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Aluminum resistance in wheat involves maintenance of leaf Ca^{2+} and Mg^{2+} content, decreased lipid peroxidation and Al accumulation, and low photosystem II excitation pressure

Julietta Moustaka · Georgia Ouzounidou · Gülriz Bayçu · Michael Moustakas

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Abstract The phytotoxic aluminum species (Al^{3+}) is considered as the primary factor limiting crop productivity in over 40 % of world's arable land that is acidic. We evaluated the responses of two wheat cultivars (*Triticum aestivum* L.) with differential Al resistance, cv. Yecora E (Al-resistant) and cv. Dio (Al-sensitive), exposed to 0, 37, 74 and 148 μM Al for 14 days in hydroponic culture at pH 4.5. With increasing Al concentration, leaf Ca^{2+} and Mg^{2+} content decreased, as well as the effective quantum yield of photosystem II (PSII) photochemistry (Φ_{PSII}), while a gradual increase in leaf membrane lipid peroxidation, Al accumulation, photoinhibition (estimated as F_v/F_m), and PSII excitation pressure ($1 - q_p$) occurred. However, the Al-resistant cultivar with lower Al accumulation, retained larger concentrations of Ca^{2+} and Mg^{2+} in the leaves and kept a larger fraction of the PSII reaction centres (RCs) in an open configuration, i.e. a higher ratio of oxidized to reduced quinone A (Q_A), than plants of the Al-sensitive cultivar. Four times higher Al concentration in the nutrient solution was required for Al-resistant plants (148 μM Al) than for Al-sensitive (37 μM Al), in order to establish the same closed RCs. Yet, the decline in photosynthetic efficiency in the cultivar Dio was not only due to closure of PSII RCs but also to a decrease in the quantum yield of the open RCs. We suggest that Al^{3+} toxicity may be mediated by nutrient deficiency and oxidative stress, and that Al-resistance of the wheat cultivar Yecora E, may be due at least partially, from the decreased Al accumulation that resulted to decreased reactive oxygen species (ROS) formation. However, under equal internal Al accumulation (exposure Al concentration: Dio 74 μM, Yecora E 148 μM) that resulted to the same oxidative stress, the reduced PSII excitation pressure and the better PSII functioning of the Al-resistant cultivar was probably due to the larger concentrations of Ca^{2+} and Mg^{2+} in the leaves. We propose that the different sensitivities of wheat cultivars to Al^{3+} toxicity can be correlated to differences in the redox state of Q_A. Thus, chlorophyll fluorescence measurements can be a

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J. Moustaka · M. Moustakas (✉)
Department of Botany, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
e-mail: moustak@bio.auth.gr

J. Moustaka
Department of Biology, University of Crete, Voutes University Campus, 70013 Heraklion, Crete, Greece

G. Ouzounidou
Institute of Food Technology, Hellenic Agricultural Organization-Demeter, 1 S. Venizelou str., 14123 Lycovrissi, Greece

G. Bayçu · M. Moustakas
Division of Botany, Department of Biology, Faculty of Science, Istanbul University, 34134 Istanbul, Turkey

promising tool for rapid screening of Al resistance in wheat cultivars.

Keywords Chlorophyll fluorescence · Nutrient uptake · Oxidative stress · Quantum yield · Redox state · *Triticum aestivum*

Abbreviations

F_o, F_m, F_v	Minimal, maximal and variable fluorescence in dark adapted leaves
F_o', F_m', F_v'	Minimal, maximal and variable fluorescence in light adapted leaves
F_s	Fluorescence in steady-state
F_v/F_m	Maximum quantum efficiency of PSII photochemistry of dark-adapted leaves
F_v'/F_m'	PSII maximum efficiency of light-adapted leaves
MDA	Malondialdehyde
Q_A	The first stable quinone electron acceptor of PSII
q_N	Non-photochemical quenching
q_p	Photochemical quenching
$1 - q_p$	Excitation pressure
PSII	Photosystem II
RCs	Reaction centres
Φ_{PSII}	Actual (effective) quantum yield of PSII photochemistry

Introduction

Aluminum (Al) toxicity in acid soils limits crop production in over 40 % of world's arable non-irrigated land (von Uexküll and Mutert 1995). In acidic soils Al tends to solubilize as the trivalent cation Al^{3+}, which is phytotoxic to most plants at relatively low concentrations (Ma et al. 2014). Many of these areas are either undeveloped for agriculture or of very low productivity which is not always economically correctable with conventional liming or other soil management practices (Foy 1988). Aluminum toxicity limits crop production on acid soils through negatively affecting the nutrient uptake and other metabolic processes, especially photosynthesis (Moustakas et al. 1995; Reyes-Diaz et al. 2009; Li et al. 2012; Grevenstuk et al. 2015). Aluminum-induced inhibition of root elongation is one of the most distinct and earliest symptoms of Al^{3+}-toxicity, which occurs within hours of exposure to Al^{3+} (Gunsé et al. 1997; Barceló and Poschenrieder 2002; Čiamporová 2002; Frantzios et al. 2005; Horst et al. 2010; Zelinová et al. 2011).

Aluminum-toxicity in plant tops is often characterized by symptoms resembling those of phosphorus or calcium deficiency (Foy 1988). Phytotoxic effects of Al^{3+} on metabolism in leaf tissues include growth reductions, chlorophyll content, mineral nutrients, photosynthesis and transpiration (Moustakas et al. 1992; Pereira et al. 2000; Chen et al. 2005; Reyes-Diaz et al. 2009; Silva et al. 2012). Wheat seedlings exposed to Al^{3+}-toxicity showed significantly decreased concentrations of Ca, Mg and P (Foy 1988). This decreased nutrient uptake is accompanied by a decreased root and shoot growth (Moustakas et al. 1995). Aluminum reduces accumulation of divalent cations (especially Ca and Mg), slows root growth, and causes impairment of photosystem II (PSII) activity (Rengel 1992; Doncheva et al. 2005; Li et al. 2012; Hasni et al. 2015).

