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THE EFFECTS OF ALUMINIUM ON THE PHOTOSYNTHETIC APPARATUS OF TWO RICE CULTIVARS

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SUMMARY

We aimed to evaluate aluminium (Al) effects on the photosynthetic apparatus of two rice cultivars with contrasting tolerances to Al. Nine-days-old seedlings were exposed to 0 or 1 mM Al for 10 days, and then dry mass, Al and chloroplastidic pigment contents and photosynthetic parameters were determined. Al accumulated mainly in the roots of the Al-treated plants. In the leaves, Al increased only in the sensitive cultivar, but there was no difference between the cultivars in Al-treated plants. The root and leaf dry mass, the net carbon assimilation rate, stomatal conductance and internal CO_2 concentration were all reduced in response to Al application, but only in the sensitive cultivar. Both the initial fluorescence and potential photochemical efficiency of photosystem II were unresponsive to the Al treatments, regardless of the cultivar. In the Al-sensitive cultivar, Al provoked significant decreases in the photochemical quenching coefficient, quantum yield of photosystem II electron transport and apparent electron transport rate, in parallel to an unaltered non-photochemical quenching coefficient. All of these parameters remained at the control levels in the tolerant cultivar. The chloroplastidic pigment content increased only in the Al-tolerant cultivar, whereas it remained unaltered after Al treatment in the sensitive cultivar. In conclusion, Al induced stomatal and (most likely) photochemical constraints on photosynthesis but with no apparent signs of photoinhibition in the Al-sensitive cultivar. Despite the similar Al levels of the cultivars, unchanging biomass accumulation or photosynthetic performance in the tolerant cultivar challenged with Al highlights its higher intrinsic ability to cope with Al stress.

INTRODUCTION

Aluminium (Al) toxicity is one of the greatest limitations to plant productivity in acidic soils in the tropical and subtropical areas of the world (Kochian *et al.*, 2004). In these soils, with pH values below 5.5, toxic forms of Al, particularly Al^{3+}, become soluble and are absorbed by plants (Kochian *et al.*, 2004), resulting in growth reductions, poor plant development and low plant productivity (Chen *et al.*, 2010; Kochian *et al.*, 2004; Silva *et al.*, 2010). The primary symptom of Al toxicity is a rapid inhibition of root growth, resulting in a limited water and mineral nutrient uptake. Most of the absorbed Al remains in the roots (Kochian *et al.*, 2004), but a small proportion can be translocated to the leaves.

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Some of the effects of Al on the photosynthetic process are apparently initiated as a consequence of its toxic effects, which are primarily manifested at the root level. Several studies show that Al interferes with the absorption and/or transport of essential mineral nutrients to the leaves (Giannakoula *et al.*, 2008), resulting in low rates of net CO_2 assimilation (*A*) and reduced biomass accumulation (Jiang *et al.*, 2008). In any case, the mechanisms by which Al may affect the photosynthetic apparatus remain unclear. In fact, impairments to *A*, which can occur through stomatal and non-stomatal factors (Jiang *et al.*, 2008; Peixoto *et al.*, 2002), may vary both inter- and intra-specifically; additionally, these impairments also depend on such factors as the age of the plant, Al concentration and exposure time to Al. In *Citrus grandis* and *Thinopyrum bessarabicum*, for example, Al treatment provoked stomatal closure and the inhibition of electron transport rates (ETRs; Jiang *et al.*, 2008; Moustakas *et al.*, 1997), suggesting both stomatal and photochemical limitations to *A*. In *Citrus reshni*, Al led to decreases in *A*, but, intriguingly, Al increased or did not affect the activity of the enzymes of the Calvin cycle (Chen *et al.*, 2005). In this case, it is likely that the main toxic effects of Al were manifested in the chloroplast ultrastructure (Moustakas *et al.*, 1997) rather than in the CO_2-fixation enzymes *per se*, which ultimately could result in depressed ETRs. Decreases in the ETRs were also observed in isolated chloroplasts from Al-treated corn hybrids (Mihailovic *et al.*, 2008). The ETR was depressed in *Citrus grandis* exposed to varying Al concentrations, with no effect on the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; Jiang *et al.*, 2008), findings that are in contrast to the results obtained with rye in which the Rubisco activity was impaired due to Al application (Silva *et al.*, 2012).

