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Light Sensitivity of Plastids and Plastid Pigments Present in the Albescent Maize Mutant[1]

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Abstract. **Dark grown albescent corn seedlings are deficient in colored carotenoids but accumulate phytoene, phytofluene and an unidentified substance in the carotenol fraction. They bleach upon exposure to bright light and appear albino. Seedlings grown under low level incandescent light are normal in appearance and contain almost as much colored carotenoid as control seedlings. The existing leaf tissue of seedlings grown under low level light does not bleach upon exposure to bright light. The enhanced carotenoid synthesis and stabilization of plastids is not affected by brief illumination with red light but requires several hours of low level incandescent light.**

The serial production of tissue with apparently normal chloroplasts and of albino tissue derived from the same meristem is of considerable interest from the standpoint of plastid development. Such a trait is found in the homozygous recessive albescent (*al*) maize mutant. These plants, when grown in the field, may emerge as albino or green seedlings. Green seedlings within a week generally display albino tissue in the youngest leaves; and subsequent growth often exhibits alternate green and albino transverse banding followed by only albino tissue (9). The homozygous albescent genotype (*al/al*) has been associated with a pale endosperm (9) suggesting a relationship with a number of albino maize mutants possessing carotenoid-deficient grains (11). Carotenoid deficiencies in a number of maize mutants result in albino plants, as was first shown with the ω_3 mutant (1,7). Albescent differs from these mutants by the presence, under some conditions, of apparently normal green tissue in the homozygous plants.

It appeared that environmental conditions determine whether green or albino tissue is produced by genetically identical tissue derived from a common meristem. The object of this investigation was to determine the environmental conditions under which green or albino tissue would be produced and to provide preliminary information on the plastid pigments in the developing tissues.

Materials and Methods

Plant Materials. The stock of albescent maize was initially obtained from the Maize Genetics Cooperative Collection (Dr. E. B. Patterson, University of Illinois) in an M14 background. This stock was outcrossed to Oh51A and maintained by pollinating known heterozygotes (+/*al*) with pollen from sibling plants of genotype *al/al*. Homozygous albescent grains were selected as the pale seeds from the resulting segregating ears and yellow (+/*al*) grains from the same ear served as the normal control seed in all cases. Less than 0.5% of the grains produced seedlings which were not of the predicted phenotype, and such seedlings were discarded.

Growth and Light Treatments. The seedlings were germinated in moist vermiculite at 25° ± 2° in the dark. Seedlings (5–7 days old) were then transferred either to continuous incandescent light at 27° or to a growth chamber (2000 ft-c incandescent-fluorescent light, 12 hr photoperiod and 21° day/18° night). In some experiments after exposure to dim incandescent light for 72 hours, seedlings were transferred to the growth chamber. Light intensity measurements were made with a thermister radiometer (YSI-Kettering Model 65) or with a cadmium sulfide photometer (Grossen-Lunisix). The red or far red light source was a cellophane filtered tungsten light (3) with intensities of 2.9×10^4 ergs/cm^2 × sec and 4.0×10^4 ergs/cm^2 × sec respectively. In some instances seedlings with or without prior red light treatment (5 min) were exposed to dim light (7½ watt bulb) at 90 cm, 1.6×10^3 ergs/cm^2 × sec for several hours.

Pigment Analysis. Seedling leaves and coleoptiles were weighed and stored in plastic bags at −20° until extraction. The tissue was ground with

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sand in a mortar with 4 volumes of acetone and a trace of calcium carbonate; the residue, following filtration, was washed with acetone until colorless. The combined acetone extracts were reduced in volume under reduced pressure and the visible absorption spectra were determined with a (Beckman DB) spectrophotometer. In some cases the pigments were transferred to hexane by addition of hexane and 2 volumes of 10 % sodium chloride; the hexane soluble material was saponified by treatment with 30 % methanolic KOH at 37° for 2 hours. Following saponification the pigments were again transferred to hexane (2 volumes of 10 % sodium chloride) and the carotenols removed by 90 % methyl alcohol partition (6). The carotenol fraction was transferred back to hexane by addition of 2 volumes of 10 % sodium chloride. The visible and ultraviolet spectra of the carotene and carotenol fractions in hexane were determined.

