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ANTHOCYANIN FORMATION IN MAIZE ROOTS

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SUMMARY

Roots of maize seedlings grown under continuous broad spectrum far-red (FR) light accumulated large quantities of anthocyanins (larger than those observed in shoots). Similar results were also obtained under continuous broad spectrum blue (B) light. Excision of the shoot at different ages and subsequent illumination with continuous-FR (c-FR) or continuous-B (c-B) of the 'root + endosperm' system caused an anthocyanin accumulation significantly higher than that observed in roots of intact plants. Intermittent monochromatic red (i-R) (cycles of 2 h) promoted anthocyanin appearance while its action was reversed when monochromatic short FR irradiation followed each R one. The data presented suggests the presence and function of phytochrome in maize roots and seem to be a final negative answer to the controversial contribution of photosynthesis to HIR of anthocyanin accumulation.

INTRODUCTION

Even though the metabolic and physiological role of anthocyanins is still disputable, it is well known that anthocyanin synthesis in a wide range of tissues and plant species is promoted by light [1]. The light promotion has been proved to be mediated by at least two photoreactions: (a) a low energy, R/FR reversible, phytochrome-controlled reaction and (b) a 'high energy' one, most effective at the B and FR spectral regions. The HIR of anthocyanin accumulation has been usually investigated and interpreted either in terms of phytochrome [2,3] or of another, yet unknown, photoreceptor [4]. Fur-

Abbreviations: B, blue; c-, continuous-; D, dark; FR, far-red; HIR, high irradiance response; i-, intermittent-; IP, intact plant; PAL, phenylalanine ammonia-lyase; R, red; R + E, root + endosperm.

thermore, it has been suggested [5,6] that photosynthesis may play a role in the HIR of anthocyanin synthesis of young, dark-grown seedlings, exposed to c-FR irradiation. However much evidence based on experiments with either metabolic inhibitors [7,8] or plant material lacking, genetically or mechanically, photosynthetic capabilities [9] does not support this suggestion.

Roots major non-photosynthetic organs, have not been used up to now as experimental material for a strict study of light-promoted anthocyanin formation, in contrast to young leaves, cotyledons, hypocotyls, stems and fruit cuticles [10]. Development of anthocyanin pigments in roots exposed to daylight has been observed in *Salix* [11,12] and *Zea* [13,14], about a century ago. Recent studies [9,15] have confirmed the appearance of anthocyanins and revealed the presence and function of PAL in maize roots exposed to white light. In this work, further study of anthocyanin formation in maize roots is undertaken, in order to investigate: (a) the mode of light action; (b) the probable contribution of photosynthesis; (c) the possibility of providing an experimental system completely devoid of other pigments.

MATERIALS AND METHODS

Zea mays L. (greek hybrid IΣ 400, harvest 1975) were grown in plastic boxes (8 cm × 8 cm × 6 cm) containing 15 ml deionised H_2O, each. Temperature was kept at 25 ± 1°C throughout all the experimental procedure. Excisions of plant segments took place under dim green safelight, immediately before illumination.

The light sources used were: (a) broad spectrum FR: 10 white incadescent tubes Philips Philinea 6276x, 60 W; 1 red and 2 blue plexiglass filters (Röhm, 501 and 627 respectively, thickness 3 mm); water bath (5 cm); 700—800 nm; $I = 5 \times 10^3$ erg/cm^2/s; (b) broad spectrum B: 8 blue fluorescent tubes Philips TL 20W/18; 1 blue plexiglass filter; 400—500 nm; $I = 2.5 \times 10^2$ erg/cm^2/s;(c) monochromatic R: 662 nm interference filter (Schott, Mainz); $I = 3.5 \times 10^2$ erg/cm^2/s; (d) monochromatic FR: 728 nm interference filter (Schott, Mainz); $I = 2.5 \times 10^2$ erg/cm^2/s.

Anthocyanin absorbance was determined in the extract (final volume 25 ml) of 15 segments extracted with 1% w/v HCl in CH_3OH. Measurements were performed in a C. Zeiss PMQ II spectrophotometer and values given represent the A at 530 nm of the final extract and are means of at least 5 replications; vertical lines (in Figures) and ± numbers (in Tables) are standard errors.

RESULTS

Figure 1 shows the anthocyanin accumulation in maize roots and shoots under c-FR and c-B, respectively. The quantities measured increase with age and after the 3rd day are significantly higher in roots than in shoots. It must

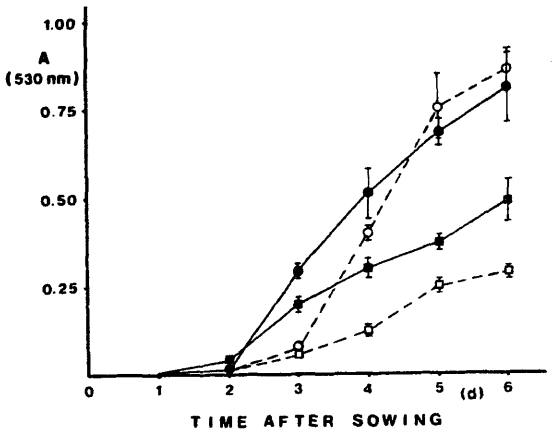

Fig. 1. Anthocyanin amounts in maize seedlings (roots ○,●; shoots □,■) grown under c-FR (solid line) and c-B (broken line).

also be noted that even at the 2nd day (when germination has just been completed and the length of roots and shoots never exceeds 2 cm) a certain amount of anthocyanins is already present.

