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Characterization of leaf apoplastic peroxidases and metabolites in *Vigna unguiculata* in response to toxic manganese supply and silicon

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Previous work suggested that the apoplastic phenol composition and its interaction with apoplastic class III peroxidases (PODs) are decisive in the development or avoidance of manganese (Mn) toxicity in cowpea (*Vigna unguiculata* L.). This study characterizes apoplastic PODs with particular emphasis on the activities of specific isoenzymes and their modulation by phenols in the Mn-sensitive cowpea cultivar TVu 91 as affected by Mn and silicon (Si) supply. Si reduced Mn-induced toxicity symptoms without affecting the Mn uptake. Blue Native-PAGE combined with Nano-LC-MS/MS allowed identification of a range of POD isoenzymes in the apoplastic washing fluid (AWF). In Si-treated plants Mn-mediated induction of POD activity was delayed. Four POD isoenzymes eluted from the BN gels catalysed both H_2O_2-consuming and H_2O_2-producing activity with pH optima at 6.5 and 5.5, respectively. Four phenols enhanced NADH-*per*oxidase activity of these isoenzymes in the presence of Mn^{2+} (p-coumaric>>vanillic>>benzoic>ferulic acid). p-Coumaric acid-enhanced NADH-*per*oxidase activity was inhibited by ferulic acid (50%) and five other phenols (50–90%). An independent component analysis (ICA) of the total and apoplastic GC-MS-based metabolome profile showed that Mn, Si supply, and the AWF fraction (AWF_{H2O}, AWF_{NaCl}) significantly changed the metabolite composition. Extracting non-polar metabolites from the AWF allowed the identification of phenols. Predominantly NADH-*per*oxidase activity-inhibiting ferulic acid appeared to be down-regulated in Mn-sensitive (+Mn, –Si) and up-regulated in Mn-tolerant (+Si) leaf tissue. The results presented here support the previously hypothesized role of apoplastic NADH-*per*oxidase and its activity-modulating phenols in Mn toxicity and Si-enhanced Mn tolerance.