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ON THE AFFINITY OF THE CULTIVATED VARIETIES OF RICE PLANTS, *ORYZA SATIVA* L[1]

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I. INTRODUCTION

In the study of the relationships of various types of plants, some scientists (4, 8, 12, 15, 20, 21 etc.) have attempted to take advantage of the serodiagnostic characters of plant proteins. However, it is well known, that the fertility of hybrid plants is higher or lower according as the parent plants are more or less closely allied (1, 3, 6, 9, 19 etc.). Indeed, in the case when the parents are very distantly allied, the hybrids often show sterility, and it is a matter of common knowledge that in such a case the formation of pollen in the F_1 plants becomes very abnormal. Cytological investigations have shown that the abnormality of pollen formation in the F_1 plants is due either to the disharmony of their respective chromosomes, or to the difference of physiological processes of pollen formation from the pollen mother-cell (5, 7, 10 etc.). If the above may be recognized as a general fact prevailing in the vegetable kingdom, the degree of affinity of certain plants may be deduced from a serodiagnostic investigation of them, an examination of the degree of fertility of their F_1 plants, or a cytological investigation of their pollen formation. TSCHERMAK (19), for instance, has already observed that the degree of fertility of the F_1 plants is fairly in accordance with the systematic relationship of the genus *Triticum*, which ZADE (21) has serodiagnostically and SCHULZ (18) morphologically elucidated.

If we consider the manner of classification of cultivated rice plants in use by many agronomists (11, 13 etc.), we shall find that all of them are based solely on the plant form, the characters which appear during cultivation, the use, or the morphological differences. These methods do not, however, take into consideration the facts concerning their affinity. This being the case, the authors collected during the last few years cultivated varieties of rice from the chief rice producing regions of the world. Then, wishing to find out their affinity, they attempted, firstly, a classification of varieties from the morphological standpoint; secondly, they carried out experiments on the fertility of their hybrid plants; thirdly, they made a cytological as well as a serodiagnostic investgation; and fourthly, they examined whether the results of the study of the affinity based upon the second and third methods above mentioned were in accord with those as deduced from the morphological character of the plants. It was established that, on the whole, they are in accord. Further, the relationships determined by means of the hybridization experiment (9) were nearly in accord with those determined by serodiagnostic investigation (8) nearly for the same material; and again the cytological inquiry confirmed the above results.

II. CLASSIFICATION OF THE VARIETIES OF RICE PLANTS FROM THE MORPHOLOGICAL STANDPOINT

The material used for the experiments consisted of glutinous, non-glutinous, scented, red, or long-glumed varieties of lowland and upland rice, collected from Japan Proper, Korea, Formosa, China, Java, India, Hawaii, the United States, etc., in 1923, and cultivated in the College of Agriculture in the Imperial University of Kyushu. Of these, about 100 representative varieties were used for the experiments.

A close scrutiny of every character of these varieties from the morphological standpoint shows that the characters of the plants are extremely varied; more particularly, 1) the shape and color of the leaf, 2) the angle formed by the uppermost leaf and the stem, 3) the shape of the grain, 4) the presence or absence of the awn, or its amount when present, 5) the length and amount of hairs on the glume, etc. The characteristics have been made the main points of classification, by means of which the rice varieties may be grouped under two types, one of which the authors have named "Japonica" and the other "Indica." The differentiating morphological features of these types may be summed up as follows:

Characters	Differentiating features	
	Varieties belonging to the "Japonica" type	Varieties belonging to the "Indica" type
1) Shape and color of the leaf:	Narrow; deep green.	Broad; light green.
2) Angle formed by the uppermost leaf and the stem:	Wide.	Narrow.
3) Forms of grains:	Broad and thick. Cross section markedly roundish. Among foreign varieties, belonging to the "Japonica," some are rarely long, but as compared with those, belonging to the "Indica" type, they are broad, and moreover, the transverse section is remarkably roundish.	Generally slender and somewhat flat.

4) Presence or absence of the awn, or its degree when present :	Various gradations from long-awned to awnless.	A large majority are awnless; though awned varieties are seldem met with, when met their awns are short.
5) Length and amount of hairs on the glume :	Hairs are thick and comparatively long.	Compared with the varieties, belonging to the "Japonica" type, hairs are thin and short.

Table I

Classification of the varieties of rice plants from the morphological standpoint

Varieties of the "Japonica" type		Varieties of the "Indica" type	
Name	Native place	Name	Native place
Wase-Shinriki (早生神力)	Japan	Tan-ko-fo-ira (短廣花螺)	Formosa
Nakate-Shinriki (中生神力)	"	Sui-yu (清油)	"
Okute-Shinriki (晩生神力)	"	Ha-koku-fo-ira (白穀花螺)	"
Omachi (雄町)	"	Pu-chiang-san-pe-li-keng-tao (浦口三百粒粳稻)	China
Aikoku (愛國)	"	Chiang-nan tao (江南稻)	"
Kameji (龜治)	"	Chan-shou-sien-tao (常熟秈稻)	"
Hinode (日ノ出)	"	Pa-shih-jih-sien (八十日秈)	"
Hinomoto (日ノ本)	"	Fung-ku-fung-tao (紅穀紅稻)	China (Chekiang)
Sekiyama (關山)	"	Fung-hsüeh-nuo (紅血糯)	China
Nishitaku-Mochi (glutinous variety) (ニシタク糯)	"	Hunan-sien (湖南秈)	"
Genki-Mochi (glutinous variety) (元氣糯)	"	Lu shih-jih-sien-tao (六十日秈稻)	China (Chekiang)
Kinen-Mochi (glutinous variety) (紀念糯)	"	Juug-hê-tao (容河稻)	China (Kiangsu)
Kuromoro (red variety) (黑諸)	"	Huug-tao keng-tao (紅稻粳稻)	China (Chekiang)
Chôeito (long-glumed variety) (長穎稻)	"	Ya-i tao (雅衣稻)	" (")
Saigoku-kawachi (scented variety) (西國河內)	"	Hsiao-li-fung-ken-tao (小粒紅粳稻)	" (")
Hiderishirazu (upland non-glutinous variety) (旱不知)	"	Fung-tsui-yü-keng tao (紅嘴玉粳稻)	China (Szech-wan)
Tamasari (upland non-glutinous variety) (田優)	"	Ping-yang-pê-sien-tao (平陽白秈稻)	China (Chekiang)
Gifu Mochi (upland glutinous variety) (岐阜糯)	"	Li-lung-ku-keng-tao (裹輪谷粳稻)	" (")
Tataso (多々租)	Korea	Huang-pi-keng-tao (黃皮粳稻)	" (")
Salpei (growing wild) (サルペー)	"	Fên-shui-san-pê-li keng tao (三百粒粳稻)	" (")
Seiso (晴租)	"	Chi-ching tao-keng tao (赤莖稻粳稻)	" (")

Chang shou-chên-mi (常熟貢米)	China	Ai-keng-tao (矮稻粳稻)	China (Chekiang)
Chang-shou-wan tao (常熟晚稻)	,,	Mang-ku-keng-tao (芒穀粳稻)	,, (,,)
Hsiang-tao (香稻)	,,	Ta-ku-keng-tao (大穀粳稻)	,, (,,)
Hsiao-pê-mang shui-tao (小白芒水稻)	,, (Chili)	Sien-tao-wu-wei (秈稻無僞)	China (Kiangsu)
San-li-tsun-muo-tao (三粒寸糯)	China	Yang-sien-tao (洋秈稻)	,, (,,)
Nanking-hsian-tao (南京香稻)	China (Nanking Univ.)	Tao-jen-chiao (道人橋)	China
Chang-li (長粒)	China (Wuchang)	Chiang-nan-tao (江南稻)	China (Anwhei)
Keng-tao (粳稻)	China (Changchow)	Tsao sien-tao (早秈稻)	China (Tônan Univ.)
Lo han-huang (羅漢黃)	China (Kiangsu)	Huang-kan-sien (黃幹秈)	,, (,,)
Keng-tao (Wu-hsi hsüeh-yen-chiao) (粳稻)(無錫雪堰橋)	,, (,,)	Mao-tzu tou-sien-tao (帽子頭秈稻)	,, (,,)
Ma tsung-nuo (馬鬃糯)	China	Huo-pe keng-tao (禾白粳稻)	China (Chekiang)
Ning-tu (寧都)	,,	Fung-ku-keng-tao (紅穀粳稻)	,, (,,)
Huo pe-ku (大白谷)	,,	Wu pe li-keng-tao (五百粒粳稻)	,, (,,)
Keng-tao-chang-chou (粳稻常州)	,,	Si-yeh-tsing-keng-tao (細葉青粳稻)	,, (,,)
Nakamura 17 (中村 17 號)	Formosa	Ta-tsao keng tao (大糙粳稻)	,, (,,)
Hawaii no. 158	Hawaii	Chang-kan-wan kang-tao (長幹晚粳稻)	China
Fortuna	U. S. A.	Meng-kan peng keng-tao (蒙幹蓬粳稻)	,,
Texas Fortuna	,,	Wu-ku jsian-chi (烏穀軟枝)	Formosa
Honduras	,,	Vellisenatti	Java
Prace no. 6	,,	Gangasale Bhatta	Southern India
Katete	Brazil	Red Kangro	Western India
Amareria	,,	Seenaddy	Ceylon
Buranko	,,	Karaffa cheenatti	,,
		Hawaii no. 17	Hawaii
		Carolina Gold	U.S.A.
		Hatadavi	Ceylon
		Basmati	India
		Black Seenaddy	Ceylon

