Aluminum toxicity and resistance in *Phaseolus vulgaris* - physiology drives molecular biology.

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Abstract

In common bean (*Phaseolus vulgaris*) aluminium (Al) inhibits root elongation not only when applied to the transition zone but also to the elongation zone. After a very Al-sensitive initial response (0-4 h), acquired genotypic specific Al resistance (8-24h) is related to the sustained release from the root apices of particularly citrate. This requires both the expression/activation of a citrate permease and the maintenance of the cytosolic citrate concentration through up-regulation of citrate synthesis and down-regulation of its degradation. Proteomic and transcriptomic studies focussed on the dynamics of the expression of Al resistance in an Al-resistant genotype allowed to pinpoint some of the decisive genes.

Common bean is the most important food legume for more than 300 million people, most of them in the developing world. About 40 % of the bean-growing area in Latin America and in central, eastern, and southern Africa are affected by aluminium (Al) toxicity resulting in yield reduction up to 60 %. Correction of acidity-related soil constraints using lime and phosphate fertilizers are beyond the capacity of resource-poor farmers. They need to make use of the variation existing for adaptation to acid soils and Al resistance among common bean genotypes. Knowledge on the physiological and molecular mechanisms of Al toxicity and Al resistance can contribute to the development of rapid and reliable screening procedures needed for accelerated conventional and molecular breeding for Al resistance. This paper focuses initially on the temporal and spatial effects of Al on root growth, Al accumulation and cellular localization, and Al exclusion mediated by the release of organic acid anions.

In agreement with Cumming et al. (1992) our results comparing two genotypes differing in Al resistance clearly corroborated that Al resistance in common bean is an Al-inducible trait. Root elongation of both genotypes was severely inhibited during the first 4 h of Al treatment. The analysis of spatial growth profiles revealed that the initial inhibition of root elongation by Al resulted from a generalized effect along the entire elongation zone (EZ). This was reflected by a reduced maximal rate of relative elongation, without changing the shape or the length of the entire EZ. Localized application of Al to specific zones of the root apex showed that in both genotypes application of Al to the transition zone (TZ) resulted in root-growth inhibition to the same extent as if the whole root tip would have been treated with Al. These results confirmed studies with maize, reporting that the TZ is the most Al-sensitive apical root zone). However, in contrast to maize (Kollmeier et al., 2000) application of Al to the EZ also reduced root growth in both common bean genotypes, though to a lesser extent than when applied to the TZ. After the initial growth inhibition, both genotypes showed gradual recovery. However, this recovery continued in genotype Quimbaya (Al-resistant) until the root-elongation rate reached the level of the control (without Al), while the genotype VAX-1 (Al-sensitive) was increasingly damaged by Al after 12 h of Al treatment.

The observed Al-induced inhibition of root elongation corresponded with an enhanced Al accumulation in the TZ owing to higher pectin contents of this zone. Additionally, the higher initial Al accumulation of cv Quimbaya in the root apex compared to VAX-1 could be related to its higher content of unmethylated pectin and thus higher negativity of the CW. Aluminium treatment enhanced the root-tip pectin-content in both genotypes and decreased its degree of methylation thus enhancing the overall negativity of the CW.

The monitoring of the binding state and cellular distribution of Al in the root apices during the initial inhibition of root elongation and subsequent recovery in genotype Quimbaya revealed that the root elongation-rate was significantly negatively correlated with the free apoplastic and the stable-bound, not citrate-exchangeable cell-wall Al representing the most important Al fraction in the root apex (80%), but not with the symplastic and the labile-bound, citrate-exchangeable cell-wall Al. It is concluded that the induced and sustained Al resistance in the Al-resistant genotype Quimbaya is mediated by reducing the stable-bound Al in the apoplast thus allowing cell elongation and division to resume. Greater accumulation

