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Research article

Impaired leaf CO_2 diffusion mediates Cd-induced inhibition of photosynthesis in the Zn/Cd hyperaccumulator *Picris divaricata*

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ABSTRACT

Mechanisms of cadmium (Cd)-induced inhibition of photosynthesis in the Zn/Cd hyperaccumulator *Picris divaricata* were investigated using photosynthesis limitation analysis. *P. divaricata* seedlings were grown in nutrient solution containing 0, 5, 10, 25, 50, or 75 μM Cd for 2 weeks. Total limitations to photosynthesis (T_L) increased from 0% at 5 μM Cd to 68.8% at 75 μM Cd. CO_2 diffusional limitation (D_L) made the largest contribution to T_L, accounting for 93–98% of T_L in the three highest Cd treatments, compared to just 2–7% of T_L attributable to biochemical limitation (B_L). Microscopic imaging revealed significantly decreased stomatal density and mesophyll thickness in the three highest Cd treatments. Chlorophyll fluorescence parameters related to photosynthetic biochemistry (F_v/F_m, NPQ, Φ_{PSII}, and q_P) were not significantly decreased by increased Cd supply. Our results suggest that increased D_L in leaves is the main cause of Cd-induced inhibition of photosynthesis in *P. divaricata*, possibly due to suppressed function of mesophyll and stomata. Analysis of chlorophyll fluorescence showed that Cd supply had little effect on photochemistry parameters, suggesting that the PSII reaction centers are not a main target of Cd inhibition of photosynthesis in *P. divaricata*.

1. Introduction

Cadmium (Cd) is generally toxic to photosynthesis in higher plants. The mechanism by which Cd brings about inhibitions of photosynthesis in plants may arise from two aspects. On one hand, high Cd concentrations in the leaf tissue can directly damage the structure, composition and functionality of PSII or PSI [1,2]. It has also been reported that Cd can modify the supramolecular conformation of the light-harvesting pigment–protein complex of PSII (LHCII) isolated from rye seedlings [2]. Moreover, it has been suggested that Cd could influence the chlorophyll content via disturbing the proteins (enzymes) about the pigment synthesis pathway [3], inducing oxidative stress, causing deficiency in Fe or Mg, substitution of the central Mg^{2+} in the chlorophyll molecules

Abbreviations: A_N, net photosynthetic rate; B_L, D_L, MC_L and S_L, biochemical conductance, CO_2 diffusional conductance, mesophyll conductance and stomatal limitations, respectively; CA, carbonic anhydrase; C_a and C_i, reference atmospheric and intercellular CO_2 concentrations; D, light fraction used for heat dissipation; E, transpiration rate; E_x, fraction of absorbed light defines excess energy; Φ_{PSII}, effective quantum yield of photochemical energy conversion in actinic light; F_o, minimal fluorescence yield; F_m, maximum fluorescence yield; F_s, steady-state fluorescence; F'_m, maximal fluorescence yield under illumination; F'_o, minimal fluorescence yield under illumination; F'_v/F'_m, efficiency of open PSII centers; F_v/F_m, maximum quantum efficiency of PSII primary photochemistry; g_m and g_s, CO_2 mesophyll and stomatal conductances, respectively; MES, 2-morpholinoethanesulfonic acid; NPQ, non-photochemical quenching; P, light fraction used for PSII photochemistry; PPFD, photosynthetic photon flux density; PSI and PSII, photosystem I and photosystem II, respectively; q_P, photochemical quenching efficient; ROS, reactive oxygen species; $V_{c,max}$, in vivo maximum rate of Rubisco carboxylation.

