114. Mutations in Einkorn Wheat Induced by X-rays. II. *Virido-albina* Mutants^{*)}

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Chlorophyll mutants, besides often occurring spontaneously, are induced frequently by ionizing radiation. Most of them are Mendelian recessives. Comprehensive studies in this field were carried out by Gustafsson and his co-workers (Gustafsson 1940, 1947 and others). The majority of mutations in diploid plants are chlorophyll mutants. According to Gustafsson and Nybom (unpubl.) and MacKey (unpubl.) chlorophyll mutants amounted to about 90% of all mutations in the X_2 -generation obtained by X-irradiation and neutron bombardment of barley seeds (Holm, 1954). Similar results were obtained with X-rays by Smith (1950) in barley and Matsumura and Fujii (1955) in wheat. Holm (1954) investigated the physiological properties and genetics of chlorophyll mutations in barley induced by X-rays.

Matsumura and Fujii (1956) found several chlorophyll mutants, such as albina, chlorina, virido-albina, basi-viridis, striata, etc. from X-irradiation of Einkorn wheat. Among them the virido-albina mutant resumed growth in the phytotron (air-controlled greenhouse) and recovered the full normal chlorophyll content but it was lethal in the field. The author reports in this paper the results of his study of morphological and biochemical differences between the normals and the virido-albina mutant. Also, some characteristics of chlorophyll formation in the mutant are discussed. The chlorophyll mutants are classified according to Gustafsson (1940).

Materials and Method

Triticum monococcum L. var. *flavescens* KÖRN. was used in this study. Virido-albina mutant appeared in the X_2 -generation from seeds treated with 8,100 r dose, at 180 KVP, 3 mA tube current, 13 cm target distance, without filter. Albina, chlorina, basi-viridis, and striata mutants were obtained by similar treatments. Exposed to X-rays were dry dormant seeds in 1951. The characteristics of these mutants were recognizable already at the seedling stage. All virido-albina plants died in the field during the period of low temperature. About 3/4 of the base of all leaves of this mutant were white, while the rest was light green. The plants were placed in a dark room of

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the phytotron (20°C constant) and daily illuminated for 14 hrs. with fluorescent light (about 4,000 lux). About a month later, they became green and developed a normal amount of chlorophyll. Morphological and biochemical observations were carried out in the recovered plants.

In the examination of the chlorophyll content fresh leaves were used. Extinction of chlorophyll components was measured with Beckman-spectrophotometer. Oxygen consumption or respiration rate and activity of cytochrome oxidase were measured with Warburg constant volume respirometer (cf. Fujii, 1955b).

Results

(1) Recovery of green color and morphological observation

The mutant in the field had about four or five leaves, but all plants died out between December and February. When the mutant was placed in the phytotron, the leaves became gradually green starting at the veins, and about 15 days later it resembled morphologically the basi-viridis mutant which has a light green leaf base. It required about further 30 days of treatment to recover to chlorophyll content similar to that of the normals. On the other hand, when kept in the same phytotron in natural light it increased gradually the chlorophyll content, reaching the condition of basi-viridis mutant about 30 days later, and it continued to grow slowly until heading. Non-heading of the mutant when given only fluorescent light could be due to insufficient light quantity. When the mutant was transferred from fluorescent light to day light after having developed the flag leaves, it was able to head and to produce ripe seeds. Time of heading and ripening was delayed about 15 days, and plant height, number of tillers, and fertility were significantly reduced in comparison with the normals (Table I). When the recovered virido-albina were

Table I. Morphological differences between normals and several chlorophyll mutants (seeds sown on 19th Oct., 1954)

		Plant height (cm)	No. of tillers	Time of heading	Fertility (%)	Survival rate (%)
In the field	Normal	131.8	50.1	15/V	91.6	97.5
	Albina	Died out about 10 days after germination				
	Chlorina	117.4	39.1	25/V	68.1	85.5
	Striata	76.5	10.5	5/VI	50.5	44.4
	Basi-viridis I	90.3	10.2	30/V	2.8	81.8
	„ II	68.0	2.2	5/VI	59.3	30.7
	Virido-albina	Died out in winter				
In the phytotron	Normal	78.2	7.8	5/III	52.6	100.0
	Virido-albina	60.1	3.8	20/III	32.1	90.0

returned to the field, the subsequent leaves showed again virido-albina character and the mutant died out in winter. Seeds of the recovered virido-albina showed a relatively high germination rate of about 80%. The whole offsprings were virido-albina.