Much research has been focused at present to elucidate the mechanism of Al^{3+}-toxicity and resistance (Poschenrieder et al. 2008; Giannakoula et al. 2010; Horst et al. 2010; Huang et al. 2014; Sade et al. 2016). However, despite the extensive research on Al-phytotoxicity on roots, Al-resistant factors remain unclear or are not well defined (Horst et al. 2010; Zhu et al. 2013; Sade et al. 2016). Comparatively less information exists about the effects of Al^{3+} on aboveground tissues (Reyes-Diaz et al. 2009).

Plants differ in their reaction to Al^{3+}-toxicity, and variability is found between plant species, as well as between cultivars or genotypes (Foy 1988; Moustakas et al. 1992; Bona et al. 1993). The strong relationship between Al^{3+}-resistance determined via visual and chlorophyll fluorescence measurements has been shown to reflect actual tissue injury (Moustakas et al. 1993). Analysis of chlorophyll fluorescence has provided considerable qualitative information on the organization and functioning of the photosynthetic apparatus (Krause and Weis 1991; Sperdouli and Moustakas 2012a; Guidi and Calatayud 2014; Sperdouli and Moustakas 2015). PSII as the most sensitive component of the photosynthetic apparatus is considered to play key roles in the photosynthetic responses to environmental perturbations (Li et al. 2012; Moustaka and Moustakas 2014; Sperdouli and Moustakas 2014a).

Our group (Moustakas and Ouzounidou 1994) has suggested chloroplast elemental loss and concomitant intrathylakoid acidification as mediating mechanisms for Al^{3+}-stress induced inhibition of photosynthesis. Al-toxicity inhibits photosynthesis as a result of partial inhibition of photosynthetic electron transport rate (ETR) at PSII and closure of PSII reaction centres (RCs) (Moustakas and Ouzounidou 1994; Moustakas et al. 1997; Hasni et al. 2015). Thus, Al-toxicity increases the proportion of closed RCs and slows down the rate of photosynthesis resulting in reduced growth and development (Li et al. 2012). By measuring photochemical fluorescence quenching it is possible to measure the fraction of open RCs (i.e. the relative oxidation state of Q_A, the first electron acceptor of PSII), or close (i.e. the relative reduction state of PSII). By this way we can estimate the relative PSII excitation pressure to Al^{3+} exposure.

Wheat cultivars that acclimate to Al phytotoxicity and are able to grow and complete their life cycle may be exploited to increase production in acid soils and be used to understand the mechanisms of Al resistance. Thus, we evaluated the responses of two wheat cultivars with contrasting Al sensitivity, to understand the mechanisms of Al resistance. In this work, we wanted to test the hypothesis that the Al resistant wheat cultivar has the ability to maintain a better reactive oxygen species (ROS) homeostasis, resulting in a decreased lipid peroxidation, and an increased fraction of open RCs, than the sensitive one. In addition, we evaluated under Al^{3+} treatments the leaf concentration of the divalent cations Ca and Mg that their accumulation is reduced more by Al toxicity (Rengel 1992).

Experimental

Plant material and growth conditions

The two wheat cultivars (*Triticum aestivum* L. cv. Yecora E and cv. Dio) that were used in the present work differ in their Al resistance (Moustakas et al. 2004). Seeds of the Al-resistant (cv. Yecora E) and the Al-sensitive (cv. Dio) wheat (*Triticum aestivum* L.) cultivars, provided by the Cereal Institute of Thessaloniki, germinated on moist filter paper in Petri dishes for 2 days. The germinated seeds were mounted on nylon-mesh floats on plastic vessels filled with continuously aerated and frequently replenished modified Hoagland solution (Moustakas et al. 1992). The seedlings were grown in a growth chamber with controlled environmental conditions under a long day photoperiod of 14/10 h, with $60 \pm 5/70 \pm 5$ % humidity, temperature of $22 \pm 1/18 \pm 1$ °C, and light intensity of 180 ± 20 μmol (photons) m^{-2} s^{-1}. All growth solutions were acidified initially to pH 4.5 with HCl (Moustakas et al. 1993).

Al treatments

A randomized block, factorial design with two cultivars, four Al treatments (0, 37, 74 and 148 μM Al) and two replicates was used. Al was supplied as $KAl(SO_4)_2 12H_2O$ for 14 days. According to the GEOCHEM-EZ speciation programme (Shaff et al. 2010) the free Al^{3+} activities in the nutrient solutions were 0, 4.3, 8.5 and 16.8 μM, respectively.

Elemental analysis

After 14 days of Al treatment the second leaves of both cultivars were oven dried for 24 h at 80 °C and elemental analysis was performed as described by Giannakoula et al. (2008). To prepare samples for inductively coupled plasma atomic emission spectrometry (ICP-AES analysis), leaves were digested in a nitric acid/perchloric acid solution ($HNO_3/HClO_4$) 4:1 (v/v). Al, Ca and Mg levels were determined by ICP-AES analysis (Perkin-Elmer Optima 3300XL Perkin-Elmer, USA).

Lipid peroxidation measurements

The level of lipid peroxidation in the second leaves of both wheat cultivars after 14-day of Al^{3+} treatment was measured as malondialdehyde (MDA) content, determined by reaction with 2-thiobarbituric acid reactive substances, as described by Moustakas et al. (2011), according to Heath and Packer (1968). Tissue was homogenized in 0.3 % TBA in 10 % trichloracetic acid at 4 °C and centrifuged for 10 min at 10,000*g*. The concentration of MDA was calculated from the difference of the absorbance at 532 and 600 nm spectrophotometrically (PharmaSpec UV-1700; Shimadzu, Tokyo, Japan), using the extinction coefficient of 155 $mmol^{-1}$ cm^{-1} and expressed as nmol (MDA) g^{-1} fresh weight.

