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VIOLAXANTHIN, THE MAJOR CAROTENOID PIGMENT IN *ZEA MAYS* ROOT CAP DURING SEED GERMINATION

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Abstract—Violaxanthin (5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol) was shown to be the major carotenoid in maize root cap during seed germination according to chromatographic and spectroscopic studies. The biosynthesis of this pigment is not influenced by light and the biological significance of this carotenoid in maize root tip is briefly discussed.

INTRODUCTION

Apart from the roots of carrot and sweet potato, which represent fairly good carotenogenic systems, the carotenoid content of roots has not been studied extensively [1]. However the unique apocartotenoid azafrin was shown to be the major pigment in the root of *Escobedia scabrifolia* [2]. Although carotenoids are present only in small amounts in many seeds, maize has larger amounts and has been examined in detail previously [3]. Nevertheless, the carotenoid composition of maize roots has not yet been unambiguously established.

It is clear from experiments with radioactive precursors that the maize root cap synthesizes a slime containing mainly an acidic polysaccharide material with a high proportion of galactose and fucose [4]. The mucilage secretion of the outer root cap cells of *Zea mays* has also been investigated by means of several ultrastructural cytochemical methods [5]. It has been shown that these cells from wheat roots contained hypertrophied Golgi bodies that were involved in the secretion process and probably the synthesis of a slime material that accumulated around the cap [6]. The present paper reports the occurrence of a major yellow pigment in the maize root tip during seed germination.

RESULTS AND DISCUSSION

During the germination of maize seeds, either in the dark or in the light, anatomical changes, associated with a cellular differentiation, occurred along with the formation and elongation of the root. During the very early steps of the germination, a significant yellow colour developed only in the root cap region. As the root continued to grow the distinct yellow colour persisted for several days.

After solvent extraction, the unsaponifiable materials were subjected to column chromatography on an alumina column. The first compound A exhibited absorption maxima in petrol at (425), 448 and 475 nm and was eluted with β-carotene when chromatographed with authentic β-carotene from higher plant leaves. The minor pigment was thus identified as β-carotene. The β-carotene content of maize root tips was as low as 4.30 μg/g dry wt of biological material.

The xanthophyll fraction B was further chromatographed through a MgO–Hyflo Super Cel column. The major pigment B_1 had absorption maxima at 419, 441, 466 and 417, 440, 460 nm in petrol and absolute ethanol, respectively. When a drop of concentrated hydrochloric acid was added to an ethanolic solution of B_1, a large hypsochromic shift of 40 nm was immediately observed which is in good agreement with the existence of a 5,6,5′,6′-diepoxide structure. Consequently the epoxide furanoid rearrangement gave rise to absorption maxima in acidic ethanolic solution at 378, 400 and 426 nm. The strong molecular ion at *m/e* 600 and the mass fragmentation pattern of pigment B_1 were in perfect agreement with those described for violaxanthin [7]. The MS chromatographic behaviour and visible spectra of pigment B_1 are consistent with the structure 5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol. The amount of violaxanthin, which represents the major pigment of maize root tips, was estimated to be about 22 μg/g dry wt corresponding to as much as 84% of the total extracted pigments.

The major compound B_1 exhibited CD signals which were in good agreement with the violaxanthin CD spectra described by Moss *et al.* [8, 9]. At the absorption maxima between 350 and 470 nm, a positive dichroic multiplet was obtained with a slight bathochromic shift of 2–3 nm. At λ (nm) 471, 443, 420, 325 and 267, the Cotton effects Δε + 2.7, +3.2, + 1.4, +2.9 and −14 were respectively obtained. By using standard violaxanthin isolated from rice leaves, the CD spectrum of compound B_1 was confirmed. However the major diepoxide compound from rice leaves exhibited a CD spectrum quite close to that exhibited by violeoxanthin (9-*cis* violaxanthin) previously described by Moss *et al.* In this case, at λ (nm) 468, 439, 415, 391, 328, 268, 260 and 230, the following Δε's were respectively obtained: +10, +12, +5, +2, −2, +5, +3 and −8. The mass fragmentation pattern of the violeoxanthin from rice leaves was in perfect agreement with the fragmentation of 5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol.

The dichroic signals at long wavelengths could be due to the intrinsic asymmetry of the polyene chain. In the crystalline state, X-ray studies have shown that

the polyene chain is not exactly planar but affected by a slight S-shaped bending as in canthaxanthin [10] or amphotericin B [11]. However the observed conformation in the crystalline lattice would not be maintained in solution where aggregative phenomena could interfere [12, 13].

In the *cis*-peak region, the CD signal is more important and with opposite value in violeoxanthin compared to that of the all-*trans* compound. At wavelength 267 nm, violaxanthin absorption is less intense than the violeoxanthin one and the Cotton effect is negative for the all-*trans* compound but positive for the mono-*cis* violeoxanthin. It is to be noted that the slight differences observed between our results and the data of the literature concerning the Cotton effect intensities could be due to concentration or solvents effects.

