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Sunlight-induced anthocyanin pigmentation in maize vegetative tissues

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Abstract

Although, in maize, sunlight-regulated anthocyanin formation in vegetative tissues is observed only in the cultivars harbouring homozygous recessive *pl* loci, the identity of the photoreceptor mediating this process is not yet fully established. In this study the nature of photoreceptor(s) mediating this response was examined using an Indian hybrid maize cultivar (Kanchan-521). The etiolated maize seedlings of this cultivar on exposure to sunlight formed anthocyanin in all vegetative organs. Sunlight elicited photoinduction of anthocyanin with a slow increase between 4–16 h after the sunlight exposure, followed by a rapid increase between 16–24 h. The photoinduction of anthocyanin was primarily mediated by the UV-B component of sunlight and could be elicited by exposure to an artificial UV-B light source. The sunlight-mediated induction of anthocyanin was reduced if the sunlight exposure was terminated with a far-red pulse before transfer to darkness, indicating a coaction of phytochrome in this photoresponse. Exposure to sunlight also stimulated phenylalanine ammonia lyase (PAL) activity in all organs with two temporally separated peaks. The first peak of PAL between 4–12 h was induced by phytochrome, and the second peak of PAL between 12–24 h was induced by UV-B light. These results indicate that the photoinduction of anthocyanin in maize is mediated by a coaction of UV-B light and phytochrome.

Key words: Maize, anthocyanin, UV-B, phytochrome, sunlight.

Introduction

In several plant species anthocyanin production is potentiated by light. Studies on the photoregulation of anthocyanin formation have highlighted that multiple photoreceptors, such as phytochrome, blue/UV-A photoreceptor and UV-B photoreceptor mediate the light action (Beggs and Wellmann, 1985; Beggs *et al*., 1986). It is assumed that these photoreceptors alter the expression of the anthocyanin regulatory and structural genes to induce the accumulation of anthocyanin. The extensive genetic analysis conducted, particularly in maize, have shown that anthocyanin formation is regulated by the action of more than 20 genes, which govern the temporal and spatial regulation of anthocyanin formation in different organs (Coe *et al*., 1988; Dooner *et al*., 1991). Several genes encoding either the structural genes or the regulatory genes of the anthocyanin pathway have been cloned (Cone, 1994) and their expression pattern have been determined during development of maize plants (Dooner *et al*., 1991; Holton and Cornish, 1995).

In maize, genetic analysis revealed that anthocyanin formation is primarily regulated by genes such as the *R* gene and its variant and also *B*, *C1* and *Pl* genes (Coe, 1994). These genes code for transcriptional activators which show homology with *myb* and *myc* oncogenes (Consonni *et al*., 1993; Goff *et al*., 1992). The transformation of tobacco and *Arabidopsis* with the maize *R* gene increased the pigmentation showing that this gene can also regulate anthocyanin biosynthesis in other species, emphasizing that the above gene may bear homology with similar genes from dicot species (Lloyd *et al*., 1992).

It has been observed that several maize cultivars show a 'sun-red' phenotype, where exposure to sunlight induces the accumulation of anthocyanin in the vegetative tissues (Coe *et al*., 1988). Using inbred varieties of maize and genetic segregation analysis, it has been shown that the sun-red phenotype appears only in those plants which bear recessive *pl* gene loci in the homologous state (Coe, 1994). In these plants, exposure to sunlight stimulates the

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Abbreviations: WG, sunlight filtered through window glass; RL, red light; FR, far-red light; BL, blue light; PAL, phenylalanine ammonia lyase.

production of the *pl* mRNA and protein, which in turn induces the formation of anthocyanin (Cone *et al.*, 1993). Since it is known that the dominant allele *Pl* bypasses the requirement for light in the induction of the anthocyanin, it is assumed that the product of *Pl*, a regulatory gene, may substitute for light for transcription of anthocyanin biosynthesis genes. In maize, it has also been shown that the photoinduced anthocyanin formation is regulated by the action of the *R* gene (Taylor and Briggs, 1990; Tonelli *et al.*, 1994), where high fluence of white light enhances the expression of alleles of *R* genes.