The classification of the varieties according to the morphological method has shown that the varieties native to Japan Proper, Korea, and Northern China belong to the "Japonica" type without a single exception; that those native to Southern China, Formosa, Java, India, Ceylon, etc. belong, as a rule, to the "Indica" type; and that some varieties in Central China, Hawaii, the United States of America, Brazil, etc. belong either to the "Japonica" or to the "Indica" type (see Table I). The Tan-ko-fo-ira, native to Formosa, closely resembling the Hunan-sien, belongs to the "Indica" type and yet the roundish shape of its grain reminds one somewhat of the "Japonica" type.

III. AFFINITY OF THE VARIETIES OF RICE PLANTS AS SHOWN BY THE FERTILITY OF THE F_1 PLANTS

1. Methods of investigation

For investigating the fertility of hybrid plants, a great number of the cultivated varieties of rice plant used in the aforesaid morphological study was employed. Special care was taken in these experiment concerning the following 12 varieties: 1) Wase-Shinriki, 2) Nakate-Shinriki, 3) Sekiyama, 4) Omachi, 5) Hiderishirazu, 6) Tataso, 7) Salpei 8) Chang-shou-shên-mi, 9) Chang-shou-wan-tao, and 10) Hsiang-tao (all belonging to "Japonica" type); 11) Hunan-sien and 12) Tan-ko-fo-ira (the latter two belong to "Indica" type). The F_1 plants were obtained by artificial crossing, firstly between several varieties belonging either to the "Japonica" or the "Indica" type (hybridization within the same type) and secondly between those of the "Japonica" and the "Indica" type (hybridization between the different types). All seeds of the F_1 plants were sown at the same time with the parent seeds, in the following year, under similar conditions. These plants were partly allowed to flower and bear seeds, while spikes of some plants which were covered with paraffin paper bags during the blooming period to prevent their natural hybridization, were studied at maturity as to the fertility of their spikes. Though the authors thus investigated the degree of the fertility of the F_1 plants, they have kept further the F_2 plants, mainly the hybrids between the different types, under cultivation and examined the segregation of the degrees of their fertility. The method of self-fertilization and the investigation of fertility in this case was the same as in that of the F_1.

The authors distinguished between fertile and sterile seeds with the naked eye; and for the purpose of indicating the degree of fertility of each individual plant, they examined in the flowering periods 10 culms for each plant, which have tillered comparatively early, whether the spikes had been covered or left uncovered, and the degree of the fertility of the plant in question was indicated by the percentage of fertile seeds against the total. As the difference of fertility between those plants of which the spikes had been covered and those left uncovered was always very insignificant, they took the average of both to indicate the degree of fertility.

2. Experimental results

1) Results of preliminary investigation on the parent plants

When the authors, on investigating the fertility of the principal varieties cultivated in the agricultural experimental farm of the Imperial University of Kyushu, used for hybridization the above named 12 varieties, including the varieties belonging to two different types, they found that those plants whose spikes had been covered with paraffin paper bags and those uncovered gave about the same degree of fertility. When we show the degree in question by the mean of the values found by these two methods of investigation in each variety, it was 79.6 % on the average, the minimum and the maximum being 68.0 % and 86.5 % respectively. Judging from the fact that the discrepancy between the two extremes is relatively small, the degree of fertility in the parent varieties self-fertilized, no matter how different they are morphologically in their native places, is about the same in both and seems to be pretty high (see Table II).

2) Fertility of the F_1 plants within the same type and of those between the different types

The investigation of 39 F_1-hybrids, which were artificially produced from any two of them, taken at random out of the 9 varieties of the "Japonica" type (Wase-Shinriki, Nakate-Shinriki, Sekiyama, Omachi, Hiderishirazu, Tataso, Chan-shou-wan-tao, Chang-shuo-chên-mi, and Hsiang-tao) on the one hand, and the 2 varieties of the "Indica" type (Hunan-sien and Tan-ko-fo-ira) on the other, showed that in the

Table II.

Degree of fertility in the parent varieties used for hybridization (Figures are of average fertility of 30 individuals)

Material used			Degree of fertility (%)		
Type	Name	Native place	Spikes covered with paraffin paper bags	Spikes not covered with paraffin paper bags	Average
"Japonica" type	Wase-Shinriki	Japan Proper	72.4	86.9	79.4
,,	Nakate-Shinriki	,,	79.9	85.3	82.6
,,	Sekiyama	,,	85.3	86.7	86.0
,,	Omachi	,,	76.7	84.3	80.0
,,	Hiderishirazu (upland rice)	,,	80.8	84.0	82.4
,,	Tataso	Korea	85.6	84.3	85.0
,,	Salpei	,,	68.0	---	**68.0**
,,	Chang shou-chên-mi	China	77.2	87.0	82.1
,,	Chang shou-wan tao	,,	80.3	77.0	78.7
,,	Hsiang tao	,,	68.8	77.6	73.2
"Indica" type	Hunan-sien	China	66.6	73.4	70.0
,,	Tan-ko-fo-ira	Formosa	86.8	86.2	**86.5**
Average			77.4	83.0	**79.6**

hybrids of varieties of the "Japonica" type, the fertility of the crosses within the same type is 48.6 % in minimum, and 83.6 % in maximum, the average being 68.7 %. Such fertility is only slightly below that of the parent plants of the same type, grown under the same conditions. The same result was obtained in the hybrids between the varieties within the "Indica" type.

The 24 F_1 plants resulting from any two varieties belonging to different types taken at random from among the "Japonica" and "Indica" types, gave the following degrees of fertility. The varieties of the "Japonica" type crossed with those of the "Indica" type showed in their fertility the minimum 0.1 %, and the maximum 26.2 %, the average being 14.1 %. The varieties of the "Indica" type crossed with those of the "Japonica" showed in this respect the minimum 0.0 %, and the maximum 33.4 %, the average being 13.6 %. There was a remarkable difference of fertility between the hybrids resulting from the different types in both cases, on one hand, and the parent plants grown under the same conditions, on the other.

However, when varieties belonging to the "Japonica" type were hybridized with the Hunan-sien, and the same varieties with the Tan-ko-fo-ira, the results did not seem to be in complete accord. In the former case, the minimum fertility was 0.1 %, the maximum, 13.9 %, and the average, 9.6 %, while in the latter, that is, when the Tan-ko-fo-ira was used for the male plant, the fertility was 10.7 %, 26.2 % and 17.5 %, respectively, which means that the hybrids in the latter case had a somewhat greater fertility. The same phenomena were manifested when the Hunan-sien and Tan-ko-fo-ira were used for the mother plants (Table III).

According to the above results, the F_1 plants produced by the hybridization of varieties, belonging to the same type, the "Indica" as well as the "Japonica," show a comparatively high degree of fertility; while, on the other hand, those produced by hybridizing different types appear to show much lower fertility.

In order to ascertain whether the facts shown by the above 12 varieties, according to the above stated method, would be applicable to other rice varieties, the authors examined 96 other varieties of cultivated rice, including glutinous and non-glutinous varieties, both upland as well as lowland kinds, red rice, and scented rice (all of them have a relatively high degree of fertility) concerning the degree of fertility of the hybrids within the same type and in those between the different types. The result showed just as in the case of the 12 varieties that the degree of fertility of the F_1 plants obtained by means of hybridization of the varieties belonging to the different types, was indeed very small (see Table IV).

