Movement of chloroplasts in mesophyll cells of *Garcinia indica* in response to UV-B radiation

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The effect of UV-B radiation (280–320 nm) alone with reference to photosynthesis was studied in *Garcinia indica*. One year-old glasshouse-grown plants were transferred to a growth chamber fitted with UV-B source. Plants were exposed to 1 mW/sq. cm of UV-B radiation for three days. Although photosynthesis was not completely inhibited due to UV-B treatment, it declined by nearly 74% compared to glasshouse-grown plants. In other words, photosynthesis was being carried out, albeit at a much lesser level. Horizontal arrangement of chloroplasts in all layers of mesophyll cells was observed in treated leaves. Spectroscopic measurements showed that plants accumulated flavone with λ_{max} at 230 nm, which was seen to be at the cost of a decrease in another flavone with λ_{max} at 215 nm. Paper chromatographic analysis also showed accumulation of flavone and anthocyanin in treated plants compared to control. It is proposed that UV-B radiation (280–320 nm) may act as a signal for chloroplast movement under *in vivo* conditions. Whether the flavone with λ_{max} at 230 nm has any cause and effect relationship with respect to UV-B-induced phototropic movement of chloroplasts in the mesophyll cells, remains to be investigated.

Keywords: Chloroplasts, *Garcinia indica*, mesophyll cells, UV-B radiation.

CHLOROPLASTS are organelles in plant cells functioning as factories producing organic substances and oxygen through photosynthesis. To obtain higher efficiency of photosynthesis or to avoid photodamage, chloroplasts change their location along the cell surface, adjacent to the cell wall. Chloroplasts move up to the periclinal walls of palisade cells when the incident light is not strong, i.e. a low fluence rate (LFR) response. Under strong light conditions, however, chloroplasts move to the anticlinal walls to avoid photodamage due to light, and this is referred to as high fluence rate (HFR) response[1]. Chloroplast accumulation response has been thought to function to maximize light absorption in order to optimize photosynthesis[2].

The exact biochemical identity of the sensory pigments responsible for blue light-induced chloroplast relocation

was considered a major unsolved problem in plant biology until recently. However, the scenario has changed rather dramatically in the last nearly five years. Initially, to elucidate the nature of the photoreceptor(s) involved, like other blue light-induced responses, action spectra for blue light-induced chloroplast movement were obtained and compared with the absorption spectra of known compounds[3,4]. This strategy did not yield conclusive results, probably because the absorption of pure chromophores and that of chromophores bound to intracellular substances is not necessarily the same; the absorption spectrum of a compound can dramatically change by its micro-environment. However, action spectra for LFR and HFR in various plants were found to be similar to the absorption spectrum of a flavin[5]. It is only recently that unequivocal evidence for the blue light photoreceptors involved in regulating chloroplast movement has emerged[2,6–8]. It has been established that phototropins, PHOT1 and PHOT2, are the photoreceptors responsible for chloroplast accumulation, while PHOT2 alone controls chloroplast avoidance response. The chloroplast accumulation/avoidance response can be separated into three parts; photoperception, signal transfer from photoreceptor to chloroplasts, and movement of the chloroplasts[9].

Although the photoreceptors for blue light-induced chloroplast movements have been identified, little is known about the underlying photochemical events and the signalling components. Why the phototropins harbour two chromophores, how they orchestrate the signalling networks, and how these blue light receptors crosstalk among themselves to eventually realize the response to blue and UV-A radiation, remains to be unravelled[3,10]. The precise mechanisms of chloroplast movement during LFR and HFR also need to be unravelled.

In this study, we show that irradiation of seedlings of *Garcinia indica* to UV-B radiation (280–320 nm) with λ_{max} at 312 nm, results in chloroplast accumulation movement (LFR movement). Accumulation of flavonoid (flavone) with λ_{max} at 230 nm as well as anthocyanin seems to be associated, directly or indirectly, with the LFR movement of chloroplasts.

The seedlings were shifted from a glasshouse (light intensity, 250 µmol/sq. m/s) and treated with UV-B radiation (280–320 nm) with λ_{max} at 312 nm (Vilbour-Lourmat, France, T-6M having filters to cut-off UV-A and UV-C radiations), in a plant growth chamber with no stray light, maintained at a day–night temperature of $28 \pm 2°C$ and 80% RH. Plants were exposed to the radiation for 6 h (10:00–16:00 h) daily for three days. A set of plants was kept under total darkness at room temperature for the same duration.

