Analysis of Self-Incompatibility Alleles of Major Varieties of Tea

By YASUMOTO FUCHINOUE

Saitama Prefecture Tea Experiment Station

Self-incompatibility of tea (*Thea sinensis* L) was observed by Tomo[6] with Japanese varieties, by Shimura[5] with Chinese varieties and Indian hybrids, and by Wu[4] with Taiwan varieties and Manipuri varieties, each from the standpoint of fertilization physiology. However, as far as the present author knows, genetics of self-incompatibility of tea has not been studied so far.

In 1961 to 1964, the author carried out a number of inbreeding and cross-breeding with related varieties, using F_1 plants obtained from all the cross-combinations of three major varieties of tea to examine the degree of compatibility, and found out an effective method to analyze self-incompatibility alleles, and also made clear the mode of heredity of self-incompatibility[2,3].

Analysis of self-incompatibility alleles by progeny tests

Using F_1 plants obtained by crosses among three major varieties, Sayamamidori, Yamatomidori and Yabukita, progeny tests, such as sib-cross, half-sib-cross, and back-cross, were carried out, and incompatibility group was identified. An example of the actual procedure is given in Figs, 1-a and 1-b, in which compatibility and incompatibility expressed in sib-crosses and back-crosses of F_1 plants of Sayamamidori × Yamatomidori are shown.

Tea plants require a long period, 1 year, from the flowering to the fruit maturity. During that period, physiological fruit drop occurs to a great extent and with great varietal differences so that the fruit-set percentage can not be taken as related to the fertilization percentage. Therefore, the author tried to find out simple and accurate methods for determining the fertilization percentage (fertility). As a result, the microscopic examination on percentage of developing ovules at about 40 days after the pollination, with free-hand sections, was proved to be the best method, and hence employed in the present study. At the peak flowering stage of the maternal plant in a cross, 15–20 flowers are pollinated, and at about 40th day after the pollination, when developing ovules become to be easily recognized, their ovaries are cross-sectioned by free hands, stained with Delafield's haematoxylin, and percentage of ovules with developing endosperm to the total number of ovules is determined. The percentage is then expressed in terms of index, i.e., the ratio of the percentage to the percentage shown in a cross with a distant variety (the same maternal plant is pollinated with a variety, Indo, in this case). Thus, the degree of compatibility is given by the index for each combination. As the distribution of the degree of compatibility (the index) showed a bimodal curve, as given in Fig. 1-a, in all cases, the curve was divided into two parts taking its lowest point as a boundary, i.e., the part with high indexes and that with low indexes, each representing the compatible group and the incompatible group, respectively.

In this case of F_1 plants of Sayamamidori × Yamatomidori, it was made clear that the incompatible group can further be divided into two sub-groups, one of which is incompatible

♀ \ ♂	Sayamamidori×Yamatomidori F_1 plants ind. No											
Sayamamidori×Yamatomidori F_1 plants ind. No.	124	125	144	150	145	128	134	137	138	127	133	142
124												
125	99											
144	97	46										
150	124	13	3									
145	29	69	91	126								
128	15	76	90	—	24							
134	116	20	34	7	150	96						
137	107	8	30	—	90	61	7					
138	136	15	42	17	120	75	0	18				
127	11	89	67	94	15	18	96	109	126			
133	134	21	33	5	130	121	5	—	—	—		
142	6	94	129	116	24	11	98	100	79	53	95	
Sayamamidori	103	100	118	115	113	115	92	105	105	96	88	107
Yamatomidori	83	35	19	35	62	81	47	10	35	101	8	64

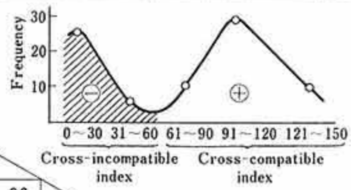


Fig. 1-a. Cross-compatibility or cross incompatibility in the sib-crosses using Sayamamidori×Yamatomidori F_1 individual and in the back crosses using them as male.
Notes ; ▭ : Cross-compatible index and ▭ : Cross-incompatible index. Year : 1961

