BRIEF COMMUNICATION

Modulation of photosystem 2 reactions mediated by aluminium toxicity in *Zea mays*

F.C. LIDON*, J.C. RAMALHO**, M.G. BARREIRO*** and J.A. LAURIANO+

*Plant Biology Unit, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825 Monte da Caparica, Portugal**
*Instituto de Investigação Científica Tropical-CEPTA, Tapada da Ajuda, Apartado 3014, 1301 Lisboa Codex, Portugal***
*Plant Physiology Department, Estação Agronómica Nacional, Quinta do Marquês, 2780 Oeiras, Portugal****
Faculdade de Ciências Agrárias, Universidade Agostinho Neto, P.O.Box 236, Huambo, Angola+

Abstract

Two weeks old maize (*Zea mays* L. cv. XL-72.3) plants were submitted to 0 to 81 g m^{-3} Al for 20 d in a growth medium of low ionic strength. The increasing Al concentrations sharply increased chlorophyll (Chl) concentrations. The rates of photosystem 2 activities ($H_2O \rightarrow DCPIP$ and $DPC \rightarrow DCPIP$) increased at 9 g(Al) m^{-3} but at higher Al doses they decreased again. A slight decrease of q_E and q_N coupled to an increase of q_P was also observed until the 27 g m^{-3} Al. The Al-induced decline in cytochrome (cyt) *b* contents per Chl unit was parallel for the $b559_{LP}$ and cyt $b559_{HP}$ forms, but on a leaf area basis more or less opposite trend in both these cyt forms was observed. Increased Al concentrations also decreased carotene and zeaxanthin contents.

Additional key-words: Al; carotenoids; chlorophyll; fluorescence induction; Hill reaction; maize; singlet oxygen.

Aluminium toxicity mostly inhibits the photosynthetic apparatus (*e.g.*, McLean 1979,

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*Fax: 351129544461.
Abbreviations: Chl = chlorophyll; cyt = cytochrome; DCPIP = 2,6-dichlorophenolindophenol; DPC = 1,5-diphenyl carbohydrazide; F_M = maximum fluorescence; F_0 = initial minimal or basal fluorescence; PS = photosystem; Q_A = quinone A; q_E = energy dependent quenching; q_N = non-photochemical quenching; q_P = photochemical quenching.
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Cambraia and Calbo 1980, Foy 1984, Haug 1984, Ohki 1986, Moustakas and Ouzounidou 1994, Lorenc-Plucińska and Ziegler 1996). The inhibition of PS2 may be associated with increased production of singlet oxygen through photodynamic activation (Takahama and Nishimura 1975, 1976, Foote 1979, Durrant *et al.* 1990), which may be overcome by the action of the xanthophyll cycle (Björkman 1987, Demmig *et al.* 1987, Demmig-Adams *et al.* 1989, Demmig-Adams 1990) involving zeaxanthin in particular. The aim of this study was to evaluate the inhibition of PS2 in maize plants submitted to Al toxicity.

Maize (cv. XL-72.3) seeds were washed in distilled water, sterilized by immersion in a 0.1 % $HgCl_2$ solution for 2 min, washed 5 times in deionized water, and dried at 28 °C for 24 h. The seedlings were grown in a glasshouse (30/37-15/19 °C day/night temperature), in pots filled with a mixture of vermiculite and *Trio-hum* tray substrate (2:3) for two weeks, and then for 20 d in solutions with 0, 9, 27 and 81 g(Al) m^{-3}, pH 4.0 (in media of low ionic strength, to keep the activity of Al at its highest level - Khashawneh 1971, Foy 1978, Pavan and Bingham 1982). Afterwards, second youngest leaves of plants were used for analyses. Chl *a* fluorescence induction was measured using the PAM Chl fluorescence system (*Walz*, Effeltrich, Germany) in leaf discs placed in an *LD2/2* oxygen electrode chamber (*Hansatech*, Kings Lynn, U.K.) at 25 °C with a CO_2 saturated atmosphere (7 %) as described by Walker (1988). Photochemical and non-photochemical quenching parameters were calculated according to van Kooten and Snel (1990) using irradiances of 450 μmol m^{-2} s^{-1} for actinic radiation and of about 4200 μmol m^{-2} s^{-1} for saturating flashes given every 60 s, with a duration of 0.6 s. The high energy dependent quenching, q_E, was determined according to Quick *et al.* (1992). Determination of Hill reaction rates (H_2O→DCPIP and DPC→DCPIP) as well as preparation of subchloroplast fractions followed the methods described by Droppa *et al.* (1987). Extraction and quantitative determination of carotene and zeaxanthin from isolated chloroplasts was done according to Hager and Meyer-Bertenrath (1966). Chl content was determined according to Arnon (1949).

