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BRIEF COMMUNICATION

Photosynthetic and anatomic responses of peanut leaves to cadmium stress

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Abstract

Cadmium (Cd) treatments caused an inhibition in the net photosynthetic rate (P_N) of peanut (*Arachis hypogaea*) plants, due to the reduction of stomatal conductance (g_s) and photosynthetic pigment contents, as well as the alteration in leaf structure. The decrease of the transpiration rate and g_s might result from the Cd-induced xerophyte anatomic features of leaves (*i.e.* thick lamina, upper epidermis, palisade mesophyll, high palisade to spongy thickness ratio, as well as abundant and small stomata). The decline of P_N was independent of the impairment in photosystem 2.

Additional key words: anatomic traits; *Arachis hypogaea*; carotenoids; chlorophyll fluorescence; intercellular CO_2 concentration; leaf structure; palisade and spongy mesophyll; stomata dimensions; stomatal conductance; transpiration rate.

Cadmium (Cd), a non-essential element that can be highly phytotoxic at low concentrations, has been ranked No. 7 among the top 20 toxins (Yang *et al.* 2004). In plants, exposure to Cd causes reductions of biomass production and nutritional quality, and inhibition of photosynthesis (Baszyński *et al.* 1980, Burzyński and Żurek 2007), stomatal conductance (Burzyński and Żurek 2007), transpiration rate (Bazzaz *et al.* 1974), saccharide metabolism (Moya *et al.* 1993), and other metabolic activities (Van Assche *et al.* 1988, Sharma *et al.* 1998).

Photosynthesis is very sensitive to heavy metals in higher plants (Lu and Zhang 2000, Lu *et al.* 2000, Tanyolaç *et al.* 2007, Ekmekçi *et al.* 2008). Previous studies have demonstrated that the decline of the photosynthetic rates under Cd stress results from the distorted chloroplast ultrastructure, the restrained synthesis of chlorophyll (Chl) and carotenoids (Car), the obstructed electron transport, the inhibited enzyme activities of the Calvin cycle, and CO_2 deficiency due to stomatal closure (Seregin and Ivanov 2001). Little information is known about the role of the leaf anatomic structure in photosynthetic performance under Cd stress. We used seedlings of peanut (*Arachis hypogaea* cv. Luhua 11) to evaluate the effect of Cd toxicity on photosynthetic performance and anatomic structure of leaves, and to determine the relationships of these characteristics.

14-d-old peanut seedlings were cultivated hydroponically in a Hoagland nutrient solution (pH = 6.5) containing 0, 10, 50, or 100 µM Cd (four replicates, two uniform plants per replicate). The nutrient solution was renewed every 2 d. After 21 d of Cd treatments, the net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were determined using a portable photosynthesis system (*LiCor-6400*; *LiCor,* Lincoln, NE, USA) equipped with a LED source leaf chamber (*6400-02b*). This experiment was conducted at photosynthetic photon flux density (PPFD) of 1 000 µmol(photon) m^{-2} s^{-1}, leaf temperature of 25 ºC, and constant [CO_2] of 380±5 µmol(CO_2) mol^{-1} in the sample chamber provided with buffer volume. The Chl *a* fluorescence parameters were performed using the *Mini PAM* (*Walz*, Effeltrich, Germany). After dark-adaptation for 30 min, the minimal fluorescence (F_0), the maximal fluorescence (F_m), the variable to maximum fluorescence ratio (F_v/F_m), and the variable to initial fluorescence ratio (F_v/F_0) were determined.

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Abbreviations: C_i – intercellular CO_2 concentration; Car – carotenoids; Chl – chlorophyll; E – transpiration rate; F_0 – minimal fluorescence; F_m – maximal fluorescence; F_v – variable fluorescence; g_s – stomatal conductance; P_N – net photosynthetic rate; PPFD − photosynthetic photon flux density; PS2 – photosystem 2.

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The youngest fully expanded mature leaflets from each plant were sampled and a 5×10 mm section was excised from the middle of the lamina, along with the mid-rib, and fixed in FAA (formaldehyde : acetic acid : 50 % ethanol, 5 : 5 : 90). Samples were dehydrated in an increasing alcohol gradient, embedded in paraffin, sectioned using an ultra microtome (12 µm thick), and stained with fast green. The density and length of stomata for the ad- and ab-axial surfaces of each leaf were determined using prints made with nail varnish. All anatomical characteristics were measured using the *PHMIAS 2003* software with a *ME200* photomicroscope.

