Proteomic and metabolomic analysis of manganese toxicity in cowpea (*Vigna unguiculata* L.)

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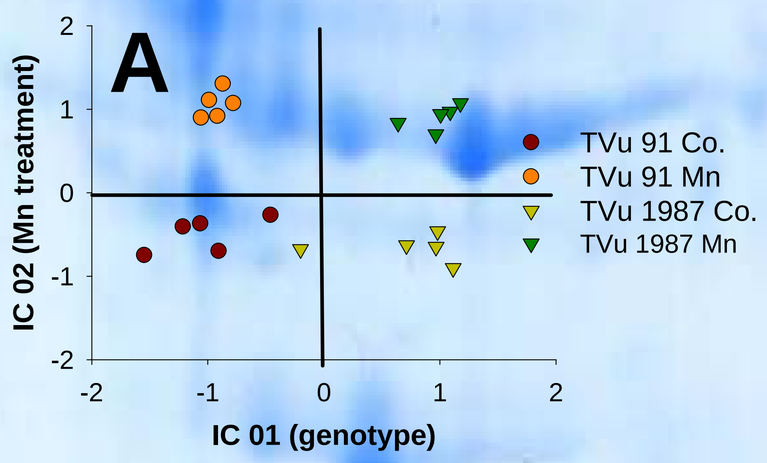
Introduction: Previous studies have shown that the leaf apoplast is the decisive leaf compartment for the expression of manganese (Mn) toxicity in cowpea (Fecht-Christoffers et al., 2003). The effect of the apoplastic metabolome on the apoplastic proteome seemed to be a key factor (Fecht-Christoffers et al., 2006). This may contribute to the understanding of genotypic differences in Mn tolerance and Si-enhanced Mn tolerance (Iwasaki et al., 2002). Here we present techniques to analyse the total proteome (DIGE) and metabolome (GC-MS and Independent Component Analysis, ICA) in cowpea bulk-leaf extracts and in the leaf apoplast.

Results:

1.1. The differences between the samples of the bulk-leaf metabolome are mainly characterized by the genotypes (IC01) and Mn treatments (IC02) as they are the Independent Components.



1.2. The AWF of both genotypes revealed the infiltration solution (IC01) and, within the ionically bound fraction, the Mn treatment (IC02) as the two most important Independent Components explaining the differences between samples.

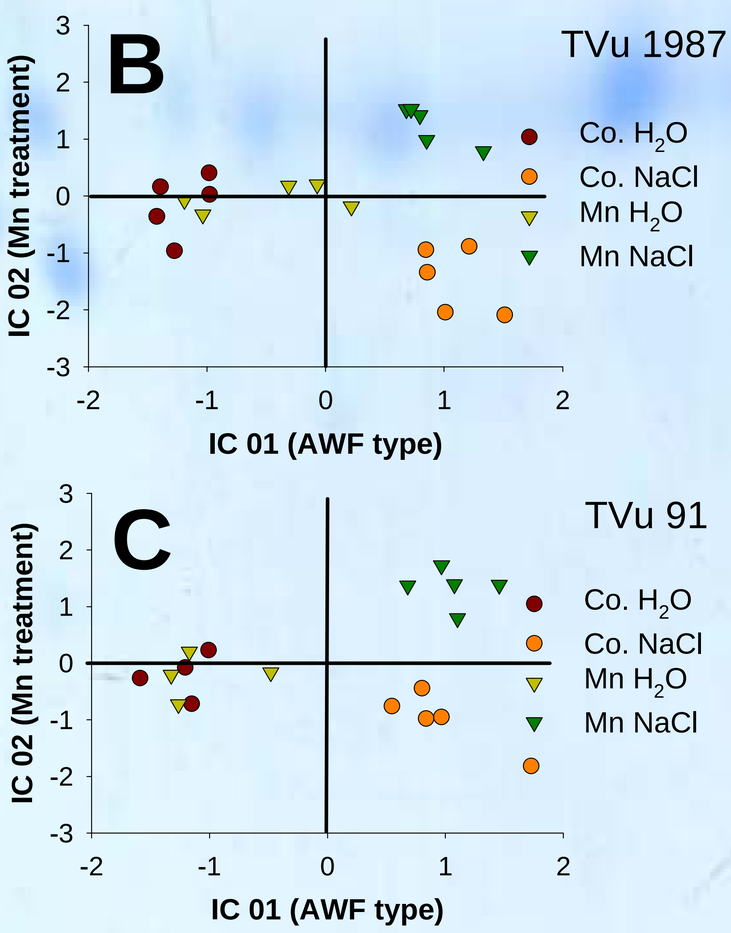


Fig.1. *ICA plot of the first two ICs of the metabolome of (**A**) the homogenate, (**B**) the AWF of the Mn-tolerant cv TVu 1987 and (**C**) the Mn-sensitive cv TVu 91. H_2O and NaCl mark the infiltration solution to extract apoplastic metabolites*

Materials and methods: Apoplastic Washing Fluid (AWF) was extracted using an infiltration/centrifugation technique (Fecht-Christoffers et al., 2003). **GC-MS analysis** of the bulk-leaf metabolome and AWF was carried out according to http://www.mpimp-golm.mpg.de/mms-library/details-e.html (Nikiforova et al., 2005) with modifications for the AWF. **DIGE technology** was applied for bulk-leaf proteome analysis according to the manufacturers instructions using paper-bridge loading (Sabounchi-Sch¸tt et al., 2000). Gel analysis was carried out using DeCyder Software (GE Healthcare).