3) Degree of the fertility of the F_2 plants

Basing on the difference of fertility of hybrids of plants within the same type and those between the different types, the authors have found in the aforesaid experiments that the "Japonica" and "Indica" types, which are two morphological divisions of cultivated rice plants, had a

Table III.

Fertility of the F_1 plants within the same type and between the different types
(Figures indicate the average of from 20 to 50 individuals)

Type of parent plant	Name of plant		(♂) "Japonica" type										(♂) "Indica" type		
			Wase-Shinriki	Nakate-Shinriki	Seki-yama	Omachi	Hiderishirazu (upland non-glutinous variety)	Tataso	Salpei	Chang-shou-wan-tao	Hsiang-tao	Average	Hunan-sien	Tan-ko-fo-ira	Average
		⊕	79.4	82.6	86.0	80.5	82.4	85.0	68.0	73.2	78.7	**79.5**	70.0	86.5	**78.3**
(♀)	Wase-Shinriki	79.4			63.3		66.0	65.5	59.5	60.7	68.8	64.0	**0.1**	10.7	5.4
"Japonica" type	Sekiyama	86.0	75.7	74.2		80.1	61.6		82.7	62.6	74.3	73.0	8.5		8.5
	Omachi	80.5						62.5	**48.6**	72.2	69.0	63.1	11.6	13.9	12.8
	Hiderishirazu (upland non-glutinous variety)	82.4			**83.6**						75.0	79.3	12.6	20.2	16.4
	Tataso	85.0	79.9		67.0				71.9	73.0	77.7	73.9			
	Chang-shou-wan-tao	73.2					69.6	58.2			66.5	64.8	13.9	**26.2**	20.1
	Chang-shou-chên-mi	82.1		79.0		62.2	53.6			74.2		67.3	8.7	21.5	14.6
	Hsiang-tao	78.7	62.2		60.4	75.7	54.1			69.7		64.4	12.1	12.6	12.4
	Average	**80.9**	72.6	76.6	68.6	72.7	61.0	62.1	65.7	68.7	71.9	**68.7**	9.6	17.5	**14.1**
(♀)	Hunan-sien	70.0	0.3		8.9		16.2	4.5	**0.0**	3.8		5.6		68.7	68.7
"Indica" typpe	Tan-ko-fo-ira	86.5	21.9		**33.4**			22.8		19.0	18.4	23.1	71.4		71.4
	Average	**78.3**	11.1		21.2		16.2	13.7	0.0	11.4	18.4	**13.6**	71.4	68.7	**70.5**

⊕ Denotes the fertility of the plants by self-fertilization.

Table IV.

Fertility of the F_1 plants obtained from many different varieties of cultivated rice plants

A. Fertility of the hybrids within the same type of the "Japonica"

Vareity of the "Japonica" type used for the female plant			Variety of the "Japonica" type used for the male plant			Fertility of the F_1 plants
Name	Native place	Fertility by self-pollination	Name	Native place	Fertility by self-pollination	
Wase-Shinriki	Japan	79.4 %	Hawaii no. 158	Hawaii	80.0 %	66.3 %
Sekiyama	"	86.0	Chang-shou-chên-mi	China	82.1	60.1
"	"	"	Hsiao-pê-mang-shui-tao	China (Chili)	93.7	91.6
Aikoku	"	89.3	Chang-li	China (Wuchang)	86.1	86.7
Hinode	"	90.6	Keng-tao	China (Changchow)	91.8	88.9
"	"	"	Lo-han-huang	China (Kiangsu)	92.8	74.9
"	"	"	Keng-tao (Wu-hsi-hsüeh-yen-chiao)	China (")	86.8	74.9
"	"	"	Ma-tsung-nuo	" (")	88.2	67.4
"	"	"	Ning-tu	" (")	94.1	89.1
"	"	"	Huo-pê-ku	" (")	85.2	86.2
Hinomoto	"	77.3	Nanking-hsian-tao	China (Tônan Univ.)	86.0	83.8
"	"	"	San-li-tsun-nuo-tao	China	83.0	81.4
"	"	"	Nakamura 17	Formosa	90.8	78.2
Hinode	"	90.6	Honduras	U. S. A.	88.7	70.7
"	"	"	Prace no. 6	"	81.6	70.4
"	"	"	Katete	Brazil	—	84.8
"	"	"	Amareria	"	52.2	79.1
"	"	"	Buranko	"	81.7	83.2
Kinen-Mochi	"	83.6	Okute-Shinriki	Japan	83.1	74.2
"	"	"	Hsiang-tao	China	78.7	70.3
"	"	"	Hawaii no. 158	Hawaii	80.0	78.7
Hawaii no. 158	Hawaii	80.0	Wase-Shinriki	Japan	79.4	64.3
"	"	"	Seiso	Korea	82.0	89.2
"	"	"	Hsiang-tao	China	78.7	72.4
Nanking-hsian-tao	China	77.9	Hawaii no. 158	Hawaii	80.0	79.1
Average		**83.9**	Average		**83.6**	**81.2**

B. Fertility of the hybrids within the same type of the "Indica"

Variety of the "Indica" type used for the female plant			Variety of the "Indica" type used for the male plant			Fertility of the F_1 plants
Name	Native place	Fertility by self-pollination	Name	Native place	Fertility by self-pollination	
Pu-chiang san-pe-li-keng-tao	China	83.8 %	Chang-shou-sien-tao	China	85.6 %	84.9 %
Chiang-nan-tao	"	90.9	Pa-shih-sien	"	87.2	86.2
Average		**87.4**	Average		**86.4**	**85.6**

C. Fertility of the F_1 plants between the different types, that is, between the "Japonica" and the "Indica" type

Variety of the "Japonica" type used for the female plant			Variety of the "Indica" type used for the male plant			Fertility of the F_1 plants
Name	Native place	Fertility by self-pollination	Name	Native place	Fertility by self-pollination	
Okute-Shinriki	Japan	83.1 %	Tan-ko-fo-ira	Formosa	86.5 %	3.0 %
Sekiyama	"	86.0	Sei-yu	"	86.0	8.8
Hinomoto	"	77.3	Ha-koku-fo-ira	"	74.2	0.7
Wase-Shinriki	"	79.4	Pu-chiang-san-pe-li-keng-tao	China	83.8	5.5
Nakate-Shinriki	"	82.6	Fung-ku-fung-tao	"	77.2	1.8
Sekiyama	"	86.0	Lu-shih-jih-sien-tao	China (Chekiang)	80.5	24.7
"	"	86.0	Jung-hê-tao	China (Kiangsu)	72.6	13.2
Aikoku	"	89.3	Fung-ku-fung-tao	China (Chekiang)	77.9	19.6
"	"	89.3	Ya-i-tao	" (")	91.9	1.1
Kameji	"	90.7	Hsiao-li-fung-keng-tao	" (")	76.6	0.4
"	"	90.7	Pu-chiang-san-pe-li-keng-tao	" (")	61.6	8.3
Hinode	"	90.6	Fung-tsui-yü-keng-tao	China (Szechwan)	82.6	0.5
"	"	90.6	Ping-yang-pê-sien tao	China (Chekiang)	75.9	6.5
"	"	90.6	Li-lun-ku-keng-tao	" (")	82.5	0.5
"	"	90.6	Huang-pi-keng-tao	" (")	72.6	1.6
"	"	90.6	Fên-shui-san-pê-li-keng-tao	" (")	71.2	13.2
"	"	90.6	Chi-ching-tao-keng-tao	" (")	71.1	0
"	"	90.6	Ai-keng-tao	" (")	72.5	0

