Temperature Effect on the Accumulation of Plastid Pigments in Mutants of Einkorn Wheat

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December, 1960

Reprinted from the Memoirs of the Faculty of Agriculture, Kinki University, No. 1, 1960

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It has been reported by many authors that due to genetical deficiencies plants lack plastid pigments even under favorable conditions of temperature, nutrition and illumination. Those plants have provided useful materials not only for the genetic studies[1] but also for the studies of photosynthesis,[2,6] photomorphogenesis[14] and others.

The present paper deals chiefly with the experimental results on the plastid pigment accumulation under the experimental temperature conditions as well as the analysis of the pigments in two types of X-ray induced mutants of *Triticum monococcum.*

Materials and Methods

Two types of chlorophyll mutants of *Triticum monococcum* induced by X-ray treatment, namely albino and carotina, have been used in the present investigation. Under the normal conditions, the albino seedlings look red or white in color and the carotina seedlings do orange. Those mutants are nonviable in field, while their heterozygotes are viable and segregate normal green and albino or carotina in a Mendelian ratio of three to one (Table I).

Table I. Mendelian segregations in the strains for respective mutants

	1956			1957			1958		
	Mutant	Normal	Total	Mutant	Normal	Total	Mutant	Normal	Total
Albino strains	60	181	241	27	86	113	198	640	838
Carotina strains	29	94	123	34	93	127	39	112	151

In a preliminary experiment, the albino seedlings showed green pigmentation when they were grown at 1°C for about 60 days from right after the

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germination and then were kept one day at 25°C in a light room, while they remained red or white when they were grown at 25°C, 20°C, 15°C and slightly green at 10°C. The green pigment accumulation in the albino seedlings under the low temperature condition was localized in the lower portion of the leaves which seemed to have developed during the low temperature condition (Table II). The green pigment once accumulated did not easily disappear as long as the leaves remained fresh even when the plants were placed under the higher temperature condition.

Table II. Accumulation of the green pigment in the leaves of albino seedlings treated by the low temperature at 1°C for 60 days

Days after the low temperature treatment	1st leaf			2nd leaf			3rd leaf		
	G	P-R	Total length	G	P-R	Total length	G	P-R	Total length
2	30.0	7.5	37.5	—	—	22.5	—	—	—
10	34.9	6.4	41.3	4.4	62.5	66.9	0	50.3	50.3

G : The length of the green portion in mm (mean values of 18 plants).
P-R : The length of the pale or red portion in mm.

For further experimentations of the low temperature effect on the pigment accumulation in the albino seedlings, 100 heterozygous seeds were sown on wet filter paper in six centimeter Petri-dishes. After being kept about one day at 25°C for germination, the dishes were placed in a dark room controlled at 1±1°C for 10, 16, 20, 26, 36 and 46 days respectively. Water was supplied at an interval of two weeks. Immediately after the end of the low temperature treatment the plants were transferred into the room controlled at 25±2°C and illuminated at about 2500 lux on the plant level by the fluorescent day light tubes and 100 watt incandescent lamps. The pigmentation in the mutant seedlings was observed after one week.

Table III. Effect of low temperature on the green pigmentation in albino seedling.

Days of the low temperature treatment at 1°C	0	10	16*	20	26*	36*	46*	60
Pigmentation	−	−	−	−	−	+	+	+

+ : The pigmentation occurred and a part of the leaves became green.
− : The seedling remained red or pale in color.
* : A parallel experiment at 5°C showed the same results.

As it was observed that a considerable amount of the green pigment was accumulated in the carotina seedlings and the leaves became yellow green in summer, the following experiments have been carried out. Namely, a hundred heterozygous seeds were sown on wet filter paper in six centimeter Petri-dishes. They were kept in the illuminated rooms as described above with varing temperature of 10 ± 2°C, 20 ± 2°C, 26 ± 2°C, and 32 ± 2°C, respectively. Observation of the pigmentation in the carotina seedlings was made in 10 days after germination.

