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Studies on the Character Manifestation in Chlorophyll Mutants of Rice

I. Virescent Mutants Sensitive to Low Temperature

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Three kinds of virescent mutant, v_1 , v_2 and v_3 , were caused the deficiencies in both chlorophyll and carotenoid, and the abnormality in ultrastructure of chloroplast by low temperature. When they were grown in White's medium below 22, 20 and 30°C, respectively, their chlorophyll contents were negligible. In this respect, they are said to be the mutants sensitive to low temperature. The chlorophyll content of a certain matured leaf was determined by the temperature only at the early elongating stage of the leaf. The chlorophyll content varied inversely as the amount of nitrogen nutrients, thus threshold temperature preventing chlorophyll formation shifted to higher temperature by increasing application of nitrogen nutrient. It was found by δ -aminolevulinic acid (ALA) feeding that the mutants could synthesize protochlorophyll(ide) from ALA even they were grown at 20°C. The chloroplasts in the mutants grown at low temperature remained proplastid and contained no ribosome. These facts are suggestive to make clear where is blocked by these genes in the normal pathways of chlorophyll and carotenoid biosyntheses and of chloroplast formation.

INTRODUCTION

Various kinds of mutants having the defect in chlorophyll biosynthesis or chloroplast morphogenesis have been known in many plant species. Some of them show stable phenotype under various environments, and the others are modified in their phenotype by environmental factors such as temperature, light and nutrients (Wallis, 1967, 1971).

In rice, many chlorophyll deficient mutants have been described, however, studies on the interaction between genotype and environment in the expression of gene are a few (Omura and Tanaka, 1959). Hundreds of chlorophyll deficient mutants are preserved in Plant Breeding Laboratory, Faculty of Agriculture, Kyushu University. The phenotypes of some chlorophyll mutants grown under the field condition are often changed by sowing time, climate and others, consequently, it made the identification of mutants difficult. However, the use of advanced biotron, which is able to control precisely over temperature, moisture,

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light and other environmental factors, enables us to analyze the influence of environmental factors on the character manifestation. Then, studies on the influence of environmental factors on the character manifestation have been undertaken. In this paper, strong interactions between genotype and temperature and nitrogen nutrient on the phenotypic expression of three virescent genes are clarified.

MATERIALS AND METHODS

Materials

Three kinds of the virescent lines, F1191, **HO 799** and CM 13 were used in these experiments. F1 191 is a hybrid progeny of an American rice variety "Arkrose Virescent" introduced from Dr. Jodon of U. S. D. A. HO 799 sent from Emeritus Prof. Takahashi, Okayama University is a spontaneous mutant from a Japanese paddy rice variety "Yaeho." **CM 13** is a mutant induced from a paddy rice variety "Kinmaze" by *n*-nitroso-*n*-methylurea. The genes for the virescent of F1 191, HO 799 and CM 13 have been designated as v_1 , v_2 and v_3 , respectively, and v_1 and v_2 belong to the linkage group XI (Iwata and Omura, 1977; Omura and Iwata, 1972) and v_3 to the group I (unpublished).

The phenotypes of the mutant seedlings grown under field condition are remarkably varied with sowing time. When they are sown in April, the third leaf shows nearly perfect white, and the fourth and fifth leaves manifest white in the upper half. The third leaf decreases its white part according to the delayed sowing, and become whole green when seeds are sown at the beginning of July. These virescents are often hard to distinguish each other, showing similar phenotype.

Culture conditions

All the experiments were conducted in artificially lit growth cabinets in Biotrpn Institute, Kyushu University. Seedlings were grown at given temperature with precision of $\pm 0.5^\circ\text{C}$ in continuous light of 2,200–2,500 lux, supplied by day light fluorescent tubes (40 W x2). Unless otherwise mentioned, the culture medium used was a modified White's medium containing $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 739.5 mg, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 287.8 mg, $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ 453.8 mg, KNO₃ 80.0 mg, KCl 65.0 mg, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 21.4 mg, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 7.18 mg, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 2.16 mg, H_3BO_3 1.5 mg, KI 0.75 mg, Fe-citrate 15 mg, agar 6 g and distilled water 1,000 ml. Glass pots or test tubes containing the culture medium were autoclaved at 120°C for 150 min.

Hulled seeds were surface-sterilized by immersing them in 80 % ethanol for 3 min, in 10 % chlorinated limes for 20 min, in 3 % hydrogen peroxide for 20 min, and then rinsed several times in sterilized water. The seeds germinated at 25°C for 48 hr were sown on the culture medium and grown in the cabinets.

Chlorophyll determination

The content of chlorophyll a and b in the third leaf were used as an index of phenotype. Three to five leaves of mutant seedlings were weighted and ground in a mortar with 80 % acetone. The extract was centrifuged at low

temperature for 13 min at 12,000 g. The supernatant was used for the determination of chlorophyll a and b contents. The contents were determined spectrophotometrically (Hitachi EPS-033 spectrophotometer) using specific absorption coefficients of Mackinney (1941).

