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Studies on the Chlorosis Expressed under Low Temperature Condition in Rice, *Oryza sativa* L.

I. Classification and Relationships with Other Characaters.

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Based on the degree of chlorosis expressed at 17°C. cultivated rice varieties were classified into 5 types. Of these, 3 types expressed clear chlorosis. 2 types did not. Based on the fact that chlorosis expressed in great majority among *indica* varieties and did not among *japonica* varieties, it can be considered as a characteristic of *indica* subspecies. This is convinced by the coincidence of chlorosis with other three characters which distributed in high frequency among the *indica* subspecies namely C pattern of acid phosphatase zymogram. positive reaction to phenol and C type of grain shape. No close linkage relation between chlorosis and the germination ability under low temperature condition as well as the three characters above mentioned was revealed.

INTRODUCTION

During the course of working on the cultivated rice, *Oryza sativa* **L.,** for a long time, we realized that many varieties originated from the tropical regions manifested the symptom of chlorophyll deficiency on the leaves of young seedlings when they were grown on early or mid-April. This suggests us to study furthermore this phenomenon. The lack of chlorophyll has been reported much in wheat, mainly in artificial mutation (Gustaffson et al., 1970; Gilmore and Tuleen, 1973), in barley (Highkin, 1950; Robertson, 1967; Boardman and Highkin, 1966), and in maize (Millerd *et al.,* 1969). In rice, Omura *et al.* (1977) described 3 kinds of virescent mutants performed their various phenotypic expression depending on the temperature of the environment.

The chlorosis in rice express their phenotypes under low temperature condition in various aspects. First of all, we attempt to classify chlorosis according to their phenotypes and then investigate the relationships with some other characters which are used as characteristics of *indica* and *japonica* subspecies.

MATERIALS AND METHODS

Materials used in this study were 341 varieties chosen among the world rice stock of the Plant Breeding Laboratory, Faculty of Agriculture, Kyushu

University, including 44 Japanese varieties and 297 foreign varieties. The modified White's solution in agar (Omura *et al.*, 1977) was used in order to prevent the variation of chlorosis phenotype by the fluctuation of nutrituous elements. The seeds germinated in darkness at 30°C for 3 days were sown on the culture media and grown in the incubator set at 17°C with the precision of ±1°C in continuous illumination of about 2000 lux supplied by fluorescent lamps.

Chlorosis were observed on the third leaves of the young seedlings after they fully emerged from the second leaf sheath.

Phenol reaction test was carried out by the usual method, soaking unhulled seeds in 1% phenol solution at 25°C for 24 hours and then drying, described by Morinaga et al. (1943). Grain shape was grouped by the mean values of length and width of 10 unhulled grains into 3 types described by Matsuo (1952) as A type or short type, B type or large type and C type or long type. Acid phosphatase isozyme pattern of the rice stock of the laboratory was investigated by Chern and Katayama (1975) as C pattern, a characteristic of ***indica*** subspecies, and A pattern, that of *japonica* subspecies. The result of the investigation was used in the study.

For the test of the germination ability under low temperature condition, fifty well matured seeds of each varieties were sterilized by 0.2 % Benret for 12 hours, washed twice with tap water then directly sown on filter paper in Petri dishes and incubated at 15°C for germination. The germination was observed at the fourteenth day after sowing. Seeds were considered as germinated when the coleoptile tips broke out of the husks. For statistical analysis, the germination percentage was transformed to arcsine.

RESULTS

Classification of chlorosis

Above 25°C, all of the varieties used in the experiment showed no sign of chlorosis. But when grown under low temperature condition, below 20°C, the symptom of chlorophyll deficiency started to appear. Some varieties showed a little change in color, some showed remarkable deficiency in chlorophyll on the second and the third leaf blades of the seedlings. The chlorosis appear under various aspects ranging from yellow pale to whole white like albino. Some varieties started to express their chlorotic phenotypes at the demarcation of 20°C, some remained still green, and at 17°C, many varieties showed chlorosis and the varietal difference became clear as shown in Fig. 1. Therefore, based on the phenotype expressed at this temperature, chlorosis were classified into 5 types.