C. Continued

Hinode	Japan	90.6 %	Mang-ku-keng-tao	China (Chekiang)	81.4	0.9
,,	,,	90.6	Ta-ku-keng-tao	,, (,,)	81.0	0
,,	,,	90.6	Sien-tao-wa-mei	China (Kiangsu)	81.0	1.4
,,	,,	90.6	Yang-sien-tao	,, (,,)	76.3	1.5
Hinomoto	,,	77.3	Tao-jen-chiao	China	86.2	0
,,	,,	77.3	Chiang-nan-tao	China (Anwhei)	89.8	4.4
,,	,,	77.3	Tsao-sien-tao	China (Tô-nan Univ.)	86.8	0
,,	,,	77.3	Pa-shih-tzu-sien	,, (,,)	72.2	1.4
,,	,,	77.3	Huang-kan-sien	,, (,,)	87.0	2.2
,,	,,	77.3	Mao-tzu-tou-sien-tao	,, (,,)	85.7	0
,,	,,	77.3	Lu-shih-jih-sien-tao	China (Chekiang)	80.5	6.6
,,	,,	77.3	Huo-pe keng-tao	,, (,,)	85.3	0
,,	,,	77.3	Fung-ku-keng-tao	,, (,,)	65.1	0.2
,,	,,	77.3	Wu-pe-li-keng tao	,, (,,)	69.9	0.3
,,	,,	77.3	Si-yeh-tsing-keng-tao	,, (,,)	91.8	1.5
Hinode	,,	90.6	Ta-tsao-keng-tao	,, (,,)	84.0	0
Nishitaku-Mochi	,,	78.2	Chang-ken-wan-keng-tao	China	88.9	3.3
Nanking-ksian tao	China	77.9	Meng-kan-peng-keng-tao	,,	84.9	8.7
Keng-tao-chang-chou	China (Kiangsu)	91.8	Kôkyaku-fo-ira	Formosa	84.6	2.7
,,	,,	91.8	Ukoku-nanshi	,,	81.1	7.4
Nishitaku-Mochi	Japan	78.2	Vellisenatti	Java	87.5	2.4
Hinode	,,	90.6	Gangasale Bhatta	Southern India	55.9	0
,,	,,	90.6	Red Kangro	Western India	81.0	20.3
Kameji	,,	90.7	Seenaddy	Ceylon	78.0	6.0
Hinode	,,	90.6	Karaffa cheenatti	,,	75.1	0
,,	,,	90.6	Hawaii no. 17	Hawaii	—	29.9
,,	,,	90.6	Carolina Gold	U.S.A.	93.6	1.4
Keng-tao-chang-chou	China (Kiangsu)	91.8	,,	,,	93.6	16.2
Average		**85.4**	Average		**79.8**	**4.7**

D. Fertility of the F_1 plants between the different types, that is, between the "Indica" and the "Japonica" type

Variety of the "Indica" type used for the female plant			Variety of the "Japonica" type used for the male plant			Fertility of the F_1 plants
Name	Native place	Fertility by self-pollination	Name	Native place	Fertility by self-pollination	
Pu-chiang-san-pe-li-keng-tao	China (Chekiang)	83.8 %	Wase Shinriki	Japan	79.4 %	3.6 %
Fung-ku-fung-tao	„ („)	77.2	Nakate-Shinriki	„	82.6	1.9
„	„ („)	77.2	Keng-tao-chang-chou	China (Kiangsu)	92.4	2.3
To-tsao-keng-tao	„ („)	84.0	Nakamura 17	Formosa	90.8	0
„	„ („)	84.0	Fortuna	U.S.A.	54.2	3.5
„	„ („)	84.0	Texas Fortuna	„	81.5	1.7
Average		**81.7**	Average		**80.2**	**2.2**

very poor sexual affinity. Not satisfied with such results alone, however, they self-pollinated the chief F_1 plants of both kinds and obtained seeds. They examined the fertility of the F_2 plants in the same manner as in the F_1, and found that in the F_2 plants derived from the hybrids between the different types, the lines of various degrees of fertility was segregated out, ranging from a high degree of fertility to absolute sterility, the mean of fertility being 26.2 % in minimum, and 59.9 % in maximum, and 39.9 % on the average. The deviation of fertility in every line was, when the mean is put 100, ± 39.1 for the minimum, and ± 85.9 for the maximum, and ± 59.7 for the average.

On the other hand, they observed that every line produced from hybrids within the same type was very fertile. Taking Tataso × Wase-Shinriki and Tan-ko-fo-ira × Hunan-sien for comparison, they found that the mean of fertility was 87.3 % in the former and 74.4 % in the latter, the average being 80.9 %, while the deviation in both cases was only ± 8.6, when the mean was 100. In other words, the F_2 plants resulting from hybrids between the different types, as compared with those within the same type, were small as to the mean of fertility, and consequently much greater in deviation (see Table V).

Table V.

Fertility and deviation of fertility of the F_2 plants derived from the hybrids between the different types and within the same type

Combination	F_1				F_2		
	The female plant		The male plant		Total no. of individuals in every strain	Mean of fertility (%)	Deviation of fertility as the mean 100
	Tpye	Name of variety	Type	Name of variety			
Hybrids between the different types	The "Japonica"	Wase-Shinriki	The "Indica"	Tan-ko-fo-ira	135	31.1	±75.9
	,,	Okate-Shinriki	,,	,,	126	51.2	±46.5
	,,	Omachi	,,	,,	190	27.4	±78.8
	,,	Hiderishirazu (upland non-glutinous variety)	,,	,,	218	55.0	±**39.1**
	,,	Chang-shou-chên-mi	,,	,,	353	29.9	±78.1
	,,	Tataso	,,	,,	98	52.3	±44.9
	,,	Chang-shou-wan-tao	,,	,,	193	**59.9**	±39.2
	,,	Hsiang tao	,,	,,	208	38.4	±57.2
	Sum or Average				1521	42.7	±57.5
	The "Indica"	Tan-ko-fo-ira	The "Japonica"	Wase-Shinriki	196	45.4	±45.6
	,,	,,	,,	Sekiyama	192	51.2	±40.2
	,,	,,	,,	Salpei	271	34.6	±55.5
	,,	,,	,,	Chang-shou-wan-tao	295	50.2	±44.8
	,,	,,	,,	Hsiang-tao	377	34.2	±58.8
	Sum or Average				1331	43.1	±49.0
	The "Japonica"	Omachi	The "Indica"	Hunan sien	220	**26.2**	±**85.9**
	,,	Hiderishirazu (upland non-glutinous variety)	,,	,,	82	28.2	±80.5
	,,	Chang-shou-chên-mi	,,	,,	81	48.5	±61.9
	,,	Chang-shou-wan-tao	,,	,,	199	33.7	±66.5
	,,	Hsiang-tao	,,	,,	92	36.2	±66.1
	Sum or Average				674	34.6	±71.2

Table V. Continued

Hybrids between the different types	The "Indica"	Hunan-sien	The "Japonica"	Sekiyama	161	39.0	±61.3
	"	"	"	Hiderishirazu (upland non-glutinous variety)	162	42.1	±53.9
	"	"	"	Tataso	43	29.9	±75.9
	"	"	"	Chang-shou-wan-tao	76	34.1	±60.7
	Sum or Average				442	36.3	±63.0
	Grand sum or Average				3968	**39.9**	**±59.7**
Hybrids within the same type	The "Japonica"	Tataso	The "Japonica"	Wase-Shinriki	86	87.3	± 8.6
	The "Indica"	Tan-ko-fo-ira	The "Indica"	Hunan-sien	229	74.4	± 8.6
	Grand sum or Average				315	**80.9**	**± 8.6**

Seemingly, there is also a slight difference in the segregation of degrees of fertility between the hybrids of the different types when the Hunan-sien is used for a parent and those of the different types when the Tan-ko-fo-ira is used for one. To speak more specifically, in the former case, the mean of fertility was 36.3 % and the deviation, ± 63.0 on the average, the mean being 100, when the Hunan-sien was used for the female plant; when it was used for the male plant, the mean of fertility was 34.6 % and the deviation, ± 71.2 (mean = 100). Averaging the above values, we get 35.5 % for the mean of fertility and ± 67.1 for the deviation, the mean being again 100. In the latter case, however, the mean of fertility was 43.1 % and the deviation ± 49.0 when the Tan-ko-fo-ira was used for the female plant; when it was used for the male plant, the mean of fertility, 42.7 % and the deviation, ± 57.5 (mean = 100). Averaging the above values, we get 42.9 % for the mean of fertility, and ± 53.3 the deviation with the mean 100. Judging from these facts, the fertility of the F_2 plants of the different types, when the Hunan-sien is used, seems to be smaller in the mean of fertility and greater in the deviation than that of the F_2 plants of the different types, when the Tan-ko-fo-ira is used.

IV. AFFINITY OF THE VARIETIES OF RICE PLANTS FROM THE SERODIAGNOSTIC STANDPOINT

1. Methods of investigation

For the serodiagnostic investigation fresh materials were always used. They consisted of the principal varieties used in the experiments of fertility, namely 12 varieties, from 1) Wase-Shinriki to 12) Tan-ko-fo-ira. The other additional five varieties were Kuromoro, Chôeito, Saigoku-kawachi, Genki-Mochi (these are of the "Japonica" type) and Fung-hsüeh-nuo (this is of the "Indica" type). Previous to this investigation on rice, the authors carried on a comparative study with 7 graminaceous crops, viz. rice, Italian millet, Sawa millet, maize, barley, wheat, and corakan. The authors prepared antigen in the serodiagnostic reaction, following the method of GOHLKE (4), and made use of the solution, obtained by extracting their seed powder in physiological salt solution. However, for preparing the solution for the precipitin reaction, they firstly made the protein content uniform according to the MISAO's method (16) and then according to the KOKETSU's method (12), added normal rabbit serum to get off the pseudo-precipitate which was produced thereby. This solution was used as the precipitinogen.