Chloroplast extraction for cyt $b559_{HP}$ and $b559_{LP}$ determination followed the method of Spiller and Terry (1980), with minor modifications. Leaves were ground for 1 min in a Waring blendor in a preparative solution consisting of 0.4 M sucrose, 20 mM Tricine-KOH (pH 8.0), 10 mM NaCl, and 30 mM sodium ascorbate. The suspension was filtered through six layers of cheesecloth, and centrifuged at 300×*g* for 2 min at 4 °C. The supernatant was further centrifuged at 30 000×*g* for 5 min at 4 °C. The pellet resuspended in 25 cm^3 of wash solution consisting of 20 mM Tricine-KOH (pH 8.0), 10 mM NaCl, and 30 mM sodium ascorbate, was centrifuged at 30 000×*g* for 10 min at 4 °C. This pellet, resuspended in a second wash solution of 20 mM Tricine-KOH (pH 8.0) and 10 mM NaCl, was centrifuged at 30 000×*g* for 15 min to remove sodium ascorbate. The pellet was then resuspended in 40 mM Hepes (pH 7.5), and cyt $b559_{HP}$ and $b559_{LP}$ concentrations were determined after Houchins and Hind (1984), assuming an extinction coefficient of 20 mM cm^{-1}. Using the 545 nm as the reference wavelength, the cyt contents were determined by measuring reduced-*minus*-oxidized difference spectra. Absorbance changes were measured

relative to a straight line drawn through the 548-568 nm isosbests. The cyt $b559_{HP}$ content was determined from the ascorbate-*minus*-hydroquinone difference spectrum, whereas the menadiol-*minus*-ascorbate spectrum was assumed to consist of 60 % of cyt $b559_{LP}$.

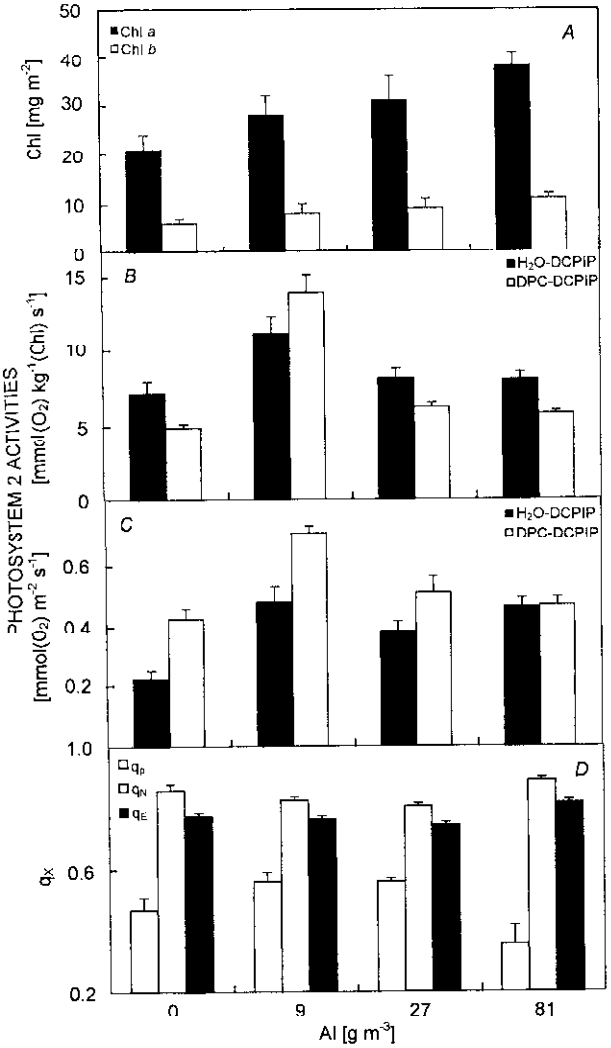

Fig. 1. Chlorophyll (Chl) concentrations (*A*), photosystem 2 reaction activities per unit chlorophyll (*B*) or leaf area (*C*), and fluorescence quenching parameters q_E, q_N and q_P (*D*) of maize plants submitted to four doses of Al for 20 d. Each value is the mean ± S.E. based on three replicates.

