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Changes in the chlorophyll fluorescence characteristics of chloroplasts from intact pumpkin cotyledons, caused by organ excision and kinetin treatment

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Abstract

The chlorophyll (Chl) fluorescence emission as well as excitation and polarization characteristics of chloroplasts from intact cotyledons were determined in pumpkin seedlings after removal of one cotyledon (co-cotyledon) or apical bud or primary root, or after kinetin treatment of derooted seedlings. Qualitatively, the fluorescence emission and excitation spectra of chloroplasts were similar. The fluorescence emission spectra showed a maximum at 685 (F_{685}) and a hump at 735 nm (F_{735}), whereas the excitation spectra showed peaks at 439, 471, 485, and 676 nm. The fluorescence intensities at F_{685} and F_{735} differed in various groups of seedlings, as indicated by changes in their ratios. Similarly, the ratios of 471/439, 485/439, and 676/439 nm were also different. Variability in the Chl fluorescence intensity values and the fluorescence polarization of chloroplasts prepared from various seedling types may suggest a different degree of binding between the pigment complexes and light-harvesting Chl-protein (LHCP), resulting in different rates of photoexcitation energy loss in the form of fluorescence emission. Kinetin treatment improved the coupling of pigment complexes with reaction centre, as indicated by low polarization values in derooted and kinetin-treated seedlings, which suggests the development of a suntype chloroplast.

Additional key words: apical bud; cotyledon development; *Cucurbita maxima*; fluorescence absorption and emission spectra; fluorescence polarization; root.

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Abbreviations: Chl - chlorophyll; DB - debudded; DCPIP - 2,6-dichlorophenol indophenol; DR - derooted; DRK - derooted plus kinetin-treated; LHCP - light-harvesting chlorophyll protein; PS - photosystem; SOC - seedling with one cotyledon.

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Introduction

Regulation of physiological activities by different organs is important for the plant development. These organs include the hypocotyl hook and embryonic axis (Biswal 1985), root (Dhillon 1977), apical bud (Van Staden and Carmi 1982, Ïordanov and Manolov 1986), other leaves (Rasulov 1986), and reproductive organs such as flowers and fruits (Ho *et al.* 1987). As we have shown in our earlier communication (Behera and Choudhury 1990), in developing cotyledons from a pumpkin the Chl content and photochemical activities of chloroplasts are regulated by the co-cotyledon, root, or apical bud.

In the present study we examined effects on the Chl fluorescence emission, excitation and polarization characteristics of the chloroplasts during development of pumpkin cotyledons after the removal of root (DR plants), apical bud (DB plants) or co-cotyledon (SOC plants). Kinetin, a plant growth regulator which helps in the membrane organization and functioning of cellular organelles, was applied to the DR seedlings to see whether the inter-organ correlation could be explained through this phytohormone.

Materials and methods

Plants: Pumpkin (*Cucurbita maxima* L. cv. Baldyabati) seeds were obtained from a local farm. Plants were grown in a culture room on sterile cotton in Petri dishes under a continuous "white light" (12 W m^{-2}) at 25±2 °C. On the day of its emergence out of the seed coat, one cotyledon was excised from each in a set of seedlings. Likewise, the primary root or apical bud was removed from other sets. Kinetin solution (50 μM) was being sprayed onto a group of seedlings without root at 24 h intervals. Cotyledons were regularly harvested every 24 h for various experiments.

Isolation of chloroplasts: Five cotyledons were homogenized in a chilled mortar and pestle in cold isolation medium containing 0.4 M sucrose, 0.01 M EDTA-Na, and 0.1 M phosphate buffer (pH 7.8). Chloroplasts were isolated following the method of Swain *et al.* (1990). Chl concentration of the chloroplast suspension was estimated using chilled 80 % acetone, according to Arnon (1949).

Measuring of Chl fluorescence emission and excitation spectra, and fluorescence polarization: The room temperature fluorescence emission specra of chloroplasts isolated from fully mature and senescent cotyledons were measured with a fluorescence spectrometer (*Hitachi* model *640-40*, Japan), following the procedure of Mishra *et al.* (1989). For all scannings a slit of 5 mm width and the same recorder scale were used. The samples were excited at 450 nm, and emission was recorded from 600 to 750 nm. The samples contained chloroplasts equivalent to 6 g m^{-3} of Chl in 0.1 M phosphate buffer (pH 7.8).