Although an extensive knowledge has been gathered about the molecular–genetic regulation of anthocyanin in maize seedlings, information about the photoreceptors controlling sunlight-dependent anthocyanin formation is limited. In the present study, the sunlight-mediated photoinduction of anthocyanin in different organs of hybrid maize seedlings (cultivar Kanchan-521) was examined. It is shown that the above photoinduction of anthocyanin is mediated by a coaction of the UV-B photoreceptor and phytochrome. Moreover, each of these photoreceptors regulates a specific phase of phenylalanine ammonia lyase (PAL) biosynthesis.

Materials and methods

Plant material

Maize seeds (*Zea mays* L.) of hybrid cultivar Kanchan-521 were obtained from Andhra Pradesh State Seed Development Corporation, Hyderabad. Though its genotype is not disclosed by the supplier, the above cultivar was selected among locally available cultivars for maximal induction of anthocyanin under sunlight. Maize seeds were first soaked in distilled water for 12 h, and then were sown on four layers of moist germination paper. Seeds were sown on germination paper with the embryo side down and were watered with distilled water. Seedlings were grown in black cardboard boxes in a darkroom at 25 ± 1 °C. An examination of the effect of age on the photoinduction of anthocyanin revealed that 6-d-old etiolated seedlings were most photoresponsive; therefore, all experiments were conducted with 6-d-old seedlings. Seedlings were exposed to different lights for varied durations before being returned to darkness. The estimation of anthocyanin and phenylalanine ammonia lyase (PAL) activity was done after the indicated intervals of darkness. Each experiment was repeated 3–5 times independently and the mean and SE values were determined.

Light treatments

Six-d-old etiolated seedlings were exposed to the midday sun (1100 h–1500 h) by opening the boxes in the sunlight (*c.* 2800 μmol m^{-2} s^{-1}). Sunlight free of UV-B (>320 nm, 2600 μmol m^{-2} s^{-1}) was obtained by filtering the sunlight through a 4 mm thick window glass plate (Klein, 1979). The experiments were conducted on different days and therefore the total sunlight quanta received by seedlings were not uniform. The temperature of the box was maintained at 25 °C. Blue light (λ_{max} 450 nm, 1.0 μmol m^{-2} s^{-1}) was obtained by passing the output of two cool-white fluorescent tubelights through a blue plastic filter (Manga and Sharma, 1988). The red light (λ_{max} 650 nm, 2.8 μmol m^{-2} s^{-1}) was obtained using a set-up similar to that for blue light but using a red plastic sheet. The long wavelength far-red light (FR) (λ_{max} 756 nm, 16 μmol m^{-2} s^{-1}) was obtained by passing light from a halogen lamp (150 W) through a Schott interference filter. White light (WL) (100 μmol m^{-2} s^{-1}) was obtained by using four cool-white tubelights. The UV-B (0.6 W m^{-2} s^{-1}) source consisted of two Phillips UV-B tubelights (TL40/12 Holland). The photon fluence rate of light was measured by using a SKY quantum photometer (UK).

To find out the duration of sunlight exposure needed to elicit maximum anthocyanin induction, the 6-d-old etiolated seedlings were irradiated with different durations of sunlight or other artificial light and were then transferred to the darkness for 24 h. At that time point the seedlings were harvested and excised into different organs for the determination of anthocyanin (Fig. 1). For all other experiments the 6-d-old seedlings were exposed either to 4 h of sunlight or to other artificial light and then returned to the darkness. The time point of transfer to the darkness was taken as zero time for conducting time-course analysis. For time-course analysis, the seedlings were harvested at different time intervals after transfer to darkness for the estimation of anthocyanin or phenylalanine ammonia lyase activity.