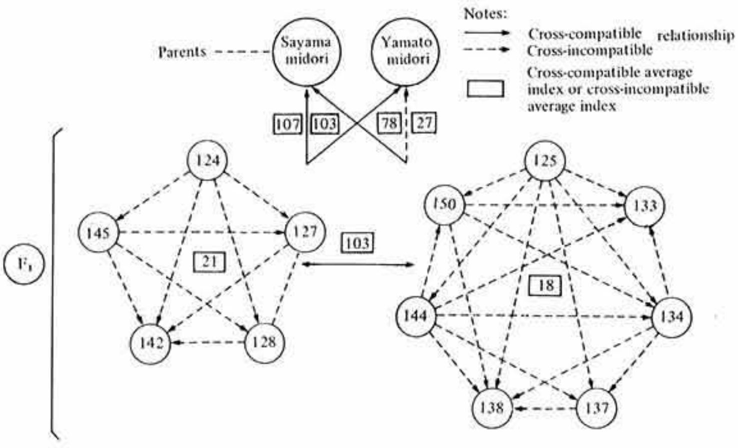


Fig. 1-b. Intra-sterile and inter-fertile relations among the F_1 individuals, grouped in Fig. 1-a.

to the pollen parent.

Using such a method of progeny test, the degree of incompatibility was examined in 1961 to 1964 for all cases of back-cross, sib-cross and half-sib-cross of six groups of F_1 plants obtained among three major varieties, Sayamamidori, Yamatomidori, and Yabukita. The results are summarized in Fig. 2, which indicates the followings:

With F_1 plants between Sayamamidori and Yamatomidori, it was shown, irrespective of either of the reciprocal crosses, that the incompatible group identified in the progeny test can be further divided into two groups, and one of them was incompatible to the father plant. With F_1 plants obtained by reciprocal crosses between Sayamamidori and Yabukita, and between Yabukita and Yamatomidori, the incompatible groups was divided into 4 sub-groups. Although all of them were

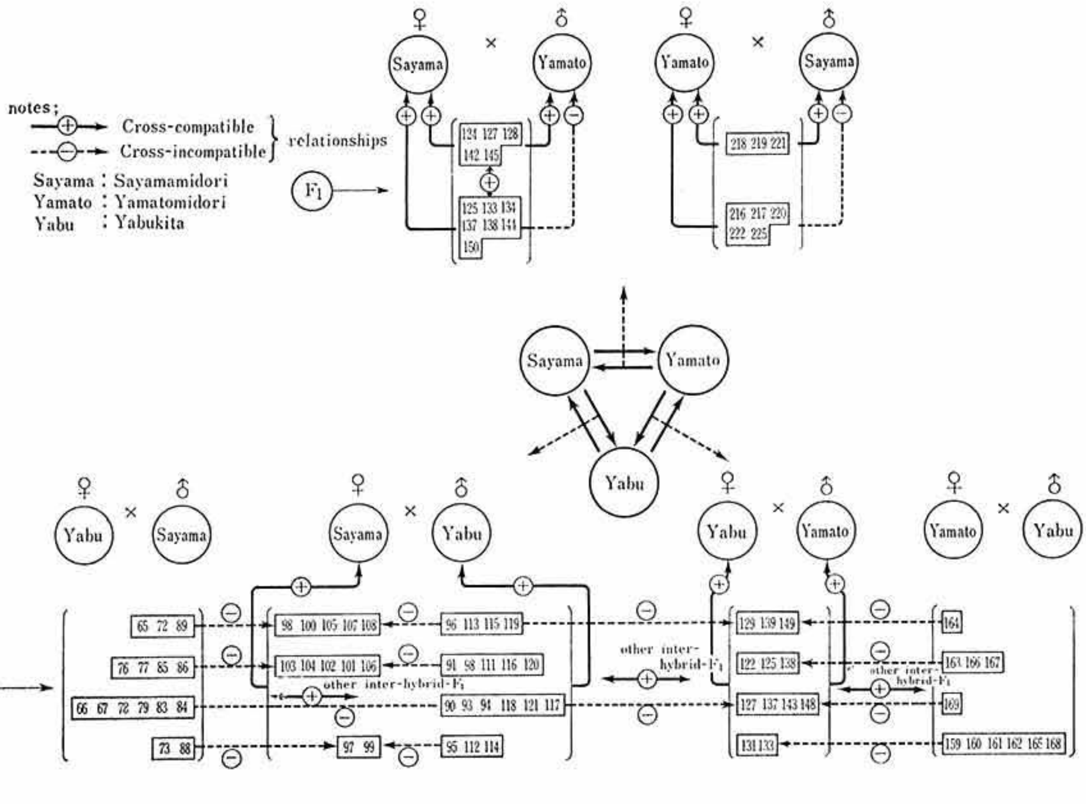


Fig. 2. Diagrams of results obtained in 1961~64

compatible to both parents, there were two groups which showed the incompatibility between both groups of F_1 plants, i.e., Sayamamidori × Yabukita *vs* Yabukita × Yamatomidori.