Protochlorophyll determination

The hulled seeds of v_2 , sterilized as mentioned previously, were sown on the modified White's agar medium in a Petri dish and grown at 20°C and 30°C in the dark until the third leaf fully emerged.

The etiolated leaves were cut pieces of 1 or 2 mm off under a safety green light, put them on a solution of δ -aminolevulinic acid (ALA) (100 μ g/ml in distilled water) in a Petri dish and incubated at 30°C for 36 hr in the dark. After incubation, protochlorophyll(ide) was extracted from the leaf pieces by grinding with 80 % acetone in a mortar and transferred to an ethyl ether layer in a separate funnel.

Accumulation of protochlorophyll(ide) was measured in the ether solution spectrophotometrically by using the equation of Koski (1950). In control experiment, leaf piece incubation was performed in a distilled water, without ALA.

Electron microscopy

The small pieces of leaf tissues from the seedlings of v_1 grown at 20°C (v_1 -20), v_2 grown at 20°C and 30°C (v_2 -20 and v_2 -30) and v_3 at 30°C (v_3 -30) were fixed in 2.5 % glutaraldehyde followed by 2 % osmium tetroxide. Both solutions were buffered with 0.1 M phosphate, pH 7.2. Fixed tissues were dehydrated in a graded ethanol series, transferred to QY-1 (n-butyl glycidyl ether) and embedded in Epon 812. Sections were obtained using glass knives on a Sorval Porter Blum MT-1 ultramicrotome and stained with 2 % aqueous uranyl acetate and lead acetate. Specimens were examined in a JEM-7A electron microscope.

RESULTS

1. Modification of phenotype by temperature

The chlorophyll contents of the virescents grown at 20, 25 and 30°C are shown in Fig. 1. Only v_3 was grown also at 35°C, because it remained white below 30°C. Higher chlorophyll content was observed at higher temperature in all mutants, but the effect of temperature differed among mutants. In v_1 seedlings, chlorophyll could not be traced at 20°C, however, at 25°C, the content reached 2.22 mg/g.f.w., nearly the same at 30°C, and the leaf appeared almost whole green. In v_2 seedlings, the content and the appearance at 20°C were almost the same as those of v_1 seedlings grown at 20°C. At 25°C the content of 1.78 mg/g.f.w. was, different from v_1 , merely 70 % of that at 30°C, and the tip of the third leaf remained white. At 30°C the content increased to 2.32 mg/g.f.w. and the leaf appeared nearly perfect green. In v_3 seedlings, differing remarkably from the above mutants, chlorophyll content was less than 0.5 mg/g.f.w. even at 25°C or 30°C, and 1.6 mg/g.f.w. at 35°C.

The ratio of chlorophyll a to b was about 3 : 1 in all of the virescents independently of the chlorophyll content. It is probable that the carotenoid content

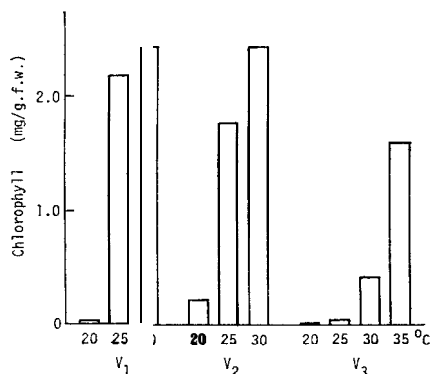


Fig. 1. Chlorophyll content of three virescent mutants at different temperature.

changed always in proportion to the chlorophyll content, because the absorption spectrum in visible ray showed the same pattern in every case. It is a reason why chlorophyll content was used as a indicator of phenotype in the virescents.

As the chlorophyll content of v_1 seedlings grown at 20°C extremely differed from those grown at 25°C, the influence of temperature on the chlorophyll content was examined in detail. As shown in Fig. 2, the content was negligible below 22°C, however, it began to increase rapidly at 22°C and attained the plateau at 25°C. Therefore, the threshold temperature preventing chlorophyll synthesis is estimated at 22°C in v_1 . Similarly, those of v_2 and v_3 are estimated at 20°C and slightly below 30°C, respectively.

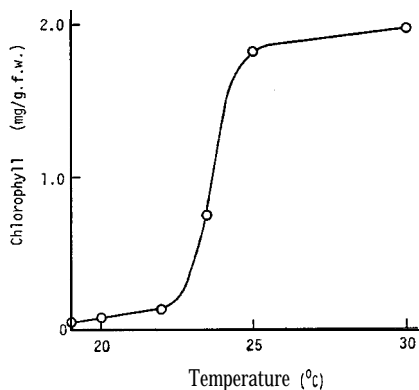


Fig. 2. Chlorophyll content of the third leaves of v_1 seedlings grown at various temperatures from 19°C until 30°C.