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[4] and so on [5,6]. Apart from impairments of chlorophyll and PSII, other symptoms of Cd toxicity on photosynthesis include the impaired properties of the photosynthetic apparatus such as chloroplast structure [7] and enzymatic activities of the Calvin cycle [8]. On the other hand, high Cd exposure can also result in morphological alternations and stunned development in leaves (*e.g.*, the stunned mesophyll and the close stoma) [9–11], all of which may interfere with the stomatal conductance (g_s) and mesophyll conductance (g_m) [12], further decreasing the efficiency of CO_2 diffusion, thus restricting the photosynthetic rate indirectly. It has been suggested that the Cd-induced inhibition of leaf growth can result from a variety of physiological disruptions, *e.g.*, generation of oxidative stress [13], disruption of nutrient uptake [14], interference with the homeostasis of the essential elements like Zn, Ca and Fe [15,16] and so on [17].

However, a number of higher plants, termed Zn/Cd hyperaccumulators, have developed distinct strategies to accumulate Cd in leaves to an extraordinarily high level without suffering serious toxicity with regard to some photosynthetic parameters [18–20]. In the case of *Sedum alfredii* exposed to nutrient solution containing 100 μM Cd, chloroplasts appeared relatively normal in shape, with clear and regular thylakoid membranes, although chloroplast size was reduced [18]. Similarly, typical ellipsoidal chloroplasts with an intact ultrastructure were observed in *Picris divaricata* exposed to a 75 μM Cd treatment [20]. Interestingly, for *Noccaea caerulescens* [19] and *P. divaricata* [20], Cd exposure significantly increased the activity of carbonic anhydrase (CA), a ubiquitous photosynthetic enzyme in C_3 and C_4 plants [21]. All of these results suggest that in some Zn/Cd hyperaccumulators, many or even most steps in photosynthesis may be Cd tolerant.

Despite the Cd tolerance exhibited by the photosynthetic systems of a few Zn/Cd hyperaccumulators, inhibitory effects of high Cd concentration on photosynthetic reactions have been observed in some studies on hyperaccumulators [18,20,22,23]. However, the primary mechanism of Cd-induced inhibition of photosynthesis in Zn/Cd hyperaccumulators is a matter of controversy. In *N. caerulescens*, the potential efficiency of PSII photochemistry (F_v/F_m) and the effective quantum yield of photochemical energy conversion in actinic light (Φ_{PSII}) have been found to decrease with increasing Cd exposure, suggesting that damage to the PSII photosynthetic reaction center (PSII RC) may be the main site of inhibition under Cd stress [23]. However, in the Zn/Cd hyperaccumulator *S. alfredii*, the value of F_v/F_m was nearly unchanged even in the presence of 400 μM Cd, suggesting that Cd-induced inhibition of growth in *S. alfredii* was not primarily due to PSII inhibition [22]. The difference in the photochemical response to Cd stress between *S. alfredii* and *N. caerulescens* suggests that the photochemical function of PSII is not the primary target for Cd toxicity to photosynthesis in all Zn/Cd hyperaccumulators. In other words, Cd stress may inhibit photosynthesis through a variety of mechanisms in different Zn/Cd hyperaccumulators species. To date, however, the relative contributions of the various factors that may be involved in Cd-induced inhibition of photosynthetic activity in Zn/Cd hyperaccumulators have not been completely quantified.

In numerous studies on water stress and leaf aging [24,25], limitations to photosynthesis are partitioned into different functional components related to stomatal (S_L) and mesophyll conductance (MC_L) and leaf biochemical characteristics (B_L). Sagardoy et al. [26] modified this method to investigate the effects of zinc (Zn) toxicity on photosynthesis rates in the model plant sugar beet (*Beta vulgaris*) and concluded that decreases in stomatal (g_s) and mesophyll conductances (g_m) to CO_2 are the main cause of impaired photosynthesis in plants grown in the presence of excess Zn. The possibility that Cd may act similarly to indirectly affect the photosynthetic rate in Zn/Cd hyperaccumulators via limitation of CO_2 diffusion (D_L) has been ignored in previous studies [20,22,23].