Table IV. Effect of temperature on the chlorophyll accumulation in carotina seedling

Temperature	10±2°C	20±2°C	26±2°C	32±2°C
Chlorophyll accumulation	−	±	+	+

\+ The accumulation occurred and the leaves became yellow green in color.

− The accumulation did not occur and the leaves remained orange in color.

For the pigment analysis, the method by Koski et al[10] was adopted. The leaves of the seedlings which were kept in ten days after the low temperature treatments for the albino and after the germination for the carotina were ground in a mortar with a small amount of silica sand and 25 ml of 85 per cent acetone, and the pigments were extracted in 25 ml of

Table. V. Comparison of chlorophyll content in the first leaves of albino and normal seedlings germinated and grown at 1°C for various durations

($\times 10^{-3}$ mg/gm of fresh weight)

Duration		Protochlorophyll	Chlorophyll a	Chlorophyll b
46 days	albino	22.0	82.6	72.8
	normal	42.4	613.6	547.7
36 days	albino	12.7	27.7	21.0
	normal	44.1	524.0	455.2
26 days	albino	0	0	0
	normal	49.3	553.6	592.9
16 days	albino	0	0	0
	normal	50.0	637.3	625.0
0 days	albino	0	0	0
	normal	25.0	508.6	451.2

* Entire leaf was used for measuring chlorophylls. Therefore, the actual content of the pigments in the green portion is larger than the values presented.

ether. A mixture of ether and acetone extracts were filtered, and acetone was then removed by washing distilled water in a separatory funnel. The ether extract was messed up to a volume of 25 ml and kept in a refrigerator for two hours or more. The extract was then analyzed in a Beckmann's photoelectric spectrophotometer. Optical density was measured between 340–700 mμ of wave length, and especially at 663, 664 and 624 mμ which are known to be the positions of maximum absorption of chlorophyll a, chlorophyll b and protochlorophyll, respectively. The amount of pigments was calculated according to the formula presented by the above mentioned authors.

