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Effect of aluminium exposure on the release of organic acids and genistein from the roots of *Lupinus albus* L. plants

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ABSTRACT

Aluminum (Al) toxicity is one of the main factors limiting crop productivity in strongly acidic soils. Plant tolerance to Al toxicity has been widely studied even if the mechanisms involved in the plant response are yet not fully elucidated. White lupin is well known to release organic acids and flavonoids under nutrient deficiency, while less is known about its response to elevated Al concentrations. The aim of this work was therefore to shed light to the adaptive response of white lupin to Al toxicity, analysing the root exudate pattern. A pH buffer (MES) or inhibitor compounds were used in order to further investigate the mechanisms adopted by white lupin to release root exudates as response to Al toxicity. The results showed that not only organic acids but also phenolic compounds are involved in the response to elevated concentrations of Al together with the alkalinisation of the growth medium.

Aluminum (Al) toxicity is one of the main factors limiting crop productivity in strongly acidic soils (Kochian et al., 2005) not only for its high release from minerals but also for its high toxicity for roots even at μM concentration (Ryan et al., 1993). Plant tolerance is mainly based on two distinct strategies: (a) Al-translocation to the shoots and, then, to the Al-deposit (as Al-organic acid complexes) in the vacuoles in Al-accumulating plant species (Shen et al., 2002); (b) root release of Al-chelating compounds to prevent Al uptake in Al-excluding plant species (Pellet et al., 1997). This latter strategy has been described for several crops (Jones, 1998; Ma, 2007) and involves the release of organic acids characterized for their Al-binding capacity (citrate > oxalate > malate). In this context it is interesting to note that some plant species are depicted for their release also of phenolic compounds as a consequence of Al-toxicity (Kidd et al., 2001; Tolrà et al., 2009). While the role played by this class of compounds has been extensively reviewed for their impact in plant nutrient acquisition (Cesco et al., 2012), their implication in Al-tolerance has received up to now only little attention. Therefore, this work is aimed at shedding light to the adaptive response of plants to Al toxicity with particular emphasis on the root-exudate pattern of organic acids and flavonoids. The experiments were performed using plants of white lupin (*Lupinus albus* L.), crop well known for its huge releases in the rhizosphere of these compounds under different nutritional disorders (Valentinuzzi et al., 2015a). A pH buffer (MES) or inhibitors such as Anthracene-9-carboxylic acid (inhibitor of anion channels) or vanadate (specific inhibitor of the plasma membrane [PM]-H^+-ATPase) were used in order to further investigate the mechanisms underlying the root exudation. For the exudates collection, roots of intact plants, grown as described in Valentinuzzi et al. (2015a), were transferred in pots containing 100 mL of: H_2O MQ (Control), $Al(NO_3)_3$ 100 μM (+Al), $Al(NO_3)_3$ 100 μM + MES-KOH (2-(N-morpholino)ethanesulfonic acid) 10 mM pH 6 (+Al +MES), $Al(NO_3)_3$ 100 μM + V_2O_5 500 μM (+Al +Van), $Al(NO_3)_3$ 100 μM + Anthracene 50 μM (+Al +Anthr). The collection lasted 2 or 24-h; thereafter, roots were removed, weighed and stored at –80 °C while the solutions filtered at 0.45 μm and lyophilized. Root contents of organic acids and flavonoids were analysed *via* a root-extraction with 100% (v/v) methanol (1-h). The achieved solutions, once filtered (0.45 μm), were evaporated. The content of organic acids and flavonoids both in lyophilized exudates and roots extracts were analysed by HPLC after their re-suspension in distilled water or in methanol for organic acids or phenolic compounds, respectively. Results (means of 3 replicates ± SD) were statistically analysed using Statgraphics (Statpoint technologies, INC., Warrenton, VA, USA). Analysis of variance (ANOVA) was carried out and means were compared using LSD test at $P < 0.05$.

Results confirm the increase of citrate release by roots of Al-treated plants after 2-h (Fig. 1A). The limitation of citrate exudation for the addition of the pH buffer as well as of vanadate corroborates the idea of the involvement of the PM-H^+-ATPase in the