To give a genetic explanation on these results, the author proposes the following assumptions:

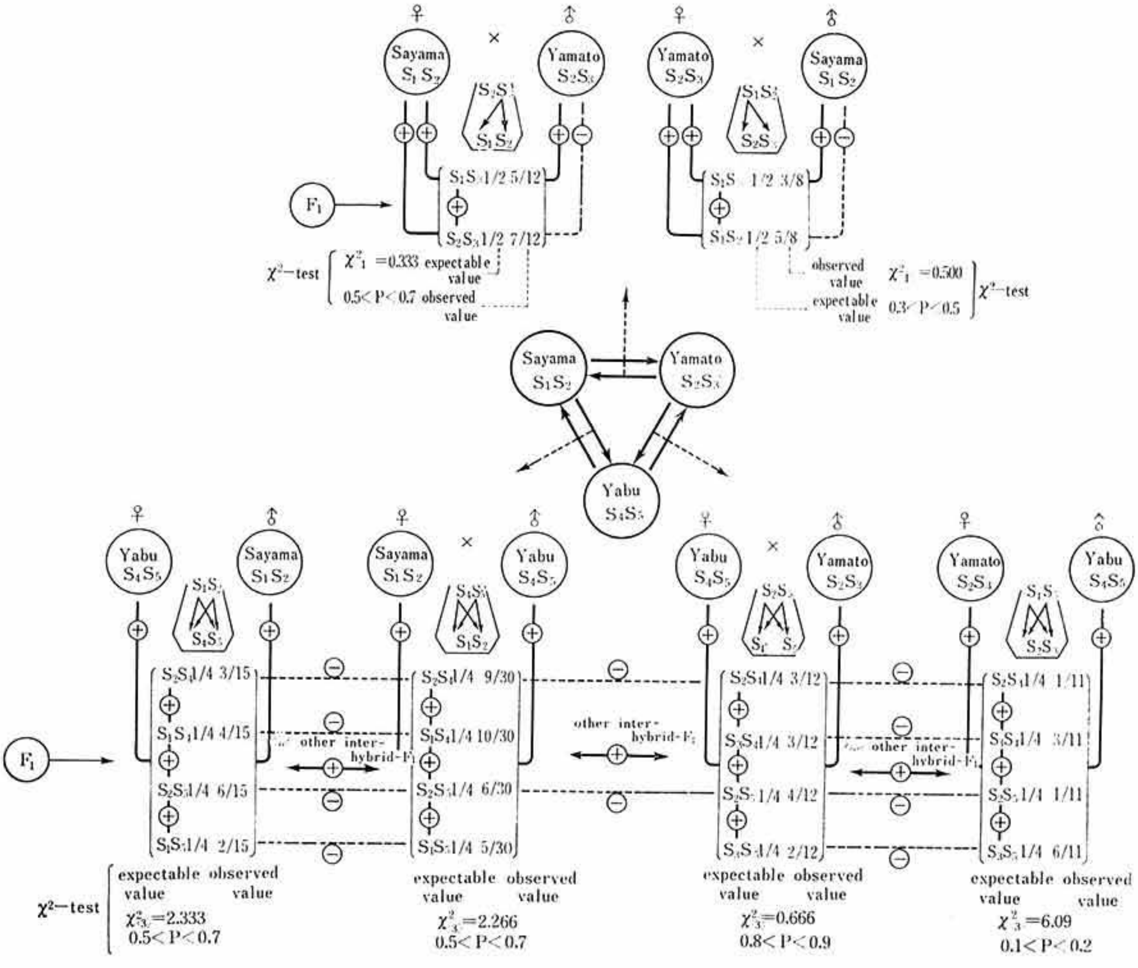
a) Self-incompatibility alleles of these varieties are composed of 5 multiple oppositional alleles, S_1 to S_5, as shown in Fig. 3. Sayamamidori has S_1 and S_2, Yamatomidori S_2 and S_3, and Yabukita S_4 and S_5.

b) The incompatibility is caused by the mutual relation between genotype of pollen and that of style, i.e., when the genotype of pollen is common to that of style, no fertilization takes place (a so-called gametic reaction)[1].

Based on this hypothesis, all the aspects of behavior of the incompatibility alleles, genotypes of F_1 plants and their frequency of occurrence and the pattern of compatible and incompatible expressions mentioned above can be explained quite well.