2. Determination of temperature-sensitive growing stage on the phenotypic expression of genes

As mentioned above, the phenotypes of the virescents were strongly influenced by temperature. Then, it was first investigated whether the fully expanded leaves changed in their phenotype or not by transferring the seedlings from

20°C to 30°C, and conversely from 30°C to 20°C. The phenotype already appeared was never changed by this treatment. It suggests that the phenotype is determined by the temperature only at a certain early developmental stage of the leaf.

In respect of the stage, more detailed experiments were conducted as follows. The v_1 and v_2 seedlings grown at 20°C were transferred to the growth cabinet controlled at 30°C every day from 1 to 9 days after germination. After the third leaves expanded, the chlorophyll content was determined with 3 to 5 replications. In the case of v_1 , seeds were sown directly at 20°C.

The results are shown in Fig. 3. The chlorophyll content of v_1 seedlings grown at 20°C for 1 day was 2.57 mg/g.f.w., nearly the same as that grown at 30°C from the beginning, but it decreased rapidly with increasing the period at 20°C, and reached about 0.5 mg/g.f.w. when seedlings grew at 20°C for 5 days. The growing stages of the seedlings at 1 day and at 5 days after germination were at the second leaf and the third leaf emergence, respectively. The length of the third leaf at these stages were 1.6 mm and 14.4 mm, respectively. Similar result was obtained in the case of v_2 .

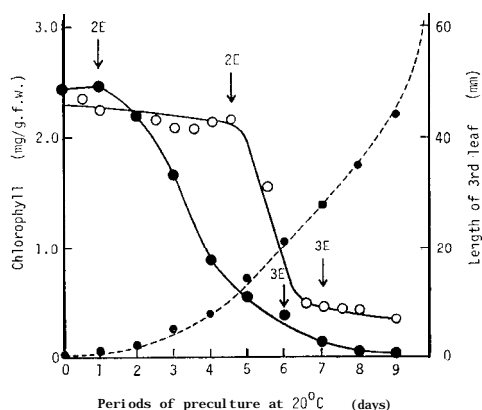


Fig. 3. Chlorophyll contents of the third leaves of v_1 and v_2 which are transferred to 30°C after different periods of preculture at 20°C, and the length of third leaf of v_1 . —●—, chlorophyll content of v_1 ; —○—, that of v_2 ; —●—, length of third leaf of v_1 ; 2 E and 3 E, stages at second and third leaf emergence, respectively.

The result of transferring v_1 seedling from 30°C to 20°C is shown in Fig. 4. The chlorophyll content was, contrary to Fig. 3, very low when the seedlings were transferred before the second leaf emergence, and reached maximum when they were transferred after the third leaf emergence. The appearances of leaves subjected these treatments were shown diagrammatically in Fig. 5. In the former case, transferring from 20°C to 30°C, a white region was only limited at the tip of leaf when seedlings were grown at 20°C for 1 day, however, it was expanded gradually to the bottom with the increase in the period of preculture at 20°C, and finally became whole white. In the latter case, contrary to the former case, a green region was expanded from the tip to the bottom with prolonging

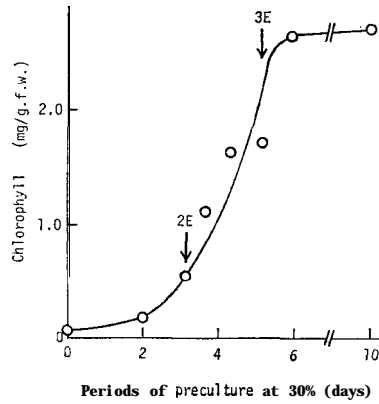


Fig. 4. Chlorophyll content of ν_1 which is transferred to 20°C after different periods of preculture at 30°C. 2E and 3E indicate the stage of emergence of second and third leaves, respectively.

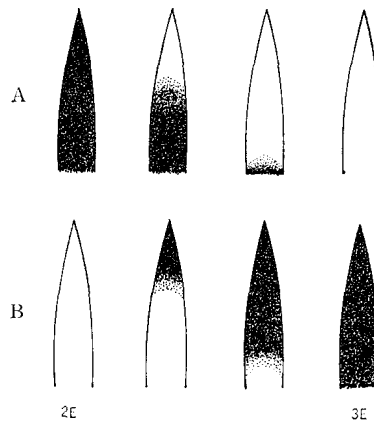


Fig. 5. Diagram showing appearance of third leaf of ν_1 which is transferred at different growing stage of seedling between second leaf emergence and third leaf emergence. A, transferring from 20°C to 30°C; B, from 30°C to 20°C; 2E and 3E, second and third leaf emergence, respectively.

the period of preculture at 30°C. Therefore, it is considered that the temperature at this early developmental stage of the third leaf, its elongating stage, affects their chlorophyll content, and that the effect always appeared from the tip.