Estimation of anthocyanin

For anthocyanin estimation maize seedlings were excised into root, mesocotyl, coleoptile, and first leaf. Anthocyanins was extracted by submerging the organs in 3 ml of acidified (1% HCl, v/v) methanol for 24 h at 4 °C with continual shaking. A Folsch partitioning was performed to remove chlorophyll and other contaminating pigments. To 2 ml of the anthocyanin extract, 1.5 ml of water and 2.5 ml of chloroform were added and centrifuged at 1600 *g* for 20 min at 4 °C. After centrifugation, the anthocyanin content in the supernatant was estimated by measuring absorbance at 535 nm (Adamse, 1988).

Phenylalanine ammonia lyase assay

Maize seedlings were excised into root, mesocotyl, coleoptile, and first leaf. The excised organs were homogenized at 4 °C in a pre-cooled mortar and pestle with 200 mg of sea sand and 150 mg of polyvinylpolypyrrolidone in 3 ml of 0.1 M borate buffer (pH 8.8) containing 50 mM 2-mercaptoethanol. The homogenate was centrifuged at 18 200 *g* for 30 min at 4 °C and the supernatant was applied to a Sephadex G-25 column equilibrated with 0.1 M borate buffer (pH 8.8). The fractions constituting void volume were pooled together and were used for assay. The PAL assay was performed at 25 °C in an assay mixture consisting of 1 ml of enzyme extract and 0.5 ml of 50 mM L-phenylalanine. The PAL activity was assayed by monitoring the increase in A_{290} against a control without phenylalanine over a period of 4 h at 1 h intervals. The rate of appearance of *trans*-cinammic acid was taken as a measure of enzyme activity using an increase of 0.01 A_{290} equal to 3.09 nmol of *trans*-cinammic acid formed (Saunders and McClure, 1975). The PAL activity is expressed in nkat (nmol *trans*-cinammic acid formed s^{-1}) g^{-1} of tissue.

Results

In etiolated maize seedlings transferred to sunlight, the photoinduction of anthocyanin was observed in all the organs, whereas seedlings grown in total darkness completely lack anthocyanin. Since sunlight exposure activ-

ates every photoreceptor such as UV-B, UV-A/blue and phytochrome, it is difficult to ascribe sunlight-induced anthocyanin formation to a particular photoreceptor. In view of this, the relative effectiveness of different wavebands of lights to induce anthocyanin was compared with that of sunlight. Figure 1A shows that sunlight was most effective in inducing anthocyanin accumulation in roots, and 4 h of sunlight exposure followed by 24 h darkness elicited the maximal induction of anthocyanin in the maize seedlings. By contrast, the seedlings exposed to sunlight filtered through window glass (WG) showed much lower induction of anthocyanin. Using the artificial light sources such as WL, RL and BL only a marginal induction of anthocyanin accumulation was elicited. Sunlight was also found to be most effective in inducing anthocyanin production in mesocotyl (Fig. 1B). In coleoptiles too, sunlight induced maximal production of anthocyanin (Fig. 1C). In both mesocotyl and coleoptile other lights such as WL, WG, RL, and BL, all showed only a marginal effect on anthocyanin production. However in leaf, sunlight, WL, and RL were equally effective in promoting anthocyanin formation for first 3 h (Fig. 1D). In the root, mesocotyl and coleoptile, the higher induction of anthocyanin in sunlight-exposed organs was observed only when the exposure to sunlight was longer than 2 h, whereas in the leaf, sunlight exposure of longer than 3 h showed a higher photoinduction of anthocyanin.