2. Separation of proteins of the bulk-leaf proteome using DIGE technology showed several spots differing in the expression level due to Mn treatment for both genotypes.

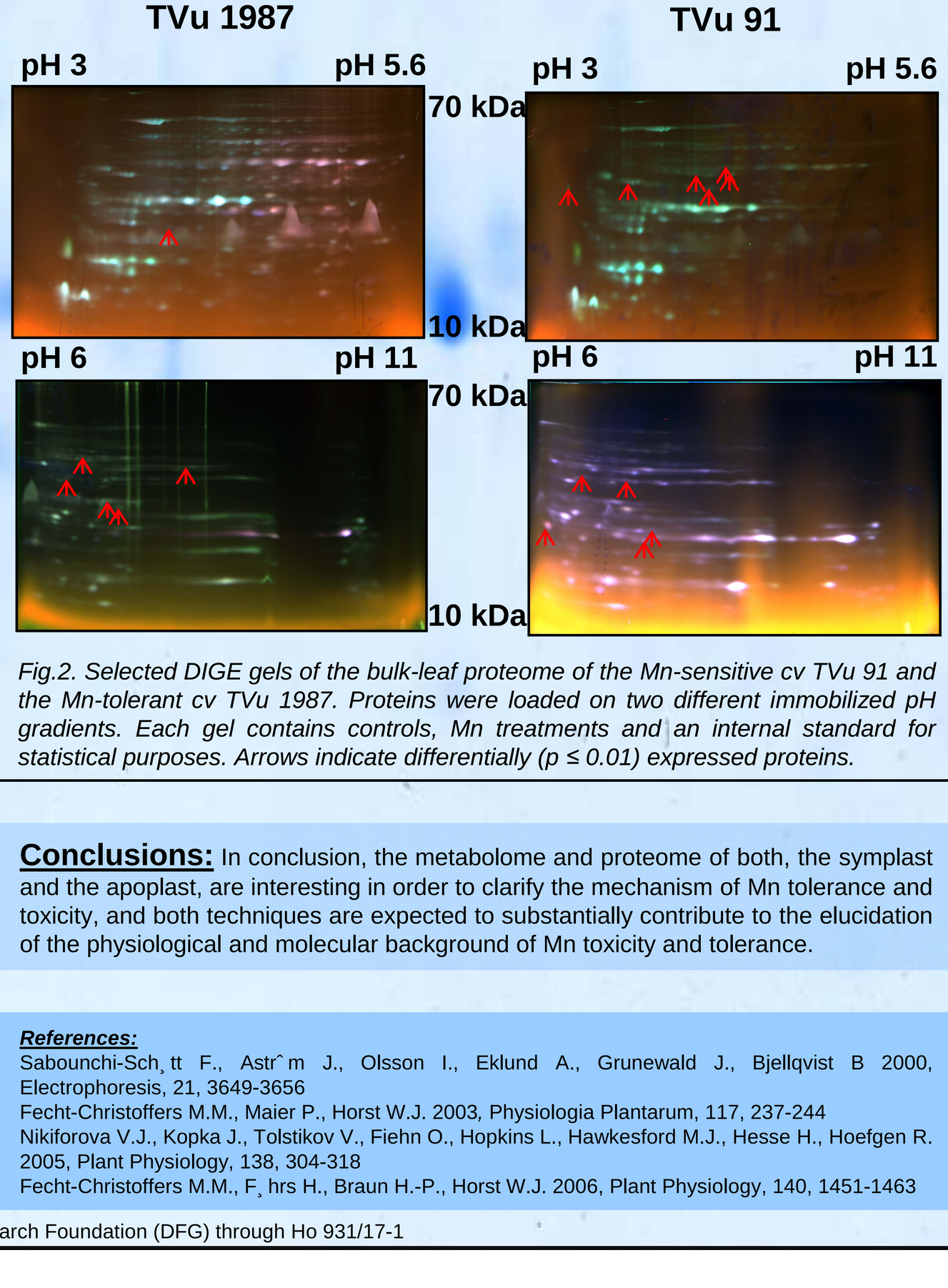


Fig.2. Selected DIGE gels of the bulk-leaf proteome of the Mn-sensitive cv TVu 91 and the Mn-tolerant cv TVu 1987. Proteins were loaded on two different immobilized pH gradients. Each gel contains controls, Mn treatments and an internal standard for statistical purposes. Arrows indicate differentially ($p \leq 0.01$) expressed proteins.

Conclusions: In conclusion, the metabolome and proteome of both, the symplast and the apoplast, are interesting in order to clarify the mechanism of Mn tolerance and toxicity, and both techniques are expected to substantially contribute to the elucidation of the physiological and molecular background of Mn toxicity and tolerance.

References:
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