Type 0: Normal green, this type appeared almost in Japanese varieties and those originated from the temperate regions (Fig. 1-A).

Type I: The tnain feature of this type was pale green. It did not show any variegation in color on the leaf blade. This type generally appeared in the ***indica*** varieties. Some were easily distinguishable from type 0 by their

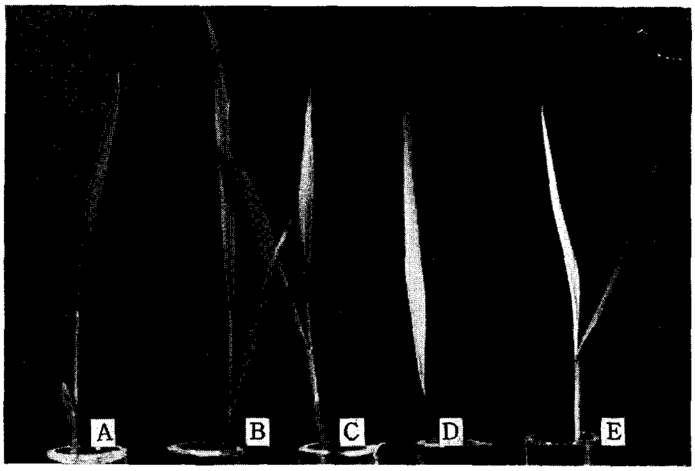

Fig. 1. Chlorosis types expressed at 17°C. A, type 0; B, type I; C, type II; D, type III; E, type IV.

pale green or sometimes yellowish green in color but some were difficult to distinguish. Type I did not change its phenotype even till later stage of the full expansion of the third leaf and even under high temperature condition. Thus, type I is considered as a normal type of the *indica* subspecies (Fig. 1-B).

Type II: Type II was characterized by the non-homogeneity of color on the leaf blade with the appearance of white or greenish white part on the tip or on almost the whole leaf blade (Fig. I-C). The white part of type II might express under various form. Most of type II showed a white portion in the upper part of the third leaf and almost green in the lower part. In some cases, the white portion extended along the leaf edges and ended in the lowermost part of the leaf blade, and in other cases, white stripes appeared along the length of the leaf blade (Fig. 2). In another case, type II also expressed chlorosis as whitish pale color on the whole leaf blade.

Type III: Type III expressed its phenotype as viridoalbino leaf. The tip of the third leaf still remained in green and the lower part in white. The green tip temporarily existed and reverted in white when the third leaf expanded in full size and thereafter the leaf gradually turned in green from the lower part (Fig. 1-D).

Type IV: Type IV showed the highest degree of chlorophyll deficiency. It was all white, very similar to the albino leaf (Fig. 1-E). Though its phenotype was very close to type III at the stage when the third leaf expanded in full size, the recovery of type IV into green was slower than that of type III.

Thus, the progressive order of chlorophyll deficiency of 5 types of chlorosis was $0 < I < II < III < IV$. Among those, type III and type IV were easily distinguishable from the others by their clear appearances in white or albino-like. Due to the absence of clear chlorotic symptom, type I along with type 0 were classified as normal green class in contrast with types II, III and IV

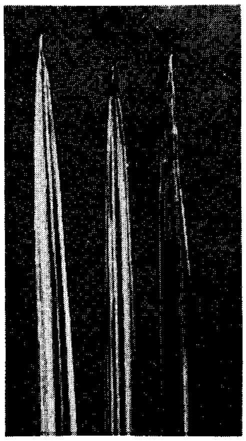

Fig. 2. Another form of type II expressed at 17°C.

as chlorosis class.

Chlorosis did not appear among the Japanese varieties used in this study while all types of chlorosis appeared among foreign varieties with the frequency of 37.9 %, including types II, III and IV with the frequencies of 14.7, 18.8 and 4.4 %, respectively (Table 1). Moreover, chlorosis expressed mostly in the varieties originated from the tropical regions.