The observation that sunlight filtered through a window glass is less effective than sunlight indicates that the observed sunlight effect is likely caused by UV-B photoreceptor. This is based on the fact that 4 mm thick glass completely cuts off UV-B light (Klein, 1979), and filtered sunlight induces the severely reduced formation of anthocyanin. This view was confirmed by the direct irradiation of etiolated seedlings with artificial UV-B source, which also stimulated strong induction of anthocyanin (Fig. 2), in a fashion similar to that observed under direct sunlight. However, the relative induction of anthocyanin under direct irradiation with UV-B light was lower than sunlight. It is likely that, in addition to UV-B, an additional photoreceptor may contribute to anthocyanin induction in sunlight, resulting in higher induction under the sunlight. In many species, the UV-B effect needs a coaction and/or potentiation by phytochrome, therefore, the contribution of phytochrome on UV-B-induced anthocyanin was ascertained by irradiating seedlings exposed to sunlight with a short duration of R or FR light before transfer to darkness. In roots, sunlight exposure followed by a brief exposure to artificial weak red light before transfer to darkness reduced anthocyanin level by 28%. In comparison, a brief FR exposure after sunlight inhibited anthocyanin induction by 80% (Fig. 3A). Following FR exposure with 15 min RL reversed the loss in level of anthocyanin induction, but only to 60% of sunlight level. Interestingly, following FR exposure with a 45 min RL exposure recovered anthocyanin level nearly to the sunlight control level. Figure 3B and C show the effect of RL and FR on sunlight-induced anthocyanin production in mesocotyl and coleoptile. The response of mesocotyl and coleoptile to various combinations of light was qualitatively similar to that of roots. In these organs too, FR strongly inhibited sunlight-induced anthocyanin production. In the leaf, FR was found to be less effective in reversing the sunlight effect as seen for root or mesocotyl (Fig. 3D).

The time-course of anthocyanin induction was compared in different organs of maize seedlings after a 4 h of

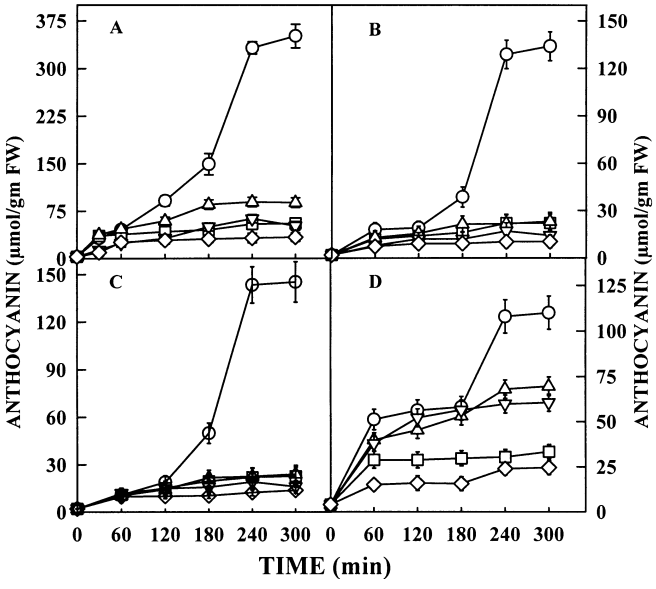

Fig. 1. Effect of different wavebands of light on anthocyanin accumulation in maize seedlings. Six-d-old dark-grown seedlings were exposed to sunlight (○), RL (▽), WL (△), BL (◇), and WG (□) for different durations indicated on the abscissa and transferred back to darkness. Anthocyanin level was measured in different organs of the maize seedlings at 24 h after the end of light treatment. Root (A), mesocotyl (B), coleoptile (C), and leaf (D).

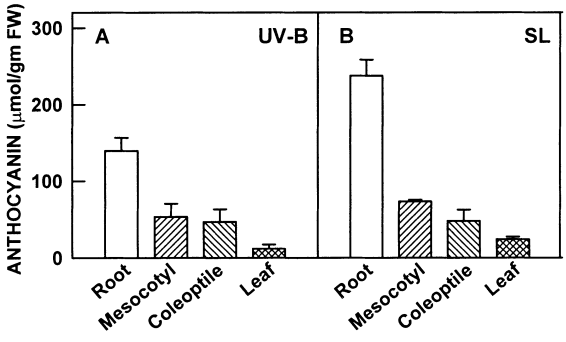

Fig. 2. Effect of UV-B (A) and sunlight (B) on induced anthocyanin accumulation in maize seedlings. Six-d-old dark grown seedlings were exposed to either UV-B light or sunlight (SL) for 4 h and transferred back to darkness. Anthocyanin level was measured in different organs of the maize seedlings at 24 h after the end of light treatment.