7 virido-albina plants appeared among 30 X_2 -plants (1952) and 37 of the same mutant among 165 X_3 -plants (1953) obtained from selfed progeny of heterozygous X_2 -plants. The mutant was crossed with various other mutants. The F_1 plants were green and in F_2 segregated in monohybrid ratio. The virido-albina mutation, therefore, is simply recessive. Chromosome conjugation of the mutants was normal, and 7_{II} were formed at metaphase in recovered virido-albina.

(2) Increase of chlorophyll content

Investigation of the difference in chlorophyll content was carried out between the normals and the mutant grown both in the field and in the phytotron. The extraction of chlorophyll components was carried out with a solvent of 3 parts methanol and 1 part acetone. 1 gram of fresh leaves was extracted at low temperature in darkness for 24 hrs. with 12 cc of the solvent. Extinction of the extracts was measured at the wave length of 4,230 Å with Beckman-spectrophotometer. In the dark phytotron illuminated by fluorescent lamps, the chlorophyll amount of the virido-albina and basi-viridis were similar to that of the normals, while in the field the amount of chlorophyll of the former was about 1/4 and that of the latter about 1/2 of the normal. On the other hand, the chlorophyll content of the chlorina mutant was about 1/2 compared with that of the normals (also Fujii, 1955a). The normals and the chlorina mutant kept in the dark phytotron in fluorescent light showed only a small increase of chlorophyll amount; such a marked increase as observed in the virido-albina and basi-viridis was never found (Table II). Albina and striata mutants showed the same tendency. Similar results with regard to the increase of chlorophyll amount in the phytotron were obtained in the basi-viridis mutant of another variety, *T. monococcum vulgare*, and in the virido-albina mutant of barley. Detailed results and the genetic analysis of the mutants will be reported elsewhere.

The above treatments were carried out under 14-hour daily illumination by fluorescent light. A similar increase of chlorophyll amount

Table II. Increase of chlorophyll content

Mutant	Extinction	
	In the field	In the phytotron
Normal	0.48	0.51
Chlorina	0.25	0.28
Basi-viridis I	0.26	0.50
„ II	0.29	0.49
Virido-albina	0.13	0.51

was also observed under 8-hour illumination. Therefore, the increase in chlorophyll content in the phytotron could hardly be due to the length of artificial or natural illumination, but was probably due to favorable temperature and light quality.

(3) Respiration rate and activity of cytochrome oxidase

Respiration rate is usually measured by the rate of oxygen consumption. 0.5 gram of fresh leaves of the normals and the mutant taken in the field and in the phytotron were used in the following measurements with Warburg-respirometer. 0.3 cc of 2N NaOH was placed in the center well of flasks as absorbent of CO₂ gas. Respiration rate of the normals and the mutant grown in the phytotron was significantly lower compared with that of plants in the field. Respiration rate of virido-albina mutant in the field was about half of that of the normals, and in the phytotron the rate recovered virido-albina was rather higher than that of the normals (Table III). The same

Table III. Respiration rate in 0.5 gram of fresh leaves (μ l)

		Time from the start of experiment in minutes	
		30	60
In the field	Normal	0.291	0.616
	Virido-albina	0.146	0.335
In the phytotron	Normal	0.104	0.215
	Virido-albina	0.162	0.324

Table IV. Respiration rate in a mixture of 0.5 gram of fresh leaves with buffer (μ l)

		Time from the start of experiment in minutes					
		10	20	30	40	50	60
In the field	Normal	0.020	0.073	0.126	0.152	0.179	0.212
	Virido-albina	0.015	0.051	0.095	0.102	0.116	0.138
In the phytotron	Normal	0.007	0.020	0.040	0.052	0.059	0.072
	Virido-albina	0.006	0.032	0.059	0.071	0.078	0.087