They used "Ring probe" for precipitin reaction: that is to say, the authors put a certain amount of immune serum into narrow tubes; then to each of these they added a certain amount of the solutions of different concentration degrees which had been made by diluting the above mentioned precipitinogen solution with the physiological salt solution twice, four times, eight times, and so on; then they determined the strength of the precipitate produced at the plane of contact. The result of this experiment was shown by the strength of reaction within two hours, and the value was denoted by the figures which indicated the minimum concentration showing the positive reaction. As it is difficult however, by this simple method of precipitin reaction alone to make the obvious distinction of rice varieties, the authors performed in addition WEICHART's "Absättigungsverfahren" modified by MISAO in the test-tube and moreover MISAO's "Absättigungsverfahren" in the living body (16) for precipitin reaction.

2. Experimental results

1) Results of investigation on the grasses used for comparative study

Before undertaking a serodiagnostic investigation of rice, the authors examined, for a comparative study, the affinity of 7 graminaceous crops, all of which belong to different genera, namely rice, Italian millet, Sawa millet, maize, barley, wheat, corakan, by "Ring probe" with each immune serum. No matter what immune serum might be used, the different genera were easily distinguished from each others. And the result seems to show that rice, Italian millet, Sawa millet, and maize are closely related; that barley and wheat are also intimately related; that the latter are distantly related to the former 4 genera (Table VI).

Table VI.

Precipitin reaction with immune serum of 7 graminaceous crops[1]

Immune serum / Precipitinogen	Anti-rice	Anti-Italian millet	Anti-Sawa millet	Anti-maize	Anti-barley	Anti wheat	Anti-corakan
Rice: *Oryza sativa* L.	9	3	4	1	0	0	0
Italian millet: *Setaria italica* BEAUVOIS	2	8	5	3	0	0	0
Sawa millet: *Panicum Crus-Galli* L.	2	3	5	2	0	0	0
Maize: *Zea mais* L.	2	3	3	6	0	0	0
Barley: *Hordeum sativum* JESS.	0	0	0	0	9	5	0
Wheat: *Triticum sativum* LAM.	0	0	0	0	6	8	0
Corakan: *Eleusine Coracana* GÄRTNER	0	0	0	0	0	0	9

[1] The varieties used in this experiment were as follows: rice, Shinriki; Italian millet, Toranoo; Sawa millet, Yamanome no. 2; maize, Hassaku; barley, Hizahachi; wheat, Akadaruma.

2) Results of investigation on the rice plants

Thanks to this comparative study, the authors clearly distinguished the graminaceous crops, belonging to different genera by the ordinary method of precipitin reaction with each immune serum. Then, adopting the same method as in this comparative study, they studied the affinity of 6 cultivated varieties of rice which belong to the same species and yet live in different regions, and which have been distinctly classified morphologically by the method of the fertility of hybrid plants. They included 5 cultivated varieties of the "Japonica" type, Shinriki, Sekiyama (these are of Japan Proper), Tataso (Korean), Chang-shou-wan-tao and Chang-shou-chên-mi (Chinese) and 1 variety of the "Indica" type, Hunan-sien (Chinese). In this case, whichever immune serum was employed, it was difficult to distinguish one from another. On the other hand, when the methods of "Absättigungsverfahren" were used, both in the test-tube and in the living body for precipitin reaction, these varieties were clearly distinguishable. The results are shown below:

Experiment I. Firstly, Shinriki, Omachi, and Sekiyama, which are common cultivated varieties of lowland rice, in Japan Proper, belonging to the "Japonica" type, were taken. Secondly, the immune serum of each variety was made, and to this "Absättigungsverfahren" in the test-tube was applied, and then "Ring probe" for precipitin reaction was tried. The varieties were in this case not distinguishable (Table VII).

Table VII.

Precipitin reaction by "Absättigungsverfahren" in the test-tube upon lowland varieties of Japan Proper, belonging to the "Japonica" type

Immune serum	Anti-Shinriki		Anti-Omachi	Anti Sekiyama
Precipitinogen for saturation / Precipitinogen for test	Sekiyama	Omachi	Shinriki	Shinriki
Shinriki	o	o	o	o
Sekiyama	o	...	...	o
Omachi	...	o	o	...

Experiment II. Following the method in experiment I, the authors studied the affinity of 7 varieties of Japan Proper, which, like those in the above experiment, belong to the "Japonica" type, and which are remarkably different from the ordinary cultivated rice from the standpoint of cultivation, use, or grains, viz. Shinriki (a non-glutinous variety), Genki-Mochi (a glutinous variety) (which are lowland rice), Tamasari (a non-glutinous variety), Gifu-Mochi (a glutinous variety) (which are upland rice), long-glumed rice, Kuromoro (red rice), Saigoku-kawachi (scented rice) (which are peculiar kinds of rice). The result was that, the Kuromoro and Saigoku-kawachi were barely distinguishable from the others, but the rest was difficult to be distinguished from one another in this case (see Table VIII).

Table VIII.

Precipitin reaction, by « Absättigungsverfahren " in the test-tube, of glutinous and non-glutinous varieties of lowland or upland rice or varieties of peculiar rice which are all of Japan Proper and of the " Japonica " type

Immune serum	Precipitinogen for test / Precipitinogen for saturation	Shinriki	Genki-Mochi	Tamasari	Gifu-Mochi	Chôeito	Kuromoro	Saigoku-kawachi
Anti-Shinriki	Genki-Mochi	o	o	...	...	...	...	...
	Tamasari	o	...	o	...	...	...	...
	Gifu-Mochi	o	...	...	o	...	...	...
	Chôeito	o	...	...	...	o	...	...
	Kuromoro	I	...	...	...	...	o	...
	Saigoku-kawachi	o	...	...	...	...	...	o
Anti-Genki-Mochi	Shinriki	o	o	...	...	...	...	...
	Tamasari	...	o	o	...	...	...	...
	Gifu-Mochi	...	o	...	o	...	...	...
	Chôeito	...	o	...	...	o	...	...
	Kuromoro	...	2	...	...	...	o	...
	Saigoku-kawachi	...	o	...	...	...	...	o
Anti-Tamasari	Shinriki	o	...	o	...	...	...	...
	Genki-Mochi	...	o	o	...	...	...	...
	Gifu Mochi	...	...	o	o	...	...	...
	Chôeito	...	...	I	...	o	...	...
	Kuromoro	...	...	I	...	...	o	...
	Saigoku-kawachi	...	...	o	...	...	...	o

Table VIII Continued

Anti-Gifu-Mochi	Shinriki	0	...	...	1	...	...	...
	Genki Mochi	...	0	...	0	...	...	...
	Tamasari	...	...	0	0	...	...	...
	Chôeito	...	...	...	0	0	...	...
	Kuromoro	...	...	...	2	...	0	...
	Saigoku-kawachi	...	...	...	2	...	...	0
Anti-Chôeito	Shinriki	0	...	...	...	0	...	...
	Genki-Mochi	...	0	...	...	0	...	...
	Tamasari	...	...	0	...	0	...	...
	Gifu-Mochi	...	...	...	0	0	...	...
	Kuromoro	...	...	...	...	0	0	...
	Saigoku-kawachi	...	...	...	...	0	...	0
Anti-Kuromoro	Shinriki	0	...	...	...	...	3	...
	Genki-Mochi	...	0	...	...	...	3	...
	Tamasari	...	...	0	...	...	3	...
	Gifu-Mochi	...	...	...	0	...	2	...
	Chôeito	...	...	...	...	0	0	...
	Saigoku-kawachi	...	...	...	...	...	2	0
Anti-Saigoku-kawachi	Shinriki	0	...	...	...	...	...	1
	Genki Mochi	...	0	...	...	...	...	2
	Tamasari	...	...	0	...	...	...	2
	Gifu-Mochi	...	...	...	0	...	...	2
	Chôeito	...	...	...	...	0	...	1
	Kuromoro	...	...	...	...	...	0	2

Experiment III. From the morphological standpoint, the following 4 varieties, Shinriki (of Japan Proper), Salpei (Korean), and Chang-shou-wan-tao (Chinese) of the "Japonica" type and Hunan-sien (Chinese) of the "Indica" type, were investigated in the manners as described in experiment I. In this case, the above varieties were clearly distinguishable from one another. Taking all facts into consideration, Shinriki and Chang-shou-wan-tao are related while Hunan-sien is fairly distinct. Salpei, on the other hand, belongs to the "Japonica" type, yet it is also related, according to this experiment, to Hunan-sien of the "Indica" type (see Table IX)

Table IX.