The pigments were separated by thin layer chromatography on activated silica gel plates (Adsorbosil -2, Applied Science Inc., State College, Pa.) according to Davies (6) using either hexane (carotenes) or hexane: *n*-propanol (20/1, v/v) (carotenols and chlorophylls).

In several cases the total carotenoid content has been measured by reading the optical density of acetone extracts at 475 mμ where interference due to the chlorophyll *a* or *b* blue bands is minimal.

Results

Preliminary Experiments and Observations. An attempt was made to simulate the spring temperature and photoperiod conditions of the field when banding of albescent plants was evident. Seedlings germinated in quartz sand were exposed to 16 hour days and cool nights (5°–15°). All seedlings appeared albino except for the tips of the first leaves which were green. A faint anthocyanin banding was noted in some seedlings.

It was observed that when albescent seedlings germinated under low light intensity they were visually like control seedlings. When these seedlings were transferred to the controlled environment chamber, the existing leaf tissue became green and appeared normal. However, all subsequent leaf tissue was albino. Dark-grown albescent seedlings were pale bluish-green in appearance and when transferred to a growth chamber they became albino. It was possible to produce green tissue in bright light if the meristem was wrapped with aluminum foil (12). This could be demonstrated in the growth chamber or the field. If green albescent seedlings in the field were uncovered to the seed level all leaf tissue subsequently produced was white.

Although the white tissue is referred to as albino, plastids are present in the guard cells of the epidermis. Also, small "islands" of plastid-containing cells are sometimes found in the leaf tissue by microscopic examination.

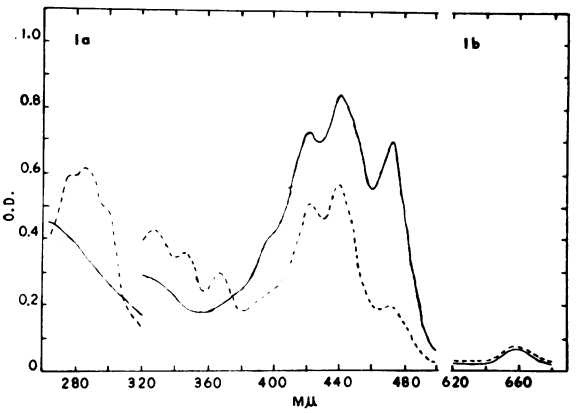

FIG. 1. The absorption spectra of extracts from 10-day-old dark grown normal (————) and albescent (--------) corn leaves. Figure 1a. Hexane soluble pigments from normal (0.1 g/ml) and albescent (0.5 g/ml) corn leaves. Figure 1b. Acetone extracts at 0.2 g fresh wt/ml.

Pigments of the Dark Grown Seedlings. Dark grown albescent seedlings contained as much protochlorophyll as did normal seedlings, and following exposure to a few minutes of fluorescent room light (about 100 ft-c) it was converted to chlorophyll (fig 1b). The carotenoid pigments of the albescent mutant appeared to be both qualitatively and quantitatively different from carotenoids in normal dark grown seedlings (fig 1a). There was a deficiency of colored carotenoids in the 430 to 475 mμ region (fig 1a) and peaks in the near ultraviolet (6) suggested the accumulation of phytofluene (329, 348, 368 mμ) and phytoene (298 mμ). This was confirmed by saponification and methanol partition to obtain the carotene fraction (fig 2). Phytofluene

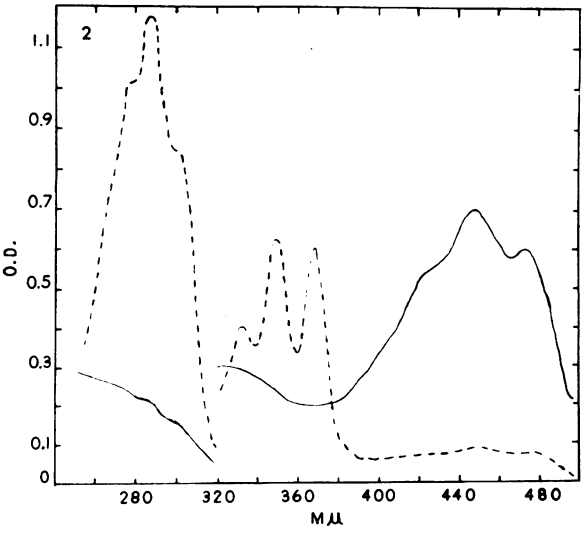

FIG. 2. The absorption spectra of the carotene fractions in hexane of normal (————) and albescent (-------) 10-day-old dark grown corn leaves. Concentrations are 2.0 g fresh wt/ml from 320 to 500 mμ and 0.4 g fresh wt/ml from 260 to 320 mμ.