The same chloroplast suspension was used to measure the fluorescence excitation spectra. The samples were excited from 400-700 nm, and the emission was recorded at 735 nm. For measuring the fluorescence polarization, the polarization accessories of the equipment were used, and the chloroplast suspension was excited at 620 nm. The polarization value (P) was calculated using the formula of Swain *et al.* (1990)

$$P = \frac{F_{\parallel} - F_{\perp}}{F_{\parallel} + F_{\perp}}$$

where $F_{\parallel}$ is the intensity of fluorescence polarization in the same direction, and $F_{\perp}$ the intensity of fluorescence perpendicular to the polarization of incidental radiation.

Results

Chl fluorescence emission spectra of chloroplasts prepared from the cotyledons of different seedlings showed a peak at 685 (F_{685}) and a hump at 735 (F_{735}) nm in both

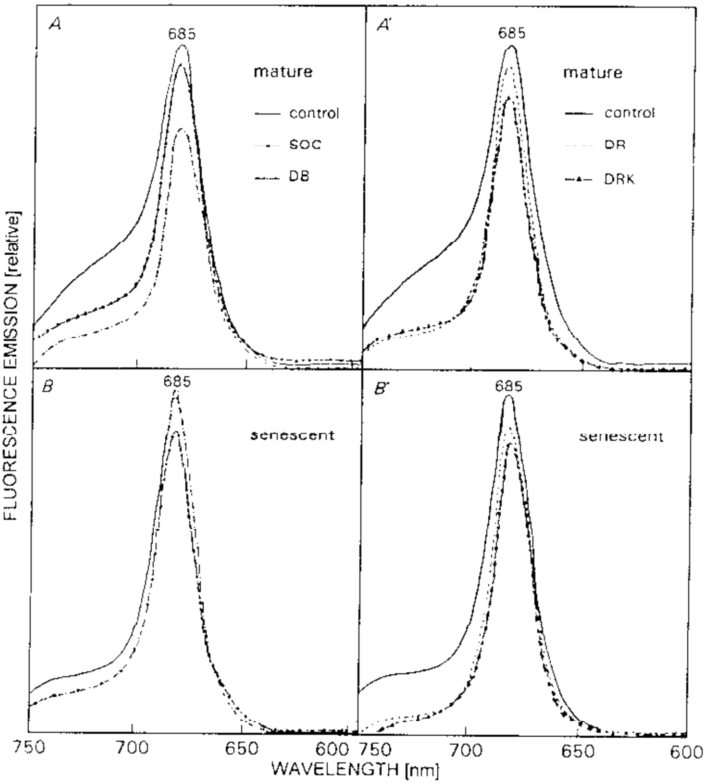

Fig. 1. Changes in chlorophyll fluorescence emission spectra of chloroplasts isolated from mature (*A* and *A'*) and senescent (*B* and *B'*) cotyledons of control (with both cotyledons), SOC (with one cotyledon), DB (debudded), DR (derooted), and DRK (derooted + kinetin-treated) seedlings. The samples were excited at 450 nm, and emission was recorded between 600 and 750 nm. For other details see Materials and methods.

mature and senescent cotyledons (Fig. 1). Qualitatively, the spectra were similar in different groups of seedlings. An increase was observed in the fluorescence ratio of F_{685}/F_{735} in chloroplasts of senescent cotyledons compared to respective cotyledons of mature stages (Table 1), indicating a relative increase in F_{685} in different types of seedlings. The increase was maximum in the DRK but minimum in DB seedlings.

Table 1. The chlorophyll fluorescence emission ratio (F_{685}/F_{735}) of chloroplasts prepared from mature and senescent cotyledons of control (with both cotyledons), SOC (with one cotyledon), DB (debudded), DR (derooted), and DRK (derooted + kinetin-treated) seedlings. SD < 5 %, *n* = 5.

Seedling	F_{685}/F_{735} mature	senescent
Control	6.13 ± 0.29	6.81 ± 0.31
SOC	6.53 ± 0.30	7.98 ± 0.34
DB	7.42 ± 0.31	7.94 ± 0.33
DR	7.04 ± 0.32	9.11 ± 0.41
DRK	7.47 ± 0.33	10.30 ± 0.49

Chl fluorescence excitation spectra for F_{735} emission of chloroplasts prepared from different types of seedlings showed peaks at 439, 471, 485 and 676 nm, and several humps in both mature and senescent cotyledons (Fig. 2). Qualitatively, the spectra were similar except varying relative peak heights. In mature cotyledons, the ratios of peak height of 471/439 and 485/439 nm were almost similar in the control and DRK seedlings, while those of the SOC, DB and DR seedlings were less than the controls (Table 2). On the other hand, the ratio of 676/439 nm was higher in the controls than in any other seedling types. In comparison with the values of mature cotyledons, the ratios of peak heights in senescent cotyledons declined in all types of seedlings.

