Effects of Light and Darkness on Biosynthesis of Carotenoid Pigments in Wheat Seedlings [1]

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The seeds of most higher plants contain relatively small quantities of carotenoid pigments. Upon germination and early seedling growth in the absence of light, more carotenoids are synthesized. Both in seeds (4) and in dark-grown seedlings (3, 14, 2), the carotenoid fraction has been found to consist principally of xanthophylls, with carotenes being present in much smaller amounts. The biosynthesis of carotenoid pigments, which can occur in total darkness, is greatly stimulated in light, much of the increase occurring in the carotene fraction (1, 2). The role of light in carotenoid biosynthesis has been studied intensively in a variety of plant materials by earlier investigators (10, 11, 7, 5).

The study of Kay and Phinney (7) with corn indicated that lutein was the major constituent of dark-grown seedlings. On illumination, large increases occurred in β-carotene and neo β-carotene. In French bean, *Phaseolus vulgaris,* Goodwin and Phagpolngarm (5) observed large increases in β-carotene and lutein in both cotyledons and leaves upon illumination of dark grown plants.

It is the purpose of this paper to present the results of analyses of the carotenoid pigments of wheat seedlings grown in light and in darkness.

Materials and Methods

The wheat used was obtained from a local distributor. The grains were dispensed, 16 grains per dish, into petri dishes containing filter paper. One lot of seedlings was grown in a dark box, in which a constant temperature of 24° was maintained, and was watered at daily intervals under minimal exposure to a green safelight of low intensity. Another lot of seedlings was grown in a Percival compact plant growth chamber under a 14-hour photoperiod, with a light intensity of 1000 ft-c, a day temperature of 24° and a night temperature of 19°.

Plants were harvested after 7 to 10 days, and the dark-grown plants were immersed briefly in boiling water before further treatment. The roots were removed and discarded. Fresh weights of the shoots were determined on an analytical balance. The plant material obtained from each dish was cut into small pieces with a scissors, and the pigments were extracted by grinding in 85 % aqueous acetone in a Waring blendor. After suction filtration to remove cell debris, the volume of the acetone solution was restored to 100 ml, and total carotenoids were estimated spectrophotometrically, using a Beckman DU spectrophotometer and the equations of Röbbelen (12).

The acetone solution of the pigments was then placed in a separatory funnel with a small quantity of petroleum ether (bp 30–60°) and the pigments were transferred to the petroleum ether layer upon the addition of water. The petroleum ether solution of the pigments was carefully washed at least 20 times with water.

Aliquots of the petroleum ether extract were then spotted onto 7 × 7 inch sheets of Whatman No. 3 MM filter paper. Development of the chromatograms was done following the technique of Lind, Lane, and Gleason (9) and Khudairi (8) using petroleum ether: *n*-propanol 99:1 (v/v) in the first direction, and petroleum ether: chloroform 75:25 (v/v) in the second. The chromatograms were developed at 4°, in darkness. Customarily, identical spots from 2 papers, prepared from the same sample and developed simultaneously, were pooled. The various xanthophyll spots were eluted from the papers with 5 ml quantities of petroleum ether containing 1 % methanol, while 5 ml portions of carbon disulfide were used to elute the carotenes. Optical densities of each of the resulting solutions were rapidly determined at the absorption maximum of each pigment.

The various pigments were identified by comparison of the R_F values obtained with those of Khudairi (8), by determination of the absorption maxima in a number of different solvents and comparison of these values with those reported in the literature (15, 6, 4), and by exposing the papers to HCl vapor, which produces a blue to green color in xanthophylls having an epoxide structure. From the optical density values, the percentage of the total carotenoids represented by each component was calculated, and, by reference to the original fresh weights of the plants, the quantities of each pigment in μg/g were determined. Samples of the dry ungerminated grain were also extracted and chromatographed in the manner just described.

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Results

The average of 3 determinations of the total carotenoid content of dry ungerminated wheat grain was 4.2 μg/g. When extracts were chromatographed, 3 spots were found, corresponding to lutein, lutein epoxide, and carotenes. Lutein was the principal constituent, equivalent to 1.9 μg/g, while lutein epoxide was present to the extent of 1.6 μg/g and carotenes to the extent of 0.7 μg/g. No other carotenes were detected, even upon extraction of large amounts of material. It is apparent that the quantity of carotenoids in the ungerminated grain is quite low, and that xanthophylls predominate.

