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Comparative study of responses in four *Datura* species to a zinc stress

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Abstract

The effects of zinc toxicity on the growth and the photosynthetic activities of four *Datura* species (*Datura metel*, *Datura innoxia*, *Datura sanguinea*, *Datura tatula*) were studied using various $ZnSO_4$ concentrations (0, 1, 2.5 and 5 mM) added in the Coïc Lessaint solution. Growth, photosynthesis, chlorophyll fluorescence and chlorophyll concentration were measured after 20 days of zinc stress. These parameters were severely reduced by this heavy metal. The zinc excess involves the stomate closing, the increase of CO_2 concentration in the leaves, the inhibition of certain enzyme of the Calvin cycle, a degradation of photosystem and the chlorophyll decomposition. These phenomena allow the decrease of the net photosynthesis to be partially explained. These key parameters to assess photosynthetic performance allow the plants to be classified according to their resistance to zinc. Compared with the three other species, *D. innoxia* showed a very strong capacity to protect itself against toxic zinc concentrations; a large amount of $ZnSO_4$ (5 mM) was required to inhibit 43% of the photosynthesis.

Keywords: Chlorophyll; *Datura* spp; Heavy metal; Photosystem II; Photosynthesis; Zinc

1. Introduction

Datura is a perennial plant, a herbaceous species, belonging to the *Solanaceae* family. It is distributed in warm regions of the world (Drake et al., 1996). There are various species of *Datura* which are cultivated for the production of secondary metabolites. The leaves of *Datura* are an important source of tropane alkaloids: atropine, hyoscyamine and scopolamine. The economic importance of these molecule relies on their medicinal

Abbreviations: F_0, minimum fluorescence of PSII; F_m, maximum fluorescence of PSII; F_v/F_m, maximal photochemical yield of PSII in dark-adapted leaves; PSII, photosystem II; ΦPSII, photochemical yield of open PSII reaction centres in light; RuBisCO, ribulose-bisphosphate carboxylase/oxygenase; RuBp, ribulose-1,5-bisphosphate

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applications (Bruneton, 1993). These alkaloids are used as parasympathicolitics because of their ability to suppress the activity of the parasympathic nerve system. The sole source for these compounds are plants and they are of commercial interest to the pharmaceutical industry (Griffin and Lin, 2000). *Datura* was chosen because it has shown ability to accumulate various heavy metals with adequate capacities and the feasibility to be cultured with high biomass production (Lin and Rayson, 1998).

The phytotoxicity of the heavy metals due to industrial pollution has serious implications in soil degradation (Friedland, 1990). This may reduce both the quality and productivity of plants. Heavy metals differ according to their role in metabolic functions. Microelements, such as zinc, are essential and are involved in numerous physiological processes (Rengel, 1999); however at high concentrations, they are strongly toxic and impair plant growth. Heavy metals such as cadmium or mercury are very toxic even at low concentrations (Marschner, 1999). Zinc is a major industrial pollutant of the terrestrial and aquatic environment (Barak and Helmke, 1993). Zinc toxicity leads to chlorosis in young leaves, and inhibits photosynthesis at various steps and through different mechanisms. Zinc show a specific effect on the Calvin cycle (Chaney, 1993) and photosystem activities (Van Assche and Clijsters, 1986).

The objective of this work is to research plants were able to tolerate and/or accumulate higher concentrations of metal. We investigated the effect of an additional supply of zinc on the photosynthetic activity of four *Datura* species. The net photosynthetic rate, the chlorophyll *a* fluorescence and the chlorophyll content were measured to determine the physiological modifications induced by zinc excess.

2. Materials and methods

2.1. Plant material and treatments

The seeds of *Datura* plants (*D. innoxia*, *D. metel*, *D. sanguinea*, *D. tatula*) were germinated in vermiculite at 25 °C for 7 days. The seedling were cultivated in plastic boxes containing vermiculite as substrate and irrigated every 2 days with a Coïc and Lessaint (1973) nutrient solution, in a temperature-controlled chamber with a 14 h photoperiod and an irradiance of 400 μmol m^{-2} s^{-1} of photosynthetically active radiations (measured with a quantum sensor LI-COR, Lincoln, NE, USA) at the plant level. Day/night temperatures were 23 ± 2 °C/18 ± 2 °C and the relative humidity was 60 ± 5%. Five plants were grown for each treatment. After 45 days of culture in the nutrient solution, zinc stress was imposed for 20 days by adding 1, 2.5 and 5 mM $ZnSO_4 \cdot 7H_2O$ to the nutrient solution.

2.2. Growth measurements

At the beginning and at the end of the experimentation, five plants per treatment were harvested, separated into shoot and root parts, and their fresh weights were determined. The dry weights (DW) were obtained by drying the plant at 85 °C to constant weight.

2.3. Chlorophyll fluorescence

Chlorophyll *a* fluorescence transients were determined by a pulse amplitude modulation (PAM FMS1, Hansatech Instruments Ltd., Norfolk, UK). During the whole experiment, the measurements were always made on the adaxial side on the central parts of the young leaves. The leaves used for the measurements were pre-conditioned in the dark. The initial fluorescence (F_0) was obtained after 2 h of dark adaptation. The maximal fluorescence (F_m) was obtained with a saturating flash (1 s, 13 000 μmol m^{-2} s^{-1}). The ration of variable to maximal fluorescence (F_v/F_m) and F_v/F_0 (= $F_v/F_m/[1 - F_v/F_m]$) were calculated. When coupled with measurement of the light-adapted variable to maximal fluorescence ratio they afford the PSII quantum efficiency: ΦPSII (quantum yield of electron flow throughout PSII) using the model of Genty et al. (1989). ΦPSII represents the number of electrons transported by a PSII reaction centre per mole of quanta absorbed by PSII. Five plants for treatment were used and three measurements per plant were made.

2.4. Gas exchange

After 20 days of treatment with $ZnSO_4$, the net photosynthetic rate (P_n), the stomatal conductance (g_s), the substomatal CO_2 concentration (C_i) and the transpiration rate (T) were measured using a portable infrared gas analyser (LI-Cor Model 6400, Lincoln, NE, USA). It was equipped with a clamp-on leaf cuvette that exposed 6 cm^2 of leaf area. Light, temperature and humidity were 400 μmol m^{-2} s^{-1}, 23 ± 1 °C and 30%, respectively. CO_2 was maintained at a constant level of 360 μmol l^{-1} using an LI-6400-01 CO_2 injector (LI-Cor Lincoln, NE, USA) with a high pressure liquefied CO_2 cartridge source. From these data, the water use efficiency was estimated (WUE = P_n/T).

2.5. Chlorophyll content

The total chlorophyll was extracted in an aliquot of 80% acetone and estimated as described by Lichtenthaler and Welburn (1983).