Table 2. Chlorophyll excitation peak ratios of chloroplasts prepared from mature and senescent cotyledons of control (with both cotyledons), SOC (with one cotyledon), DB (debudded), DR (derooted) and DRK (derooted + kinetin-treated) seedlings. SD < 5 %, *n* = 5.

Seedling	Ratio of peak heights 471/439 mature	senescent	485/439 mature	senescent	676/439 mature	senescent
Control	1.45 ± 0.07	1.23 ± 0.06	1.30 ± 0.05	1.13 ± 0.04	1.88 ± 0.08	1.22 ± 0.06
SOC	1.14 ± 0.05	1.02 ± 0.05	1.02 ± 0.05	0.97 ± 0.03	1.20 ± 0.05	0.94 ± 0.04
DB	1.20 ± 0.06	1.03 ± 0.05	1.09 ± 0.04	0.85 ± 0.03	1.25 ± 0.05	1.20 ± 0.06
DR	1.16 ± 0.05	0.99 ± 0.05	1.04 ± 0.04	0.80 ± 0.03	1.30 ± 0.06	0.86 ± 0.03
DRK	1.41 ± 0.07	1.20 ± 0.06	1.31 ± 0.06	0.83 ± 0.03	1.42 ± 0.06	0.86 ± 0.03

Chl fluorescence polarization of chloroplasts from mature cotyledons of the control seedlings showed a polarization value of 0.128 (Table 3). With the exception of SOC seedlings possessing a higher polarization value, the values for DB, DR, and DRK seedlings were lower than those in the controls. The polarization values increased in senescent cotyledons of all seedling categories compared to the corresponding mature ones. The increase was minimum in the control and maximum in the DR seedlings.

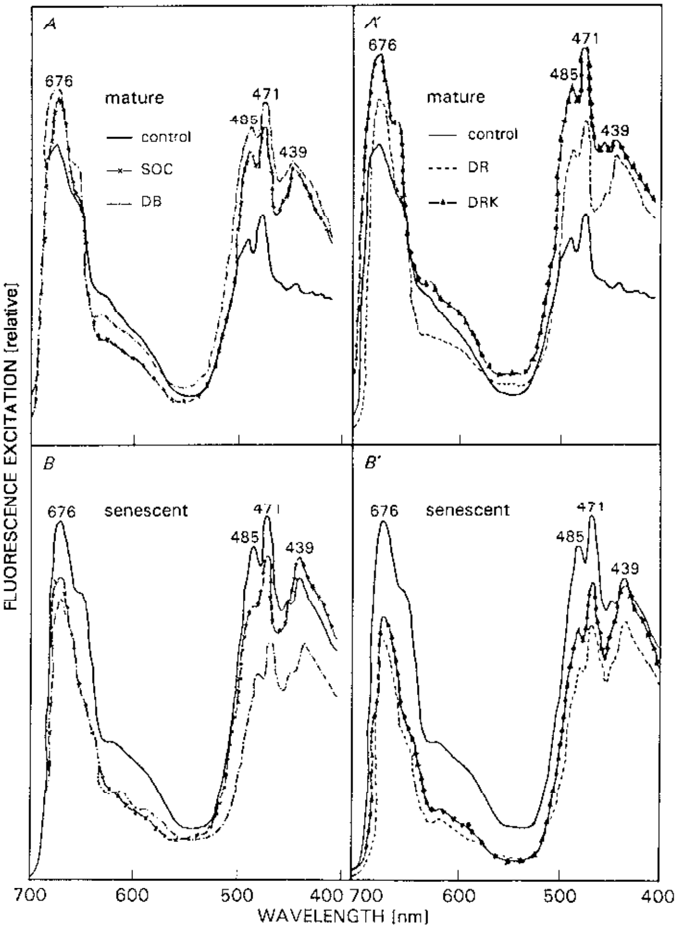

Fig. 2. Changes in chlorophyll fluorescence excitation spectra of chloroplasts isolated from mature (*A* and *A'*) and senescent (*B* and *B'*) cotyledons of control (with both cotyledons), SOC (with one cotyledon), DB (debudded), DR (derooted), and DRK (derooted + kinetin-treated) seedlings. The samples were excited from 400-700 nm, and the emission was recorded at 735 nm. For other details see Materials and methods.

Discussion

In our earlier communication (Behera and Choudhury 1990), we observed different Chl concentrations as well as DCPIP photoreduction activity in mature cotyledons from different groups of seedlings. DRK seedlings showed the highest values both of

Chl concentration and DCPIP photoreduction, whereas SOC showed the lowest ones. In the SOC, with their Chl concentration comparable to that of the controls, a lower value of F_{685} (as indicated by the F_{685}/F_{735} ratio) in mature cotyledons (Table 1) suggested a radiationless decay of the excitation energy (Lichtenthaler *et al.* 1982, Swain *et al.* 1990, Horton and Ruban 1992, Ruban *et al.* 1992). A lowered DCPIP reduction in these seedlings (Behera and Choudhury 1990) further suggested the radiationless decay of excitation energy and/or the setting of an early senescence in the cotyledons.