Precipitin reaction by "Absättigungsverfahren" in the test tube, of varieties, belonging either to the "Japonica" or the "Indica" type, and differing in native place

Immune serum	Anti-Shinriki			Anti-Salpei			Anti-Hunan-sien			Anti-Chang-shou-wan-tao		
Precipitinogen for saturation / Precipitinogen for test	Salpei	Hunan-sien	Chang-shou-wan-tao	Shinriki	Hunan-sien	Chang-shou-wan-tao	Shinriki	Salpei	Chang-shou-wan-tao	Shinriki	Salpei	Hunan-sien
Shinriki	7	5	1	2	0	1	2	1	2	2	6	2
Salpei	2	1	4	5	1	4	4	2	3	4	2	3
Hunan sien	5	1	2	5	0	4	5	2	4	5	7	0
Chang-shou-wan-tao	7	2	1	3	0	2	2	1	1	2	7	1

Experiment IV. As in the experiment III, the degree of relationship of 7 varieties, Shinriki, Sekiyama, Kinen-Mochi (a glutinous variety), Hiderishirazu (upland rice), (all of these are from Japan Proper), Tataso (Korean rice), Chang-shou-wan-tao (Chinese rice) belonging to the "Japonica" type and the Tan-ko-fo-ira (Formosan rice) belonging to the "Indica" type, was examined by using "Ring probe" for precipitin reaction with immune serum obtained by "Absättingungsverfahren" in the living body of rabbits immunized with the solution of each of the above varieties. The result was as in the case tried with "Absättingungsverfahren" in the test-tube, i.e. one could not distinguish Shinriki from Sekiyama, while the other varieties could be distinguished from one another, though in varying degrees. It was determined that Shinriki is closely related to the other varieties of the "Japonica" type, while it is very remotely related to Tan-ko-fo-ira (see Table X).

Table X.

Precipitin reaction tried "Absättigungsverfahren" in the living body with the cultivated varieties of rice, belonging to the "Japonica" or the "Indica" type

Immune serum	Anti-Shinriki						Anti-Sekiyama	Anti-Hideri-shirazu	Anti-Kinen-Mochi	Anti-Tan-ko-fo-ira	Anti-Tataso	Anti-Chang-shou-wan-tao
Precipitinogen for saturation / Precipitinogen for test	Sekiyama	Hideri-shirazu	Kinen-Mochi	Tan-ko-fo ira	Tataso	Chang-shou-wan-tao	Shinriki	Shinriki	Shinriki	Shinriki	Shinriki	Shinriki
Shinriki	4	12	8	8	11	5	23	8	2	3	11	4
Sekiyama	4	...	...	...	...	...	23	...	...	...	...	...
Hiderishirazu	...	11	...	...	...	...	...	11	...	...	...	...
Kinen Mochi	...	...	7	...	...	...	..	...	4	...	...	...
Tan-ko-fo-ira	...	...	...	4	...	...	...	...	...	8	...	...
Tataso	...	...	...	...	8	...	...	...	...	...	15	...
Chang-shou-wan-tao	...	...	...	...	...	4	...	...	...	...	...	7
Difference of reaction	0	1	1	4	3	1	0	3	2	5	4	3

Table XI.

Precipitin reaction tried "Absättigungsverfahren" in the living body with the cultivated varieties of rice, belonging to the "Japonica" or the "Indica" type

Immune serum	Anti-Hunan-sien						Anti-Sekiyama	Anti Hideri-shirazu	Anti-Kinen-Mochi	Anti-Tan-ko-fo-ira	Anti-Tataso	Anti-Chang-shou-wan tao
Precipitinogen for saturation / Precipitinogen for test	Sekiyama	Hideri-shirazu	Kinen-Mochi	Tan-ko-fo-ira	Tataso	Chang-shou-wan tao	Hunan-sien	Hunan-sien	Hunan-sien	Hunan-sien	Hunan-sien	Hunan-sien
Hunan-sien	8	15	8	8	12	15	4	5	1	1	1	11
Sekiyama	1	...	...	...	...	...	15	...	...	...	...	...
Hiderishirazu	...	8	...	...	...	...	...	15	...	...	...	...
Kinen-Mochi	...	...	1	...	...	...	...	...	12	...	...	...
Tan ko--ifora	...	...	...	4	...	...	...	...	...	8	...	...
Tataso	...	...	...	...	4	...	...	...	...	...	8	...
Chang-shon-wan-tao	...	...	...	...	...	8	...	...	...	...	...	19
Difference of reaction	7	7	7	4	8	7	11	10	11	7	7	8

Experiment V. As in the experiment IV, the affinity of the under-named varieties, belonging to the "Japonica" type or the "Indica" type, viz. Sekiyama, Hiderishirazu (upland rice), Kinen-Mochi (glutinous rice), Tataso, Chang-shou-wan-tao (the above are of the "Japonia" type), Hunan-sien and Tan-ko-fo-ira (the above are of the "Indica" type), by using the immune serum which had been studied by means of "Absättigungsverfahren" in the living body. It was found that the varieties of the "Indica" type were closely related to one another and that they were rather distantly related with those of the "Japonica" type (see Table XI).

According to the above results, the cultivated rice of Japan Proper, Korea, Formosa, China are more or less distinguishable by "Ring probe" for precipitin reaction, using "Absättingungsverfahren" either in the test-tube or in the living body. And on the whole, regardless of native regions, the varieties of "Japonica" and "Indica" types which are morphologically quite distinct appear to be closely related among themselves, while those of the "Japonica" and the "Indica" type seem to be very distantly related.

V. AFFINITY OF THE VARIETIES OF RICE PLANTS, VIEWED FROM THE CYTOLOGICAL STANDPOINT

1. Methods of investigation

Before conducting a cytological investigation, the authors examined, as a preliminary experiment, the formation of pollen in 15 F_1 hybrids, namely 8 within the same type and 7 between the different types, which were the same as those used for the investigation of fertility. They observed fresh pollen without fixing, and judged the success or failure of pollen development by its form, color, the presence of content, degree of coloration with iodine solution, etc.; to indicate the degree of its success or failure, they cut 1 to 2 anthers from each of 3 to 5 florets, taken at random, from an ear of each plant produced at a comparatively early period, then all pollen grains of each anther were scattered on one slide as evenly as possible, and they counted the percentage of perfect pollen grains as compared with their total number within a certain definite area under the microscope.

As the material for the cytological research, the authors have taken the chief varieties used in the study of fertility and serodiagnostics.

They are Shinriki, scented rice, Salpei, in the case of the "Japonica" type; the Hunan-sien, Fung-hsüeh-nuo, and Tan-ko-fo-ira in the case of the "Indica" type. For the examination of the F_1 plants, they used, besides the hybrids from these parent plants, the chief hybrids used in the investigation of fertility, making a total of 11 kinds, that is, Nakate-Shinriki × Fung-hsüeh-nuo, Hinode × Fung-tsui-yü-keng-tao, Hinode × Hatadavi, Hinode × Basmati, Hinomoto × Huo-pe-keng-tao, Hinomoto × Pu-chiang-sang-pe-li-ken-tao, Aikoku × Tsao-sien-tao, Kameji × Black Seenaddy, Hunan-sien × Nakate-Shinriki, Fung-hsüeh-nuo × Nakate-Shinriki (crosses between the different types), Sei-yu × Fung-hsüeh-nuo (crosses within the same type).

The authors observed the shape and behavior of the chromosomes in the cells of root-tips and in the pollen mother-cells of the parents as well as the F_1 plants, and furthermore, the general development during pollen formation in the F_1 plants. For the fixation of the cells of root-tips, they used FLEMMING's solution and for that of the pollen mother-cells, chiefly BOUIN's solution, but sometimes CARNOY's or FLEMMING's solution. The coloration was made with HEIDENHEIN's iron-alum-hematoxylin. For the observation of the general development during pollen formation, the fixation and the coloration were done as above at its early stages; at the stages later than the formation of tetrads, fresh materials were examined, without fixing. The method used in the determination of pollen development was the same as in the preliminary investigation.