Measurements of chlorophyll a fluorescence

Chlorophyll a fluorescence was measured at room temperature in dark-adapted (20 min) leaf samples, from the upper surface of the attached second leaf of wheat seedlings grown under control and Al stress conditions, using a pulse amplitude modulation fluorometer (PAM-101/103, *Walz, Effeltrich*, Germany), as described by Moustakas et al. (1996). Initially, the measuring modulated beam was switched on to determine the dark-adapted F_o. Fluorescence (F_o level) was excited by light pulses from a light emitting diode (LED, peak wavelength at 650 nm) applied at a frequency of 1.6 kHz. The dark-adapted F_m was then measured by exposing the leaf to the "saturating light pulse". After determination of the initial F_o and F_m, the actinic light (870 μmol m^{-2} s^{-1}) was switched on and the leaf was left to reach F_s at steady-state photosynthesis. The saturation level (F_m') was obtained by applying short pulses (1 s) of actinic white light of saturating intensity (8000 μmol m^{-2} s^{-1}) supplied by a Schott KL 1500 light source triggered by the PAM 103. The fluorescence changes induced by the actinic light were recorded at 100 kHz modulation pulse frequency in order to improve the signal/noise ratio as well as the time resolution. After F_s and photosynthesis had returned to the steady state, the actinic light was switched off and the leaf was exposed to the far-red light. The fluorescence yield dropped to a minimum, which is the F_o'. The variable fluorescence at steady state is given by $F_v' = F_{m-} - F_o'$. The effective quantum yield of PSII photochemistry, Φ_{PSII}, is defined by $(F_m' - F_s)/F_m'$ (Genty et al. 1989). The photochemical quenching (q_p) was calculated as $(F_m' - F_s)/(F_m' - F_o')$ and the non-photochemical quenching (q_N) as $1 - [(F_m' - F_o')/(F_m - F_o)] = 1 - (F_v'/F_v)$.

Data treatment

Each treatment was analyzed with at least five replicates and data are expressed as mean ± SD. One-way ANOVA was carried out using the StatView computer package (Abacus Concepts, Inc. Berkley, Berkley, CA, USA) and means were separated at a level of $P < 0.05$. For the estimation of the relationships among the measured parameters a regression analysis was also performed (Sperdouli and Moustakas 2012b).

Results

Decreased calcium and magnesium uptake after aluminum exposure

The leaf Ca^{2+} and Mg^{2+} content of the two wheat cultivars, differing in their resistance to Al, exhibited a significant decrease to Al-treatment (Fig. 1). Under Al-exposure, the resistant cultivar Yecora E retained, at 148 μM Al, higher concentrations of Mg in the second leaf (34 %), as a percentage of the control (−Al), than the cultivar Dio (19 %). The decrease of Mg concentrations in both cultivars was significant ($P < 0.01$) at 37 μM Al in the nutrient solution (Fig. 1a).

The decrease of Ca concentration in the leaves of "Dio" was significant ($P < 0.01$) at 37 μM Al, but in "Yecora E" the decrease of Ca concentration was significant ($P < 0.001$) only at 74 μM Al (Fig. 1b). The resistant cultivar Yecora E retained at 148 μM Al larger amounts of Ca (57 %), than the cultivar Dio (23 %), compared with control (−Al).

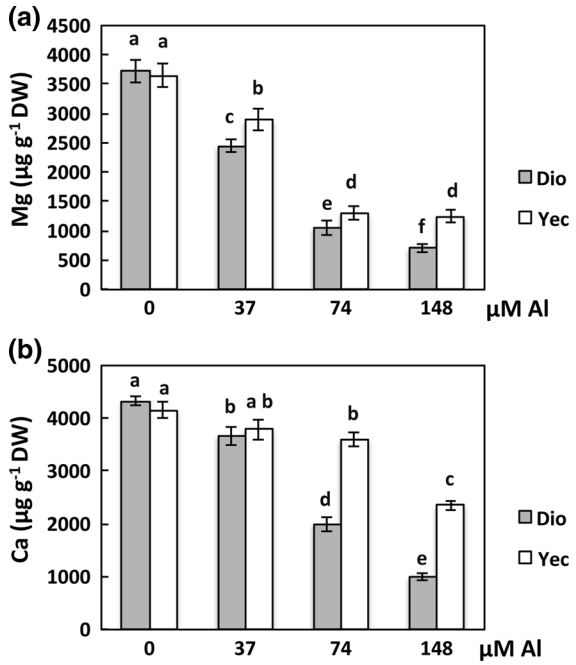

Fig. 1 Changes in **a** magnesium and **b** calcium concentrations, measured as μg g^{-1} dry weight, in the leaves of the Al-resistant cultivar Yecora E and Al-sensitive cultivar Dio, after exposure to 0, 37, 74 and 148 μM Al. Data shown are means and SD that is indicated by *bars*. *Bars* with different lowercase letters are significantly different ($P < 0.05$)

Lower aluminum accumulation and oxidative stress in the resistant wheat cultivar in response to aluminum

Al concentration increased significantly ($P < 0.01$) in the leaves of both cultivars at 74 μM Al (Fig. 2a). After 14 days of exposure to 148 μM Al, the concentration of Al in the leaves of "Dio" was almost 1.5 times higher than in "Yecora E".

Following Al exposure an increase of the oxidative stress occurred due to ROS formation that was estimated by measuring MDA accumulation, an indicator of the extent of lipid peroxidation. The level of lipid peroxidation increased significantly ($P < 0.01$) in both cultivars at 74 μM Al, but remained significantly higher in the sensitive cultivar Dio (Fig. 2b). After 14 days of exposure to 148 μM Al, the level of lipid peroxidation in the sensitive cultivar Dio was 14 times higher than control and 8.7 times higher in the resistant cultivar Yecora E.