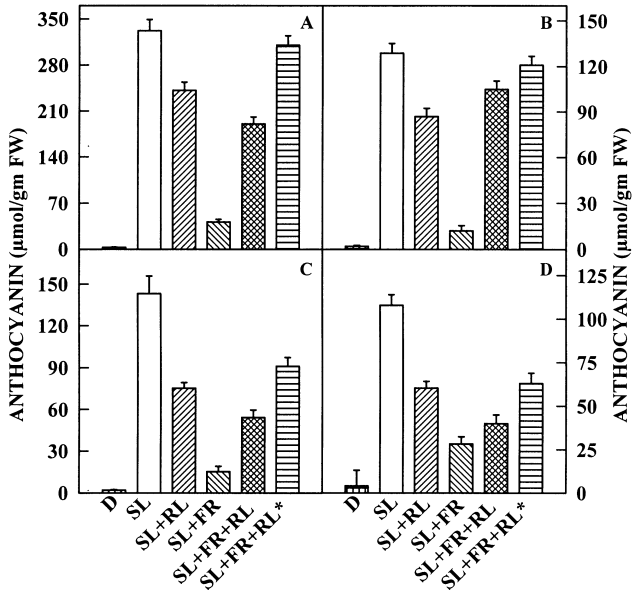

Fig. 3. Photoreversion of sunlight-induced anthocyanin accumulation by RL and FR in maize seedlings. Six-d-old dark grown seedlings were exposed to sunlight (SL) for 4 h. At the end of sunlight treatment, seedlings were irradiated with 15 min of RL or FR and transferred back to darkness. In case of SL + FR + RL*, after 15 min of FR treatment seedlings were exposed to 45 min of RL. Anthocyanin level was measured in different organs of maize seedling at 24 h from the end of light treatments. Root (A), mesocotyl (B), coleoptile (C), and leaf (D).

sunlight exposure and transfer to the darkness. Figure 4 shows that in the above seedlings the maximal rise in the photoinduced anthocyanin level was observed in the root followed by the coleoptile, mesocotyl and leaf in decreasing order. In the root and in the mesocotyl 4 h of sunlight exposure induced a slow rise in anthocyanin level up to 16 h, followed by a rapid increase in the anthocyanin level. Similarly, in the coleoptile, a rapid increase in anthocyanin was observed, but only after 20 h. The

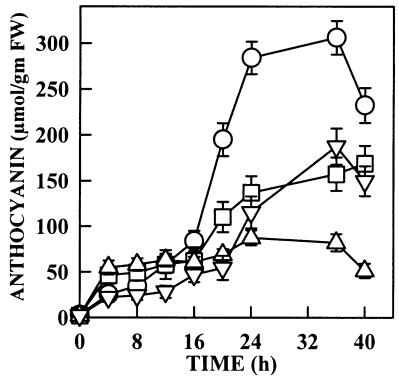

Fig. 4. Time-course of anthocyanin accumulation in various organs of the maize seedling. Six-d-old dark-grown seedlings were exposed to sunlight for 4 h and transferred back to darkness. The dark-transferred seedlings were harvested at the time intervals indicated on the abscissa and anthocyanin level was estimated in different organs of maize seedlings. Root (○), mesocotyl (□), coleoptile (△), and leaf (▽).

photoinduced increase in the anthocyanin content in the leaf was nearly 5-fold less in comparison to that in roots. In the root, mesocotyl and leaf, maximal anthocyanin induction reached a peak level at 24 h, whereas in the coleoptile, the increase in anthocyanin level continued up to 36 h after transfer to darkness.