Rice (*Oryza sativa*) has been reported to be the most Al-tolerant cereal crop under field conditions and is capable of withstanding significantly higher concentrations of Al than other major cereals (Foy, 1988). Although the genetic variability of some rice cultivars in terms of Al toxicity has been documented (Mendonça *et al.*, 2005), the mechanisms for the high Al resistance of rice are not well understood (Famoso *et al.*, 2010; Ryan and Delhaize, 2010). Thus, there is an urgent need to increase our understanding of the mechanisms that govern rice tolerance to Al stress. The objective of this work was, therefore, to evaluate the effects of Al on the photosynthetic apparatus in two rice cultivars with contrasting tolerances to Al.

MATERIAL AND METHODS

Plant material and growth conditions

Two rice cultivars (*Oryza sativa* L.), Fernandes (CNA-1158) and Maravilha (CNA-6843-1), considered tolerant and sensitive to Al, respectively, were used in this study. Seeds, obtained from the Brazilian Center for Rice and Bean Research (EMBRAPA, Goiânia, GO, Brazil), were selected for size uniformity and form and were treated with concentrated sulphuric acid for 1 min. After being washed in running water, the seeds were surface sterilized with 2% (v/v) sodium hypochlorite for 15 min and washed again in running, deionized water. The seeds were germinated in rolls of neutral paper dipped in Clark's nutrient solution (Clark, 1975), pH 4.0, at one-third of the

original ionic strength under continuous aeration (Mendonça *et al.*, 2005). After nine days, the seedlings were selected and transplanted into polyethylene pots containing 1.8 L of Clark's nutrient solution (pH 4.0) and then exposed to 0 and 1.0 mM Al, applied as anhydrous $AlCl_3$. The nutrient solutions were maintained under continuous aeration, and the pH was adjusted daily to 4.0 using NaOH 0.1 N or HCl 0.1 N. The experiment was performed in a temperature-controlled growth chamber (25 ± 3 °C), under a photosynthetic photon flux of 230 μmol m^{-2} s^{-1} and a 16 h photoperiod. After applying the Al treatments for 10 days, the photosynthetic parameters were measured in the early morning (see below), and the plants were harvested and washed thoroughly in deionized water for biomass determination and further analyses.

Al content

The dry, powdered plant material was mineralized in a nitric-perchloric mixture (3:1, v/v). The Al content was determined using a modified aluminon method (Wang and Wood, 1973).

Biomass

The plant tissues were oven-dried at 70 °C for 72 h, and the dry weights of the leaves and roots were determined.

Leaf gas exchange and chlorophyll a fluorescence parameters

The net carbon assimilation rate (A), stomatal conductance to water vapour (g_s), transpiration rate (E) and internal CO_2 concentration (C_i) were always measured on the second fully developed attached leaves in the morning (8:30–9:30 h) using a portable, open-system infrared gas analyser (Portable Photosynthesis System LI-6400, LI-COR Inc., Lincoln, NE, USA) equipped with a blue/red light source (LI-6400–02B). The measurements were made under artificial irradiance of 800 μmol photons m^{-2} s^{-1} at the leaf level and 400 μL CO_2 L^{-1} of air. All measurements were performed at 25 °C, and the vapour pressure deficit was maintained at approximately 1.0 kPa, while the amount of blue light was set to 10% of total irradiance to optimize the stomatal aperture.