Mature leaves (0.2 g) were extracted in dark at 4 ºC with 5 cm^3 mixture of acetone and ethanol (v/v = 1 : 1) until colour disappeared completely. Light absorbances at 663, 645, and 470 nm were determined by spectrophotometry. The concentrations of Chl *a* and *b* and Car were calculated using adjusted extinction coefficients (Lichtenthaler 1987). Analysis of variance (ANOVA) for all the measured variables was performed by *SPSS* version *11.5* software, and differences between means were determined using the Duncan's test.

The contents of Chl *a*, Chl *b*, Chl *a*+*b*, and Car decreased significantly by Cd treatments (Fig. 1*A*), whereas no significant difference was observed in Chl *a*/*b* and Chl/Car in different Cd treatments (Fig. 1*B*). Elevated Cd content inhibited P_N (Fig. 1*C*). This was accompanied by an increase in C_i, and a decrease in the

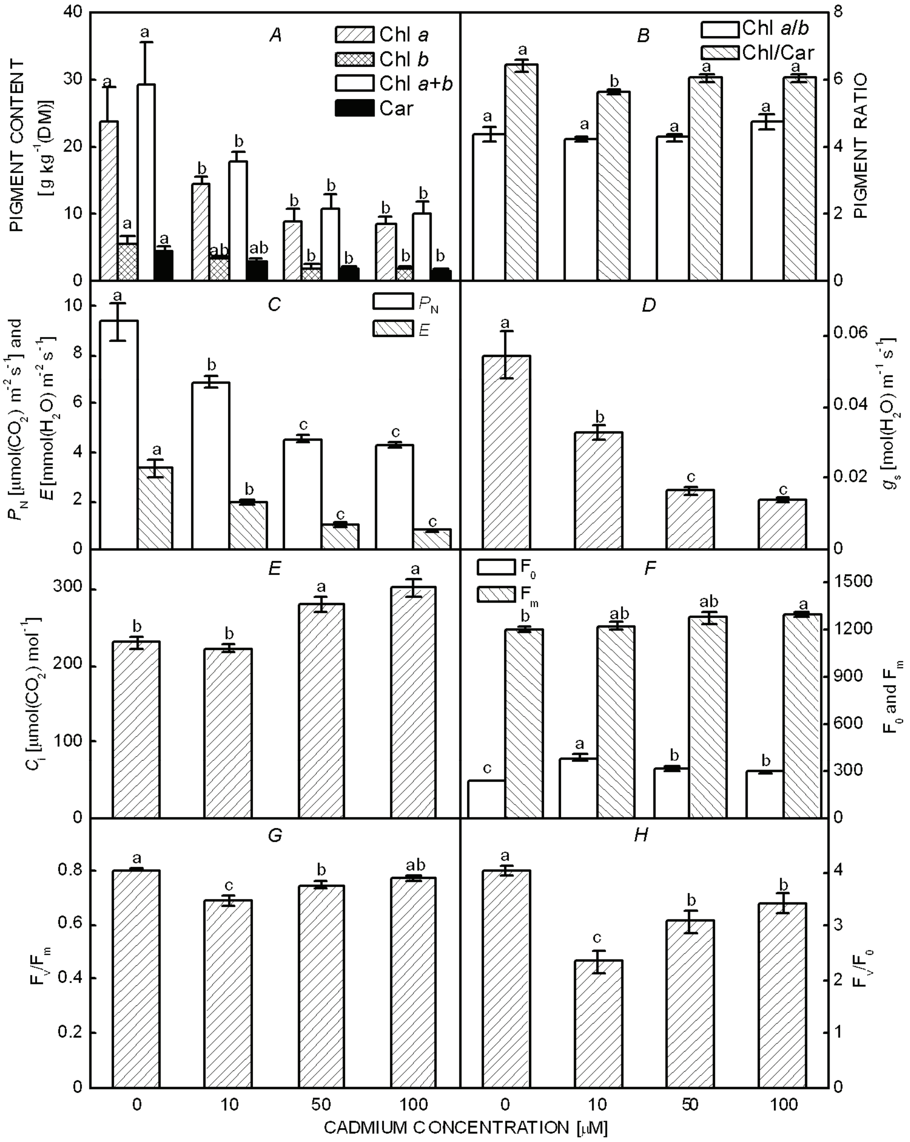

Fig. 1. Effects of Cd on (*A*) pigment contents, (*B*) Chl *a*/*b* and Car/Chl, (*C*) net photosynthetic (P_N) and transpiration (*E*) rates, (*D*) stomatal conductance (g_s), (*E*) intercellular CO_2 concentration (C_i), (*F*) F_0 and F_m, (*G*) F_v/F_m, and (*H*) F_v/F_0 in leaves of *A. hypogaea*. Means±S.E. *n* = 4 for pigment contents, *n* = 24 for gas exchange, *n* = 16 for Chl *a* fluorescence sharing the same letters above the bars are not significantly different at $p<0.05$.