Chlorophyll fluorescence was measured to determine the photosynthetic efficiency, using a pulse amplitude modulation fluorometer (PAM 101, Walz, Effelrich, Germany) as described by Sharma *et al.*[11]. The leaves were dark-adapted for 10 min and then exposed to a modu-

lated light with an intensity of 4 μmol/sq. m/s to measure initial fluorescence (Fo). This was followed by exposure to a saturating pulse of white light of 4000 μmol/sq. m/s to provide the maximum fluorescence (Fm). The variable fluorescence (Fv = Fm – Fo) and the Fv/Fm ratio were calculated.

Hand sections were taken and stained with saffranine for 2–3 min and observed using a light microscope with photographic facility (Nikon, Eclipse E800).

Plant material was weighed (2 g leaves) and ground finely using 5% methanolic HCl. (The concentration of HCl was increased to 5% in order to completely denature the pigments.) The mixture was then incubated in a water bath for 30 min and ground again. The final volume was made to 25 ml. The mixture was then homogenized using a motorized glass homogenizer and kept for extraction overnight. It was then centrifuged at 8000 g for 20 min. The supernatant was used for spectrophotometric and chromatographic studies.

Paper chromatography of phenolic compounds was carried out according to Sharma et al.[11]. A 25 μl aliquot of the extract was loaded on Whatman No. 1 filter paper and developed in n-butanol, acetic acid and water (6 : 1 : 2). Bands were visualized under an UV transilluminator after treating the chromatogram with or without ammonia fumes. The compounds were identified according to Swain[12] based on their Rf value and change in spot colour with and without ammonia fumes under UV light.

Spectroscopic observation of the methanolic-HCl extract of the plant tissue was also taken in the range 190–700 nm to further characterize the nature of the phenolic compounds. Measurements were taken using double beam spectrophotometer (model UV-2450, Shimadzu Corporation, Japan).

The rate of photosynthesis measured as the Fv/Fm ratio, an indicator of efficiency of PS II, decreased considerably (74%) in plants exposed to UV-B radiation compared to plants grown under normal light (Figure 1). Although
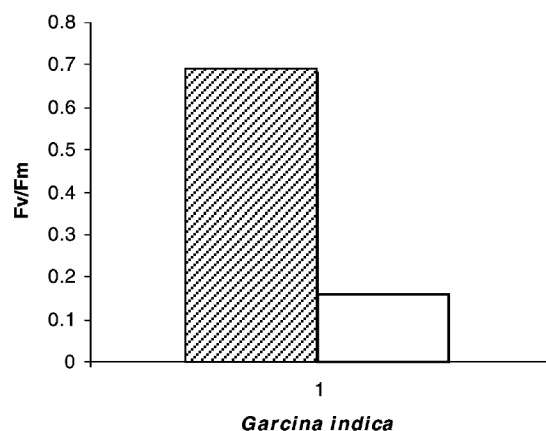
photosynthesis takes place under PAR (400–700 nm) in higher plants, the results of the present study indicate that UV-B radiation (280–320 nm) could also stimulate photosynthesis, albeit at a much lower efficiency. There have been some reports to suggest that UV-B radiation, in the absence of visual radiation, could stimulate photosynthesis to a limited extent[13].

The section of UV-B-exposed leaf showed more or less same thickness of the cuticle. However, in UV-B-exposed leaves there was slightly more accumulation of anthocyanin in the epidermal cells (Figure 2). Figure 2 shows clear accumulation movement of chloroplasts (LFR). The LFR movement of chloroplasts observed in this study under exclusive UV-B radiation might be an adaptation strategy of the plants, whereby the chloroplasts arrange themselves in an orientation to harvest the maximum possible visible light as and when it becomes available, or to absorb UV-B to carry out photosynthesis, by an unknown mechanism, to probably sustain itself on short-term basis. However, the former possibility seems more plausible as plants under normal conditions protect themselves from harmful UV-B radiation by increasing cuticle formation, in order to reflect the UV-B radiation, as well as synthesis of phenolic compounds to absorb the UV-B radiation to protect the internal sensitive tissues and macromolecules like DNA and proteins[14,15]. However, in this study, no significant increase in the thickness of the cuticle could be observed. The slight increase in anthocyanin in epidermal cells observed here may be to prevent oxidative damage as anthocyanin can quench oxygen radicals[16], or to absorb higher dosages of subsequent UV-B radiation[14]. UV-B is known to cause oxidative damage to plants[17].

In this study, we have observed accumulation of flavone and anthocyanin in plants grown under UV-B radiation. A spectral scan of the UV-B-exposed leaf extract showed an increase at 230 nm and a decrease at 215 nm, but the opposite effect was seen in leaf extracts of plants grown under normal light in the glasshouse (Figure 3). This increase in the flavone compound with λ_{max} at 230 nm, at the expense of the compound with λ_{max} at 215 nm, may act as a signal for chloroplast accumulation movement or could well be elicited in parallel, independent of chloroplast response.