3. Effect of light at the temperature-sensitive stage on chlorophyll content

As the above experiments were carried out under continuous illumination, the effect of light at the temperature-sensitive stage on the chlorophyll content was still unsettled. Then, the chlorophyll content of ν_2 seedlings which were transferred from the dark at 20°C to the light at 30°C was measured (Fig. 6). This decreasing curve of chlorophyll content was very similar to that shown in Fig. 3. Furthermore, when the ν_2 seedlings were grown under the dark at 30°C for

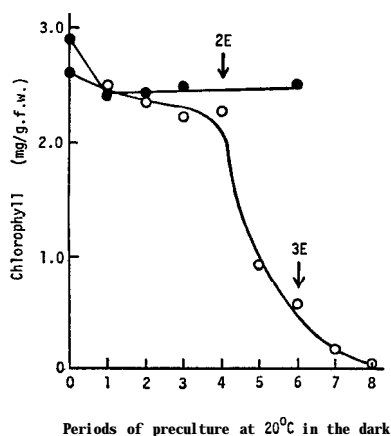


Fig. 6. Chlorophyll content of third leaf of ν_2 which is transferred to 30°C in the light after different periods of preculture at 20 or 30°C in the dark. ○, 20°C in the dark; ●, 30°C in the dark.

various durations and then transferred the light at 30°C, the chlorophyll content was the same as normal type irrespective of the duration. The results show that the light at the temperature-sensitive stage may be ineffective to the chlorophyll content.

4. Modification of phenotype by nitrogen nutrients

In the experiments hitherto mentioned, modified White's medium was used. However, it is well known that chlorophyll content is influenced by various nutrients. Therefore, the effect of nutrient on the phenotypic expression of virescent genes was examined. Seedlings which raised in the agar medium lacking all the components of White's medium except Fe-citrate (A-medium) were compared with those raised in White's medium (W-medium). Only Fe-citrate was added in A-medium because Fe-deficient medium caused extremely poor growth of seedlings and chlorophyll deficiency irrespective of genotype.

The chlorophyll contents of ν_1 and ν_2 grown in A- and W-media at various temperatures are shown in Table 1. The chlorophyll content of the virescents was higher in A-medium than in W-medium at every temperature, though that of normal type was slightly higher in W-medium than in A-medium. It was found from further experiments that only medium containing NO_3^- -compounds has the same effect as W-medium.

Table 1. Chlorophyll contents of mutants grown in W-medium and A-medium at different temperatures. (mg/g.f.w.)

Temperature °C	ν_1		ν_2		ν_3		Normal	
	W	A	W	A	W	A	W	A
20								
25	2.12 0.04	3.34 1.03	0.23 1.78	2.80 1.50	0.06 0.00	0.04 0.00	3.18 3.50	2.94 2.78
30	2.44	3.22	2.46	3.11	0.44	1.27	3.33	3.13

Then, the effects of NO_3^- on the chlorophyll content of v_1 seedlings grown at 18, 20, 24 and 26°C were examined by the application of KNO_3 . The amounts of KNO_3 were 0 mg/l (N-O), 75 mg/l (N-75), 300 mg/l (N-300) and 1200 mg/l (N-1200). The NO_3^- concentration in N-300 approximately corresponds to that in W-medium. The result is shown in Fig. 7. The effect of NO_3^- varied with the temperature, showing no effect at 18°C and the highest effect at 22°C. The chlorophyll content at 20°C was reached about 1 mg/g.f.w. only in N-O and remained very low in the others. At 22°C the content of more than 1 mg/g.f.w. was gotten in N-75, nevertheless, those in N-300 and N-1200 were still very low. At 24°C the content reached more than 2 mg/g.f.w. in all of the NO_3^- levels. These facts show that the chlorophyll content decrease against NO_3^- concentration, resultingly, the threshold temperature is shifted up by increase in the concentration of NO_3^- in medium. It was found, moreover, that ammonium sulfate, urea and glycine had the same effect as NO_3^- .

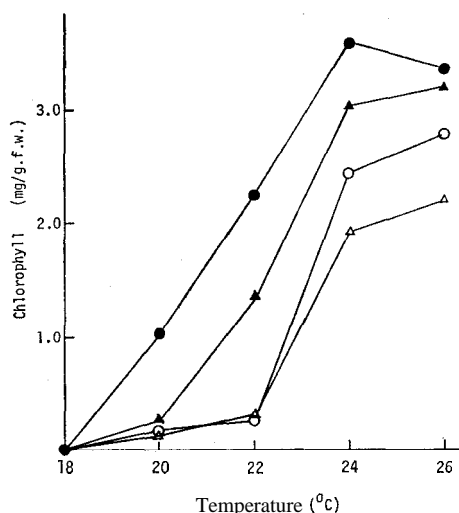


Fig. 7. Effect of nitrogen nutrient (KNO_3) on chlorophyll content of v_1 grown at different temperature. ●, 0 mg/l; ▲, 75 mg/l; ○, 300 mg/l; △, 1200 mg/l.

