Varietal Difference and Breeding Behavior of Fermentation Ability (Polyphenol Oxidase Activity) in Tea Plants

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Fermenting property is one of the most important traits in the breeding for black tea quality of tea plants; *Camellia sinensis* (L.) O. Kuntze. The quality potential of black tea is greatly influenced by fermenting property and tannin content (catechin group of polyphenols) in tea leaves.[1,3] It was previously estimated that these two variables account for about 50 to 60% of the variation of black tea quality among cultivars and selections.[5] The assessment of fermentation ability by the chloroform test[2] is more efficient than that of tannin content, as a simple method of evaluating the black tea quality. Fermentation ability can exactly be evaluated by the chloroform test, because of a high correlation coefficient (r=0.928)[4] between the polyphenol oxidase activity and the color of treated leaves. The amount of brown color developed on the leaves in a specified time is visually estimated or measured with a color and color difference meter.

This paper briefly presents some research results on the varietal difference of fermentation ability among cultivars, inheritance pattern, heritability estimates by parent-offspring relationship in the trait, and some discussions on the

Table 1. Classification of main cultivars of tea into 4 groups according to fermentation ability* evaluated by the chloroform test

	Var. *sinensis*				Var. *assamica*		Hybrid cv.	
	Japanese cv.		Chinese cv.					
Very good	Asakawa (koro)	4.66*	Miya Ck 6	4.62	Ai 106	5.00	MC 3	4.60
	Hatsumidori	4.25	Cd 31	4.50	Ak 56	4.50	•Fukuin 7	4.60
			Cd 86	4.25	•(Ak 937)**	4.66	F 839	4.60
			Cd 37	4.20	•(Ak 1440)**	4.50	Benihomare	4.32
Good	Sayamamidori	4.10	C 17	3.82	(Ak 2034)**	4.10	Tadanishiki	4.08
	Natsumidori	3.62					•Benifuji	3.95
	Tamamidori	3.50					Benihikari	3.75
	Okumusashi	3.50					MC 29	3.66
Medium	Yutakamidori	3.25	Ch 2	3.47	Ai 21	3.05	Indo	3.40
	Takachiho	3.12	Miya Ck 10	3.00	Ai 2	3.00	Benikaori	3.35
	Z 1	3.12			Ak 145	3.00	Satsumabeni	3.23
	Asatsuyu	3.00			(Ak 1510)**	3.69	Inzatsu 131	3.17
Poor	Yamatomidori	2.55	Yamanami	2.75	Ai 26	2.25	Benitachiwase	2.92
	Yabukita	2.50	Dah yeh oolong	2.61			CA 288	2.90
			Chin shin oolong	2.33			Akane	2.72
			Chin shin dan pang	2.16			CA 278	2.30
Very poor	Yayeho	1.83	Cd 78	1.00	Aj 8	1.00	Hatsumomiji	1.55
					Ai 86	0.75		

* Fermentation ability was rated from 1 (very poor) to 5 (very good).
** The cultivar considered as Assam hybrid.
• The cultivar is or may be a progeny of "Benihomare".