2. Experimental results

1) Pollen formation in the F_1 plants

In the fertility experiment of hybrids, it has been already found that the F_1 plants within the same type, be it the "Japonica" or the "Indica," are comparatively very fertile while those between the different types are markedly infertile. Now the investigations of the formation of pollen in 8 F_1 plants within the same type and of 7 F_1 ones between the different types, have taught us that the pollen in hybrids within the same type, is nearly perfect; while that between the different types is quite imperfect: imperfect ones are 55.7 % in minimum and of 75.7 % in maximum, the average being 66.1 %. The formation of pollen is notably abnormal.

This being the case, one of the causes of poor fertility of crosses between the unlike types may be at least the formation of imperfect pollen (see Table XII).

Table XII.

Developmental condition of pollen in the F_1 plants within the same type and between the different types (Figures in the table indicate the average value of 3 individuals examined)

Hybrids	The Female Plant		The Male Plant		Percentage of imperfect ones in the total number of pollen grains in the F_1 plants
	Type	Name of Variety	Type	Name of Variety	
Hybrids within the same type	"Japonica"	Wase-Shinriki	"Japonica"	Hiderishirazu	0
	,,	,,	,,	Salpei	0
	,,	Sekiyama	,,	Omachi	0
	,,	,,	,,	Chang-shou-chên-mi	0
	,,	,,	,,	Chang-shou-wan-tao	0
	,,	Tataso	,,	Salpei	0
	,,	Hsiang-tao	,,	Sekiyama	0
	,,	,,	,,	Salpei	0
	Average				**0**
Hybrids between the different types	"Japonica"	Wase-Shinriki	"Indica"	Tan-ko-fo-ira	65.8
	,,	Omachi	,,	,,	**55.7**
	,,	Hsiang-tao	,,	,,	71.7
	,,	Nakate-Shinriki	,,	Hunan sien	**75.7**
	,,	Sekiyama	,,	,,	57.3
	,,	Tataso	,,	,,	73.9
	"Indica"	Tan-ko-fo-ira	"Japonica"	Hsiang-tao	62.6
	Average				**66.1**

2) Results of cytological research

The above preliminary investigation of pollen in the F_1 plants has revealed the fact that the formation of pollen in the hybrids between the different types becomes very abnormal. In what stage of pollen formation, then, does this abnormality take place? Is the cause of this abnormality due to the chromosomes of the parent varieties? To answer these questions, the authors made an inquiry into the number and behavior of the chromosomes in the root-tip and the pollen mother-cells in the 7 varieties, namely Nakate-Shinriki, Okute-Shinriki, scented rice, and Salpei, belonging to the "Japonica" type, and the Hunan-sien, Fung-hsüeh-nuo, and Tan-ko-fo-ira, belonging to the "Indica" type. They found that the chromosomes were 12 in the haploid and 24 in the diploid number in every one of the varieties, which agrees with the results of previous studies (5, 14, 17). Again, a close examination of the shape and size of the chromosomes has failed to discover any differences among the varieties. Nor have the authors been able to find any irregularity in the behavior of chromosomes in the pollen mother-cells and the development of pollen, following the tetrad formation.

The authors then took the 11 chief F_1 plants out of the hybrids between these 7 varieties and those varieties used in the fertility experiment, i e., 1 hybrid within the same type and 10 hybrids between the different types. Then, they examined the number, shape, and behavior of chromosomes in the root-tip and pollen mother-cells in the same manner as in the parent varieties, and found that in the hyprids within the same type the points under consideration were essentially the same as in the above mentioned 7 varieties.

On the other hand, in the hybrids between the different types, the number and shape of chromosomes and the course up to the formation of the tetrad in the pollen mother-cells were almost the same as in the hybrids within the same type. There was a great amount of abnormalities in the development of pollen after the tetrad formation. To go into detail, following the formation of tetrad, each cell which is separated out from an other, becomes round. In this initial stage of growth of the pollen grain, the authors have observed abnormality in the shape of the grain. And moreover at the middle stage of pollen development, when the growth of grains advances further and the content became visible in them, they not only saw that there are some grains of abnormal shape, but also that such grains are never colored with iodine solution, and even among those of normal shape, there are a lot of uncolored ones. And at the period when the plants were about to flower, after the full development of pollen (the stage of maturity), pollen grains of imperfect shape, those lacking content though normal in shape, those which were very slightly colored with iodine solution or those which were thought to be imperfectly developed, were found in great numbers and the degree of imperfect development was approximately identical with that at the middle stage. To illustrate this, in the crosses, Hunan-sien × Nakate-Shinriki and Fung-hsüeh-nuo × Nakate-Shinriki, the pollen grains of imperfect shape at the initial, middle and maturing stages, ones insufficiently colored with iodine solution, and those which were considered to be imperfectly developed, were found on investigation to be as follows:—at the initial

Table XIII.

Abnormality in the course of pollen development in the F_1 plants

Name of the F_1 plants		Percentage of imperfect grains in the total number of pollen grains[1]		
		Initial stage of development	Middle stage of development	Maturing stage of development
Hybrids between the different types	Hunan-sien × Nakate-Shinriki	22.3	59.9	53.9
	Fung-hsüeh-nuo × Nakate-Shinriki	13.1	55.3	56.4
	Average	**17.7**	**57.6**	**55.2**
Hybrids within the same type	Sei-yu × Fung-hsüeh-nuo	0	0	0

[1] Imperfect pollen grains mean, at the initial stage, those which are of imperfect shape; at the middle stage, those which are of imperfect shape and insufficiently colored with iodine treatment; and at the maturing stage, those which are of imperfect shape, lacking content, insufficiently colored with iodine treatment.

stage, those abnormally shaped were 17.7 % ; at the middle stage, those insufficiently colored with iodine solution, 57.6 % ; and at the maturing stage, those considered to be incompletely developed, 55.2 % on an average.

However, in the F_1 plants within the same type (Sei-yu × Fung-hsüeh-nuo were used for comparative study), we found that imperfect pollen at the initial stage of development amounts to 0.0 %, insufficiently colored pollen with iodine treatment at the middle stage to 0.0 %, and the pollen considered incompletely developed at the maturing stage, also to 0.0 % (see Table XIII).

These results indicate that there are no differences in the shape, number, and behavior of chromosomes between the "Japonica" and the "Indica" type. This is also true of all kinds of hybrids investigated. Although the course of pollen development after the tetrad formation is quite normal in both types and also in the hybrids within the same type, but in the hybrids between the different types abnormality is observed soon after the tetrad is formed, and accordingly a great deal of imperfect pollen is to be seen at the maturing period of the pollen.

VI. GENERAL CONSIDERATION AND CONCLUSION

The above mentioned cultivated varieties of rice may be classified into the "Japonica" and "Indica" types from the morphological standpoint. And the results of experiments on fertility show that there are certain differences between both types, when even merely the fertility of the respective F_1 plants is considered. Indeed, the authors were able to find that the distinction from the morphological standpoint, and that from the degrees of the fertility of the hybrids are in full accord.

In spite of the fact that the parent plants are highly self-fertile, the phenomenon of sterility is witnessed in the F_1 plants. This is mostly due to the extreme smallness of the sexual affinity, as they are not closely related in descent. In such a case, it is a matter of common knowledge that the formation of pollen in the F_1 plants proceeds abnormally (1, 3, 6, 7, 9, 10, 19, etc.).

If this is true, then the varieties of the "Japonica" type and those of the "Indica" type could be considered distantly related; on the contrary, in the hybrids within the same type, where we observe a high degree of fertility and normal pollen formation, various varieties within either type, could be regarded as closely related to one another. Thus, in the F_2 generation, the degree of fertility of every hybrid strain within the same type was great, and was almost the same as that of the respective parent, the deviation moreover being small. On the other hand, in the hybrids between the two different types, there were various degrees of fertility, ranging from absolute sterility to a fairly high degree of fertility, and the deviation was enormously large, but the mean was smaller than that of the hybrids within the same type. The cause of this may be found in the fact that the varieties of the "Japonica" and those of the "Indica" type used for parents, are not closely allied. It should be noted here that there is a little difference in the fertility between the hybrid of the Hunan-sien, belonging to the "Indica" type, with a variety, belonging to the "Japonica" type and the hybrid of the Tan-ko-fo-ira with one, belonging to the "Japonica" type; the hybrid resulting from the Tan-ko-fo-ira and a variety of the "Japonica" type is somewhat more fertile than that derived from the Hunan-sien and a variety of the "Japonica" type. Furthermore, when segregation occurred in the F_2 generation, the F_2 plants of the one were somewhat great in the mean of fertility, compared with the F_2 of the other, the deviation being, however, small. This may mean that there is a difference between the two varieties in their affinity with the "Japonica" type, that is, Tan-ko-fo-ira as compared with Hunan-sien, may justly be regarded as a little more closely allied with the "Japonica" type. This fact may also be recognized from the morphological standpoint, though Tan-ko-fo-ira bears a close resemblance to Hunan-sien in the portion of the plant above the ground. The ratio of width to length, thickness to length of the unpolished grain of the Tan-ko-fo-ira is comparatively greater than in similar varieties, belonging to the "Japonica" type,—its cross section being roundish.

