Cadmium isotope fractionation reveals genetic variations in Cd uptake and translocation by *Theobroma cacao* and role of NRAMP5 and HMA-family transporters

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In response to new EU regulations, studies are underway to mitigate accumulation of toxic Cd in cacao. This investigation advances such work with Cd isotope analyses of 19 genetically diverse cacao clones and yeast transformed to express cacao NRAMP5 and HMA-family transporters.

The cacao plants have lighter Cd isotope compositions than the hydroponic solutions with $\Delta^{114/110}Cd_{tot\text{-}sol}$= –0.22 ± 0.08‰. Leaves show a systematic enrichment of isotopically heavy Cd relative to total plants, in accord with closed-system isotope fractionation characterized by $\Delta^{114/110}Cd_{seq\text{-}mob}$= −0.13‰. This reflects sequestration of isotopically light Cd in roots/stems and mobilisation of remaining Cd to leaves. The findings reveal that (i) transfer of Cd between roots and leaves is primarily unidirectional; (ii) different cacao clones utilise similar pathways for Cd sequestration, which are distinct from those of other studied plants; (iii) cacao clones differ significantly in the efficiency of Cd sequestration.

Transgenic yeast that expresses NRAMP5 was enriched in isotopically light Cd, indicating that NRAMP5 transporters constitute an important pathway for Cd uptake by cacao. The Cd isotope signatures of transgenic yeast expressing HMA-family proteins indicate the latter may contribute to Cd sequestration in cacao. With this work, our study is the first to apply *in vivo* techniques to characterize the isotope fractionation induced by specific transporter proteins.