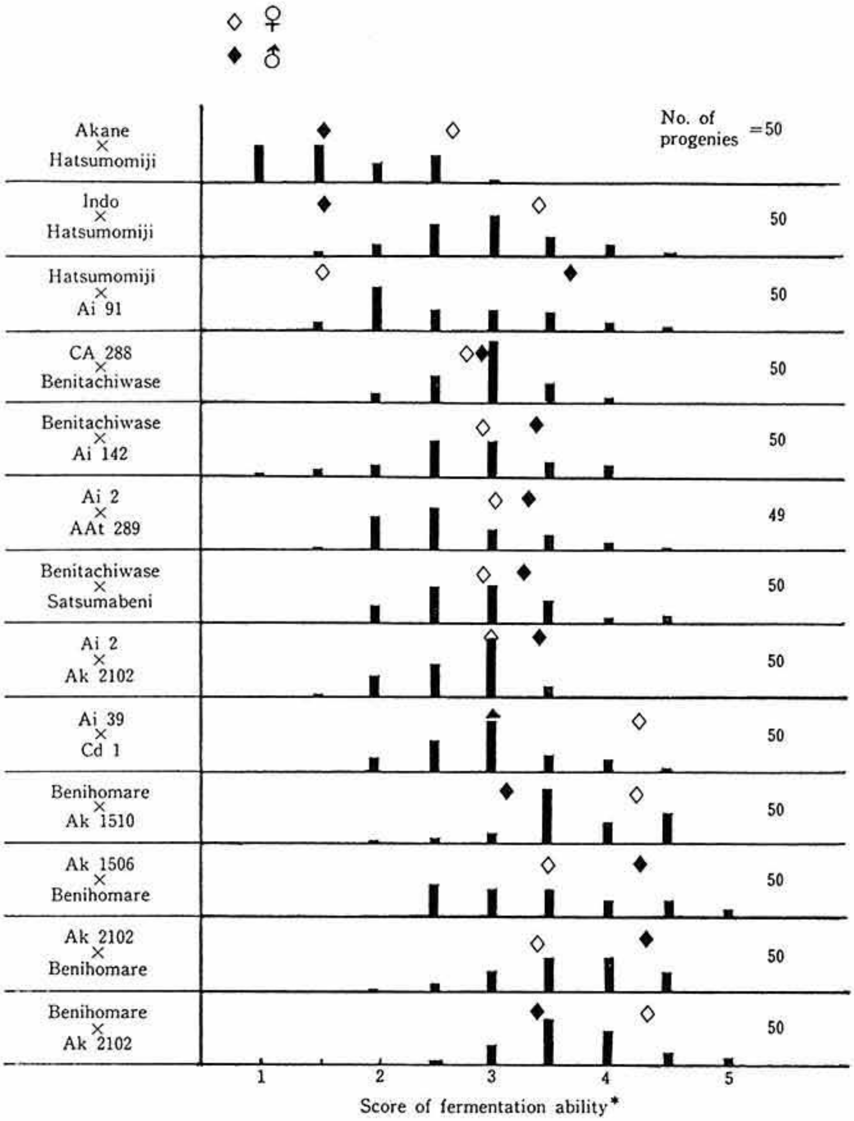


Fig. 1. Frequency distribution of progenies
Result obtained with a part of crosses in Exp. 2 is given.
* Fermentation ability was rated from 1 (very poor) to 5 (very good) in the chloroform test.

inter-varietal cross breeding for the fermentation ability.

Varietal difference of fermentation ability among cultivars and selections belonging to var. *sinensis*, var. *assamica* and their hybrids

Classification of main cultivars into five groups according to the fermentation score is summarized in Table 1.[10]

The fermentation scores of most Japanese cultivars ranked from 2.5 to 4.1. Cultivar 'Hatsumidori', 'Sayamamidori' and a part of 'Koro' type plants were found to be good fermenters, while cultivar 'Yabukita', the leading one for green tea, 'Yamatomidori', and 'Yaeho' were rather poor fermenters.

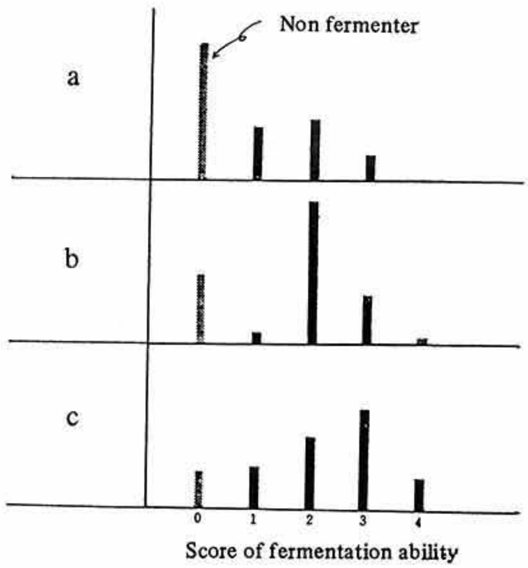


Fig. 2. Frequency distribution of non-fermenters and normal plants in three crosses
a : Hatsumomiji × F 23224
(Hatsumomiji × F 8082)
16:16(1:1), $\chi^2=0.000$
$p=1.000$
b : Hatsumomiji × F 1680
(Hatsumomiji × Akane)
25:77(1:3), $\chi^2=0.013$
$p>0.90$
c : Hatsumomiji × F 9136
(Hatsumomiji × Indo)
11 : 73(1:7), $\chi^2=0.027$
$p>0.75$

The variation in Chinese cultivars was relatively wide. 'Miya CK 6', 'Cd 31', and 'Cd 86' were ranked as good fermenters. On the other hand, 'Chin-shin oolong', 'Chin-shin dahpang', 'Dah-yeh oolong' etc., the cultivars for Oolong or Poachong tea (semi-fermenting tea) in the mainland of China and Taiwan, seemed to be nearly poor fermenters.