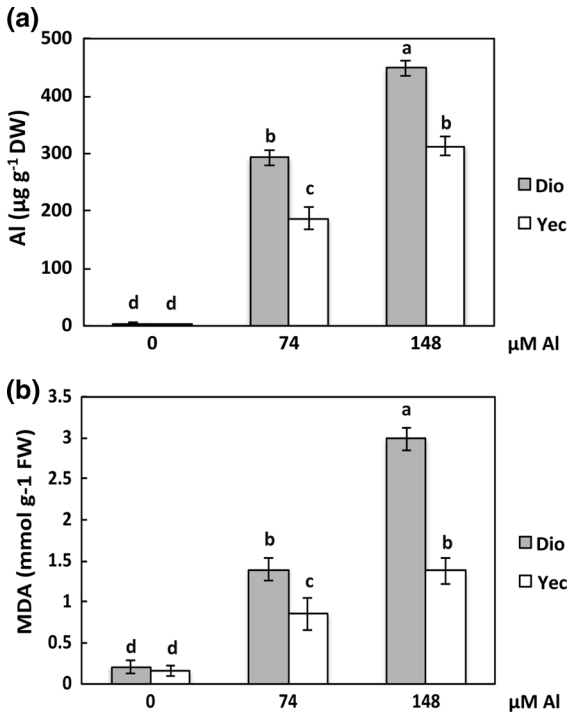

Fig. 2 Changes in **a** Al accumulation, measured as μg g^{-1} dry weight, and **b** oxidative stress, measured as mmoL (MDA) g^{-1} fresh weight, an indicator of the extent of lipid peroxidation, in the leaves of the Al-resistant cultivar Yecora E and Al-sensitive cultivar Dio, after exposure to 0, 74 and 148 μM Al. Data shown are means and SD that is indicated by *bars*. *Bars* with different lowercase letters are significantly different ($P < 0.05$)

Higher quantum yields in the resistant wheat cultivar after aluminum exposure

Exposure of wheat plants to increasing Al concentrations resulted in subsequent decreases in the ratio F_v/F_m that reflect the maximum quantum efficiency of PSII photochemistry i.e. the maximum efficiency at which light absorbed by PSII is used for reduction of Q_A and is a measure of photoinhibition. Exposure to 37 μM Al resulted in a significant decrease of F_v/F_m (i.e. increase of photoinhibition) in the cultivar Dio, while in the resistant cultivar Yecora E, F_v/F_m decreased significantly at 74 μM Al (Fig. 3a). The ratio F_v'/F_m', which reflect the photochemical yield of open, functional PSII RC decreased more than F_v/F_m in the sensitive cultivar Dio (Table 1). At 148 μM Al, the F_v/F_m ratio decreased 2 and 5 %, while the F_v'/F_m' ratio decreased 1 and 16 % in "Yecora E" and "Dio" respectively, compared to control (−Al) (Table 1).

The decrease in the photochemical quenching (q_p) and F_v'/F_m' with Al stress resulted in a decrease in the quantum yield of PSII electron transport (Φ_{PSII}) (Fig. 3b). Exposure to 37 μM Al resulted in a significant decrease of Φ_{PSII} in the cultivar "Dio", while in the resistant cultivar Yecora E decreased significantly at 74 μM Al (Fig. 3b). At 148 μM Al the quantum yield of PSII electron transport in "Yecora E" decreased 9 % while in "Dio" 33 % (Table 1).

A more oxidative state of quinone A in the resistant wheat cultivar after aluminum exposure

We estimated the redox state of PSII by measuring with modulated fluorescence the photochemical quenching (q_p). Data obtained from the two cultivars are presented in Fig. 3c. Exposure of wheat plants of both cultivars to 148 μM Al reduced the fraction of open PS II RC, (as indicated by q_p), compared to control (−Al), by 8 and 20 % in "Yecora" and "Dio E" respectively (Table 1). The fraction of closed PSII RC, i.e. Q_A reduction, was estimated as $1 - q_p$ and represents the excitation pressure on PSII (Supplementary data Fig. S1a). The depression of photosynthesis observed at the higher Al concentration resulted in a significant increase in the excitation pressure in both cultivars compared to their controls, by about 40

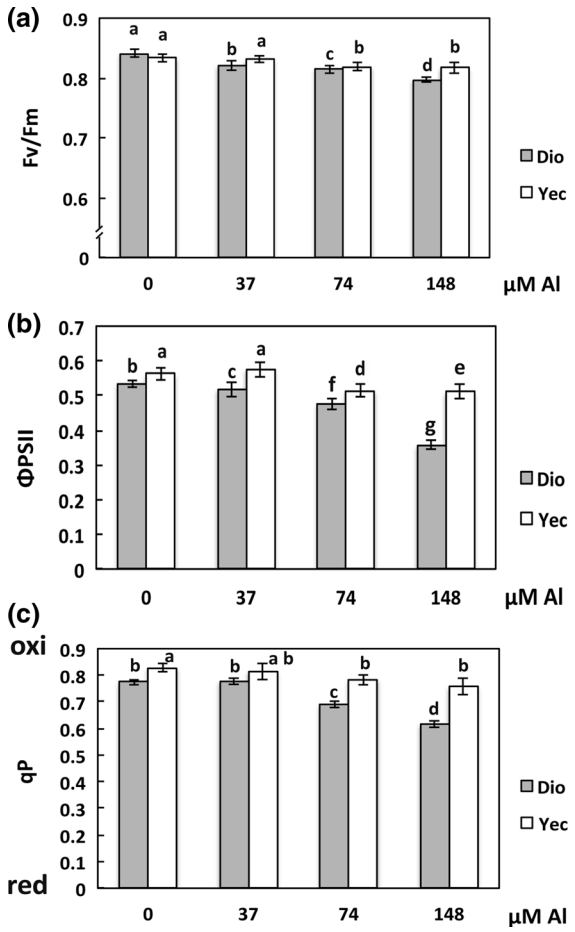

Fig. 3 Changes in **a** the maximum quantum efficiency of PSII photochemistry (F_v/F_m), **b** the effective quantum yield of PSII photochemistry (Φ_{PSII}), and **c** the photochemical quenching (q_p) that is the redox state of PSII, in the leaves of the Al-resistant cultivar Yecora E and Al-sensitive cultivar Dio, after exposure to 0, 37, 74 and 148 µM Al. Data shown are means and SD that is indicated by *bars*. *Bars* with different lowercase letters are significantly different ($P < 0.05$)