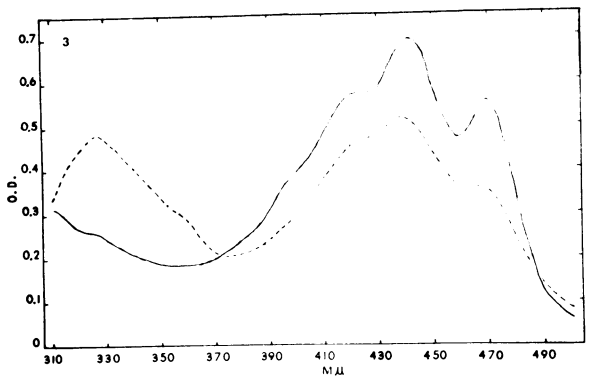

FIG. 3. The absorption spectra of the carotenol fractions in hexane of normal (————) and albescent (-------) 10-day-old dark grown corn leaves. Concentrations are 1 g fresh wt/ml for normal and 5 g fresh wt/ml for albescent.

was located on silica gel plates developed in petroleum ether by its yellowish fluorescence R_F 0.20. Phytoene was located just above the phytofluene band with I_2 vapor. After removal of these bands from the plates and elution (10 % methyl alcohol in hexane) phytofluene and phytoene spectra were obtained. In extracts from normal seedlings the major colored carotene band was at R_F 0.28 and 4 other bands were located with UV light, I_2 vapor, or visual inspection. All these components could be detected in extracts of albescent seedlings although the main carotene band was very weak.

The carotenol fraction in hexane had an absorption spectrum in the 400 to 500 mμ region similar to the normal seedlings (fig 3) except 8 to 10 times the concentration (fr wt basis) was required to get similar absorbance readings. However a major peak at 327 mμ present only in the albescent carotenol fraction has not been identified. After thin layer chromatography 7 similar bands were detected in either albescent or normal seedlings. One additional band R_F 0.87 was detected with I_2 vapor only in albescent carotenol extracts.

The apparent qualitative differences in the absorption spectra in the blue region between albescent and normal seedlings (fig 1a) are thus due to the increased chlorophyll to carotenoid ratio rather than qualitative differences in the colored carotenoids present.

Response of Albescent Seedlings to Low Intensity Light. When albescent seedlings were exposed to low intensity incandescent light for 72 hours, the carotenoids were found to increase with increasing light intensity in a manner similar to normal seedlings. At 1 to 2 foot candles, bleaching of the new leaf tissue was apparent in albescent seedlings (fig 4). Phytoene and phytofluene could still be detected, however, in albescent seedlings grown at all intensities.

The chlorophyll content of albescent seedlings grown under dim incandescent light was similar but somewhat less than that of normal seedlings. The leaf-tissue after exposure to dim incandescent light (72 hrs of 0.05–0.5 ft-c) did not bleach when transferred to the controlled environment chamber.

Several experiments were conducted to examine the red or far red light response of albescent and normal dark grown seedlings in comparison with several hours low intensity incandescent light. The results (table I) indicate that albescent seedlings do

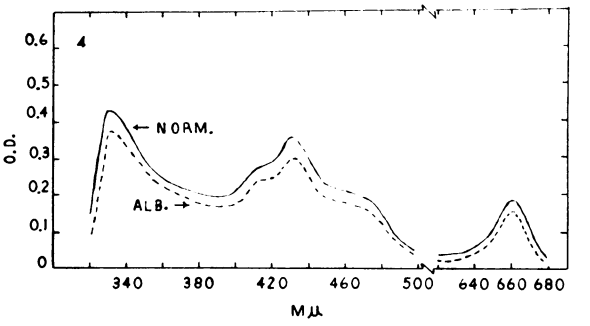

FIG. 4. The optical density (per g fr wt) at 475 mμ of acetone extracts from 10-day-old normal (-x-x-) or albescent (--o--o--) corn leaves following 72 hours exposure to various intensities of incandescent light.