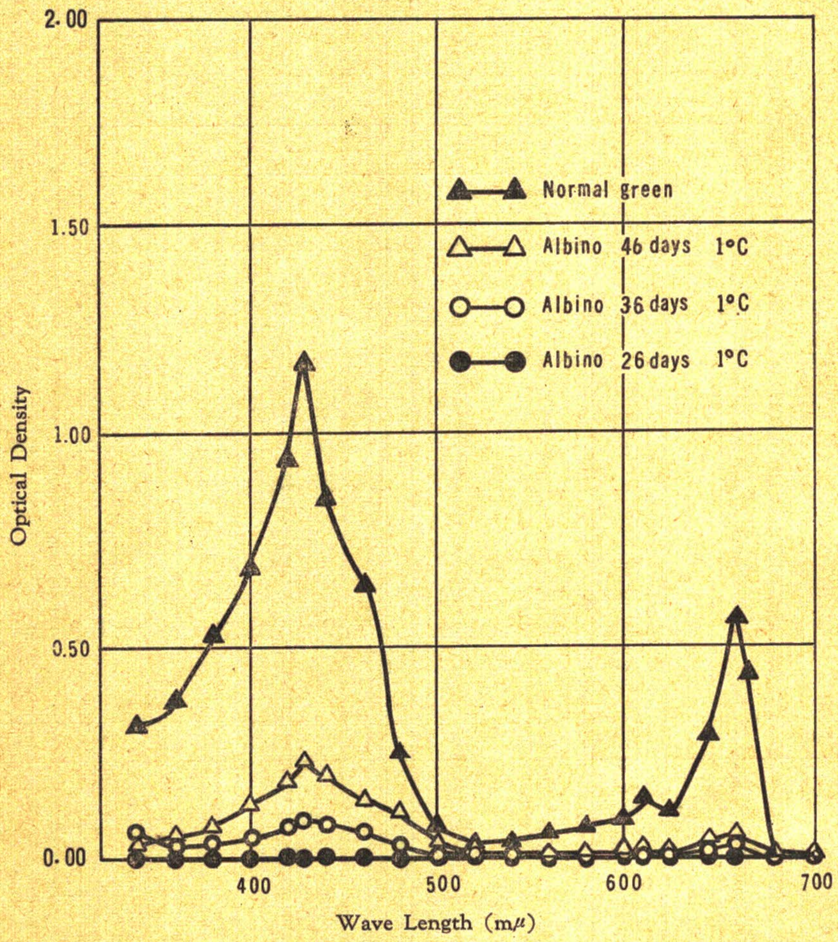

Fig. I. Absorption spectra of the pigments extracted from the albino seedlings germinated and grown under various durations at low temperature of 1°C (Solvent : Ether)

Results

1) Temperature effect on the pigment accumulation in the albino seedlings.

The albino seedlings grown at high temperature (higher than 15°C) or even exposed to the lower temperature for a short duration lack chlorophylls (Table III, V) and carotinoid pigments, as shown in Fig. I. Namely the ether extracts of the mutant leaves showed no absorption in the region of 340–700 mμ of wave length. The red pigmentation in the albino seedlings is due to anthocyanin, which was separated into the washing water from the etherial layer in the above mentioned process of extraction. The red pigment was observed more distinctly when the plant was grown under the lower temperature or on the media with the higher concentrations of sucrose. However, in the albino seedlings grown at 1°C in 36 days or more after germination the chlorophyll pigments were accumulated in the basal portion of leaves (Table III). The pigment accumulation was increased according to the duration of exposure to the low temperature (Table V). The carotinoid pigments seemed also to be accumulated by the low temperature treatment, as the absorption curves of the extracts of the plant treated at 1°C for 36 days or more indicated the similarity to that of the normal green. (Fig. I).

Table VI. Chlorophyll content in the leaves of carotina and normal grown at 26° and 10°C.

($\times 10^{-3}$ mg/gm of fresh weight)

Temperature		Protochlorophyll	Chlorophyll a	Chlorophyll b
26°C	carotina	17.7	118.1	89.6
	normal	39.6	555.1	508.1
10°C	carotina	12.3	13.7	0
	normal	202.8	439.7	427.6
*10° and 26°C	carotina	10.4	81.4	70.9
	normal	20.1	566.4	691.4

* The plants were kept firstly at 10°C for a week and then at 26°C for 2 days.

2) Temperature effect on the pigmentation in the carotina seedlings.

The carotina seedlings contained a small amount of chlorophylls except chlorophyll b in addition to the carotinoid pigments when they grew under the temperature condition of 10°C (Table VI and Fig. II). When, however, grown under the temperature conditions higher than 20°C, visible green pigments were accumulated and the whole leaves became yellow green in color (Table IV and V). This is in contrast to the case of the albino seedlings in which the pigmentation was localized in the lower portion of the leaves. When the yellow seedlings grown under the low temperature conditions were transferred to the higher temperature, for example 25°C, the entire leaves became yellow green within a day.

3) **Culture of the mutant plants**

The seeds heterozygous for albino and carotina were sown aseptically on the nutrient agar media containing five per cent of sucrose in test tubes. The details of the culture method was presented by Sugino[15] elsewhere. The mutant seedlings thus germinated and grown at 1°C in 60 days in the