and 70 % in "Yecora E" and "Dio", respectively. An approximate 23 % closure of RCs was reached at about 37 µM Al for "Dio", and 148 µM Al for "Yecora E" leaves (Fig. 3c, Supplementary data Fig. S1a). Apparently, a 4 times higher Al concentration was needed for "Yecora E" plants to close 23 % of RCs than for "Dio" leaves.

Non-photochemical quenching increased in response to aluminum exposure

Closure of PSII RCs was accompanied by increases in non-photochemical quenching (q_N) with Al-stress. At 148 µM Al q_N increased 9 % in "Dio" and 3 % in "Yecora E" (Table 1).

Relationships between maximum quantum efficiency and oxidative damage with aluminum accumulation

The maximum quantum efficiency of PSII photochemistry (F_v/F_m) determined under 0, 74 and 148 µM Al, exhibited significant negative linear correlation with Al accumulation in the leaves of both cultivars ($R^2 = 0.927$, $P < 0.05$; Fig. 4a). The level of lipid peroxidation, measured by MDA accumulation, reflects ROS formation and corresponds to the oxidative damage. Figure 4b shows the significant positive linear correlation between Al accumulation and oxidative damage ($R^2 = 0.905$, $P < 0.05$) under 0, 74 and 148 µM Al calculated from data of Fig. 2.

Relationships between excitation pressure and effective quantum yield of PSII with aluminum accumulation

Excitation pressure ($1 - q_p$) of both cultivars during Al^{3+} exposure, exhibited a significant positive linear correlation to Al accumulation ($R^2 = 0.728$, $P < 0.05$; Fig. 4c), while the Φ_{PSII} of both cultivars during Al^{3+} exposure was significant negative correlated with Al accumulation ($R^2 = 0.743$, $P < 0.05$; Fig. 4d).

Relationship between excitation pressure and effective quantum yield of PSII with calcium and magnesium content

A strong negative relationship between closure of RCs, i.e. Q_A reduction that represents the excitation pressure on PSII ($1 - q_p$), with Ca^{2+} ($R^2 = 0.826$, $P < 0.005$; Fig. 5a), and to a less extend with Mg^{2+} ($R^2 = 0.504$, $P < 0.05$; Fig. 5b) contents in the leaves of both cultivars was verified under Al stress.

The effective quantum yield of PSII, Φ_{PSII}, of both cultivars during Al^{3+} exposure was significant positive correlated with Ca^{2+} ($R^2 = 0.784$, $P < 0.005$; Fig. 5c), and also to a less extend with Mg^{2+} ($R^2 = 0.517$, $P < 0.05$; Fig. 5d) contents in the leaves.

Table 1 Chlorophyll fluorescence parameters measured on the second leaf of control (−Al) and 148 μM Al-treated wheat plants

Cultivar	Parameter	0 μM Al	148 μM Al	Change %
Yecora	F_v/F_m	0.833 ± 0.006	0.817 ± 0.009*	−2
	F_v'/F_m'	0.678 ± 0.002	0.672 ± 0.003	−1
	q_p	0.828 ± 0.017	0.758 ± 0.031*	−8
	Φ_{PSII}	0.562 ± 0.016	0.510 ± 0.021*	−9
	q_N	0.582 ± 0.008	0.597 ± 0.005*	+3
Dio	F_v/F_m	0.841 ± 0.006	0.797 ± 0.005*	−5
	F_v'/F_m'	0.690 ± 0.005	0.579 ± 0.013*	−16
	q_p	0.774 ± 0.011	0.616 ± 0.006*	−20
	Φ_{PSII}	0.535 ± 0.010	0.356 ± 0.013*	−33
	q_N	0.623 ± 0.009	0.679 ± 0.010*	+9

Data are the mean ± SD from five separate measurements. Means followed by an asterisk are significantly different from control at $P < 0.05$

