'Yabukita' and 18 $F_1$ plants were obtained. The $F_1$ plants were all resistant to the disease and were backcrossed to 'Yabukita'. Eighteen cross combinations with 'Yabukita' showed the segregation ratio 1 : 0 : 1 for resistance, moderate resistance and susceptibility. Based on Fig. 4, the $F_1$ plants showed the same genotype, $\boldsymbol{Pl_1pl_1pl_2pl_2}$. Therefore, the genotype of 'Abo 27' was estimated to be $\boldsymbol{Pl_1Pl_1pl_2pl_2}$ (Fig. 5).

It was demonstrated that the genotype of 'Beni-

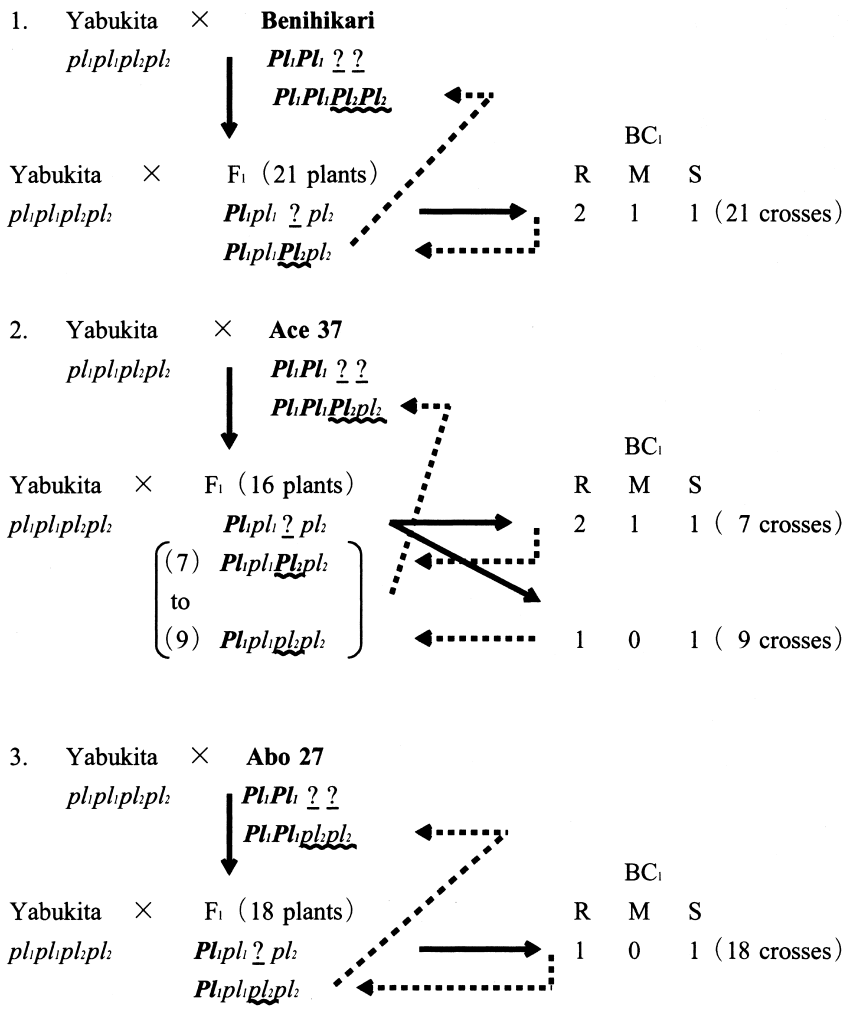


**Fig. 5. Analysis of the genotypes of 3 cultivars harboring 2 $Pl_1$ genes**

shows the genes estimated from the segregation of the plants of $F_1$ and $BC_1$ generations.

hikari', 'San-Cha-Tsi-Lan' and 'PKS 292' was $\boldsymbol{Pl_1Pl_1Pl_2Pl_2}$, while that of 'Ace 37' was $\boldsymbol{Pl_1Pl_1Pl_2}pl_2$ and that of 'Abo 27', 'IRN 17' and 'IND 75' was $\boldsymbol{Pl_1Pl_1}pl_2pl_2$. All of the 9 genotypes in combination with $\boldsymbol{Pl_1}$ and $\boldsymbol{Pl_2}$ were detected in the tea plants preserved as genetic resources. The cultivars with 2 $\boldsymbol{Pl_1}$ genes are very important materials for the breeding of resistant cultivars to the disease, because their progenies always displayed the resistance in all the cross combinations.

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