Table 3. The chlorophyll fluorescence polarization of chloroplasts prepared from mature and senescent cotyledons of control (with both cotyledons), SOC (with one cotyledon), DB (debudded), DR (derooted), and DRK (derooted + kinetin-treated) seedlings. SD < 5, *n* = 5.

Seedling	Fluorescence polarization mature	senescent
Control	0.128 ± 0.005	0.186 ± 0.009
SOC	0.133 ± 0.006	0.196 ± 0.009
DB	0.113 ± 0.005	0.180 ± 0.009
DR	0.108 ± 0.004	0.218 ± 0.011
DRK	0.102 ± 0.004	0.205 ± 0.010

A relative increase in F_{685} as indicated by the increase in F_{685}/F_{735} ratio (Table 1) in DB seedlings suggested a relatively stable PS2 photophysical process during ageing (Panda and Biswal 1989). A significant increase in the ratio of F_{685}/F_{735} during senescence in the SOC, DR, and DRK indicated that the long-wavelength absorbing species of Chl were relatively more susceptible to ageing in these seedlings (Lebedev *et al.* 1986, Swain *et al.* 1990).

The peaks of excitation spectra at 439, 471, 485, and 676 nm are attributed to Chl *a*, Chl *b*, carotenoids, and Chl *a* red band, respectively (Kramer *et al.* 1981, Lebedev *et al.* 1985). Therefore, a change in the peak heights reflects alterations in the pigment complexes of thylakoid membranes (Panda and Biswal 1989, Mishra *et al.* 1990). The excitation spectra of chloroplasts isolated from mature cotyledons in all categories of seedlings showed significant changes in the relative peak heights, as reflected in alteration of the ratios (Table 2). A high value for 471/439 nm is attributed to a strong coupling of Chl and carotenoids (Panda and Biswal 1989, 1990). Higher values of ratios in the controls and DRK indicated a strong coupling between the pigments, whereas lower values in the SOC and DR seedlings indicated a weak coupling. The low value of 471/439 nm in the DR was, however, enhanced in the DRK seedlings due to the kinetin treatment (Table 2).

The fluorescence polarization measurements revealed an increase in polarization values during senescence of chloroplasts in all categories of seedlings, suggesting an ageing-induced conversion of thylakoid membrane to the gel phase. This process was also indicated by uncoupling of LHC II from the reaction centre (Biswal *et al.* 1989, Swain *et al.* 1990), ultimately causing a drop of DCPIP photoreduction activity in the chloroplasts during senescence (Behera and Choudhury 1990).

With the DB seedlings there was a marked change in the relative height of 439 nm peak as compared to the controls (Fig. 2, Table 2). The removal of epicotyl from these seedlings increased the pigment concentration, but with a marginal increase in DCPIP reduction (Behera and Choudhury 1990) instead of a concomitant one in the fluorescence emission ratios (Table 1) it showed a decrease in the values both of mature and senescent cotyledons. This indicated the radiationless decay of excitation energy in the DB seedlings. The aggregation of LHC II leads to a non-photochemical quenching of Chl fluorescence in isolated thylakoids (Horton and Ruban 1992, Ruban *et al.* 1992). Our observation tallies with that of Ruban and his co-workers.

During senescence, the highest (35 %) decline in ratio of 476/439 nm was suffered by the control seedlings and the lowest (4 %) by DB, indicating a relatively weaker coupling of Chl and carotenoids in the former and a stronger coupling in the latter type of plants (Ortiz *et al.* 1985, Panda and Biswal 1990, Swain *et al.* 1990). The higher DCPIP reduction activity in DB seedlings (Behera and Choudhury 1990) supports the above mentioned proposition.

Removal of the co-cotyledon, apical bud, root, or kinetin treatment as a substitute for the root (in derooted plants) apparently modulates the Chl fluorescence emission, excitation, and polarization characteristics of chloroplasts during development of a pumpkin cotyledon. The kinetin treatment of inact wheat leaves increased the efficiency of xanthophyll cycle, a characteristic feature of suntype leaves (Choudhury and Choe 1996). An increase in the number of photosynthetic reaction centres that ultimately led to a higher photosynthetic efficiency has also been shown in kinetin-treated plants (Powles 1984). Certainly the hormone is an important agent through which the influence of an organ is exerted during the cotyledon development by induction of suntype chloroplasts.

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