P. divaricata is an ideal test species for investigating the questions raised above about the effects of Cd on photosynthesis in Zn/Cd hyperaccumulators. We have previously shown that photosynthesis in *P. divaricata* is Cd tolerant, as measured by parameters such as chloroplast ultrastructure and carbon assimilation enzymes [20]. In the same study, leaf chlorosis was not observed in this plant species under Cd stress, even at the highest Cd treatment (75 μM). However, net photosynthetic rate (A_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were decreased, by 44.2%, 73.9%, 83.2%, and 20.2%, respectively, in the 75 μM Cd treatment compared with the control. These results suggest that PSII is not the main site of Cd inhibition of photosynthesis in *P. divaricata*, which was shown previously for *S. alfredii* [22]. Nevertheless, Cd does inhibit photosynthesis in *P. divaricata* at high concentrations, possibly due to factors related to CO_2 diffusion. In the present study, we used photosynthesis limitation analysis to assess the relative contributions of diffusional and biochemical factors to Cd-induced inhibition of photosynthesis in the Zn/Cd hyperaccumulator *P. divaricata*.

2. Results

2.1. Limitations to photosynthesis

P. divaricata plants supplied with 5 μM Cd had the highest rate of photosynthesis (Table S1); therefore this treatment was used as a reference, in which all limitations to photosynthesis were regarded as 0. Plants supplied with 0 μM and 10 μM Cd experienced a slight degree of total limitation to photosynthesis (T_L), with values of 18.7% and 15.3%, respectively (Table 1). At higher rates of Cd supply, T_L increased substantially, with values of 29.2%, 44.4%, and 68.8% for the 25 μM, 50 μM, and 75 μM Cd treatments, respectively. Diffusional limitation (D_L), defined as the sum of stomatal limitation (S_L) and mesophyll limitation (MC_L), increased with Cd supply in a similar fashion, with values ranging from 28.7% to 65.7% in the three highest Cd treatments (Table 1). In contrast, biochemical limitation (B_L) was much lower than D_L at all levels of Cd supply, with values ranging from 0.5% to 3.1% in the three highest Cd treatments (Table 1). Thus, for *P. divaricata* plants supplied with 25–75 μM Cd, D_L accounted for 93–98% of T_L, while only 2–7% was attributable to B_L. Both components of D_L made major contributions to photosynthesis limitation, with S_L accounting for 44–59% of D_L in the three highest Cd treatments, while MC_L accounted for 41–56% (Table 1).

2.2. Stomatal density, pore size, and thickness of leaf cross-section

Exposure to Cd did not result in a significant decrease in stomatal pore size in most cases, with the exception of stomatal length

Table 1
Photosynthesis limitation parameters (%) in *Picris divaricata* plants grown in nutrient solution with a range of Cd concentrations.

Cd treatment (μM)	S_L	MC_L	D_L	B_L	T_L
0	9.0 ± 0.8^c	4.2 ± 1.9^c	13.6 ± 1.3^c	5.1 ± 0.7^a	18.7 ± 1.4^c
10	12.1 ± 0.2^c	2.6 ± 0.2^c	14.7 ± 0.1^c	0.6 ± 0.0^c	15.3 ± 0.1^c
25	17.0 ± 1.1^b	11.7 ± 1.2^b	28.7 ± 1.0^b	0.5 ± 0.1^c	29.2 ± 1.2^b
50	23.0 ± 0.9^b	18.5 ± 1.7^b	41.5 ± 1.9^b	2.9 ± 0.5^b	44.4 ± 2.1^b
75	28.8 ± 1.2^a	36.8 ± 4.2^a	65.7 ± 3.6^a	3.1 ± 0.2^b	68.8 ± 3.6^a

Note: 1) The 5 μM Cd treatment was taken as a reference, for which all limitations were set to 0. 2) S_L, stomatal limitation; MC_L, mesophyll conductance limitation; B_L, biochemical limitation; D_L, diffusional limitation; T_L, total limitation. 3) Data are the means ± SE of three replicates. Different letters in the same column indicate that values were significantly different at $P < 0.05$.