Paper chromatographic analysis showed an increase in anthocyanins (Rf 22 and Rf 39) in UV-B-exposed leaf, and also synthesis of another derivative of anthocyanin (Rf 59), not seen in the normal leaf extract (Figure 4). Flavonol (Rf 90) seen in normal leaf extract was not seen in UV-B-exposed leaf extracts.

Accumulation of these phenolic compounds (flavone and anthocyanin) cannot be in order to screen the UV-B radiation, as the movement of chloroplast indicates an attempt to utilize all the incorporated light, which in this case is only UV-B radiation. It is probable that flavone alone, or in combination with anthocyanin, plays a role or is associated with chloroplast movement. We have observed an
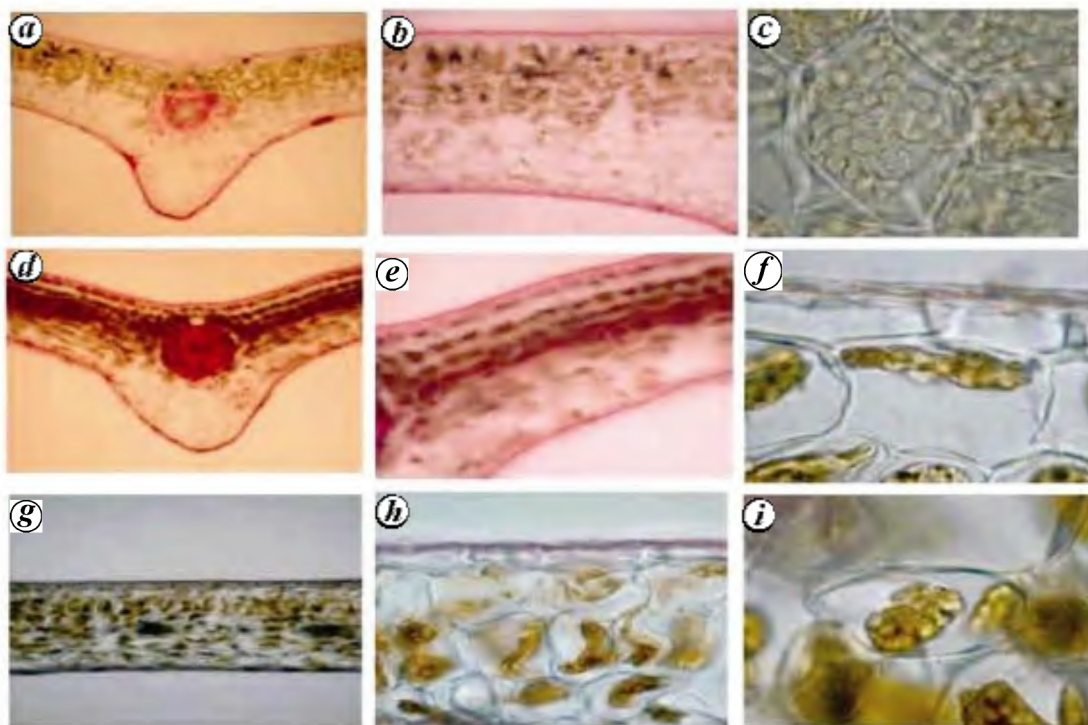
Figure 2. Effect of UV-B radiation on T.S. of leaf anatomy showing LFR movement of chloroplasts in mesophyll cells from leaves of *G. indica*. *a–c*, T.S. of control leaf under 10, 20 and 100× respectively. *d–f*, T.S. of UV-B-treated leaf under 10, 20 and 100× respectively. *g–i*, T.S. of leaf kept under dark under 10, 40 and 100× respectively. For details of experimental condition see text.
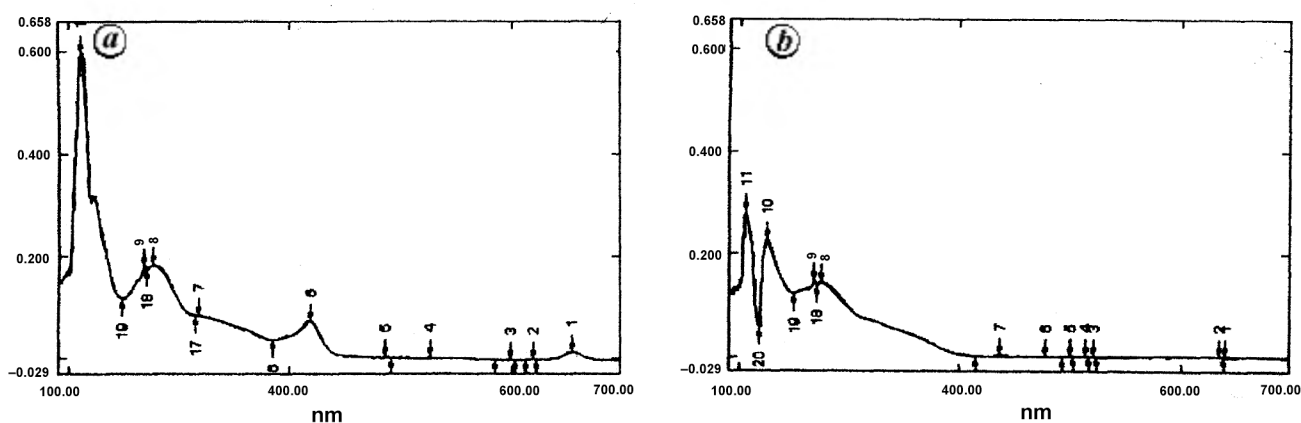