5. Protochlorophyll(ide)-synthetic ability in etiolated seedlings

In order to examine the chlorophyll-biosynthetic ability, the accumulation of protochlorophyll(ide) in the etiolated leaves of v_2 seedlings given ALA, a precursor of chlorophyll, was investigated. The result is shown in Table 2. The amounts of protochlorophyll(ide) in the seedlings grown at 20°C and 30°C reached 48.7 and 62.1 $\mu\text{g/g.f.w.}$ by ALA feeding, respectively. The result shows that both seedlings have almost the same ability to synthesize protochlorophyll(ide) from exogenous ALA. Therefore, it may be concluded that the etiolated seedlings grown at 20°C have no defect in the enzyme or enzymes participating in the pathway from ALA to protochlorophyll(ide), notwithstanding

Table 2. Effect of ALA on formation of protochlorophyll(ide) in etiolated ν_2 seedlings grown at 20°C for 30 days (A) and at 30°C for 13 days (B) in darkness.

Material	ALA 100 $\mu\text{g}/\text{ml}$	Protochlorophyll(ide) $\mu\text{g}/\text{g.f.w.}$
A	+	48.7
	—	1.3
B	+	62.1
		5.2

ing they have no ability to form chlorophyll by illumination at 30°C.

6. Comparison of chloroplast ultrastructures in virescent seedlings grown at different temperature

The ultrastructure of chloroplasts in the virescent mutants was observed in order to investigate the influence of temperature on the development of chloroplasts.

The results are summarized in Table 3. The plastids of ν_1 -20 still remained as proplastid, containing prolamellar bodies. No grana and ribosomes could be seen in their stroma which was very low electron-density (Plate I, Figs. 8 and 9). The plastids of ν_2 -20 resembled to those of ν_1 -20 in appearance, but contained often osmiophilic globles and starch grains (Plate I, Figs. 10 and 11). Whereas plastids in green leaf of ν_2 -30 contained well-developed granum (Plate II, Fig. 16). These possessed stroma substantially denser than the surrounding cytoplasm. Their appearance was similar to those of chloroplast found in normal seedlings. The green tissues in the tip of ν_3 -30 had the chloroplasts with well developed granum and immatured plastids (Plate II, Fig. 15). On the other hand, the various degrees of the formation of lamellar were observed in the plastids from the white tissues of ν_3 -30; some plastids resembled to those of ν_1 -20 and ν_2 -20 and other ones contained thylakoid stacks (Plate II, Figs. 12-14). The stroma of the latter was more compact than the former and contained many osmiophilic globles and a few ribosomes. Thus, the structural development of chloroplasts was, as well as chlorophyll content, inhibited by low temperature.

Table 3. Ultrastructure of chloroplasts from virescent mutants grown at 20°C and 30°C.

Items	ν_1 -20	ν_2 -20	ν_2 -30	ν_3 -30	
Color of third leaf	white	white	green	green	white
Grana	—		+	+	—
Prolamellar body	+	+	—	—	+
Starch	—	+	+	—	—
Ribosome		—	+	+	—
Osmiophilic globles		±	+	±	±
Stroma*	LD	LD	HD	HD	LD

* L D : Low electron-density, H D : High electron-density.

DISCUSSION

The virescents, v_1 , v_2 and v_3 , could not accumulate chlorophyll below 22, 20 and 30°C, whereas they could do above 25, 28 and 35°C, respectively (Figs. 1 and 2). In this respect, they are said to be the mutants sensitive to low temperature. Similar mutants have been detailed in maize (Millerd and McWilliam, 1968) and in barley (Miller and Zallik, 1965), but few in rice.

The growing stage of a leaf sensitive to low temperature is limited to its early elongating stage (Figs. 3 and 4). This stage is a little later than that in a maize mutant (M 11) reported by Millerd and McWilliam (1968), who showed that the primary site of low temperature sensitivity in it was the shoot apex. As shown in Fig. 5, when the duration of preculture at 20°C or 30°C was very short, 1 or 2 days in this case, the white or green part of a leaf was limited to its tip. This fact indicates that only the tip is influenced by the temperature in preculture. It agrees with the finding by Robertson and Laetsch (1974) that all of the leaf regions are not in the same stage of plastid development, but the tip region make more rapid development than the bottom. Therefore, it is considered that the temperature-sensitive stage is strictly limited at a certain developmental stage of cell, and at this stage the ability to form chloroplast in the developed leaf is determined. It is interesting that each of the virescents is sensitive to low temperature at the same growing stage, though it is controlled by non-allelic genes, v_1 , v_2 or v_3 . It is still obscure whether they regulate the same site or not. However, in either case, the genes presumably regulate a certain metabolic system for chloroplast development, which exists in this stage, and mutant genes cannot regulate the normal metabolism when temperature is lower than respective limit.