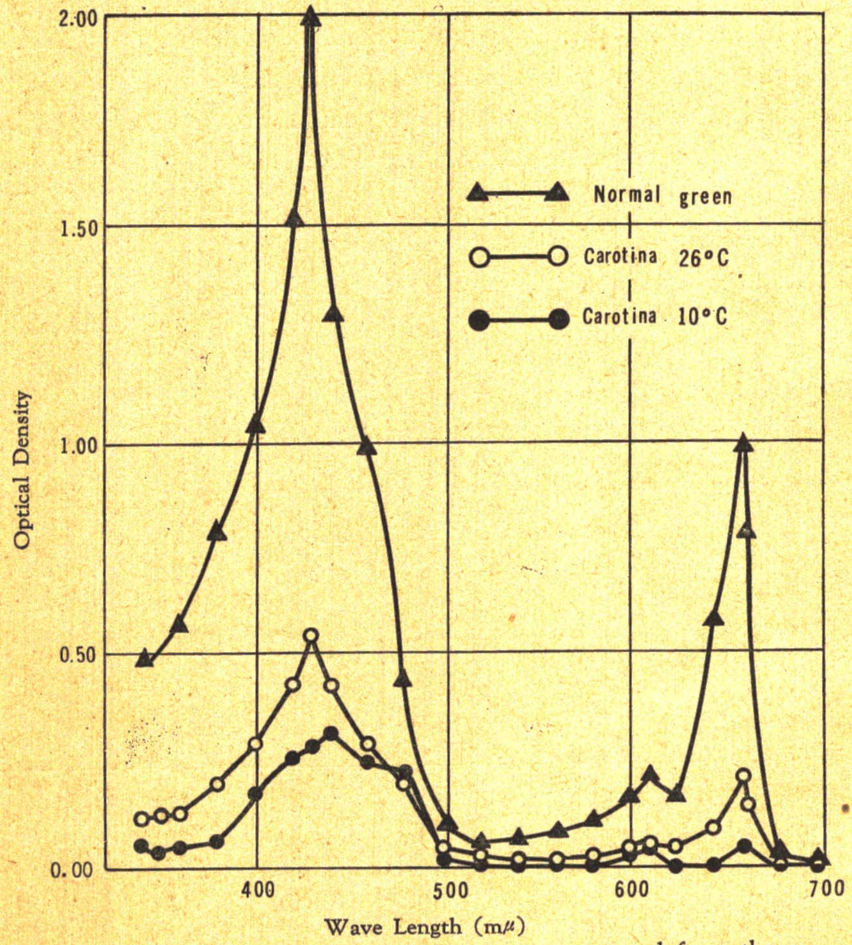

Fig. II. Absorption spectra of the pigments extracted from the carotina seedlings grown under low and high temperature conditions. (Solvent : Ether) darkness were placed in the light under the temperature condition of 25±2°C and were observed after about 96 days. The results are given in Table VII. The green pigments which were accumulated on the basal portion of leaves of the albino seedlings by the low temperature treatment (Table II) persited in

Table VII. Culture of the mutant plants in which the green pigment accumulated (The plants were kept at 1°C for 60 days and then at 25°C for 96 days.)

	Albino	Carotina	Normal
Number of plants observed	60	20	20
Number of leaves formed on the main axis	6.29	7.95	7.95
Stem length in mm	2.9	60.5	112.8
Dry weight of the shoot in mg	12.1	17.8	22.7
% of plants with flower primordia	3.3	100	100
Flowering stage	0.5	2.0	3.5
Survival state	dead	alive	alive

the following higher temperature condition but the plants were not able to sustain their growth.

In the carotina seedlings the pigment accumulation occurred on the entire leaves under the higher temperature condition so that they were able to sustain their growth longer than the albino.

Discussion and Conclusion

It is interesting to compare the plastid pigment changes occurring in the two types of the chlorophyll mutant seedlings. Namely, the albino seedlings contain none of any green pigments and carotinoids, while the carotina seedlings do carotinoids and a little amount of chlorophylls under the normal temperature conditions. In the carotina seedlings the formation of the chlorophylls is inhibited strongly under the low temperature condition, under which the normal seedlings show bright green pigmentation, while an accumulation of the pigments occurs under the high temperature condition (Table VI). In the albino seedlings the plastid pigments are accumulated only under the low temperature condition. The pigment accumulation is localized on the basal portion of leaves in the albino seedling, while the greening occurs over the entire leaves in the carotina seedling. The former process takes place in a long term about a month (Table III) in the meristematic tissues of the leaves under the low temperature condition, but the latter occurs in a short term (one day) in the mature leaves including the non-meristematic tissues under the high temperature condition.