Reddening generally appears at the outer layer of the cortex of the differentiation zone of both main and adventitious roots as well as in mesocotyl and coleoptile.

In Table I, anthocyanin formation in roots is tested under different light regimes and for a final seedling age of 5 days (selected for practical reasons). Besides intact plants, isolated roots and 'root + endosperm' (R + E) systems are used. Even though excised roots are not capable of considerable growth, a small amount of anthocyanins is formed under c-FR. The R + E system produces more anthocyanins compared to roots from intact plant (IP), under 3dD + 2dFR and 4dD + 1dFR regimes, and its decreased capability at 2dD + 3dFR can be attributed to lower growth potential. When 3dD + 2dB light regime is applied, the difference of root anthocyanins

TABLE I

ANTHOCYANINS IN MAIZE ROOTS UNDER DIFFERENT LIGHT REGIMES

Roots from intact plants isolated roots and ones from 'root + endosperm' systems are used. Numbers given are *A* values.

	5dFR	1dD + 4dFR	2dD + 3dFR	3dD + 2dFR	4dD + 1dFR	5dD
Roots of IP	0.69 ± 0.04	0.66 ± 0.03	1.20 ± 0.11	1.21 ± 0.11	0.65 ± 0.04	0.00
Roots of R + E	—	—	0.65 ± 0.03	1.63 ± 0.11	0.77 ± 0.15	—
Excised roots	—	—	0.01 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	—

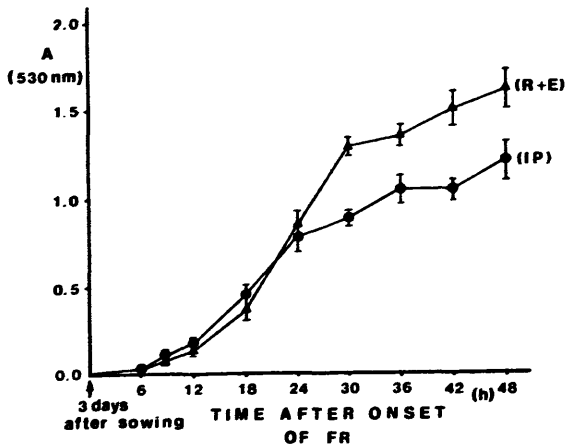

Fig. 2. Time course of anthocyanin accumulation under c-FR in maize roots of IP (•—•) and R + E (▲—▲). Seedlings were grown in darkness for 3 days before illumination.

TABLE II

ANTHOCYANINS IN MAIZE ROOTS UNDER INTERMITTENT IRRADIATION
R + E systems are isolated from dark-grown seedlings (3d) and subsequently treated for 2 days. Each monochromatic irradiation is applied for 5 min and the cycle period is 2 h. Numbers given are *A* values.

Dark controls	0.00
i-R	0.63 ± 0.04
i-FR	0.21 ± 0.04
i-R + FR	0.28 ± 0.05

between R + E and IP is more pronounced (1.71 ± 0.12 and 0.75 ± 0.11 respectively).

Therefore 3dD + 2dFR 'root + endosperm' system is chosen for studying the time-course of anthocyanin accumulation (Fig. 2). The curve obtained resembles a typical one, with a lag phase less than 6 h, a phase of rapid increase and a third one of decreased rate beginning at approx. 30 h. Comparing the kinetics between R + E and IP, it may be concluded that in the latter case appears a substrate deficiency.

Intermittent R or FR can substitute for c-FR or c-B, although the R action is significantly higher (Table II). Its reversal by immediately following FR irradiations permits the interpretation of anthocyanin accumulation in maize roots in terms of phytochrome.

DISCUSSION

The results presented offer a first study of light promoted anthocyanin formation in maize roots. Recent observations with white light [9,15] are now confirmed and extended.

Therefore, the increased anthocyanin amount in R + E system, compared to roots from IP, is due to either an excision effect or, most probably, a competition between root and shoot for substrates provided by the endosperm. The 'root + endosperm + mesocotyl' system used previously [15] seems to be less satisfactory compared to the R + E system, because mesocotyl can photosynthesise and competes also for anthocyanin substrate.

Even though i-FR can promote the formation of anthocyanins (which are never present in dark-grown maize plants), i-R is more effective (Table II), at least for the 2 h cycles used, and its action is reversed, down to the i-FR level, when each R irradiation is followed by a FR one. These observations are in agreement with the conclusion [16] that, in cabbage and mustard, i-R promotes and subsequent FR reverses when the dark interval exceeds 1 h. Concerning the mediation of R and FR light action, it may be concluded that phytochrome is the only photoreceptor acting in the way described by Mancinelli et al. [17,3]. It must be noted that phytochrome is located in the root itself which is the photosensitive organ.

The anthocyanin accumulation in maize roots is a typical HIR, as Fig. 2 shows. The lag phase is less than 6 h [18,19] and the third phase begins after approx. 30 h [20]. Therefore, the R + E system from dark-grown (3d) maize seedlings seems to be a first-class experimental material for anthyocyanin studies. Its advantages are: (a) anthocyanins are never produced in darkness, in contrast to many cruciferous plants; (b) the quantity of anthocyanins in roots of IP, is very large and becomes larger in the R + E system; (c) there is a complete independence from photosynthesis.

Finally, as far as it concerns photosynthetic involvement in HIR of anthocyanin accumulation, we can not help citing Onslow's comment. "The formation of anthocyanin in normally uncoloured roots when exposed to light appears to be the most convincing evidence at hand for the production of anthocyanin due to the direct action of light".

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