The kinetics of the chlorophyll (Chl) *a* fluorescence induction were measured using a 5.5-mm fibre optic probe interfaced with a portable pulse amplitude modulation fluorometer (MINI-PAM, Heinz WALZ, Effeltrich, Germany), in parallel to the gas exchange measurements described above (using the same leaf). The probe, conducting saturating pulses, measuring and actinic light (see below), was held 1.2 cm from the surface of leaf blades at a 60° angle using a standard leaf clip. Following a dark adaptation for 30 min using leaf clips, the leaf tissue was illuminated with a weak modulated measuring beam (0.03 μmol m^{-2} s^{-1}) to obtain the initial fluorescence (F_0). A saturating white light pulse of 6000 μmol m^{-2} s^{-1} was applied for 0.8 s to ensure the maximum fluorescence emission (F_m) from which the variable-to-maximum fluorescence ratio $F_v/F_m = [(F_m–F_0)/F_m)]$ was calculated. This ratio has been used as a measure of the potential photochemical efficiency of photosystem II (Φ_{PSII}). The

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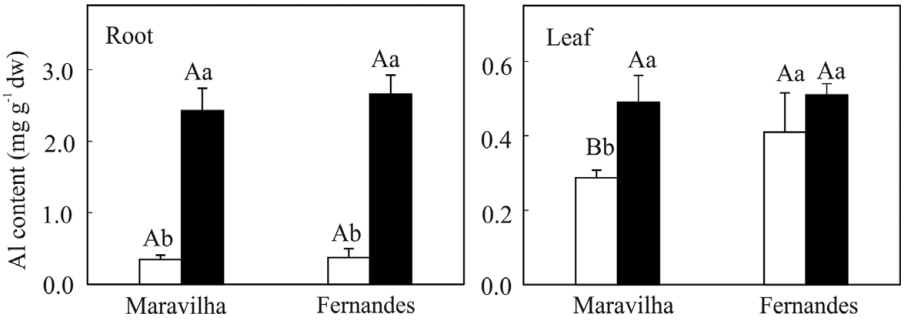

Figure 1. Aluminium content in the roots and leaves of two rice cultivars exposed to 0 (□) and 1 mM (■) aluminium for 10 days. The means followed by the same capital letter between the cultivars and the same small letter between the Al levels within each cultivar do not differ significantly ($p \geq 0.05$, F test). The bars represent the mean ± SD of triplicates.

leaf tissue was exposed to actinic photon irradiance (800 μmol m^{-2} s^{-1}) for 30 s to obtain the steady-state fluorescence yield (F_s). Subsequently, a saturating white light pulse (3000 μmol m^{-2} s^{-1}; 0.8 s) was applied to achieve the light-adapted maximum fluorescence (F_m'). The light-adapted initial fluorescence (F_0') was estimated according to Oxborough and Baker (1997). Using these parameters, the photochemical (q_P) and non-photochemical quenching (NPQ) coefficients, the quantum yield of PSII electron transport (Φ_{PSII}) and the apparent ETR were calculated, as described elsewhere (Cruz *et al.*, 2003).

Chloroplast pigments

The chlorophylls (*a* and *b*) and carotenoids were extracted by grinding the leaves in aqueous acetone 80% (v/v), and the absorbances of the extracts were spectrophotometrically measured at wavelengths of 470.0, 646.8 and 663.2 nm, according to Lichtenthaler (1987).

Statistical analysis

The treatments were arranged in randomized blocks following a 2 × 2 factorial scheme (two cultivars and two Al levels), with three plants in individual pots per treatment combination serving as conditional replicates. The data were subjected to an analysis of variance, and the means were compared using the F test at a 5% probability.

RESULTS

Compared with the control individuals, the Al-treated plants (both the Al-sensitive cv. Maravilha and the Al-tolerant cv. Fernandes) displayed dramatic increases (approximately 700%) in the Al concentration in their root tissues. In contrast, the Al concentration in the leaves increased to a lesser extent (approximately 70%) only in Maravilha (Figure 1). Notably, the Al concentrations in both the roots and leaves were essentially similar between the cultivars.