In order to determine if the sunlight-mediated induction of anthocyanin is associated with an increase in the enzymes of phenylpropanoid metabolism, the time-course of phenylalanine ammonia lyase accumulation was examined in seedlings transferred to darkness after 4 h of sunlight exposure. Figure 5A shows that in sunlight-treated roots, two distinct peaks of PAL activity are observed at 8 h and at 20 h, respectively. In comparison, light other than sunlight induced only the first peak of PAL activity. Thereafter, the activity declined and reached the dark control level by 12 h in RL- and BL-exposed seedlings. At the same time, the decline in PAL level in WG-exposed seedlings was slow and reached dark control level only after 24. Similarly, in the mesocotyl and coleoptiles (Fig. 5B, C) of sunlight-exposed seedlings photoinduction of PAL also showed two peaks at 8 h and at 20 h, respectively. In contrast, the BL-, WG-, and RL-exposed seedlings shown only the first peak of PAL activity, and thereafter PAL activity declined to control level after attaining peak. Figure 5D shows that, in the leaf of sunlight-exposed seedlings, PAL induction showed two peaks at 8 h and 16 h, respectively, whereas under

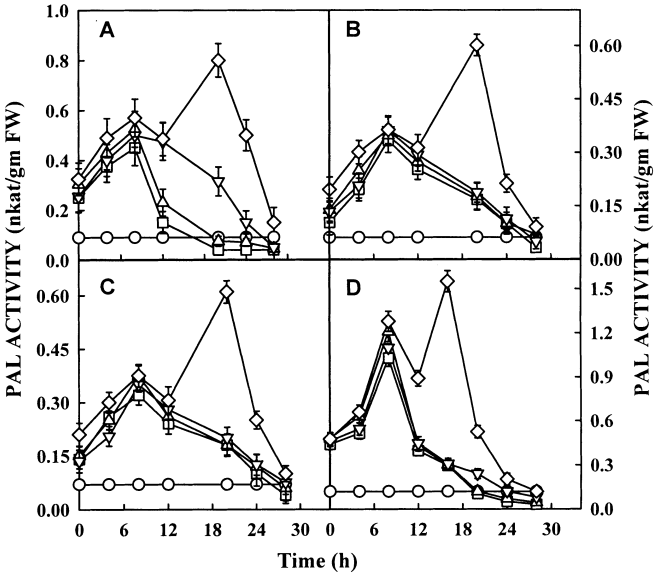

Fig. 5. Time-course of PAL activity in different organs of maize seedlings in response to different light treatments. Six-d-old dark-grown seedlings were exposed to various light treatments [sunlight (◇), RL (△), WG (▽), and BL (□)] for 4 h and transferred back to darkness. The dark-transferred seedlings were harvested at the time intervals indicated on the abscissa and PAL level was estimated in different organs of maize seedlings. A set of dark-grown seedlings (○) which was not exposed to light was used as control. Root (A), mesocotyl (B), coleoptile (C), and leaf (D).

other light such as RL, WG and BL only the first peak at 8 h is observed. Though the second peak of PAL appears in the leaf and other organs at 16 h and 20 h respectively, it is plausible that the actual peak of PAL may be between 16–20 h in all organs. Interestingly, although the leaf makes the least amount of anthocyanin the level of PAL activity in the leaf was about 2-fold higher than in the root.