Table V. Activity of cytochrome oxidase (μ l)

		Time from the start of experiment in minutes					
		10	20	30	40	50	60
In the field	Normal	0.053	0.119	0.192	0.258	0.305	0.351
	Virido-albina	0.015	0.124	0.189	0.247	0.291	0.342
In the phytotron	Normal	0.058	0.130	0.195	0.254	0.286	0.312
	Virido-albina	0.084	0.175	0.259	0.330	0.369	0.389

materials were homogenized with 5 cc of Sørensen phosphate buffer (pH 7.4) and 0.3 cc of 0.25 M sucrose. Manometric measurements were carried out with these mixtures, and similar results were obtained (Table IV).

Activity of cytochrome oxidase was measured with the following techniques. 0.18 cc of 0.114 M Na-ascorbate (pH 7.0) and 0.32 cc of 2.4×10^{-4} M cytochrome C as substrate were added to the homogenized material mentioned above. The activity of cytochrome oxidase in the mutant, grown in the field and in the phytotron, was similar to that of the normals (Table V). There was no difference in the respiration rate and activity of cytochrome oxidase between the normals and the mutant in the phytotron.

Discussion

The virido-albina mutant illuminated in the phytotron with fluorescent lamps showed a marked increase in chlorophyll content. After 30 days the color of the leaves of the mutant was normal green and it was indistinguishable from that of the normals.

The relation between pigment formation and environmental conditions has been widely studied by many investigators. All wave lengths of the visible spectrum take part in chlorophyll development. At equal irradiation, a narrow band of blue rays of about 4,450 Å is most effective in inducing chlorophyll formation in oat seedlings, and a broader wave band in the orange red rays (about 6,200–6,600 Å) being the next most effective (Frank, 1946). In general, chlorophyll synthesis can occur over a wide range of temperature. In etiolated wheat plants synthesis of chlorophyll occurs at any temperature within the range 3–48°C, but is most rapid between 20 and 30°C (Lubimenko and Hubbenet, 1932). Somewhat similar results have been obtained by Larsen (1950) who found for chlorophyll synthesis the approximate range 11–19°C. Smith (1949) pointed out, that when one part of an etiolated barley leaf is illuminated at 0–4°C, it remains yellow, while the other part of the same leaf exposed to the same light intensity at 10–12°C, attains normal green colouration. Holm (1954) reported that in chlorophyll mutants of barley induced by X-rays, strong light and moderate temperature (about 20°C) are most effective for chlorophyll formation. In some cases, the recessive homozygotes of his chlorophyll mutants were detectable in the field but not in the greenhouse; the opposite case has never occurred. Nybom (1955) compared the chlorophyll contents of several chlorophyll mutants of barley at two temperatures, 11 and 21°C. He showed that in most of these mutants the increase in chlorophyll content was greater at 21°C than at 12°C. Also, Largridge (1955) observed that by raising

the temperature virido-albina mutants induced by X-rays in a Cruciferae species (*Arabidopsis thaliana*) became green again and resumed growth. Our results, therefore, are in good accord with those mentioned above. Increase of chlorophyll content is probably due to moderate temperature and optimum quality of light.

According to my results, the respiration rate of virido-albina mutant in the field was about half of that of the normals, and the respiration rate of the recovered mutant was rather higher than that of the normals. On the other hand, there was no difference in the activity of cytochrome oxidase between the normals and the mutant either in the field or in the phytotron. The decrease of respiration rate in the phytotron, therefore, could not be in relation to the activity of cytochrome oxidase. The author reported already (1955a) a similar tendency toward a decrease of the chlorophyll content and activity of cytochrome oxidase in the chlorina mutant. The chlorina mutant did not markedly increase its chlorophyll content in the phytotron. From these results, it was assumed that a recovery of chlorophyll content without a normal activity of cytochrome oxidase could not take place. The relation between increase of chlorophyll content and enzyme activity awaits further investigation.

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