The effect of temperature on the expression of genes is influenced by the nitrogen nutrient in the manner of shifting the threshold temperature up in proportion to its amount (Table 1 and Fig. 7). However, it influences only in a certain temperature range, and does not in higher or lower temperature than the range. From this fact, it is probable that the nitrogen nutrient does not play a major role in the gene expression but modify the effect of temperature. This fact is worth notice because the influence of nutrients other than nitrogen on the gene expression have considerably reported (Nishiyama and Motoyoshi, 1962; Walles, 1963), but that of nitrogen nutrient is few.

These virescents revealed not only chlorophyll deficiency but also carotenoid deficiency and impaired chloroplast development. In regard to chlorophyll deficiency, they are blocked in the biosynthetic pathway before ALA because etiolated leaves of seedlings grown at 20°C in the dark could synthesize protochlorophyll(ide) from exogenous ALA (Table 2). The carotenoid content varied in parallel with the chlorophyll content. The chloroplast development in the virescents grown at 20°C remained proplastid and ribosome could not be detected in the stroma (Table 3 and Plates I and II). From the fact that ribosome could not be seen in the plastids of virescents grown at 20°C, the defects simultaneously occurred might be caused by the genetic block in the fundamental

step regulating the development or differentiation of photosynthetic organs. Millerd et al. (1969) have presented the same view about the impaired syntheses of the pigments and the impaired development of chloroplast in M 11 mutant of maize. On the other hand, it is said that a temperature-sensitive mutant, ν_1 , of maize (Robertson and Anderson, 1961) and a carotenoid-deficient mutant of sunflower (Wallès, 1965) were caused primarily by the impaired carotenoid synthesis and resultingly prevented chlorophyll accumulation and chloroplast formation. As regards this, clear evidence must be waited for further studies.

Many virescent mutants of rice sensitive to low or high temperature and nitrogen nutrient have been found by the authors. The genetical and physiological studies on these mutants will enable us to obtain the detailed information concerning the development of chloroplast.

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REFERENCES

- Iwata, N. and T. Omura 1977 Linkage studies in rice (*Oryza sativa* L.). On some mutants derived from chronic gamma irradiation. *J. Fac. Agr., Kyushu Univ.*, 21: 117-127
- Koski, V. M. 1950 Chlorophyll formation in seedlings of *Zea mays* L. *Archs. Biochem. Biophys.*, 29: 339-343
- Mackinney, G. 1941 Absorption of light by chlorophyll solution. *J. Biol. Chem.*, 140: 315-322
- Miller, R. A. and S. Zallik 1965 Effect of light quality, light intensity and temperature on pigment accumulation in barley seedlings. *Plant Physiol.*, 40: 569-574
- Millerd, A. and J. R. McWilliam 1968 Studies on a maize mutant sensitive to low temperature I. Influence of temperature and light on the production of chloroplast pigments. *Plant Physiol.*, 43: 1967-1972
- Millerd, A., D. J. Goodchild and D. Spencer 1969 Studies on a maize mutant sensitive to low temperature II. Chloroplast structure, development and physiology. *Plant Physiol.*, 44: 567-583
- Nishiyama, I. and F. Motoyoshi 1962 Cytogenetic studies in *Avena* X. The artificial culture of albino sand oats. *Jap. J. Genet.*, 37: 427-440
- Omura, T. and N. Iwata 1972 Linkage studies in rice. On the linkage group 8, 10 and 11. *Jap. J. Breed.*, 22: Suppl. 1 pp. 43-44 (in Japanese)
- Omura, T. and S. Tanaka 1959 Amounts of chlorophyll and carotenoid in the chlorophyll mutants, chlorina and xantha, of rice. *Report Kyushu Branch Crop Sci. Soc. Japan*, 14: 24-26 (in Japanese)
- Robertson, D. and W. M. Laetsch 1974 Structure and function of developing barley plastids. *Plant Physiol.*, 54: 148-159
- Robertson, D. S. and I. C. Anderson 1961 Temperature-sensitive alleles of the y_1 locus in maize. *J. Hered.*, 52: 53-60
- Wallès, B. 1963 Macromolecular physiology of plastid IV. On amino acid requirements of lethal chloroplast mutants in barley. *Hereditas* (Lund), 50: 317-344
- Wallès, B. 1965 Plastid structures of carotenoid-deficient mutants of sunflower (*Helianthus*

- anuus* L.) I. The white mutant. *Hereditas* (Lund), 53: 247-256
- Walles, B. 1967 Use of biochemical mutants in analysis of chloroplast morphogenesis. In "Biochemistry of Chloroplast," Vol. 2, ed. by T. W. Goodwin, Academic Press, Inc., London and New York, pp. 633-653
- Walles, B. 1971 Plastid inheritance and mutations. In "Structure and Function of Chloroplasts," ed. by M. Gibbs, Springer-Verlag, Berlin-Heidelberg-New York, pp. 51-88