Al treatments caused an increase in Chl *a* and *b* concentrations, the largest of *ca.* 82 % was induced by 81 g(Al) m^{-3} (Fig. 1*A*). Also the rates of photosynthetic electron transport rate associated with PS2 (the $H_2O \rightarrow$DCPIP and DPC$\rightarrow$DCPIP) were stimulated, especially at 9 g(Al) m^{-3} (Fig. 1*B,C*). The Al effect on Chl concentrations was different from the findings reported for wheat grown in lower Al

1994). The increasing Chl concentrations associated with the relatively lesser activities of the Hill reactions after the 9 g m^{-3} Al treatment suggested an increasing desorganization of thylakoid structure. The differences in q_E were small, declining from 0 to 27 g(Al) m^{-3} and increasing again at 81 g(Al) m^{-3} (Fig. 1*D*). q_E is linked to the intrathylakoidal proton gradients (Krause and Weis 1991) and is regarded as the most important quenching component under physiological conditions (Horton and Hague 1988). Similar differences were found in q_N; the differences in q_P were of opposite sense.

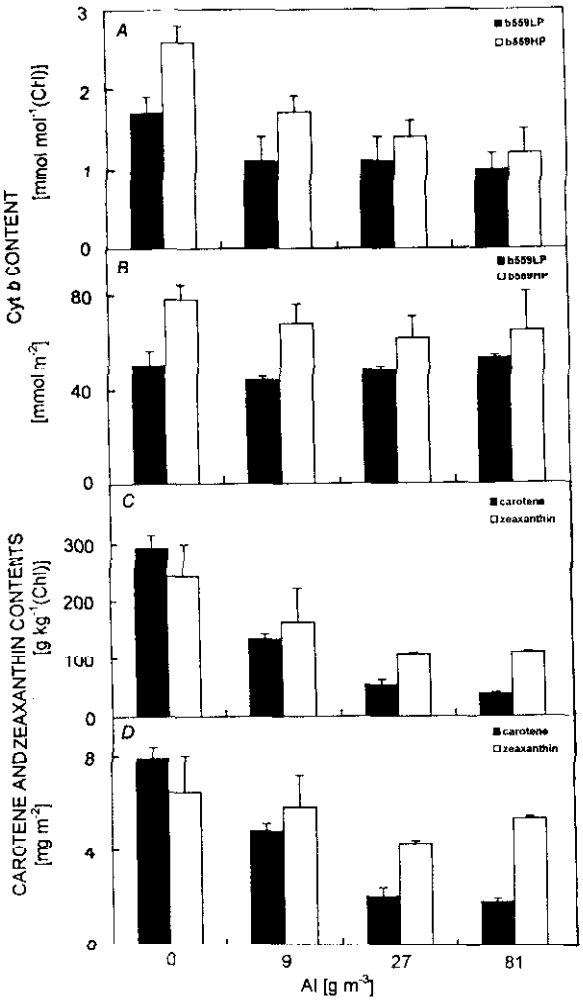

Fig. 2. Cytochrome (Cyt) *b*559 LP and HP contents per unit chlorophyll (*A*) or leaf area (*B*), and carotene and zeaxanthin concentrations per the same units (*C*, *D*) of maize plants submitted to four doses of Al for 20 d. Each value is the mean ± S.E. based on three replicates.

Calculations per leaf area unit showed above 9 g m^{-3} Al treatment a slight increase

of cyt $b559_{LP}$ concentrations, whereas these of cyt$b559_{HP}$ did not show a clear trend (Fig. 2*B*). Calculations per Chl unit showed a similar decline for both tested cyt forms (Fig. 2*A*). The control of radiationless dissipation of excitation energy involving carotene or zeaxanthin (Björkman 1987, Demmig-Adams 1990) seemed to have lower efficiency with increasing Al concentration: the carotene and zeaxanthin contents per leaf area and Chl units continuously declined, with the exception of the 81 g(Al) m^{-3} variant (Fig. 2*C,D*).

In conclusion, until the 27 g m^{-3} Al treatment, the cyclic and thereby dissipative electron transfer process around PS2, involving the cyt $b559_{LP}$, seemed to increase the efficiency of the available excitation energy used photochemically (along with the decrease of the dissipation of excitation energy in the PS2). Accordingly, the production of singlet oxygen through photodynamic activation might be limited. Thus, as the accumulation of excited states of Chl in the pigment bed would be limited, the formation of triplet Chl state might decrease the rate of the reaction with triplet oxygen giving rise to singlet oxygen. Yet, the sharp decrease of carotene and zeaxanthin concentrations did not suggest an increasing photoprotection through a direct quenching/deactivation of singlet oxygen and/or by preventing singlet oxygen formation through triplet-triplet energy transfer from the excited triplet state of Chl to carotenoids.

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