Discussion

The results obtained show that the sun-red phenotype of maize seedlings is probably caused by the activation of a UV-B specific photoreceptor under sunlight. This notion is supported by the observation that only direct sunlight exposure induces a significant amount of anthocyanin, whereas sunlight filtered through glass (WG) is comparatively ineffective. The large difference between the levels of anthocyanin in sunlight- and WG-exposed seedlings indicates that the UV-B component of sunlight plays a major role in anthocyanin induction. Moreover, the irradiation of seedlings with artificial UV-B light also elicits strong induction of anthocyanin similar to the sunlight. However, anthocyanin induction in maize seedlings can not be solely ascribed to the UV-B component of sunlight, as a terminal FR exposure reduces the anthocyanin level in sunlight-exposed seedlings. It is therefore evident that phytochrome plays a modulatory role in anthocyanin induction as well. It can be assumed that while the UV-B photoreceptor plays a major role in anthocyanin induction, it also requires a coaction of phytochrome. Intriguingly, an exposure to a weak artificial red light source also reduces the sunlight-mediated response. However, it is now known that phytochromes are encoded by a multi-gene family, therefore it is likely that sunlight may activate a high fluence-dependent phytochrome species, whose level is diminished by weak red light exposure. Nevertheless, the results obtained in this study indicate that full expression of UV-B-mediated anthocyanin induction needs the coaction of phytochrome.

In several higher plants UV-B action is seen only in the presence of active phytochrome suggesting a coaction between the two photoreceptors (Beggs *et al*., 1986; Drumm-Herrel and Mohr, 1981). For example, in parsley cell suspensions, the UV-induced flavonoid level was reduced by a subsequent FR pulse (Duell-Pfaff and Wellmann, 1982), whereas in sorghum FR completely reversed the UV-induced anthocyanin (Oelmüller and Mohr, 1985). Similarly, in rice seedlings, UV-B-induced anthocyanin production was inhibited when phytochrome was converted to the Pr form at the end of sunlight exposure (Reddy *et al*., 1994).

Although in many plants anthocyanin induction is light dependent, in maize it is not obligatorily dependent on light. In maize, where the genetics of anthocyanin induction has been extensively studied, anthocyanin formation involves the interplay of many regulatory and structural genes (Dooner *et al*., 1991; Holton and Cornish, 1995; Coe, 1994). Maize seedlings become photoresponsive only when the *Pl* gene is mutated and is in the homozygous state (*pl*,*pl*) (Cone *et al*., 1993; Coe, 1994). Studies on the regulatory role of the *Pl* gene in the photocontrol of anthocyanin pigmentation in maize seedling have led to the classification of *Pl* alleles phenotypically into two categories. One, represented by the dominant *Pl* allele, confers the light-independent formation of anthocyanin in vegetative and floral organs, and the second, the recessive *pl* allele, leads to light-dependent anthocyanin synthesis (Cone *et al*., 1993). In maize seedlings, the dominant effect of sunlight in comparison to other light becomes apparent only when sunlight exposure exceeds 2 h. Evidently in maize, anthocyanin induction is enhanced only when a certain threshold level of light exposure is surpassed. It has been proposed that the light-dependent pigmentation in *pl* plants is the result of a threshold effect (Cone *et al*., 1993), where light is needed to boost the *pl* mRNA above a crucial level to generate sufficient PL protein. The accumulation of this PL protein then activates the transcription of anthocyanin biosynthetic genes.

Although in this work it is implicitly assumed that a recessive gene of *pl* is the sole requirement for light-dependent anthocyanin induction, the currently available results also point to other alternatives. For example, it has been observed that in dominant *Pl* seedlings, light-induced accumulation of the *R* product is essential for light-dependent anthocyanin induction (Taylor and Briggs, 1990). Similarly, Dooner and his co-workers observed that if the developing ears of *B-S*, *Pl-Rh* are wrapped in aluminium foil, the cob develops only a little anthocyanin (cited in Cone *et al*., 1993). This observation strongly suggests that light is needed for anthocyanin production even in plants containing the *Pl-Rh* allele, which is presumed to be light independent. Even for the dominant *C1* locus, a requirement for light has been shown by shielding experiments. When Dooner (cited in Cone *et al*., 1993) excluded light from the *C1* ear by wrapping it in aluminium foil, the resulting seeds contained a colourless aleurone in spite of the dominant *C1* allele. In contrast, on exposing freshly harvested ears to light, anthocyanin accumulated in kernels exposed to light.

