Relationship between aluminium exclusion and accumulation in the aluminium accumulator buckwheat (*Fagopyrum esculentum* Moench)

Benjamin Klug, Walter J. Horst

Institute of Plant Nutrition, Leibniz Universität Hannover, Faculty of Natural Sciences, Klug@pflern.uni-hannover.de

Introduction:

Aluminium accumulation, Al exclusion and Al tolerance are not yet well understood in buckwheat (for further details see: Ma and Hiradate (2000) and references therein). Little is known about the membrane passage of Al in this Al accumulating plant. The free Al^{3+} is supposed to be the form which is taken up (Ma & Hiradate, 2000). The root apex is not supposed to be the site of Al uptake because the secretion of oxalate in the same root zone is suggested to exclude Al from the Al-sensitive root tip (Zheng et al., 1998). The aim of this study was to explore the interrelationship between Al exclusion and Al accumulation in the root apex of the Al accumulator buckwheat.

Results:

Al accumulation is primarily confined to the apical root tip

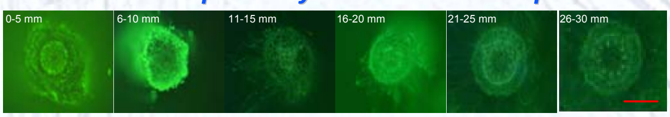

Fig. 1) Morin staining of root-tip cross-sections in different distances from the root tip after 24 h Al treatment (75 µM) in minimal nutrient solution (500 µM $CaCl_2$; 8 µM H_3BO_3; 100 µM K_2SO_4) at pH 4.3, 60 ms exposure time, 100 fold magnification, red bar represents 200 µm.

Al transport and Al resistance are colocalized in the first 10 mm of the root tip

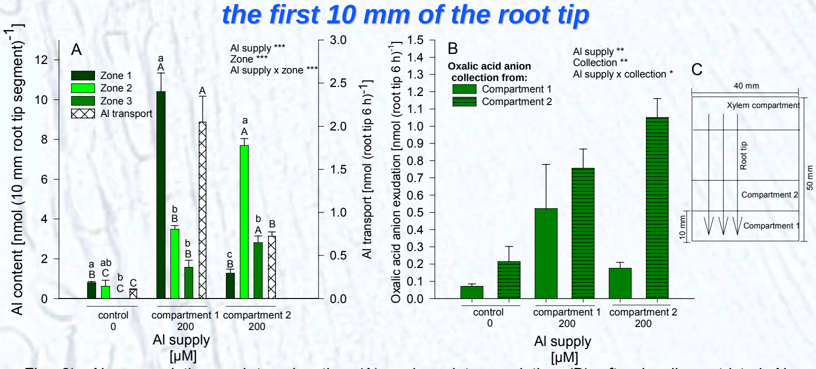

Fig. 2). Al accumulation and translocation (A) and oxalate exudation (B) after locally restricted Al application (+/- 200 µM) in micro rhizotrones (C) in minimal nutrient solution (500 µM $CaCl_2$; 8 µM H_3BO_3; 100 µM K_2SO_4) at pH 4.3 of excised adventitious buckwheat 45 mm root tips. Bars represent means +/- SE; n = 9; Different letters denote a significant difference at α = 5%.

Al loading and unloading of the symplast are active processes

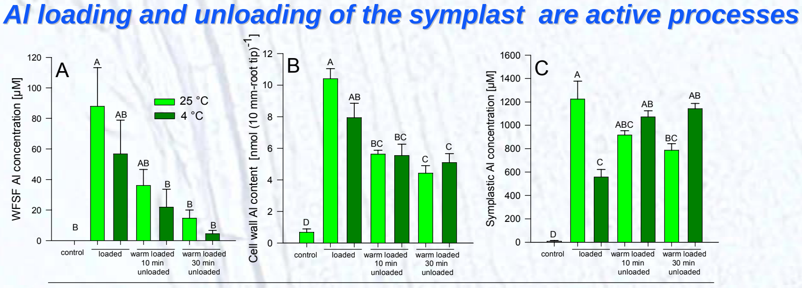

Fig. 3) Water free space (A), cell wall (B) and symplastic (C) Al concentration obtained by centrifugal extraction after warm (25°C; green bars) and cold (4°C; dark green bars) Al treatment (75 µM) for 0.5 h after different unloading durations in minimal nutrient solution (500 µM $CaCl_2$; 8 µM H_3BO_3; 100 µM K_2SO_4) at pH 4.3 of excised adventitious buckwheat 10 mm root tips. Bars represent means +/- SE; n = 5; Different letters denote a significant difference at α = 5%.