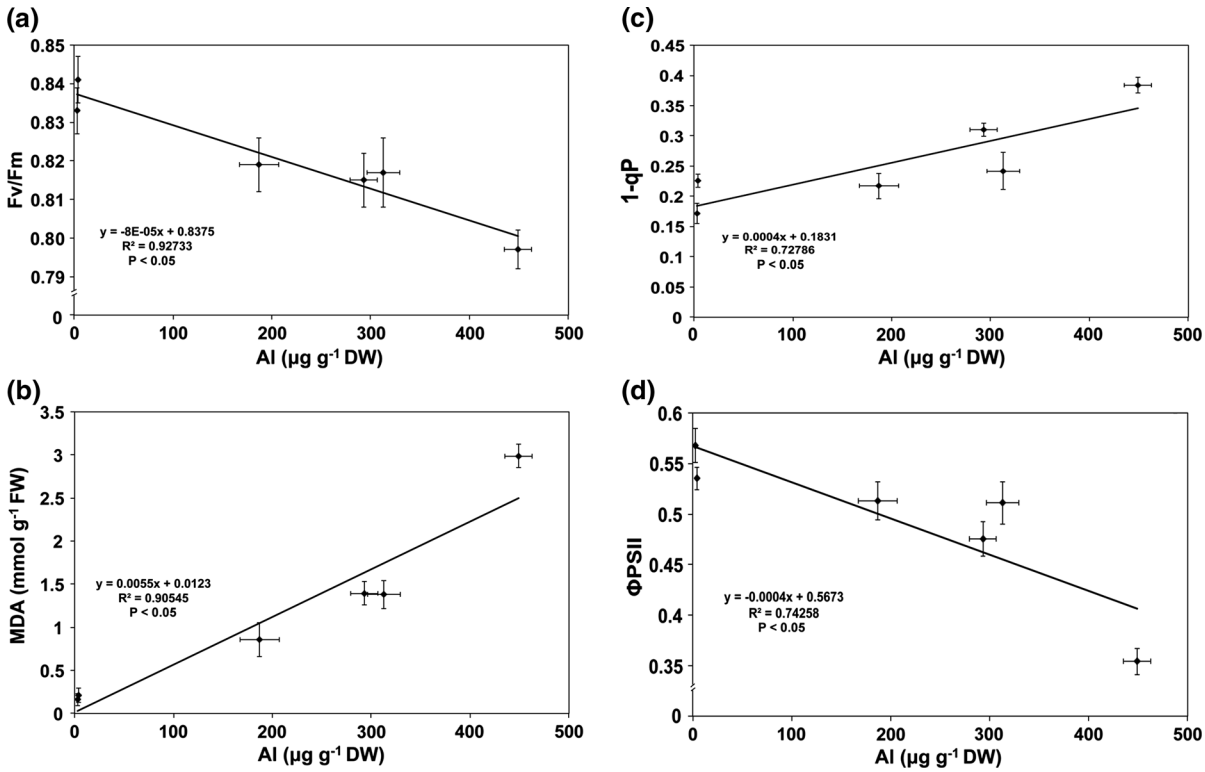

Fig. 4 Relationships between Al accumulation, measured as μg g^{-1} dry weight, with **a** the maximum quantum efficiency of PSII photochemistry (F_v/F_m), $R^2 = 0.92733$, $P < 0.05$, **b** oxidative damage, measured as mmoL MDA (malondialdehyde) g^{-1} fresh weight, $R^2 = 0.90545$, $P < 0.05$, **c** the relative reduction state of Q_A, reflecting the excitation pressure on PSII ($1 - q_p$), determined at 870 μmol photons m^{-2} s^{-1}, $R^2 = 0.72786$, $P < 0.05$, and **d** the effective quantum yield of PSII photochemistry (Φ_{PSII}), determined at 870 μmol photons m^{-2} s^{-1}, $R^2 = 0.74258$, $P < 0.05$; of the Al-resistant cultivar Yecora E and Al-sensitive cultivar Dio, after exposure to 0, 74 and 148 μM Al. SD shown was not used in regression analysis, where mean values ($n \geq 5$) were used