In their serodiagnostic investigation, the authors used the ordinary "Ring probe" for precipitin reaction to find out the relationship of 7 crops, belonging to different genera of the *Gramineae*. And these were clearly distinguishable in different degrees, and the results are in general agreement with the classification of ENGLER and PRANTL (2). The authors think that this shows serodiagnostic treatment to be of value to the study of the affinity of the *Gramineae*. Consequently, they attempted a classification of the cultivated varieties of rice by this treatment. Although they totally failed to do so by the ordinary method of "Ring probe" for precipitin reaction, they could clearly differentiate them by "Ring probe" for precipitin reaction with "Absättingungsverfahren" in the test-tube, following WEICHARDT (20), or with "Absättigungsverfahren" in the living body, following MISAO (16). This explanation may be found in the fact that, though the cultivated varieties of rice belong to the same species and have a close resemblance among themselves, there is probably a fair amount of difference in the nature of proteins contained in their respective grains. According to the result of this investigation, the commonly cultivated varieties of rice of Japan Proper cannot be distinguished at all from each other. Again, of the 7 varieties in Japan Proper used for the principal study which are different from the ordinary cultivated rice from the standpoint of cultivation and use or in the particular nature of their grains, namely lowland, upland, glutinous, non-glutinous, long-glumed, scented and red rice, the last two, namely scented and red rice alone were slightly distinct from the other 5; but the latter could never be distinguished. Then, the rice varieties living in different regions, viz. those of Japan Proper, Korea, Formosa, and China, were clearly distinguished from one another in different degrees with "Absättigungsverfahren" either in the test-tube or in the living body. To state specifically[1] on the whole, regardless of native places, the varieties, belonging to the "Japonica" type from the morphological standpoint, are allied with one another; those belonging to the "Indica" type are closely related to one another; and the varieties of the "Japonica" type and those of the "Indica" type are fairly distinct in descent. This seems to show that the varieties of the "Japonica" and those of the "Indica" type, which are different from the morphological standpoint, are not closely allied.

On comparing the results of the serodiagnostic investigation with those of the fertility experiment, the authors find that the varieties of rice of Japan Proper, Korea, and China, belonging to the "Japonica" type and those of Formosan and Chinese rice, belonging to the "Indica" type are clearly distinguishable from each other as hybrids. Thus, the fact that this distinction is in general in agreement with the results of the serodiagnostic treatment will justify the classification of cultivated rice into "Japonica" and "Indica" types.

The authors collected a great many varieties of lowland, upland, glutinous, non-glutinous, or red rice from Japan Proper, Korea, Formosa,

[1] Though belonging to the "Japonica" type, the Korean rice, Salpei is recognized to be allied with the "Indica" type, the Hunan-sien as the result of the serodiagnostic investigation.

China, Java, India, the United States of America, etc., and tentatively classified them into the "Japonica" and the "Indica" type. Then, carrying on hybridization experiments, they were able to prove that this classification which is made from the morphological standpoint was correct also from the view point of sexual disharmony of hybrids. Hence, they made inquiry into the native regions of the above varieties, and found that the rice native to Japan Proper, Korea, and Northern China all belonged to the "Japonica" type and that found in Formosa, Southern China, Java, India, Ceylon, etc., to the "Indica," while some of the varieties in Central China, Hawaii, the United States are of the "Japonica" and others, of the "Indica" type. These facts may be of significance in the study of the distribution of the species and varities of rice.

Thanks to the investigation of fertility of hybrids and serodiagnostics, the authors have learned that the two types of cultivated rice classified morphologically, the "Indica" and the "Japonica" are not closely allied. Again, in the fertility experiments, they have already seen that the formation of pollen in the F_1 plants between the two different types is markedly abnormal. Looking over the literature (5, 7, 10, etc.) on this subject, they have noticed that the cause ascribed to the abnormal formation of pollen is either the distant relationship of the parent plants and the disharmony of their chromosomes, or the occurrence of abnormality in their pollen development. In the cytological research work upon rice, the authors have found no difference between the varieties of the "Japonica" type and those of the "Indica" type, so far as chromosomes are concerned. Nor have they found any difference between the two types in the course of pollen development. This is also true of hybrids within the same type. But in the hybrids between the different types, the authors have seen much pollen which was already abnormal even at the initial stage of its development following the tetrad formation. This may mean that though the distance of the relationship between the two types, the "Japonica" and the "Indica," is not so great that some differences may be observed between the behaviors of their chromosomes, these two types are as distantly allied with each other, as there is a physiological difference in the course of pollen development.

In short, the cultivated varieties of rice belonging to the same species are morphologically classified into two groups, the "Japonica" and the "Indica" type; and these are recognized as not closely allied with each other in descent from the standpoint of the degree of sexual affinity in hybrids and of serodiagnostic reaction. Consequently, the authors have classified cultivated rice into two groups. They regard them as two subspecies under cultivated rice, *Oryza sativa* L.; and they propose to call one of them, "Japonica," and the other "Indica."

It has been customary with agronomists (11, 13, etc.), when they attempt a classification of cultivated rice, to regard long-glumed, red, and scented rice as being considerably different from the ordinary varieties, putting great weight in the character of grains. However, our investigation shows that all long-glumed varieties produced in Japan Proper belong to the "Japonica" type, showing no difference from the common rice; that red and scented rice which are found both in the "Japonica" and the "Indica" are not markedly different from the ordinary rice; that, though the distinction of glutinous and non-glutinous rice from the standpoint of use is plausible, no difference is found between them, either in the "Japonica" or the "Indica" type; and that, although rice is divided into lowland and upland varieties on account of the different method of cultivation, the upland rice of Japan Proper all belongs to the "Japonica" type and we could not find much difference from the lowland rice.

VII. SUMMARY

1) According to the morphological standpoint, the cultivated varieties of rice, including upland, lowland, glutinous, non-glutinous, long-glumed, scented, and red rice may be classified into two different types: "Japonica" and "Indica."

2) When cultivated in Japan Proper, all varieties of both types are highly self-fertile. This is also true of hybrids between varieties which belong to the same type. On the other hand, the hybrids between varieties belonging to the different types (i.e. "Japonica" and "Indica") are much less fertile.

The process of pollen formation in hybrids within the same type and in those between the different types differs very much, in as much as it proceeds normally in the former, while in the latter it is extremely abnormal, the percentage of imperfect pollen grains being markedly great. By cytological examination, it is ascertained that, in the varieties belonging to the "Japonica" and "Indica" types, there is no difference in the number and behavior of their chromosomes, and in the course

of pollen development. This holds true in the hybrids within the same type, but in the hybrids between the different types, the abnormality of pollen is already manifested at the initial stage of its development following the division of the pollen mother-cell.

3) Rice in Japan Proper, Korea, Formosa, and China, belonging to the "Japonica" or "Indica" types can be clearly differentiated in different degrees by "Ring probe" for the precipitin reaction with "Absättigungsverfahren" in the test-tube and in the living body, generally speaking, the varieties, belonging to the "Japonica" type are closely related as are those, belonging to the "Indica" type, regardless of where they come from, while the varieties between the two types are fairly distinct from each other.

4) In short, the cultivated varieties of rice are to be classified into the "Japonica" and "Indica" types from the morphological standpoint. Moreover, judging from the degree of the sexual affinity, as well as serodiagnostic reaction, these two types are considered to be distantly related in descent. Therefore, the authors divide cultivated rice into two groups, regard them as subspecies, and propose to name one "*Oryza sativa* L. subsp. *Indica* KATO" and the other "*Oryza sativa* L. subsp. *Japonica* KATO."

5) As to the distribution of subspecies "Japonica" and "Indica" of cultivated rice, we will find that the varieties indigenous to Japan Proper and Korea all belong to the one, and that those native to Formosa as well as those of Southern China, India, Java, and other countries mostly belong to the other. Although in Central China rice is, for the most part, of the "Indica" type, some varieties of the "Japonica" type are also found.

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