Aliquots of the pigments from dark-grown plants, in petroleum ether solution, were spotted on papers and chromatographed. Seven yellow to orange spots were found on the resulting chromatograms (table I). These were eluted from the papers in either petroleum ether containing a trace of methanol, or in carbon disulfide, and absorption spectra were taken in a number of solvents. Other chromatograms were exposed to HCl vapor. Spot 7, corresponding to carotenes, and spots 3, 4, and 6, corresponding to violaxanthin, lutein epoxide, and lutein, were easily identified in this way. The remaining spots proved to be somewhat more difficult to identify. Spot 1, which produced a greenish blue color on exposure to HCl vapor, was identified by its absorption maxima in petroleum ether, methanol and carbon disulfide as neoxanthin. Spot 2, which showed no color change in HCl vapor, has absorption maxima in petroleum ether at 414, 440, and 470 mμ, and in carbon disulfide at 454, 476, and 507 mμ. Efforts to identify it with any known leaf xanthophyll were unsuccessful. Spot 5, which showed absorption maxima in petroleum ether at 419, 440, and 469 mμ, in carbon disulfide at 445, 475, and 500 mμ, and in methanol at 425, 445, and 472 mμ, and gave no color change with HCl vapor, was identified as neozeaxanthin. R_F values of the pigments found in both dark-grown and light-grown wheat seedlings are presented in table I.

The quantities of the various pigments of dark-grown plants, presented in table II, are based on averages of 5 or more determinations. Lutein is the principal carotenoid, accounting for somewhat more than one-third of all the carotenoids present. Xanthophylls show a great predominance over carotenes, the latter comprising less than 10 % of the total carotenoids. Of the 6 xanthophylls present, only 2 were found in ungerminated grain, 4 more having appeared in the course of germination and early seedling growth in darkness.

Table I

R_F Values of Chloroplast Pigments of Wheat Seedlings

Pigment	Petroleum ether: *n*-propanol 99:1	Petroleum ether: chloroform 75:25
Neoxanthin	0	0.47
Unknown xanthophyll	0	0.74
Violaxanthin	0.18	0.14
Lutein epoxide	0.32	0.23
Neozeaxanthin	0.37	0.31
Lutein	0.46	0.66
Carotenes	1.00	1.00
Chlorophyll b	0.29	0
Chlorophyll a	0.61	0.31
Phaeophytin	0.66	0.97

Table II

Quantities of Carotenoid Pigments in Dark-grown and Light-grown Wheat Seedlings

Pigment	Dark-grown	Light-grown
Neoxanthin	11.9 μg/g	5.9 μg/g
Unidentified xanthophyll	14.9	13.0
Violaxanthin	6.0	5.7
Lutein epoxide	10.9	23.2
Neozeaxanthin	8.7	...
Lutein	32.3	71.2
Carotenes	7.0	61.4
Total	91.7 μg/g	180.5 μg/g

Chromatograms of light grown plants showed 3 spots attributable to substances not present in dark grown material, namely, chlorophyll a, chlorophyll b, and phaeophytins. No quantitative measurements of these constituents were made. The carotenoids present in light-grown plants were qualitatively identical with those of dark-grown material, with the exception of neozeaxanthin, which was not found in the light-grown seedlings.

The data concerned with the quantities of carotenoid constituents of light-grown seedlings (table II) are based on averages of 5 determinations. On comparison of these data with those of dark-grown seedlings, it is apparent that the total quantity of carotenoids was approximately doubled in light. As stated earlier, neozeaxanthin, which was present in etiolated material, disappears in light, and the quantity of neoxanthin has decreased. No significant change was found in the levels of violaxanthin or the unidentified xanthophyll. Significant changes do occur, however, in the levels of lutein epoxide, lutein, and carotenes. Lutein and lutein epoxide doubled in amounts, and the carotenes show a 9-fold increase. Thus the xanthophyll: carotene ratio approaches 2:1 in 1 week old wheat seedlings grown in light.

The carotene fractions from both light- and dark-grown plants were separated from other components by chromatography of a petroleum ether solution on a sucrose column. The carotene fractions collected at the foot of the column were concentrated, and chromatographed on a column of magnesia and Hyflo-Super Cel 3:1, the chromatogram being developed with petroleum ether. The carotene moved down the column as a single narrow orange band, with no resolution occurring. The eluted material gave spectra in several solvents typical of β-carotene. Alpha-carotene, which should have been detected by this procedure, was apparently lacking.

Discussion

The amount of total carotenoids found in air dry ungerminated wheat grains, namely 4.2 μg/g on a wet weight basis is comparable in magnitude to the value 8.0 μg/g on a dry weight basis reported by Barrenscheen et al. (1) for wheat. The composition of the carotenoid fraction which was found in the present study agrees well with the earlier results of Goodwin (4) though not with those of Barrenscheen et al. (1). The xanthophyll fraction of the dormant grain was found to be relatively simple, consisting of only 2 components, lutein and lutein epoxide.