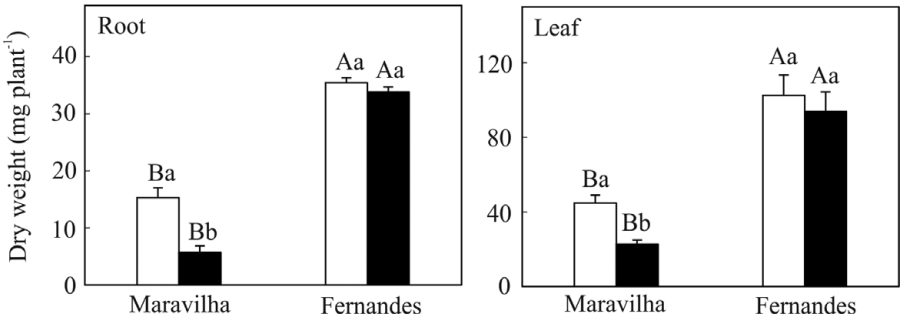

Figure 2. Root and leaf dry weights of two rice cultivars exposed to 0 (□) and 1 mM (■) aluminium for 10 days. The means followed by the same capital letter between the cultivars and the same small letter between the Al levels within each cultivar do not differ significantly ($p \geq 0.05$, F test). The bars represent the mean ± SD of triplicates.

The Al treatment resulted in significant decreases in the biomasses of the roots (62%) and leaves (50%) for Maravilha, whereas no noticeable effect of Al on the biomass was found for Fernandes (Figure 2). Irrespective of the Al treatment, Fernandes accumulated biomass in both roots and leaves to a greater extent than did Maravilha.

Under the control conditions, Maravilha showed slightly higher (though significant) values of the net carbon assimilation rate (A), stomatal conductance (g_s) and internal CO_2 concentration (C_i) compared to Fernandes, whereas the transpiration rate (E) did not differ between the cultivars (Figure 3). In response to Al treatment, these leaf gas-exchange parameters were all depressed in Maravilha yet remained unchanged in Fernandes. It should be noted that, in the former cultivar, the value of g_s decreased proportionally more than A; therefore, the observed decreases in C_i suggest stomatal limitations to photosynthesis.

Both the initial fluorescence (F_0) and potential photochemical efficiency of PSII (F_v/F_m) were unresponsive to the Al treatment, independently of the cultivar (Figures 4a and b). In Maravilha, the addition of Al provoked significant decreases in the photochemical quenching coefficient (q_P), quantum yield of PSII electron transport (Φ_{PSII}) and apparent ETR, in parallel to an unchanging NPQ coefficient (). These parameters all remained at the control levels in Fernandes (Figures 4c–f).

The Al treatment did not affect the concentrations of the photosynthetic pigments in Maravilha; in contrast, it increased the concentrations of total Chl and total carotenoids, coupled with an unaltered Chl a/b ratio, were noted in Al-treated Fernandes compared with its control (Figure 5).

DISCUSSION

The inhibition of root growth is one of the earliest and key toxic effects of Al on plants, and, therefore, most research has focused on the toxicity of Al in root tissues (Chen *et al.*, 2005). Although some recent efforts have been undertaken to improve our understanding on the effects of Al on the leaves, significant uncertainties remain whether Al may directly or indirectly affect the photosynthetic apparatus. Here, we show evidence that Al may constrain the photosynthetic performance, and

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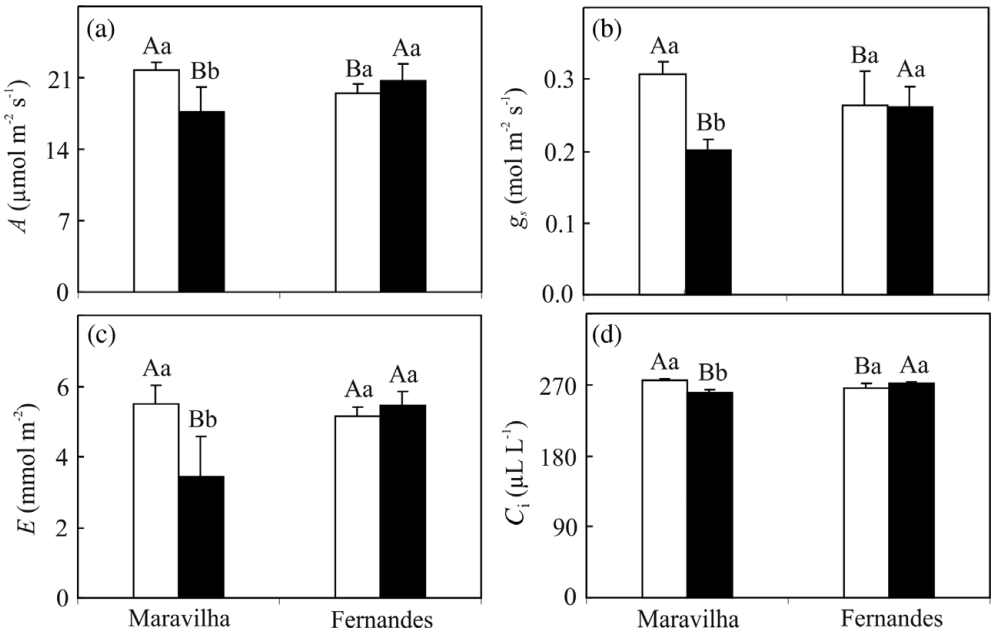