The accumulation of the plastid pigments is generally affected by the external conditions of temperature, nutrition and illumination, but the mechanism of the pigment formation is not known well. An initial conversion of protochlorophyll to chlorophyll a was fully studied by Koski and others[11,12]. A scheme of the biosynthesis of chlorophylls has been demonstrated by Granick in the mutant strains of *Chlorella* which contained intermediary porphyrin compounds in the chlorophyll formation[6].

In the present experiment, it is not clear which step in the pathway of the plastid pigment formation is blocked in the albino or the carotina plants. If, however, the lack of the pigment is due to a single gene mutation in the albino or the catotina (Table I) as suggested by Beadle[1], each of the mutant gene blocks may be operative at any step in biosynthesis of the plastid pigments.

The results are summarized as : 1) the chlorophyll formation occurs concomitantly with the carotinoid formation in the albino, 2) chlorophylls are rapidly accumulated in the whole leaves of the carotina, which contains a considerable amount of carotinoid pigment, when the plant is placed in the room with higher temperature and 3) no visible difference other than the pigmentation has been noticed in the initial growth of the seedlings between the mutant and normal plants. From these evidences it may be said that the gene block responsible for the albino or the carotina is operative in a certain pathway of the pigment formation as suggested by Frank[4,5] and Kay and Phinney[7,8]. Accordingly, if chlorophylls and carotinoids are formed from the common precursor X, it may be indicated that the albino mutant block is operative at the step before the synthesis of the precursor X and the carotina mutant block is operative after the X or at the step close to chlorophyll in the synthetic pathway. It appears that the low temperature breaks the albino gene block through some meristematic metabolism and that the high temperature breaks the carotina gene block easily but partly through non-meristematic metabolism. Another possibility that the albinism is caused by bleaching of the pigment in light[3,10,13] can not be excluded in the present investigations.

Summary

Two types of the chlorophyll mutant of *Triticum monococcum*, albino

and carotina, have been used as the experimental materials in the present investigations. The former contains no chlorophylls and carotinoids and the latter contains carotinoids and a little amount of chlorophylls under the normal condition. It has been shown that by the low temperature treatment the green pigment was accumulated in the basal portion of the leaves in the albino, but in the carotina the accumulation of chlorophylls occurred on the whole leaves under the higher temperature condition. Comparison of the pigment formation in the albino with that in the carotina suggests that the mutant gene block responsible for the albino or the carotina may be operative at the step before or after the common precursor X of both chlorophyll and carotinoid in their biosynthetic pathway.

Acknowledgement

The writers are indebted to Professor S. Imamura, Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan for his kind suggestions throughout the present investigations.

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Explanation of Plate

Fig. 1. Normal green (left) and the albino (right) seedlings grown under the normal temperature condition.

Fig. 2. Normal green (left) and the albino (middle and right) seedlings exposed to the low temperature in about six weeks.

Fig. 3. Normal green seedlings (left) and the carotina seedlings which have been kept under the high (middle) and the low (right) temperature conditions.

(Received November 1, 1960)

Fig. 1

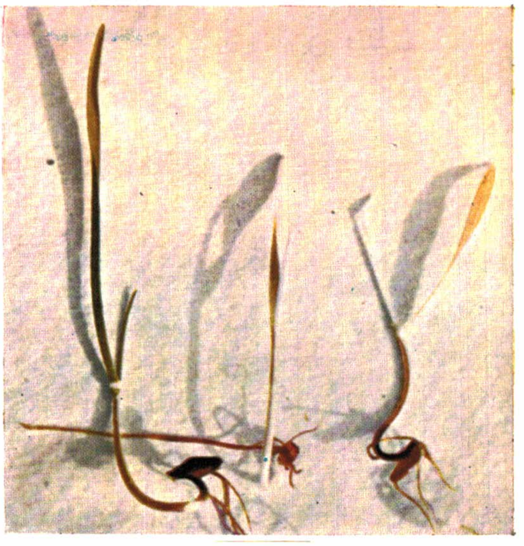

Fig. 2

Fig. 3