The carotenoid components found in dark-grown wheat seedlings include lutein, an unidentified xanthophyll, neoxanthin, lutein epoxide, neozeaxanthin, carotenes, and violaxanthin. On comparison with the xanthophylls of the ungerminated grain, it is clear that 4 new xanthophylls have been synthesized in the course of early seedling growth in darkness. Xanthophylls show a great preponderance over carotenes in dark grown seedlings, carotenes making up only 7.6 % of the total carotenoids.

On comparison of the carotenoids of light- and dark-grown wheat seedlings, the total quantity of carotenoids has approximately doubled in light. The carotenoid constituents of light-grown plants are qualitatively identical with those of dark-grown seedlings, with the single exception of neozeaxanthin, which was present only in dark-grown material. The quantity of neoxanthin declines in light, no significant change is shown in the levels of violaxanthin or the unidentified xanthophyll, and large increases in lutein, lutein epoxide, and carotene occur. With respect to the constituents which increase on illumination, general agreement with the findings of Kay and Phinney (7) in corn, and Goodwin and Phagpolngarm (5) in beans is indicated.

The changes which were found in the carotenoid constituents of wheat leaves are consistent with the scheme of carotenoid interconversions proposed by Yamamoto et al. (16), part of which is theoretical, while other parts are supported by a considerable body of evidence of several other investigators (10, 13). Since the structure of neoxanthin is not known with certainty, neither it nor the unidentified xanthophyll can be fitted into the scheme at present. The disappearance of neozeaxanthin in light, and the decrease in the quantity of neoxanthin may be interpreted either on the basis of selective bleaching in light, or that they are precursors of other pigments.

According to the scheme of Yamamoto et al. (16), all leaf xanthophylls are derivatives of either α-carotene or β-carotene. Lutein and lutein epoxide are structurally related to α-carotene. The absence of α-carotene from wheat leaves was a somewhat surprising result, which, however, is in agreement with the findings of Kay and Phinney (7), though not with those of Moster et al. (11) with corn, a result probably attributable to environmental differences in the growth conditions employed.

Beta-carotene, zeaxanthin and violaxanthin represent a second biosynthetic sequence (10, 16). Zeaxanthin was not detected in either dark- or light-grown wheat seedlings, although its isomer neozeaxanthin was found in dark-grown, though not in light-grown material. While zeaxanthin was found in corn by Moster et al. (11), it was not present in the experiments with corn reported by Kay and Phinney (7). The very large increase found in β-carotene in light is in accord with the findings of previous investigators both with wheat (1) and other plants (2, 3, 5, 7, 14). Violaxanthin could be formed either from β-carotene or through its interconvertibility with lutein (10, 13) which provides a link between the biosynthetic pathways originating with α-carotene and β-carotene respectively.

Summary

The carotenoid pigments of ungerminated wheat grain, and of dark-grown and light-grown seedlings 7 to 10 days of age have been examined by a combination of spectrophotometric and bidimensional paper chromatographic techniques. The ungerminated grain contains 4.2 μg/g of total carotenoids on a wet weight basis. Lutein, lutein epoxide, and carotene were the only pigments found. In dark-grown seedlings, the total carotenoid content was 91.7 μg/g, and includes lutein, an unidentified xanthophyll, neoxanthin, lutein epoxide, neozeaxanthin, violaxanthin, and carotene. Lutein is the principal constituent, and carotene composes only 7.6 % of the total carotenoids.

Light-grown wheat seedlings contain approximately twice as much carotenoids as dark-grown plants of equal age. The constituent pigments are qualitatively identical with those of dark-grown plants except for neozeaxanthin. The principal effects of light include a doubling in the amounts of lutein and lutein epoxide, and a 9-fold increase in carotene, so that the xanthophyll: carotene ratio approximates 2: 1. Beta-carotene is apparently the only carotene pigment present in either dark-grown or light-grown wheat seedlings.

Literature Cited

1. Barrenscheen, H. K., J. Pany, and E. Srb. 1942. Synthetische Leistungen des Keimlings. I. Mitt. Die Bildung von Ascorbinsäure und der Carotinoide in etiolierten Weizenkeimlingen. Biochem. Z. 310: 285–91.
2. Blaauw-Jansen, G., J. G. Komen, and J. B. Thomas. 1950. On the relation between the formation of assimilatory pigments and the rate of photosynthesis in etiolated oat seedlings. Biochim. Biophys. Acta 5: 179–85.
3. Euler, H. von and H. Hellström. 1929. Uber die Bildung von Xanthophyll, Carotin, und Chlorophyll in belichteten und unbelichteten Gerstenkeimlingen. Z. Physiol. Chem. 183: 177–83.