Table 1. Distribution of chlorosis types

Origin of varieties	Chlorosis type					Total
	0	I	II	III	IV	
Japanese	44	0	0	0	0	44
Foreign	142	26	50	64	15	297
Total	186	26	50	64	15	341
%	54.6	7.6	14.7	18.8	4.4	

Relationships of chlorosis with other characters

Relationship of chlorosis with phenol reaction

Of the materials used, 159 varieties or 46.6 % showed positive reaction to phenol, in which 139 varieties or 40.8 % belonged to the tropical regions. Chlorosis distributed in higher frequency (63.5 %) among the varieties showing positive reaction to phenol than the green type (36.5 %). In contrary, chlorosis appeared less frequently (15.4 %) among varieties showing negative reaction to phenol than the green type (84.6 %) (Fig. 3).

Relationship of chlorosis with grain shape

Of *338* varieties, A, B and C types of grain shape were 24.3, 30.2 and 45.6 %, respectively. Among the A type, 87.8 % of varieties expressed normal green while only 12.2 % manifested chlorosis. The B type expressed normal

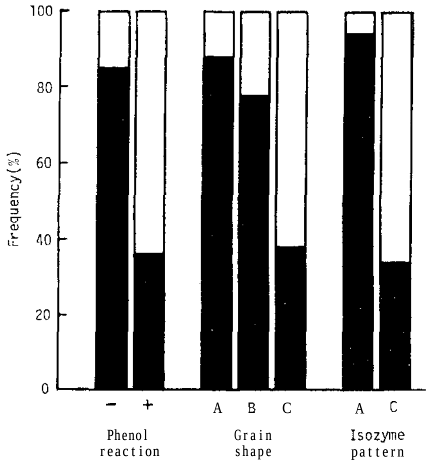

Fig. 3. Relationships of chlorosis with three characters. Black, normal green ; white, chlorosis.

green with 77.4 %, and chlorosis 22.6 %. Only the C type showed high frequency of chlorosis with 62.3 % and low frequency of normal green type with 37.7 % (Fig. 3).

Relationship of chlorosis with acid phosphatase zymogram pattern

Of the materials, 55.7% possessed the A pattern, and 44.3 % the C pattern of acid phosphatase zymogram. Among varieties belonging to the C pattern, chlorosis type appeared more frequently (66.2 %) than the green type (33.8 %). Contrary, among the varieties possessing A pattern, the frequency of chlorosis was only 5. 8 % (Fig. 3).

Combination of chlorosis with phenol reaction, grain shape and acid phosphatase zymogram pattern

Each of the above 4 characters showed discontinuous variation, namely green and chlorosis for chlorosis character, negative and positive for phenol reaction, A, B and C for grain shape, A and C for isozyme pattern. With regard to these characters, therefore, 24 kinds of combination are expected. Among these, 20 kinds of combinations were observed, but their distribution was largely biased. In the northern regions, Japan, Korea, Russia and Northern China, the combination was limitted to 10 kinds, however, in the southern regions, other than the northern regions, all of the 20 kinds were distributed. In the northern regions, the combination of green, negative reaction to phenol, A grain shape and A zymogram pattern, which were all representatives of *japonica,* dominated over the other types, showing the frequency of 41.6 % of the varieties. In the southern regions, the combination of chlorosis, positive reaction to phenol, C grain shape and C zymogram pattern were distributed with the highest frequency of 35. 9 %.

Chlorosis and the germination ability under low temperature condition

Of 341 varieties used, 264 varieties including 87 varieties of chlorosis and

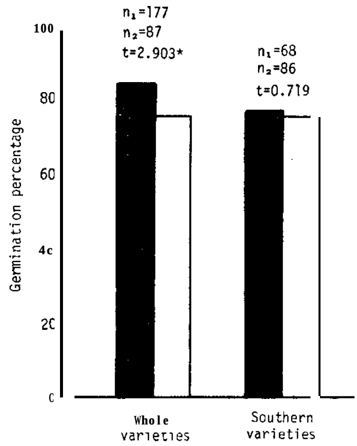

Fig. 4. Germination ability under low temperature condition. Black column, normal class ; white column, chlorosis class; n_1, number of varieties of normal class; n_2, number of varieties of chlorosis class.