Figure 3. Net carbon assimilation rate, A (a), stomatal conductance, g_s (b), transpiration rate, E (c) and internal CO_2 concentration, C_i (d) in two rice cultivars exposed to 0 (□) and 1 mM (■) aluminium for 10 days. The means followed by the same capital letter between the cultivars and the same small letter between the Al levels within each cultivar do not differ significantly ($p \geq 0.05$, F test). The bars represent the mean ± SD of triplicates.

thus biomass accumulation, through indirect factors, as the toxic effects of Al were manifested only in the Al-sensitive cultivar compared to its tolerant counterpart, despite the quite similar contents of Al in the cultivars. Therefore, others Al-tolerance mechanisms, rather than Al-exclusion mechanisms, should have played increased roles in explaining the differential genotypic abilities to cope with Al stress in this study (Ryan and Delhaize, 2010). It should be emphasized that we had already demonstrated that the cultivar Fernandes not only produces more biomass than Maravilha but it also exhibits a higher tolerance to Al under varying Al levels and exposure times (Justino *et al.*, 2006; Mendonça *et al.*, 2005).

However, the differences in A do not fully explain the genotypic differences in biomass because A decreased to a lesser extent than biomass in Al-treated Maravilha. Possibly, this cultivar possesses a lower inherent ability to redirect biomass to construct a more robust leaf area, as evidenced by its lower overall biomass accumulation, despite the larger A relative to Fernandes, as demonstrated under the control conditions.

Although the mesophyll resistance to CO_2 flux into the chloroplasts or the biochemical limitations to CO_2 fixation cannot be ruled out as factors in this study, we demonstrated that the stomatal factors played a key role in limiting A in the Al-sensitive cultivar, particularly because g_s decreased to a greater extent than A in parallel to the decreases in C_i. Aluminium-induced decreases in g_s, through an as-yet unresolved mechanism (Chen *et al.*, 2010), have been reported for other plant species