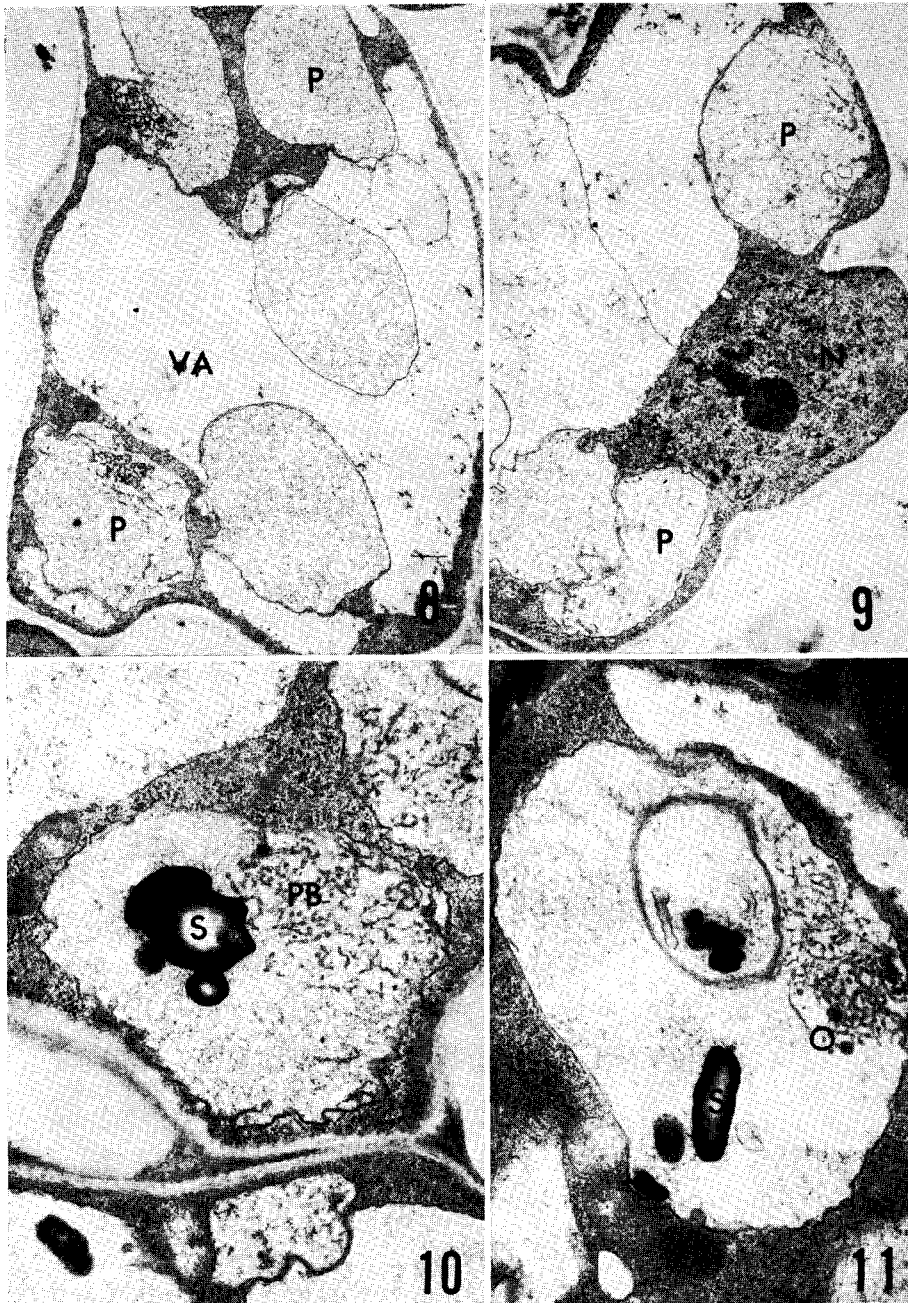
Abbreviations

CH	chloroplast	PB	prolamellar body
CW	cell wall	R	ribosome
G	granum	S	starch
N	nucleus	T	thylakoid
O	osmiophilic globule	VA	vacuole
P	plastid		

Explanation of Plate I

Figs. 8 and 9. Plastids from a white leaf of v_1-20 . Fig. 8. Section showing the typical figure of plastids observed in these tissues. $\times 5,500$. Fig. 9. Plastids containing small vesicles formed by the invagination of plastid membrane. $\times 8,000$.

Figs. 10 and 11. Plastids from a white leaf of v_2-20 . Fig. 10. Plastid containing the prolamellar body, starch grains and osmiophilic globules. $\times 12,500$. Fig. 11. Plastid containing the multimembranous structure. $\times 15,000$.