177 of normal green were tested for their germination ability under low temperature condition. The mean germination percentages were widely ranged from 4 to 100 %. The mean germination percentage of varieties originated from the northern regions was higher (82.3 %) than that of varieties from the southern regions (75.0 %). The relationship between chlorosis and the germination ability is shown in Fig. 4. The germination percentages were 83.9 and 75.4 % for normal and chlorosis classes, respectively, showing significant difference at 5 % level between them. However, when the estimation was based on the varieties from the southern regions only, excluding those from Japan, Korea, Russia and Northern China which exhibited no chlorosis and high germination percentage, the values were changed to 76.9 and 75.1 %, respectively, and there was no significant difference between the two classes.

DISCUSSION

About one third of the foreign varieties used in the experiment expressed chlorosis at 17°C. Varietal difference in the degree of chlorosis was very remarkable, so the varieties were able to divide into 5 types based on the degree of chlorosis. Because of the absence of the remarkable deficiency in chlorophyll, both types 0 and I were classified as normal green type, though type I was a little paler than type 0. Chlorosis did not appear among Japanese varieties and even among foreign varieties chlorosis also almost disappeared in varieties from the northern regions as Korea, Russia and Northern China, while all types of chlorosis appeared in the varieties from the southern regions.

Since Kato (1930) classified *Oryza sativa* L. into two subspecies, *japonica* and *indica*, many other classifications were proposed (Terao and Mizushima, 1939;

Oka, 1953; Morinaga and Kuriyama, 1955). Though the problem on classification or differentiation of 0. ***sativa*** is rather complicated, it is no doubt that japonica subspecies distributed in Japan, Korea and Northern China. Moreover, in many characters, as the grain shape (Nagamatsu, 1943; Matsuo, 1952), the phenol reaction (Oka, 1953; Kuriyama and Kudo, 1967), and the zymogram pattern of acid phosphatase (Chern and Katayama, 1975), it is known that each character is different in both subspecies or more uniform in *japonica* subspecies than in *indica* subspecies. The result of the study on chlorosis well agrees with these results. Consequently, it is suggested that chlorosis can be considered as a characteristic of ***indica*** subspecies.

A narrow variation of chlorosis types, only type 0 and type I, was observed in the northern regions, whereas a wide variation in the southern regions with all types of chlorosis. The distribution of the character combination types in the northern regions as Japan, Korea, Russia and Northern China also showed a narrow variation in contrast with that in the southern regions. This conforms with the tendency of the distribution of gametophyte genes and esterase isozyme pattern in cultivated rice (Nakagahra *et* ***al., 1972,*** 1974, 1975). The northern regions including Japan, Korea and Northern China were unique in a combination of $ga_2ga_3{}^+ga_4$ while India, Assam, Southern China possessed a wide variation of many types of the gene combination. The same result of narrow and simple genetic diversity in esterase zymogram was also revealed in the northern regions in contrast with that in the southern regions.

Based on the concept of Vavilov on the origin of the species, the primary center of a species is always characterized by a large variation in characters and by the complexity of genotypes. On the contrary, those complexes of genotypes with large variation dispersed from there to everywhere and on their way of differentiation they have their own gene pattern well adapted to the new environment (Dobzhansky, 1937). The chlorosis in this study is well fit with this concept. The study on geographical distribution of chlorosis is carrying out.

In the study on the germination behavior of cultivated rice, Nagamatsu (1943) reported that under low temperature condition, the germination ability of varieties from the tropics and subtropics was lower than that of the varieties from the temperate zone. The result on the germination ability obtained in this study coincided with the conclusion of Nagamatsu. Furthermore, significant difference in the germination percentage was obtained between normal and chlorosis classes when all varieties were used for estimation. The two results seem to suggest that the germination ability under low temperature plays a role *in* the selection of chlorosis. However, when only the varieties from the southern regions were used for analysis, there was no significant difference between the normal green and chlorosis classes (Fig. 4). This result implies that the germination ability under low temperature condition is not the agent of selection causing the absence of chlorosis in the northern regions, in other words, there is no close linkage relation between the chlorosis and the germination ability under low temperature condition nor the pleiotropic effect involved in the case. The same conclusion may be said between the chlorosis and the phenol reaction, grain shape and acid phosphatase zymogram pattern.

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