Table I. *The Carotenoid Content of Albescent or Normal Dark Grown Corn Seedlings 24 Hours after Various Light Treatments*

In experiment 1 and 3 the incandescent light treatment (1.6×10^3 ergs $cm^2 \times$ sec) was for 6 hours while in 2 and 4 it was 5 hours. Red light (2.6×10^4 ergs $cm^2 \times$ sec) and far-red light (4.0×10^4 ergs $cm^2 \times$ sec) treatments were for 5 minutes. In the red plus incandescent light treatments, the seedlings were placed in darkness for 2 hours between the light treatments.

Expt	Age	Dark		Red		Far-red		Red and incand.		Incand.	
		Alb.	Norm.	Alb.	Norm.	Alb.	Norm.	Alb.	Norm.	Alb.	Norm.
	Days					*OD 475/Gram fresh weight*					
1	8	1.11	5.08	1.09	6.33	0.95	6.19	2.06	9.40	...	...
2	8	1.10	4.20	1.06	5.65	1.56	3.07	1.38	5.56	...	...
3	6	1.24	4.22	0.93	4.47	1.16	4.00	1.96	5.74	1.76	5.14
4	7	0.60	5.45	0.78	6.40	0.51	5.82	1.45	8.19	1.05	7.60
Avg		1.01	4.74	0.96	5.71	1.04	4.77	1.71	7.22	1.40	6.37
% of dark control		100	100	95	120	103	100	164	152	139	134

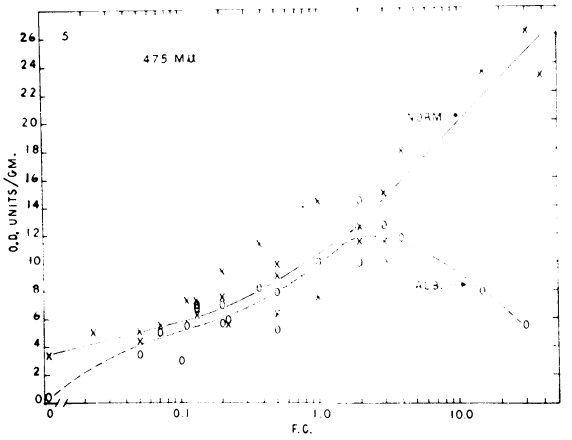

FIG. 5. The absorption spectra of acetone extracts from normal (————) and albescent (-------) corn leaves (0.08 g/ml fr wt). Eight-day-old etiolated plants were exposed to 12 hours dim incandescent light (1.6×10^3 ergs $cm^2 \times$ sec) and placed in the growth chamber for 20 hours prior to extraction.

not have more carotenoid per gram fresh weight following red light exposure, in contrast to the somewhat elevated carotenoid levels found in red light treated normal seedlings. However both normal seedlings and albescent seedlings respond in a similar manner to 5 or 6 hours low level incandescent light (with or without prior red light exposure). Albescent plastids are stabilized only by the latter treatments as indicated by lack of bleaching when such seedlings are placed under high intensity light in the growth chamber.

The Pigments of Green Albescent Tissue. Green leaf tissues from normal and albescent seedlings were extracted into 80 % acetone. The absorption spectra indicated that albescent tissues contained a complement of chlorophylls and carotenoids similar to that of the normal seedlings (fig 5). Thin layer chromatography also indicated the complement of pigments seemed to be qualitatively the same in normal and albescent seedlings.

Bleaching of Chlorophyll in Dark Grown Albescent Seedlings Following Bright Light Exposure. The bleaching of dark grown albescent seedlings when they were transferred to the growth chamber was followed by extraction of chlorophyll at various times (fig 6). The lag phase followed by rapid chlorophyll synthesis in normal seedlings may be compared to the very rapid loss of the initial chlorophyll content of albescent leaves.