The photoinduced accumulation of flavonoids is preceded by the induction of several enzymes involved in phenylpropanoid metabolism (Hahlbrock and Scheel, 1989). Photoregulation of enzymes involved in anthocyanin and other flavonoids biosynthesis including PAL, chalcone synthase, chalcone isomerase, and dihydroflavonol reductase have been studied in detail in many systems (Beggs *et al*., 1986; Kubashek *et al*., 1992). In

maize, sunlight triggered the photoinduction of PAL with two distinct peaks in all the organs of seedlings. It is likely that the first PAL peak is induced by phytochrome, because this peak is seen only in seedlings irradiated with RL, WG or BL. By contrast, the second PAL peak at 20 h seems to be induced specifically by UV-B light because it is completely missing in seedlings irradiated with WG. The observed results are reminiscent of PAL induction in rice, where sunlight also induces two peaks of PAL activity in shoots (Reddy *et al*., 1994).

The molecular events leading to the biphasic PAL induction profile under sunlight can only be speculated upon at the moment. It is possible that two peaks of PAL activity arise by the stimulation of two different genes of PAL by phytochrome and UV-B photoreceptor on different temporal scales. Since PAL is encoded by a small multi-gene family in *Arabidopsis* (Wanner *et al*., 1995) and rice (Minami *et al*., 1989), it is likely that maize PAL may also be encoded by a multi-gene family. It is possible that the individual members of the PAL gene family respond differentially to photoinduction leading to the biphasic appearance of PAL. For example, in *Arabidopsis*, transcripts encoding PAL and other enzymes of the flavonoid biosynthetic pathway are induced independently by three photoreceptors, namely, phytochrome, blue/UV-A photoreceptor and UV-B photoreceptor in a temporally determined fashion (Kubashek *et al*., 1992). The UV-B-induced PAL accumulation was similar to cold stress-treated maize seedlings, where anthocyanin induction was preceded by the modulation of *pal* transcript levels, which after onset of cold stress dramatically increased between 12–24 h and declined to pretreatment levels when the seedlings were returned to 25 °C (Christie *et al*., 1994). Interestingly, the transcripts homologous to two regulatory (*R*, *C1*) and three structural (*A1*, *A2*, and *Bz2*) anthocyanin genes also increased at least 7–10-fold during cold treatment, exhibiting similar kinetics of accumulation as observed for *pal*. It is therefore plausible that UV-B induced PAL enzyme may play a role in anthocyanin formation in maize seedlings.

Interestingly, the leaf possesses a higher PAL level when compared with the roots, but the same treatment results in the least amount of anthocyanin when compared with roots or mesocotyl and coleoptile. It is considered that, in the leaf, most of the precursors generated by PAL are diverted into other biosynthetic pathways leading to synthesis of other flavonoids. The reason for the higher production of anthocyanin in roots is not known but can be speculated. It is likely that in green tissues, other pigments such as flavonoids may provide an additional screen for UV-B radiation, and the root may offset this by producing more anthocyanin. The accumulation of anthocyanin may be of advantage to plants as anthocyanin can protect the growing meristematic zones from likely genetic damage caused by UV-radiation (Stapleton and Walbot, 1994). The studies comparing the role of anthocyanin and the DNA repair system as a protection against UV-B radiation have shown anthocyanin production to be the predominant mechanism of protection in the young plant (Hays and Pang, 1994). Studies on mutants in *A. thaliana* have shown that flavonoid-deficient mutants are more sensitive to UV-B damage (Li *et al*., 1993). Similarly, evidence has been presented that anthocyanin in maize seedlings reduces DNA damage by reducing the level of dimer formation (Stapleton and Walbot, 1994). The present study shows that maize roots, on exposure to UV-B light, accumulates a higher anthocyanin level, which may perhaps be defence response against UV-B radiation. However, more work is needed to examine this aspect.

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