4. GOODWIN, T. W. 1954. Carotinoids: Their Comparative Biochemistry. Chemical Publishing Co., Inc., N. Y. x plus 356 pp.
5. GOODWIN, T. W. AND S. PHAGPOLNGARM. 1960. Studies in carotenogenesis. 28. The effect of illumination on carotenoid synthesis in French-bean (*Phaseolus vulgaris*) seedlings. Biochem. J. 76: 197–99.
6. KARRER, P. AND E. JUCKER. 1950. Carotenoids. Elsevier Publ. Co., Inc., N. Y
7. KAY, R. E. AND B. PHINNEY. 1956. Plastid pigment changes in the early seedling leaves of *Zea mays* L. Plant Physiol. 31: 226–31.
8. KHUDAIRI, A. K. 1961. Xanthium leaf pigments and their inhibition by streptomycin. Biochim. Biophys. Acta. 46: 344–54.
9. LIND, E. F., H. C. LANE, AND L. S. GLEASON. 1953. Partial separation of the plastid pigments by paper chromatography. Plant Physiol. 28: 325–28.
10. MOSTER, J. B. AND F. W. QUACKENBUSH. 1952. The effect of temperature and light on the carotenoids of seedlings grown from 3 corn hybrids. Arch. Biochem. Biophys. 38: 297–303.
11. MOSTER, J. B., F. W. QUACKENBUSH, AND J. W. PORTER. 1952. The carotenoids of corn seedlings. Arch. Biochem. Biophys. 38: 287–96.
12. RÖBBELEN, G. 1957. Untersuchungen an Strahlen-induzierten Blattfarbmutanten von *Arabidopsis thaliana* (L.) Heynh. Z. Induktive Abstammungs-Vererbungslehre 88: 189–252.
13. SAPOZHNIKOV, D. I., Z. M. EIDEL'MAN, N. V. BAZHANOVA, T. G. MASLOVA, AND O. F. POPOVA. 1962. Participation of carotenoids in photosynthesis. Trans. Botan. Inst. Akad. Nauk SSSR, Ser. 4, Eksperim. Botan. 15: 43–52. (Chem. Abstr. 57: 11559, 1962).
14. STRAIN, H. H. 1938. Formation of carotenoids and chlorophylls in etiolated barley seedlings exposed to red light. Plant Physiol. 13: 413–18.
15. STRAIN, H. H. 1938. Leaf xanthophylls. Carnegie Inst. Washington Publ. 490: xi plus 147 pp.
16. YAMAMOTO, H. Y., T. O. M. NAKAYAMA, AND C. O. CHICHESTER. 1962. Studies on the light and dark interconversions of leaf xanthophylls. Arch. Biochem. Biophys. 97: 168–73.

Endogenous Growth Regulation in Carpophores of Agaricus bisporus [1, 2]

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The development of the fruit body or carpophore[3] in many Agaricales can be subdivided into an initial phase of cell multiplication and differentiation, and a phase of elongation marked by maximum stipe growth, by expansion of the pileus (cap) and hymenophore (lamellae), and by sporulation. The major features of mushroom growth were recognized by Schmitz (24) who also discovered that elongation was maximal in the upper stipe portion. Despite detailed descriptive work relatively few quantitative data have been published on carpophore growth. These include growth curves for *Panaeolus retirugis* by Douglas (6), for *Coprinus lagopus* by Borriss (3) and Voderberg (31), and for the cultivated Agaricus by Sacchi (23), Bonner et al. (2), and Hagimoto and Konishi (9). Bonner et al. (2) reported that cell divisions ceased in the Agaricus stipe when the fruit body was less than 2 cm long, and cell elongation was found by Hagimoto and Konishi (9) to parallel roughly the course of carpophore elongation.

The literature contains scattered and contradictory observations concerning the effect of decapitation on growth. Schmitz (24) remarked that very young carpophores of agarics continued growing after removal of a third or even half of the cap, but stopped if the whole cap was removed. According to Gräntz (7) *Coprinus stercorarius* grew normally if decapitated shortly before the onset of rapid elongation. The detached cap or parts of the cap expanded, and even the excised zone of the stipe elongation "in the usual manner". He suggested that the apparent

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[3] Following Singer (26) and others the term carpophore is preferred to sporophore or basidiocarp.