A number of superior fermenters which also have very good color and aroma were detected in Assamese tea plants and their hybrids. As some of the hybrids seemed to be derived from the progenies of a quality cultivar 'Benihomare', its genes contributing the good fermenting property might have been inherited into them. On the contrary, a famous high yielding cultivar in the southern district of Japan, 'Hatsumomiji' (F_1; Assamese × Japanese), was found to be a typical poor fermenter.

Thus, the distinct varietal difference of fermentation ability was revealed in each of three groups of tea cultivars, namely black tea, semi-fermenting tea, and green tea.

Inheritance pattern of fermentation ability and deficiency in polyphenol oxidase activity

Individual plants in each cross showed a certain variation of the fermentation score. The frequency distribution of the score in each cross demonstrated a monomial and continuous distribution in almost all the crosses (Fig. 1).[9] The results obtained in the experiments also showed no significant difference of the scores in the progenies of the reciprocal cross combinations. In addition, the distribution of scores in the progenies of each reciprocal combination showed a little variation regardless of the difference in the score of both parents and the magnitude of the parental scores. These inheritance pattern suggested that the fermentation ability was controlled by several number of nuclear genes, except in the case of non-fermenters.

The plants deficient in polyphenol oxidase activity, non-fermenters, were detected in the progenies of backcrosses between cultivar 'Hatsumomiji' and its family (Fig. 2).[11] The ratios between normal plants and non-fermenters in these progenies were shown as 3 : 1 and 7 : 1. Dominance of normal plants over non-fermenters was evident in each cross. Furthermore, the non-fermenters were segregated at the ratio of 1 : 1 in the crosses between 'Hatsumomiji' and the non-fermenters derived from the above cross experiments.

On the basis of these three segregation ratios, it was concluded that the non-fermenters were inherited as a mode of two alleles of genes. The genes might be originated from one of the Assam plants (Ai) which were introduced to Japan from India in 1929.

Heritability estimates by regression of offspring on parents in various cross combinations

Fermentation ability was studied to estimate the values of heritability in three groups of seedlings derived from artificial crossings.

Mean values of progenies in each cross closely corresponded to their mid-parental values, and accordingly the correlation coefficients as well as the regression coefficients of offsprings on their parents were considerably high in each of the three groups of seedlings (Fig. 3).[9] Correlation and regression coefficients obtained in experiment 1, 2 and 3 were 0.769, 0.717, 0.696 and 0.664, 0.878, 0.619, respectively. Next, the regression coefficients of the progenies on single parents were also obtained by means of variance-covariance analyses (Table 2). The values calculated from the regression are equivarent to the heritability estimates in the narrow sense.

The heritability estimates of the fermentation ability were high as compared with the estimates of tannin content in young shoots, length of leaf blades and shape index, respectively, and showed the same degree as the values of time of bud opening[6] and resistance to anthracnose,[7] although the values were lower than those of cold hardiness of leaves in midwinter which showed a great contrast between var. *sinensis* and var. *assamica*.[8] Therefore, the mean value of fermentation ability in the progenies can be predicted from their parental score because of the high heritability estimates.

Choice of parental cultivars by the fermenting property in cross breeding for black tea quality

The derivation of new cultivars for black tea is shown in Fig. 4 in the form of their genealogy. The project of this cross breeding was carried out