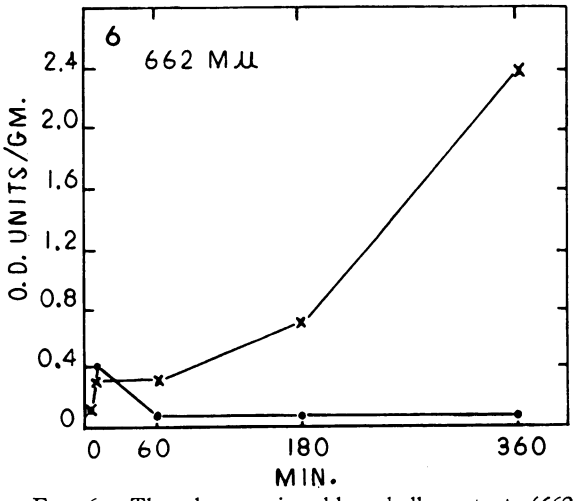

FIG. 6. The changes in chlorophyll content (662 mμ) in 9-day-old etiolated normal (-x-x-) or albescent (-o-o-) corn seedlings following transfer to a growth chamber (2000 ft-c of mixed fluorescent-incandescent light).

Discussion

A deficiency in colored carotenoid pigments is one of the most common causes of apparent albinism in maize mutants (2, 13). Anderson and Robertson (2) have indicated that albescent mutant and other albino mutants accumulated carotenoid precursors. All mutants except one formed protochlorophyll. However, the presence of normal plastid pigments had never been observed in these carotenoid deficient mutants. Even though the albescent mutant was severely deficient in carotenoids in the dark, light of low intensity for a period of several hours could enhance the carotenoid content and at the same time stabilize albescent plastids. Normal pigment accumulation in bright light could then take place.

It was found that dark grown albescent seedlings contain little or no carotene and only small quantities of carotenols. Although no attempt was made to identify all the carotenoids in normal and albescent dark grown seedlings, they were qualitatively similar except for the accumulation in albescent seedlings of phytoene, phytofluene, and the unknown substance (OD 327, carotenol fraction). It would appear that in dark grown albescent seedlings carotenoid biosynthesis was blocked at the C_{40} level. The green tissue of albescent seedlings appeared to be identical in pigment content to normal tissue.

Our data appear to be consistent with the suggestion first made by Koski and Smith (7) that carotenoids protect plastid pigments from photooxidation. In the case of the albescent mutant, it appears that there must be some threshold level of carotenoid which must be formed so that in bright light the net loss is not significant with regard to the stability of the plastid. Preliminary observations indicate that 5 hours of incandescent light is about the minimum for plastid stabilization, and the total carotenoids in albescent seedlings are 20 to 25 % of the normal control levels. It is impossible from the present study to implicate any specific carotenoid fraction in plastid stabilization, although albescent seedlings would seem to furnish ideal material for such an investigation.

Chlorophyll synthesis (10), carotenoid synthesis (5), and some aspects of plastid development (8)

are all initiated by the phytochrome system. Cohen and Goodwin (5) have reported carotenoid synthesis in dark grown corn seedlings is under phytochrome control; however, additional synthesis appeared to require additional light exposure.

A short exposure of dark grown albescent seedlings to red light was not sufficient to cause any significant increase in carotenoid content measured (24 hrs later), in contrast to normal seedlings. This indicates that not only are albescent corn seedlings blocked in carotenoid synthesis in the dark but they also lack the typical phytochrome mediated response. Our limited data (table I) do suggest that prior red light may somewhat enhance subsequent carotenoid synthesis in the light by albescent or normal seedlings as reported by Virgin (14) with wheat seedlings. Exposure of albescent seedlings to dim incandescent light for several hours enhanced carotenoid synthesis and resulted in plastid stabilization. The percentage enhancement in albescent seedlings over dark was about the same as for control seedlings, although albescent plastids are still very deficient in carotenoid. However, extremely low levels of light given over a period of many hours can bring albescent carotenoid levels up almost to normal levels (fig 5). The light quality necessary for the sustained carotenoid synthesis is unknown, however an action spectrum for a *Chlorella* mutant requiring light for carotenoid biosynthesis peaked at 670 mμ (4).

The banded appearance of many albescent seedlings in the field seems to be due to the leaf meristem being located below the soil surface. When the young leaf tissue grew through the light gradient during the day the plastids could be stabilized by low light exposure. Some of the leaf growth at night would become bleached the following day because the tissue is elevated to a position where it receives too much light before stabilization can occur. Albescent seedlings in the field uncovered to the seed level become albino. Conversely when albino plants are wrapped with aluminum foil they again produce green tissue (12). The greening of upper leaves of older foil-wrapped albescent plants indicates that the apical meristem is not the sensitive site since the apical meristem has been converted to the tassel at this stage.

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