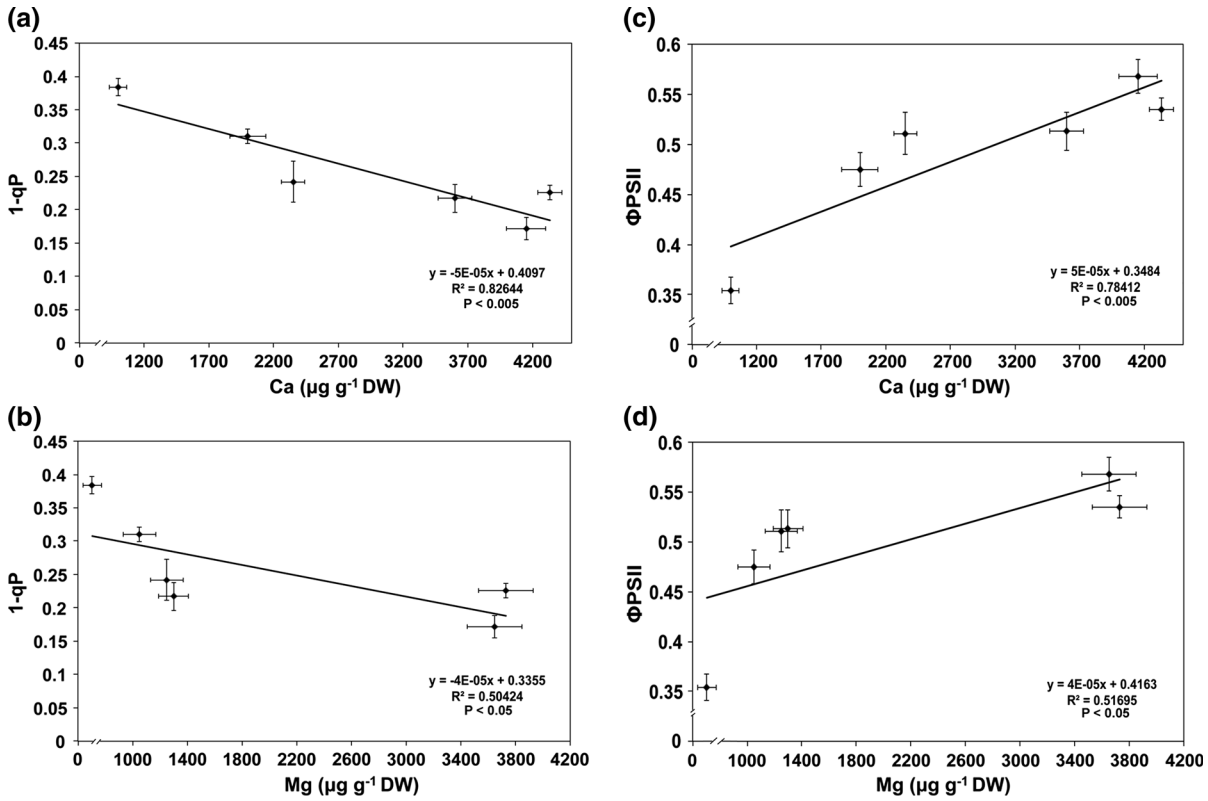

Fig. 5 Relationships between **a**, **b** excitation pressure on PSII ($1 - q_P$), determined at 870 µmol photons m^{-2} s^{-1}, with Ca^{2+} ($R^2 = 0.82644$, $P < 0.005$) (**a**), and Mg^{2+} ($R^2 = 0.50424$, $P < 0.05$) (**b**) content in the wheat leaves of both cultivars after exposure to 0, 74 and 148 µM Al; and between **c**, **d** the effective quantum yield of PSII photochemistry (Φ_{PSII}), determined at 870 µmol photons m^{-2} s^{-1}, with Ca^{2+} ($R^2 = 0.78412$, $P < 0.005$) (**c**), and Mg^{2+} ($R^2 = 0.51695$, $P < 0.05$) (**d**) content in the wheat leaves of both cultivars after exposure to 0, 74 and 148 µM Al. SD shown was not used in regression analysis, where mean values ($n \geq 5$) were used

Relationship between excitation pressure and maximum quantum efficiency

Excitation pressure on PSII ($1 - q_P$) was also negative correlated with the maximum quantum efficiency of PSII photochemistry (F_v/F_m) under Al exposure ($R^2 = 0.696$, $P < 0.05$; Supplementary data Fig. S1b).

Discussion

Al^{3+}-induced reduction in Ca^{2+} and Mg^{2+} content of the developing wheat leaves of both cultivars is a well-known phenomenon (Huang et al. 1992; Rengel 1992; Moustakas et al. 1995). Aluminum has been found to inhibit Ca^{2+} and Mg^{2+} uptake very strongly and to a greater extent than other ions (Huang et al. 1992; Rengel 1992; Mariano et al. 2015). The decreased Mg^{2+} concentration in the leaves of both wheat cultivars under Al exposure (Fig. 1a) may be due (1) to decreased Mg^{2+} absorption brought about by reduced root growth, (2) to a direct Al inhibition of Mg^{2+} uptake, or (3) to an Al^{3+} stimulated Mg^{2+} efflux from the roots as suggested by Silva et al. (2001) and Giannakoula et al. (2008). Since Al has a similar hydrated ionic radius with Mg, competes for apoplastic binding sites and plasma membrane Mg transporters (Bose et al. 2011). The ability of ryegrass cultivars to retain higher affinity for Mg^{2+} by transport proteins in the presence of Al^{3+} has been suggested as one of the mechanisms of Al resistance (Rengel and Robinson 1989). Al-resistant *Arabidopsis* genotypes maintained a higher Mg^{2+} accumulation, and had a higher Mg^{2+} influx and higher intracellular Mg^{2+} concentration than Al-sensitive genotypes (Bose et al. 2013).

Rengel (1992) found that the addition of Al^{3+} caused a large immediate reduction in Ca^{2+} influx by blocking Ca^{2+} channels. Al^{3+} due to its larger electric charge is capable of removing Ca^{2+} or any other divalent cation from plasma membranes and cell walls and to inhibit a large number of metabolic processes regulated by Ca^{2+} (Rengel and Zhang 2003; Ribeiro et al. 2013). The decrease of Ca^{2+} we observed in the leaves of both wheat cultivars exposed to Al^{3+} is attributable to either (1) an exchange reaction of Al^{3+} with Ca^{2+} in the cell wall (Reid et al. 1995), or (2) the repression of Ca^{2+} uptake by the direct blockage of Ca^{2+} channels (Rengel 1992). Aluminum is a potential inhibitor of Ca^{2+} uptake, and Al^{3+}-toxicity often induces Ca^{2+} deficiency in plants and causes disturbance of Ca^{2+} homeostasis, resulting in the inhibition of cell growth (Ishikawa et al. 2003; Rengel and Zhang 2003).

Although Al^{3+}-toxicity and other environmental stresses can have a major effect on the photosynthetic productivity of crops by influencing directly the functioning of the photosynthetic apparatus, it is possible that Al^{3+}-induced depressions in crop growth and yield can often be primarily associated with an inability of the plant to develop a fully functional photosynthetic apparatus due to modifications of the cellular ion of developing leaves (Baker and Ort 1992; Eleftheriou et al. 1993; Moustakas et al. 1997). In accordance to this, under Al exposure, we found similar significant relationships of the effective quantum yield of PSII electron transport (Φ_{PSII}), with Al accumulation (Fig. 4d, negative correlation), Ca^{2+} (Fig. 5c, positive correlation), and Mg^{2+} (Fig. 5d, positive correlation) content in the wheat leaves of both cultivars. It is postulated that Al^{3+}-induced reduction in Ca^{2+} and Mg^{2+} content of the developing leaves of both cultivars could perturb the complex series of processes associated with the development of maximal physiological function of the photosynthetic apparatus. Thus, Al^{3+}-resistance in wheat is correlated with increased levels of Ca^{2+} and Mg^{2+} content. In accordance to this, it has been pointed out that Al^{3+}-toxicity is most severe in soils poor in Ca and Mg (Abdel-Basset and Matsumoto 2008).

The fact that the resistant cultivar Yecora E accumulated in the leaves less Al than the cultivar Dio, suggests either (1) an Al exclusion mechanism from the roots (Ma et al. 2014; Kochian et al. 2015), and/or (2) an efficient endodermis barrier to Al in the root zone (Silva et al. 2010). Aluminum exclusion mechanism is an external detoxification strategy by which roots can exclude Al by secreting organic acids to bind Al^{3+} and thus reduce the amount that reaches the cell (Ma et al. 2014; Kochian et al. 2015). In most Al resistant wheat cultivars Al triggers the opening of malate-permeable channels in the plasma membrane of the root cells to secrete malate, although some cultivars also secrete citrate (Delhaize et al. 1993; Ryan et al. 2009; Ma et al. 2014). Endodermis thickening is accelerated probably as a strategy to control Al entrance in the root and transportation to the leaves in the Al-resistant wheat cultivars (Silva et al. 2010).