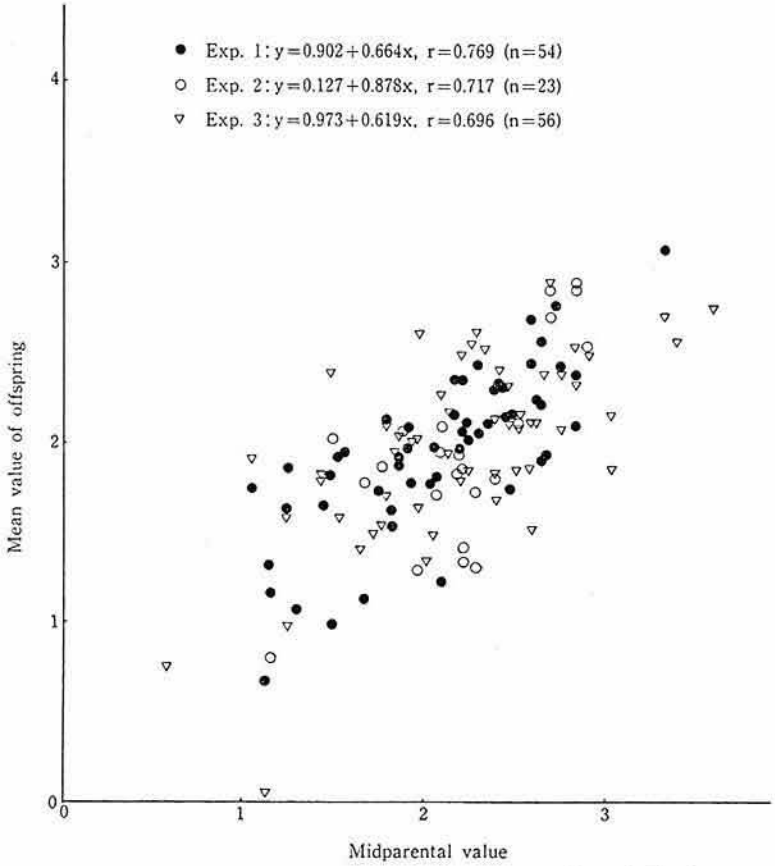


Fig. 3. Regression of mean scores of fermentation ability of offspring on midparental scores in Exp. 1, 2 and 3

Table 2. Heritability of fermentation ability estimated by regression of progeny on single parents —Variance-covariance analysis[d]—

Source	Exp. 1				Exp. 3			
	df	X[a]	XY[b]	Y[c]	df	X	XY	Y
Total	77	0.562	0.148	0.187	65	0.766	0.159	0.197
Between common parent	16	0.478	0.030	0.434	13	0.389	0.034	0.382
Within common parent	61	0.584	0.179	0.122	52	0.861	0.208	0.150
Regression coefficient	b=0.3279				b=0.2415			
Heritability	$h^2=0.6559$				$h^2=0.4830$			

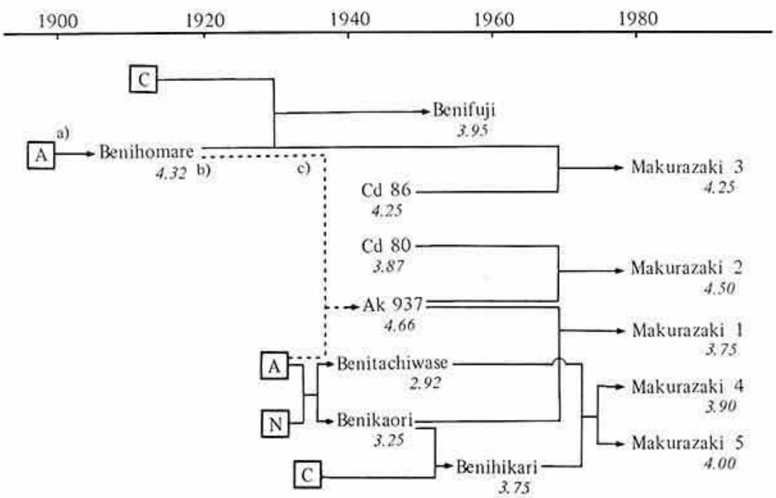
a : Variance of parental values.
b : Covariance of parent and progeny.
c : Variance of progeny.
d : Variance-covariance analysis was made using a part of crosses in Exp. 1 and Exp. 3. The data were used repeatedly regardless of parental sex.

at Makurazaki Branch, Kagoshima, Japan since 1929. The principal object was to introduce the quality and flavor of black tea and the growth vigor of var. *assamica* into Japanese cultivars.

Newly bred 'Makurazaki 1, 2, 3, 4, and 5' were selected for cold resistance, rooting ability, high yielding, resistance to diseases, and fermentation property which determines the quality and flavor of black tea. In Fig. 4, figures in italics indicate the score of fermentation ability, and those of parental cultivars show the same values as given in Table 1.

'Makurazaki 1, 2, and 3' seem to have received the genetical characteristic of fermenting property from the typical good fermenter 'Benihomare', while, 'Makurazaki 4 and 5 accumulated the genes which induce good fermenting property through two or three generations by the cross breeding.

These results support the view that the choice of parental cultivars is very important in the quality breeding of tea plants. Especially, it is



a; [A] var. *assamica*, [C] var. *sinensis* in China, [N] var. *sinensis* in Japan.
b; Score of the fermentation ability.
c; ----: Parent-offspring relation is deduced from various data.

Fig. 4. Genealogy of 5 new varieties with high fermentation ability, developed by inter-varietal cross breeding for black tea in Makurazaki Branch, National Research Institute of Tea

quite probable that the choice of parental fermentating property directly determines success or failure of a breeding project.

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