Figure 3. Effect of UV-B radiation on quantitative and qualitative changes in flavonoid content in control (*a*) and treated (*b*) *G. indica* leaves.

increase in 230 nm absorbing pigment, which is seen as a result of decrease in another pigment with λ_{max} at 215 nm. This ratio of 230 and 215 nm absorbing pigments may also be important with reference to chloroplast movement. It will indeed be interesting to find out why the absorption of a compound with λ_{max} at 230 nm is increased on exposure of plants to 280–320 nm radiation. As of now it is difficult to give a satisfactory explanation, but it has been observed[18] that glycosylation of flavonoids can cause a shift (increase) in λ_{max}. Shiono *et al.*[19] have re-

cently reported that anthocyanin in combination with a flavone can form a complex supermolecular pigment, which may also act as a blue light receptor and facilitate chloroplast movement. As mentioned earlier, Jarillo *et al.*[6] and Kagawa *et al.*[7] have also shown that chloroplast relocation movement in plants may be mediated by the blue light photoreceptor, PHOT2.

The present study clearly shows the LFR movement of chloroplasts under UV-B radiation (280–320 nm with λ_{max} at 312 nm). Accumulation of a flavone and to a limited
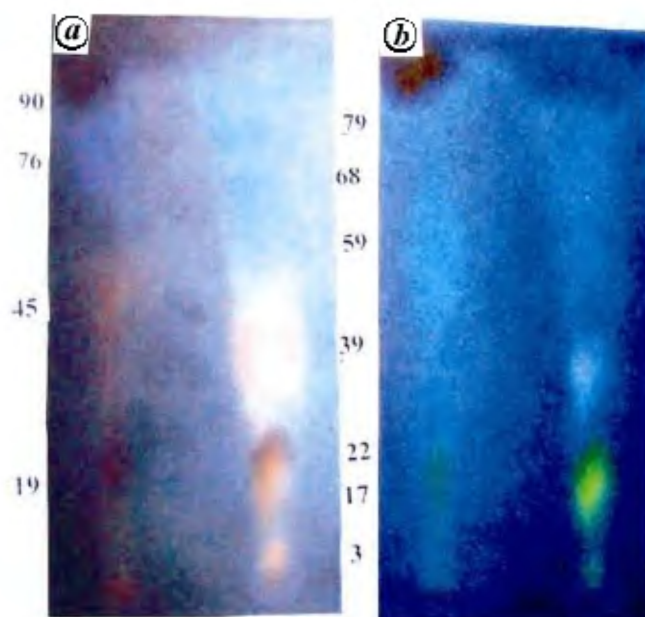
Figure 4. Paper chromatogram treated without (*a*) or with NH_3 solution (*b*) and seen under UV transilluminator, showing qualitative and quantitative changes in flavonoid content in control and UV-B treated *G. indica* leaves. *Rf* values are also given.

extent anthocyanin, individually or in combination, may play an important role in the chloroplast movement. Further characterization and conformation of the nature of the phenolic compounds (with λ_{max} at 230 nm) accumulating under UV-B radiation by GC-MS and NMR will be helpful in elucidating the mode of action of UV-B radiation in chloroplast relocation.

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Purification of DNA from chloroplast and mitochondria of sugarcane

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We report here a method for the isolation of chloroplast DNA (ctDNA) and mitochondrial DNA (mtDNA) from leaf samples of 12 elite Indian sugarcane cultivars. This method includes isolation of organelles, purification by deoxyribonuclease treatment, organelle lysis, isolation of nucleic acid with phenol–chloroform and a final DNA purification using CTAB treatment. The protocol offers pure and completely restrictable ctDNA and mtDNA without nuclear DNA contamination. Inter-organellar DNA contamination was confirmed by PCR amplification using respective organellar DNA-specific universal primers. The ctDNA and mtDNA yield was approximately 7 and 5 µg/g of leaf sample respectively. The method is useful for the isolation of ctDNA and mtDNA from plants that secrete high levels of polysaccharides and phenolic compounds.

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