Accumulation of MDA, a marker for lipid peroxidation, occurs under multiple environmental stresses (Munné-Bosch and Alegre 2003; Xu et al. 2011b; Giannakoula et al. 2008; Sperdouli and Moustakas 2012b). The present study showed that MDA accumulated to a greater extent in the leaves of the cultivar Dio than in the leaves of the cultivar Yecora E, indicating that the latter had a lower lipid peroxidation level (Fig. 2b). This was mainly due to reduced Al accumulation in the leaves of the resistant cultivar Yecora E that resulted in less oxidative stress than in the cultivar Dio. However, under equal internal Al accumulation (Dio 74 μM Al, Yecora E 148 μM Al; Fig. 2a) the level of lipid peroxidation was the same (Fig. 2b). Under the same oxidative damage, the resistant cultivar Yecora E had significant higher maximum quantum efficiency of PSII photochemistry (F_v/F_m; Fig. 3a), significant higher effective quantum yield of PSII electron transport (Φ_{PSII}; Fig. 3b) and significant increased capacity to keep quinone Q_A oxidized (Fig. 3c). Thus, under the same oxidative damage the reduced PSII excitation pressure and the better PSII functioning of the Al-resistant cultivar was probably due to the larger concentrations of Mg^{2+} (Fig. 1a) and Ca^{2+} (Fig. 1b) in the leaves than in the cultivar Dio. In accordance to this, a significant positive correlation of the effective quantum yield of PSII (Φ_{PSII}) with Ca^{2+} and Mg^{2+} (Figs. 5c, d), and a strong negative relationship between PSII excitation pressure ($1 - q_p$), with Ca^{2+} and Mg^{2+} (Figs. 5a, b) contents in the leaves of both cultivars was verified under Al stress.

High excitation pressure indicates excess excitation energy and thus an imbalance between energy supply and demand (Takahashi and Badger 2011). This

imbalance may lead to an increase in light energy that is transferred from chlorophyll to oxygen, resulting in the production of ROS and tissue damage (Dietz and Pfannschmidt 2011). Several protective mechanisms contribute to prevent this, such as, cyclic electron flow around PSI, photorespiration, non-photochemical quenching (q_N) and scavenging of the unavoidably formed ROS (Niyogi 1999; Pintó-Marijuan and Munné-Bosch 2014). The non-photochemical quenching (q_N) reflects the dissipation of excess excitation energy in the form of harmless heat, thus protecting the plant from the damaging effects of ROS (Hideg et al. 2008). It seems that under Al exposure q_N increase was not high enough for sufficient protection of wheat plants from the damaging effects of ROS. Maximal photoprotection can be achieved only if q_N is regulated in such a way that RCs remain open under given conditions (Lambrev et al. 2012). The failure of photoprotective mechanisms in both wheat cultivars to Al exposure facilitated lipid peroxidation, as evident by the accumulation of MDA (Fig. 2b), indicating the involvement of ROS in Al^{3+} toxicity of the photosynthetic apparatus of wheat plants. The increased resistance to photoinhibition of the wheat cultivar Yecora E (measured as F_v/F_m), is not caused by an increased non-photochemical quenching (q_N), but rather by an increased capacity to keep quinone Q_A oxidized (q_p, Table 1). Exposure to Al^{3+} has been shown to retard electron transfer between the quinones Q_A and Q_B, resulting in PSII photochemical damage and inhibition of the photosynthetic rate (Li et al. 2012).

Generation of ROS has been implicated as an early event and important factor in Al toxicity (Tamás et al. 2004; Xu et al. 2011a; Matsumoto and Motoda 2013; Huang et al. 2014). Aluminum-induced oxidative stress has been most commonly attributed to alterations in membrane structure, which then favours radical chain reactions mediated by Fe resulting in the formation of lipid peroxides (Yamamoto et al. 2003; Abdel-Basset and Matsumoto 2008). An increase in ROS levels can cause severe oxidation of cellular components inducing redox status changes (Jubany-Marí et al. 2010; Sperdouli and Moustakas 2014b). Decreased oxidative stress results to a higher level of oxidized state of Q_A, which alleviates the accumulation of excited species within PSII (Munné-Bosch et al. 2001). Alternatively, Liu et al. (2014) speculated that ROS production might be associated with Al signaling.

The leaves of the Al-resistant Yecora E exhibited higher values of q_p at Al concentrations above 74 μM Al than did leaves of the Al-sensitive Dio. Of importance was that q_p in the cultivar Dio did not decrease in proportion to the measured quantum yield of electron transport (Φ_{PSII}), as in the cultivar Yecora E and as would be predicted theoretically (Foyer et al. 1990). In other words, the decline in photosynthetic efficiency in the cultivar Dio was not only due to closure of RCs, but also to a decrease in the quantum yield of the open RCs. In accordance to this, the ratio F_v'/F_m' had higher decrease in the sensitive cultivar Dio (Table 1).

Since the susceptibility of PSII to Al increases as a function of RCs closure to increased Al accumulation, i.e. an increased proportion of reduced to oxidized Q_A, the more resistant cultivar with lower Al accumulation maintained a substantial higher fraction of RCs in an open state. By using wheat cultivars as experimental material we demonstrated that the different sensitivities of photosynthesis to Al^{3+} toxicity in cultivars differing in their resistance to Al^{3+} could be fully accounted by differences observed in the redox state of Q_A. Thus, chlorophyll fluorescence measurements can be checked, using a larger range of cultivars, in order to be used as a tool for rapid screening of Al resistance. We also suggest that this difference in the redox state of Q_A is largely accounted for by a higher capacity for photosynthesis in the more resistant cultivar.

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