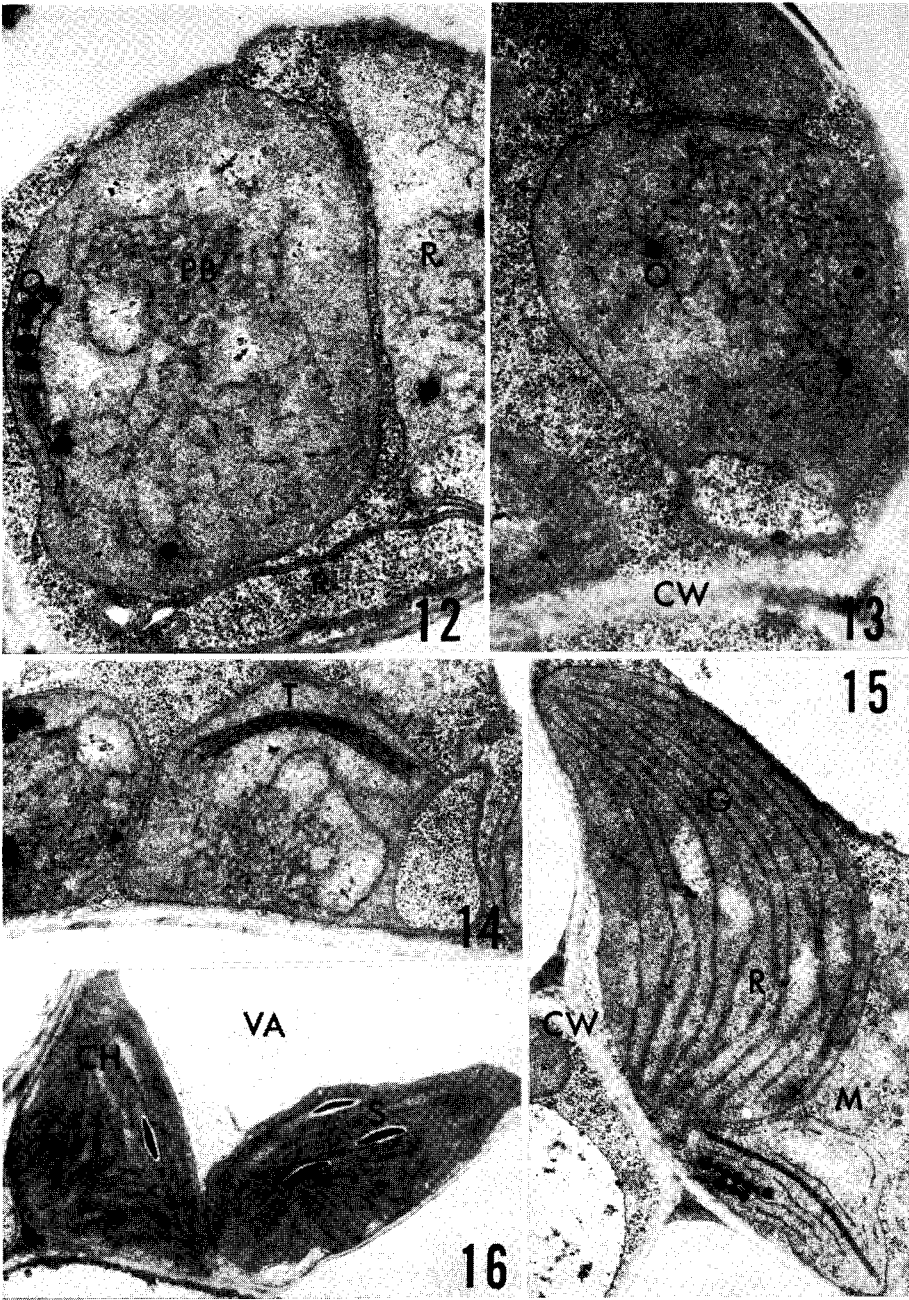
Low-Temperature-Sensitive Virescents in Rice

Explanation of Plate II

Figs. 12-14. Plastids from white tissues of v_3 -30. Fig. 12. Arrangement of osmiophilic globules between the thylakoids. A few ribosomes can be seen in the stroma, $\times 18,000$. Fig. 13. Thylakoids extending from the prolamellar body. Intrusion of cytoplasm is seen in plastid. $\times 27,000$. Fig. 14. Plastid containing the aggregation of thylakoid sheets. $\times 20,000$.

Fig. 15. Chloroplast from the green tissues in the tip of v_3 -30. Plastid with well developed grana and the immatured plastid are seen in one cell. $\times 15,000$.

Fig. 16. Chloroplasts from the green leaf of v_2 -30, showing normal appearance. $\times 5,000$.



Low-Temperature-Sensitive Virescents in Rice