The minor compound B_2, more strongly absorbed in the two chromatographic systems used, exhibited absorption maxima in ethanol solution at 400, 422 and 446 nm. By acid treatment, a 22 nm hypsochromic shift was observed, characteristic of a mono-5,6-epoxide group. Unfortunately, as B_2 was present only in traces it was impossible to carry out a further chemical structure elucidation.

In conclusion, the carotenoid content of maize root caps was *ca* 30 μg/g dry wt which represents about 10 ng of carotenoid per each young root cap. In view of the enormous quantity of polysaccharides in this root area, it is rather surprising not to have found some glycosidic carotenoids. It still remains difficult to ascertain if the lipid globules described in the literature [5] represent an artefact formed during the treatment of the root-cap cells prior to electron microscopy, or if the xanthophyll compounds exist in loose association with some membranes constituents. In addition, the carotenoid content of the entire root, except the cap, has been estimated. As a first approximation the pigment concentration above the tip is at least fifty times lower than that of the root cap region.

Maize seeds contain β-carotene, cryptoxanthin and zeaxanthin as major pigments but no violaxanthin has been detected even among the minor components [14]. The occurrence of zeaxanthin diepoxide in the root tip raises the question of the epoxidation reaction which does not seem to be affected by light or dark as the maize germination was carried out either in light or in complete darkness. However, the presence of different types of peroxidases in maize root [15] could play a role in the epoxidation of zeaxanthin, though the enzymatic mechanism of carotenoid epoxidation is still unknown. In contrast, with the epoxidation in intact *Chlorella*, where the reaction started immediately after very strong illumination [16], the epoxidation in maize root cap can take place even in complete darkness.

Although purely speculative this time, the xanthophylls present in the root caps may induce some tropism toward some receptive microorganisms of the rhizosphere, perhaps by their products of degradation. However, it seems more likely that these pigments act by their products of transformation as it has been shown that violaxanthin is converted by light to xanthoxin, a new plant growth inhibitor against root elongation [17, 18].

EXPERIMENTAL

Plant materials. Seeds of *Zea mays* var. W 64 A XW 182 E from the Wisconsin Seed Foundation (U.S.A.) were soaked in tap water and germinated under sterile conditions on an Agar Hoagland medium either in the light or in complete darkness at 25° during *ca* 65 hr [19]. Seedlings were taken when their roots were *ca* 3–4 cm long and were sectioned with a razor blade 1.5 mm from the tip to yield mainly the root cap. The rice used (*Oryza sativa*) was a Delta 77 variety from INRA (France).

Extraction and isolation of carotenoid pigments. Fresh root tips were carefully ground in cold Me_2CO–MeOH (7:2) with a Polytron homogenizer. After repeated extractions, the organic solvent extracts were combined and a mixture of petrol (40–60°) and Et_2O (peroxide free and dry) was added. The etheral epiphase was saponified with 10% KOH in EtOH in the dark overnight under argon at room temp. The unsaponifiable material was extracted with petrol–Et_2O (7:2) washed throughly and finally dried over dry Na_2SO_4. All the carotenoid solns were stored under argon at −20° in organic solvent.

Chromatography of carotenoid pigments. A first adsorption column chromatography was carried out by using Al_2O_3 Merck grade II–III according to Brockmann, eluted by increasing amounts of Et_2O in petrol and finally by increasing amounts of MeOH in Et_2O. In order to purify the xanthophyll compounds, obtained after the first alumina column, a second chromatography was carried out using MgO–Hyflo Super Cel (1:1) Johns Manville (U.S.A.) as adsorbent. The elution was achieved by increasing amounts of Me_2CO in petrol under a slight pressure of argon [20].

Identification of carotenoids. Tentative identification of chromatographically resolved carotenoids was on the basis of similarities of electronic spectra and chromatographic behaviour to well-characterized carotenoids isolated from higher plant leaves (*Oryza sativa*). The chemical structure of the major carotenoid component of the maize root caps was elucidated after analysis by MS and CD. The MS were recorded at 12 eV, with an ion source temp. of 250–270° and with the probe heater at the temp. 100–200° The CD spectra were recorded in EtOH. The absorbance of compounds used in CD has been adjusted to a value of 1 ± 0.1 either by dilution or by modifying the optical cell path. The diepoxide function was shown by adding a drop of conc. HCl to an EtOH soln of the carotenoid in a spectrophotometer cuvette and observing the resulting hypsochromic shift [8].

Quantitative estimation of carotenoids. Visible spectrophometry was used to estimate the amounts of carotenoids. The specific extinction coefficient $E^{1\%}_{1\,cm}$ values used were, respectively, 2592 for β-carotene in petrol [21] and 2550 for 5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol (violaxanthin) [22].

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