of Al in the symplastic fraction in genotype Quimbaya might by explained by the greater cell volume and thus vacuoles typical for genotypes of Andean origin in comparison to the Mesoamerican origin of VAX-1.
The initial genotype-independent Al injury was related to the absence of citrate exudation from the root tips in both genotypes in spite of high citrate contents in the root apices particularly in genotype Quimbaya. Thereafter (5-9 h), in both genotypes recovery of root elongation was related to an Al-enhanced exudation of citrate typical for pattern-II Al-induced release of organic acid anions (Ma et al., 2001). Aluminium-enhanced citrate exudation has been previously implicated in Al resistance of common bean (Miyasaka et al., 1991; Mugai et al., 2000; Shen et al., 2002) and in soybean (Silva et al., 2001; Yang et al., 2001). This citrate release requires the activation or expression of an organic anion permease in the plasma membrane and is initially mainly derived from the internal organic acid pool. In genotype VAX-1 citrate efflux could be sustained during this period by a down-regulation of the activity of NADP-isocitratedehydrogenase (ICDH) thus reducing the cytosolic turnover of citrate and a low but constant citrate synthase (CS) activity. In genotype Quimbaya, the citrate efflux was sustained by both a lower NADP-ICDH activity and a greater internal citrate pool in spite of a decreased CS activity. In genotype Quimbaya the recovery of the CS activity after 24 h Al treatment allowed sustaining an enhanced capacity to exude citrate and to restore the internal organic acid pool through maintaining a high level of PEPC activity thus remaining independent of the supply of assimilates through glycolysis as indicated by a lack of response of ATP-phosphofructokinase a key enzyme channeling assimilates into the tricarbonic acid (TCA) cycle. This led to decreasing root-tip Al contents, and thus recovery of root growth. This response suggests a similar regulation as in soybean (Ermolayev et al., 2003). In genotype VAX-1 the temporal recovery from initial Al injury through enhance release of organic acid anions could not be sustained because the inability to maintain PEPC at a higher level. A greatly stimulated ATP-PFK activity under Al stress in VAX-1 may reflect an enhanced need of C skeletons through glycolysis in order to cope with the release of OA-anions which, however, cannot be met.
Based on this detailed physiological characterization we decided to focus further studies aiming at a better understanding of the molecular basis of Al toxicity and resistance particularly on the initial Al-induced inhibition of root elongation (0-4 h Al treatment) and the acquisition of Al resistance (4-24 h Al treatment) in the genotype Quimbaya. Through a proteomic approach using 2D IEF / SDS PAGE and 1D high resolution SDS PAGE followed by Nano-LC-MS/MS peptide analysis, and a transcriptomic approach through the construction of a differential cDNA library using the “Suppression Subtractive Hybridization” (SSH) system we were able to identify a range of genes up and down regulated during the initial Al-stress perception and the acquisition of Al resistance in an identical genetic background. The expression of genes related to citrate membrane transport and maintaining cytosolic citrate concentrations were quantified using qRT-PCR allowing pinpointing some of the decisive genes involved in the build up of Al resistance in an Al resistant common bean genotype. The study demonstrates that in plants species with largely unsequenced genome and inaccessible to reverse genetics, an in-depth physiological understanding is a prerequisite for a molecular understanding of Al toxicity and resistance.

References:

Cumming JR, Buckelew Cumming A, Taylor GJ (1992) Patterns of root respiration associated with the induction of aluminium tolerance in *Phaseolus vulgaris*. Journal of Experimental Botany. 43: 1075-1081

Ermolayev V, Weschke W, Manteufel R (2003) Comparison of Al-induced gene expression in sensitive and tolerant soybean cultivars. Journal of Experimental Botany 54: 2745-2756.

Eticha D, Stass A, Horst WJ (2005b) Cell-wall pectin and its degree of methylation in the maize root-apex: significance for genotypic differences in aluminium resistance. Plant Cell and Environment. 28: 1410-1420.

Kollmeier M, Felle HH, Horst WJ (2000) Genotypical differences in aluminum resistance of maize are expressed in the distal part of the transition zone. Is reduced basipetal auxin flow involved in inhibition of root elongation by aluminum? Plant Physiology. 122: 945-956.

Ma JF, Ryan PR, Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends in Plant Science. 6: 273-278.

Miyasaka SC, Buta JG, Howell RK, Foy CD (1991) Mechanism of aluminum tolerance in snapbeans: Root exudation of citric acid. Plant Physiology 96: 737-743.

Mugai EN, Agong SG, Matsumoto H (2000) Aluminium tolerance mechanisms in Phaseolus vulgaris L.: Citrate synthase activity and TTC reduction are well correlated with citrate secretion. Soil Science and Plant Nutrition. 46: 939-950.

Silva IR, Smyth TJ, Raper CD, Carter Jr. TE, Rufty TW (2001) Differential aluminum tolerance in soybean: An evaluation of the role of organic acids. Physiologia Plantarum. 112: 200-210.

Shen H, Yan X, Wang X, Zheng S (2002a) Exudation of citrate in common bean in response to aluminum stress. Journal of Plant Nutrition. 25: 1921-1932.

Yang ZM, Nian H, Sivaguru M, Tanakamaru S, Matsumoto H (2001) Characterization of aluminium-induced citrate secretion in aluminium-tolerant soybean (*Glycine max*) plants. Physiologia Plantarum. 113: 64-71.