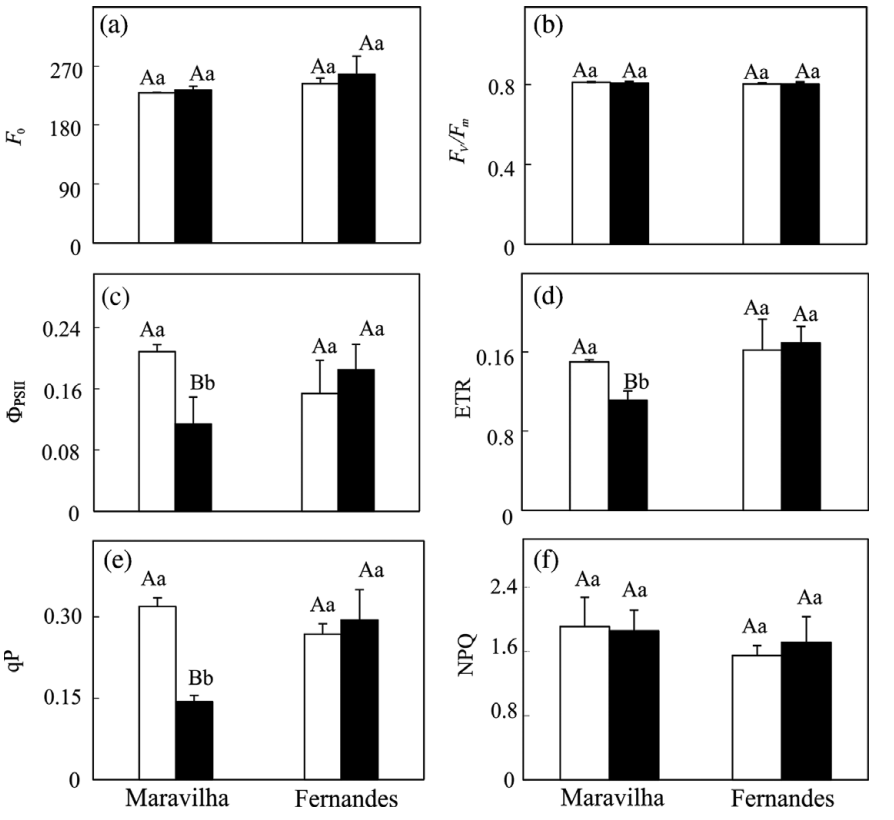

Figure 4. Minimum chlorophyll *a* fluorescence (F_0) (a), variable-to-maximum chlorophyll fluorescence ratio (F_v/F_m) (b), effective quantum yield of PSII (Φ_{PSII}) (c), electron transport rate (ETR) (d), photochemical quenching coefficient (q_p) (e) and non-photochemical quenching coefficient (NPQ) in two rice cultivars exposed to 0 (□) and 1 mM (■) aluminium for 10 days. The means followed by the same capital letter between the cultivars and the same small letter between the Al levels within each cultivar do not differ significantly ($p \geq 0.05$, F test). The bars represent the mean ± SD of triplicates.

(Akaya and Takenaka, 2001; Peixoto *et al.*, 2002). One possible mechanism concerns the reduced root water uptake that would trigger stomatal closure, as suggested for rice (Mendonça *et al.*, 2003), even though this effect has been considered unimportant in *Quercus glauca* (Akaya and Takenaka, 2001). Recent information has noted that an Al-activated malate transporter in guard cells may be involved in stomatal closure (Meyer *et al.*, 2010), suggesting a direct effect of Al on stomatal movements. In any case, the unresponsiveness of g_s to Al in the Fernandes plants may largely explain the maintenance of its gas exchange rates and biomass at the control values.

In addition to the stomatal limitations to *A*, photochemical constraints also possibly limited the actual *A* in the Al-treated Maravilha plants, particularly because Φ_{PSII} and, consequently, the ETR decreased to greater extents than *A*. Considering that carbon fixation, the usual main sink for the absorbed light in chloroplasts, was depressed in Maravilha under Al stress, adjustments in the capture, use and dissipation of light are required to provide photoprotection to the photosynthetic apparatus. Because

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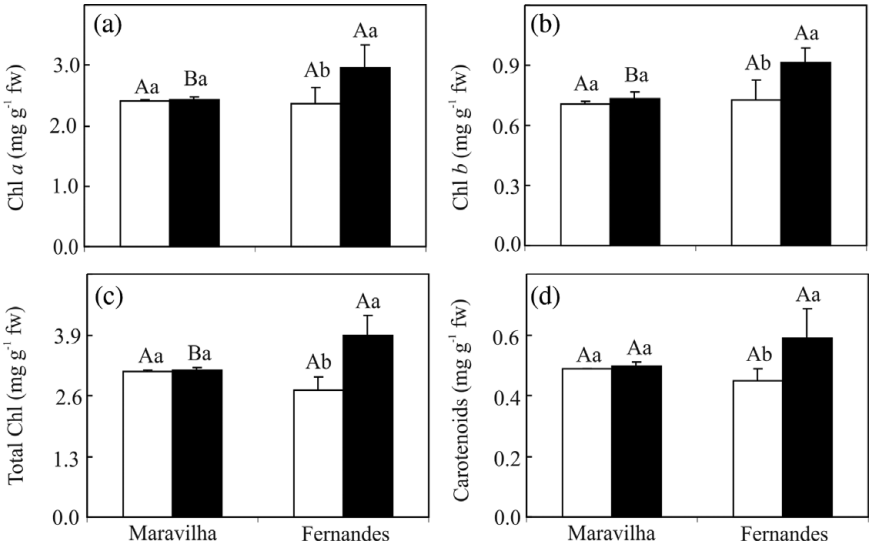