A simple method of analysis of self-incompatibility alleles using limited number of pollen

The pollination using a restricted number of pollen was tried with an aim of knowing whether this method can be used or not to identify the differences of incompatibility in selfings or in crossings with or without S gene(s) common to the style and pollen (self, cross-1, and cross-2 in Fig. 4, respectively). The result, given in Table 1, indicates clearly that the percentage of fertilized ovules to the total number of ovules in the cross-2 and that



Cross-compatible } relationships
Cross-incompatible

Sayama : Sayamamidori
Yamato : Yamatomidori
Yabu : Yabukita

Fig. 3. Genetic diagram of S genes

in the cross-1 coincided quite well to the theoretical ratio of 2:1. Thus, it was proved that the direct pollination method using a restricted number of pollen can be used to determine the gene type for the incompatibility, instead of employing the progeny test, mentioned earlier, which requires much longer time.

Determination of self-incompatibility alleles of major 12 varieties by the direct pollination method

Using 10 Japanese varieties, including 3 varieties with S genes already known as

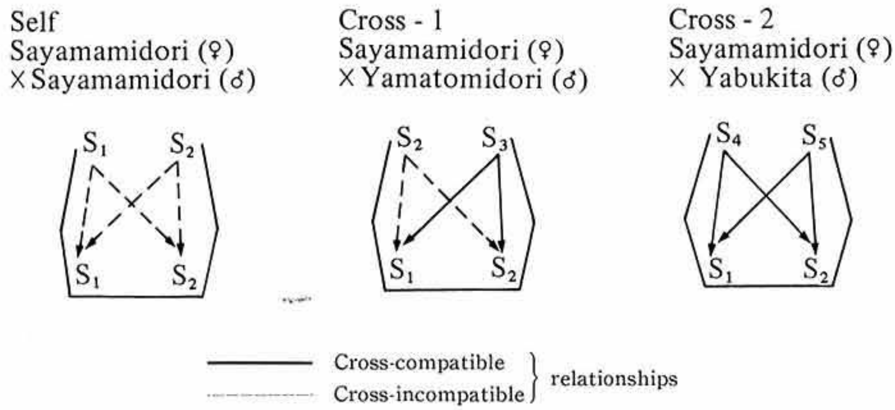


Fig. 4. Parental combination tried for the test by direct pollination

Table 1. Fitness to the ratio of number of fertilized ovules (Cross 2 : Cross 1) in the case of using small amount of pollen

Number of pollinated pollen	date of pollination	Number of fertilized ovules Observed Cross-2 : Cross-1	Number of fertilized ovules Expected Cross-2 : Cross-1 (2 : 1)	X	P
1—20	1964. Oct. 29	84 : 44	85.33 : 42.67	0.0350	0.8—0.9
	Oct. 30	98 : 48	97.33 : 48.67	0.0307	0.8—0.9
	Nov. 4	90 : 43	88.67 : 44.33	0.0339	0.8—0.8
11—60	Oct. 29	112 : 55	111.33 : 55.67	0.0268	0.8—0.9
	Oct. 30	110 : 59	112.67 : 56.33	0.2403	0.5—0.7
	Nov. 4	103 : 56	99.33 : 49.67	0.2547	0.5—0.7
41—190	Oct. 29	148 : 121	179.33 : 89.67	16.0564	0.001
	Oct. 30	158 : 119	184.67 : 92.33	11.8644	0.001
	Nov. 4	129 : 86	143.33 : 71.67	4.0928	0.02—0.05

Table 2. S gene type of main varieties

Variety	S. gene	Notes
Sayamamidori, Tamamidori	S_1 S_2	with S_2 gene in common
Yamatomidori	S_2 S_3	
Yabukita, Omune	S_4 S_5	with S_5 gene in common
Indo	S_5 S_6	
Benihomare	S_7 S_8	
Natsumidori	S_9 S_{10}	
Takachiho	S_{11} S_{12}	
Rokuro, Koyanishi	S_{13} S_{14}	
Asatsuyu	S_{15} S_{16}	

above, and 2 Indian hybrids, the pollination with a restricted number of pollens was made for all combinations of these 12 varieties with 30 flowers used per combination in Autumn of 1965. Average number of fertilized ovules per fruit was measured, and compared by the t-test to that obtained in the cross-2, as follows:

a) When the former is markedly less than the latter, the gene type common to style and pollen exists.

b) When the former is slightly less than the latter, and it's percentage of fertilized ovules to the total number of ovules is close to 1/2 of that of the cross-2 at a high probability, one gene is common to style and pollen.

c) When the former shows no significant difference, by the t-test, from the latter and the fitness of the percentage of fertilized ovules to the 1/2 value of the cross-2 is very low, the gene type is entirely different between style and pollen.

The result indicates 16 multiple oppositional alleles, as shown in Table 2. Of 12 varieties, there are 3 pairs of varieties having the same gene type for each pair, and 2 pairs of varieties with one gene in common in each pair.

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