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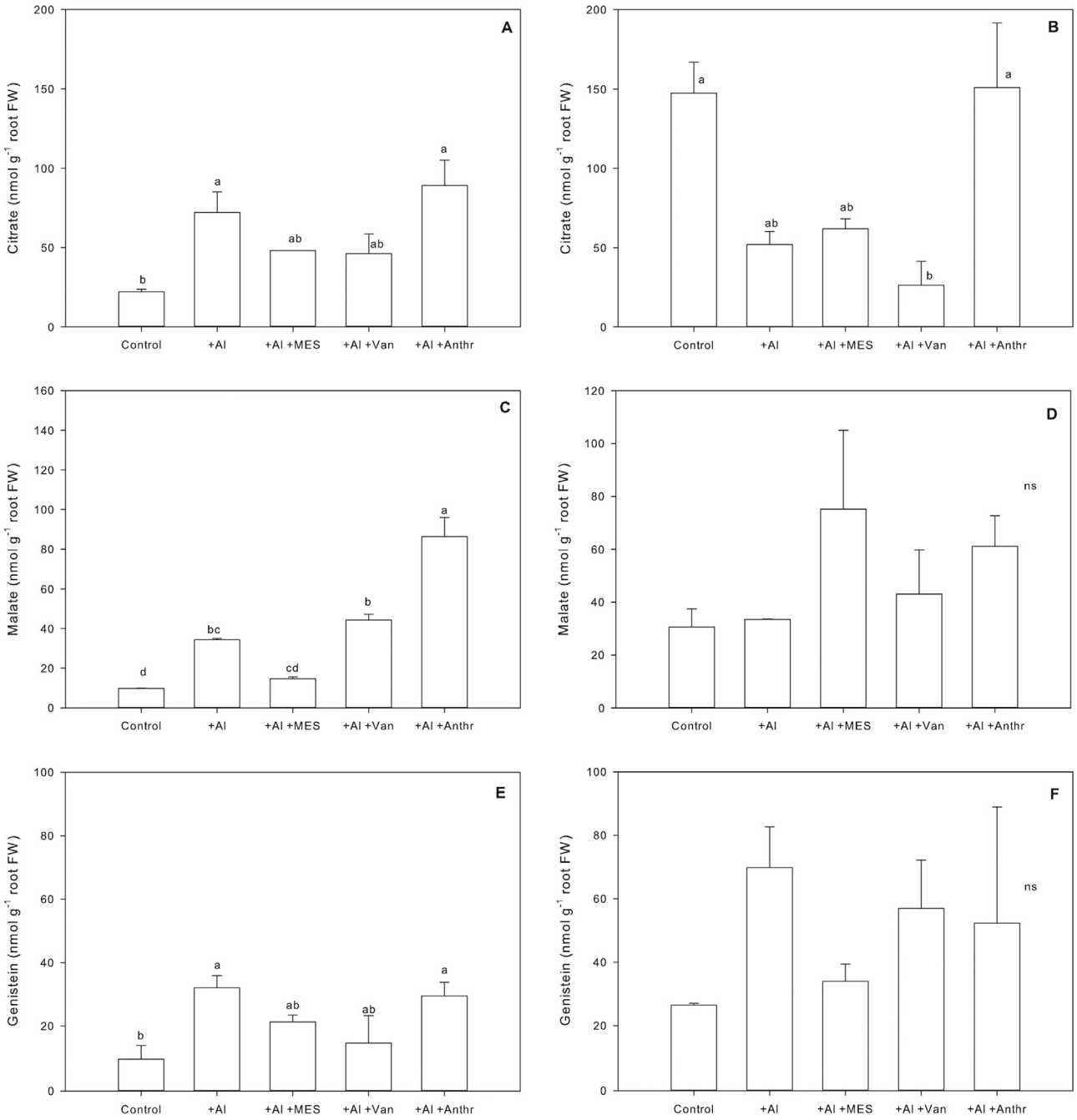

Fig. 1. Citrate, malate and genistein detected in root exudates collected from white lupin roots at 2 (A, C, E) and 24 h (B, D, F) respectively, in the following release solutions: H_2O MQ (Control), $Al(NO_3)_3$ 100 μM (+Al), $Al(NO_3)_3$ 100 μM+MES-KOH (2-(N-morpholino)ethanesulfonic acid) 10 mM pH 6 (+Al +MES), $Al(NO_3)_3$ 100 μM+V_2O_5 500 μM (+Al +Van), $Al(NO_3)_3$ 100 μM + Anthracene-9-carboxylic acid 50 μM (+Al +Anthr); (mean ± SD, n=3); different letters indicate significant differences at $P < 0.05$ as measured by an LSD test.

organic acid release, as described in P-deficiency (Tomasi et al., 2009). In fact, it is supposed that the citrate release is carried out by specific transporters belonging to MATE family (Valentinuzzi et al., 2015b; Wang et al., 2014) *via* an antiport mechanism with H^+. Therefore, an alteration of the transmembrane H^+-gradient could surely compromise the functionality of these mechanisms. In addition, no effect of Anthracene was observed, differently from what described by Zhu et al. (2005) and Neumann and Römheld (1999). However, the different experimental conditions adopted in these three studies make their results difficult to compare. Prolonging the exudate collection up to 24-h, the citrate release and its content in roots of Al-treated plants were lower than in control plants suggesting the functionality of an Al-detoxification mechanism (Mimmo et al., 2013) based on the translocation to the shoot and allocation into the vacuoles of the Al-citrate complexes (Shen et al., 2002).

The root-exudate analysis showed for the first time a triggered (three times higher) release of malate (Fig. 1C, D) as a consequence of the plant Al-exposure, differently from what previously described by Wang et al. (2006) in white lupin. The Al-dependent malate-efflux from root apices has been described as response to Al-resistance in different plant species (Inostroza-Blancheteau et al., 2012). Moreover, the addition of Anthracene further increased the release of malate in Al-treated plants, differently to what described by Zhu et al. (2005). Considering what experienced by Ligaba et al. (2004) with *Lupinus pilosus* plants, this effect