Figure 5. Total chlorophylls, Chl (a + b), Chl a/b ratio (a–c) and carotenoid concentration (d) in two rice cultivars exposed to 0 (□) and 1 mM (■) aluminium for 10 days. The means followed by the same capital letter between the cultivars and the same small letter between the Al levels within each cultivar do not differ significantly ($p \geq 0.05$, F test). The bars represent the mean ± SD of triplicates.

the Chl pools (largely associated with light capture) were unaltered in response to Al in Maravilha, the decreases in A should lead to a surplus excitation energy, that could potentially lead to photoinhibition given the limited ability of Maravilha to safely dissipated such excess as heat, as evidenced by its unchanged NPQ values in response to Al stress (Krause and Weis, 1991). In addition, the portion of oxidized Q_A (analysed as q_p) decreased remarkably in Al-treated Maravilha, thus representing a fraction of PSII centres prone to suffer photoinhibitory damage (Lima *et al.*, 2002). Irrespective of these facts, the F_v/F_m ratio was maintained at high values (~0.80), coupled with an unchanging F_0 and, therefore, we argue against the possibility of occurrence of photoinhibitory damages under the present experimental conditions. The unresponsiveness of both F_v/F_m and F_0 to the Al stress contrasts with the results reported for *Thinopyrum bessarabicum* (a wild relative of wheat; Moustakas *et al.*, 1997) and sorghum (Peixoto *et al.*, 2002) plants (that were grown under light intensities similar to those of this current study) in which these parameters decreased in response to Al. In any case, it must be emphasized that the rice plants were grown under relatively low photon irradiances; had they been grown under field conditions where irradiances can reach values higher than 2000 μmol photons m^{-2} s^{-1}, photoinhibitory damages caused by Al stress should be expected.

In contrast to several plant species (e.g., sorghum (Peixoto *et al.*, 2002), soybean (Milivojevic and Stojanovic, 2003) and corn (Mihailovic *et al.*, 2008)) in which a decrease in the concentrations of chloroplastidic pigments has been noted due to Al stress, we found unaltered (Maravilha) or enhanced (Fernandes) pigment concentrations. The reported decreases in pigment pools have often been associated

with the impacts of Al on the uptake and/or transport of several essential mineral nutrients required for chloroplastidic pigment biosynthesis (Giannakoula *et al.*, 2008). Nonetheless, in Fernandes leaves, Justino *et al.* (2006) have previously demonstrated an increase in the nitrogen content in response to Al, which could circumstantially explain the increased Chl concentrations, considering that Chl biosynthesis is highly responsive to the nitrogen content in the leaves (Mihailovic *et al.*, 2008). Furthermore, studying the effects of Al on the same rice cultivars used in this study, Mendonça *et al.* (2003) found an improved macronutrient (Ca, Mg, P and K) use efficiency in Fernandes compared to Maravilha, lending additional support to explain the increases in Chl in Fernandes.

CONCLUSION

In summary, our data indicate that Al affects both the growth of the roots and also the growth of the leaves. In the Al-sensitive Maravilha, Al induced stomatal, and most likely photochemical, constraints on photosynthesis, with no apparent signs of photoinhibition. In contrast, no alterations in biomass accumulation and photosynthetic performance due to the Al supplementation were evident in the Al-tolerant Fernandes, despite the similar Al levels of the cultivars. These results highlight a higher intrinsic ability to cope with Al stress in Fernandes.

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