Table 1. Leaf anatomical characteristics of *A. hypogaea* exposed to different concentrations of Cd. Means±S.E. ($n = 80$ for stomatal characters and $n = 32$ for others). Means in the same row followed by the same letter are not significantly different at $p<0.05$ based on Duncan's test.

Anatomical characteristics	Cd concentration [µM] 0	10	50	100
Stomata density in upper epidermis [mm^{-2}]	190±3c	201±3c	232±3b	286±7a
Stomata density in lower epidermis [mm^{-2}]	167±2c	179±3b	186±3b	207±3a
Stomata length in upper epidermis [µm]	26.3±0.3a	25.3±0.2b	24.3±0.2c	24.1±0.2c
Stomata length in lower epidermis [µm]	26.5±0.2a	25.6±0.2b	26.6±0.2a	26.3±0.2a
Upper epidermis thickness [µm]	17.4±0.8b	18.7±0.5ab	19.2±0.6a	17.2±0.4b
Lower epidermis thickness [µm]	17.3±0.5a	15.0±0.4b	15.1±0.4b	15.5±0.4b
Palisade tissue thickness [µm]	74.5±2.1b	100.7±2.4a	98.4±1.9a	101.9±1.8a
Spongy tissue thickness [µm]	44.0±1.9b	47.7±1.4b	47.1±1.3b	52.1±1.5a
Palisade to spongy mesophyll thickness ratio	1.85±0.11b	2.22±0.09a	2.19±0.09a	2.02±0.06ab
Lamina thickness [µm]	153.7±2.6b	182.1±2.8a	179.7±2.2a	186.7±2.8a

g_s, E, and Chl and Car contents (Fig. 1*A−E*). The parallel change of P_N and g_s showed that the photosynthetic response of peanut leaves to Cd stress could be mainly attributed to the alteration of the pigment contents and g_s. However, the increase of C_i suggests that enzymatic dark reaction of photosynthesis was affected (Sheoran *et al.* 1990, Sas *et al.* 2006).

Photosynthetic activity can be evaluated by Chl *a* fluorescence measurements (Sayed 2003). In peanut plants, the dramatic increase of F_0 was observed in low Cd condition (10 µM), which could be mainly responsible for the decrease of F_v/F_m and F_v/F_0 (Fig. 1*F−H*). The increase in F_0 indicates an impaired energy trapping efficiency in the photosystem 2 (PS2) reaction centres or a partial disconnection of the antennae from the centres (Ralph and Burchett 1998). Decreased F_v/F_m and F_v/F_0 as well as parallel change in Chl/Car (Fig. 1*B*) suggest a dislocation of the pigments in thylakoid complexes, and this might lead to a loss in the photochemical potential of thylakoid (Gruszecki *et al.* 1991). Hence the decline of P_N in peanut plants treated with Cd might be independent of the inhibition of PS2.

We found that Cd caused an increase in the stomatal density either in upper or lower epidermis, and a decrease in the stomatal length in upper epidermis (Table 1). Small but abundant stomata maximize the rate of carbon gain while minimizing water loss, which is usually considered as a xerophytic characteristic of leaves (Fahn 1964, Sundberg 1986, Bosabalidis and Kofidis 2002).

Apart from the stomata, gas exchange was also affected by the other anatomic traits of leaves. Abundant palisade tissue is believed to increase the CO_2 absorbing surface of the mesophyll (Rhizopoulou and Psaras 2003). The mesophyll compartmentalization protects the leaf against water stress (Terashima 1992), but, unavoidably, increases the CO_2 diffusion resistance of the tissue (Miyazawa and Terashima 2001). As seen in Table 1, Cd treatments result in an increase of lamina, upper epidermis, and palisade thickness, as well as the palisade to spongy thickness ratio. These traits, as well as stomata characters mentioned above, were considered as xerophyte features (Fahn 1964). The features might contribute to the decline in the g_s and E under Cd stress, and consequently cause a decrease in P_N.

In conclusion, exposure to Cd inhibits P_N and E, changes leaf anatomic structure, and decreases pigment contents. The restriction of P_N by Cd results from the reductions in g_s and Chl and Car contents. It may be Cd-induced xerophyte characteristics of leaves, characterized as thick lamina, upper epidermis, palisade mesophyll, as well as abundant and small stomata, that contribute to the reduction of E and P_N in peanut plants treated with Cd.

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