Conclusions:

Al resistance and tolerance mechanisms are spatiotemporally interrelated. Al uptake and xylem loading are energy-consuming processes. Excess supply of oxalate to Al in the external solution reduces the Al-induced inhibition of root elongation by reducing Al concentrations in the apoplast. The application of an anion channel inhibitor enhances the Al induced inhibitory effects. However, the translocation is not affected and again only excess oxalate concentrations lead to a collapse of symplastic uptake and translocation of Al. **This is interpreted as circumstantial evidence that Al is transported into the symplast as $(Al\text{-}Ox_1)^+$ complex.**

References:
Ma & Hiradate, 2000, Planta 211, 355-360 ; Zheng et al. 1998, Plant Physiol. 117, 745–751 ;Yu et al. 1999, New Phytol. 143, 299-304 ; Wang et al. 2004, Plant Phys. 136, 3762–3770

Material & Methods:

Fractionation of Al and organic acid anions: A fractionated extraction procedure was applied following the methodology suggested by Yu et al. (1999) and modified by Wang et al. (2004). *Al determination:* Al was determined by GF-AAS after digestion of root tips with ultra-pure nitric acid. *Determination of organic acids:* The organic acid concentrations in the nutrient solution were analyzed by HPLC. *Al loading and unloading of root tips:* 30 root tips (10 mm length) per sample were collected in ice cold nutrient solution at pH 4.3. This solution was replaced three times. All root tips, except the control treatment, were transferred to either a warm or a cold Al containing solution. Only warm loaded root tips were transferred to either warm or cold unloading minimal nutrient solution without Al for 10 or 30 min. Anion-channel inhibitor and oxalate applications: Plants were exposed to Al, phenylglyoxal (PG) and oxalic acid in different ratios in nutrient solution. Plants were pre-treated with 75 µM Al for 15 min prior to the Al/oxalate treatment in order to trigger Al-induced oxalate exudation.

The Al to oxalate ratio is decisive for Al uptake

Anion channel inhibition   Oxalic acid effect

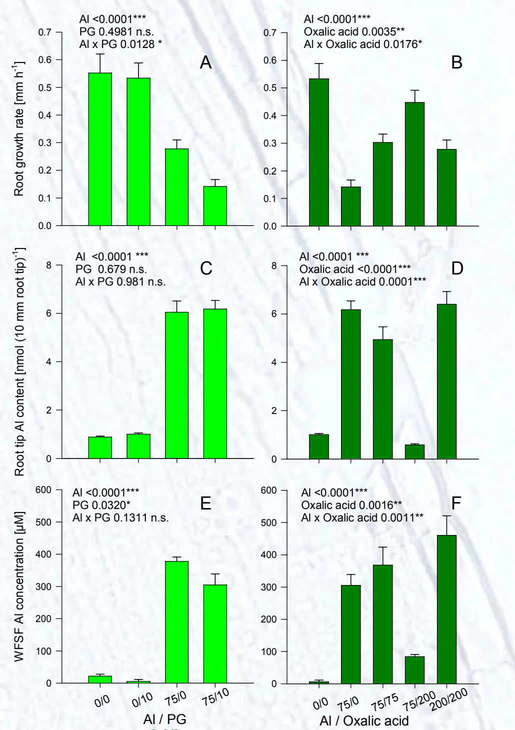

Fig. 4) Root growth rate (A+B), root tip Al content (C+D) and WFSF Al concentration (E+F) after 24 h treatment in differentially composed nutrient solution of 500 µM $CaCl_2$, 8 µM H_3BO_3, 100 µM K_2SO_4 at pH 4.3, +/- 75 or 200 µM $AlCl_3$, +/- 10 µM PG and +/- 75 or 200 µM oxalic acid. Bars represent means +/-SE; n = 4, Different letters denote a significant difference at α = 5 %.