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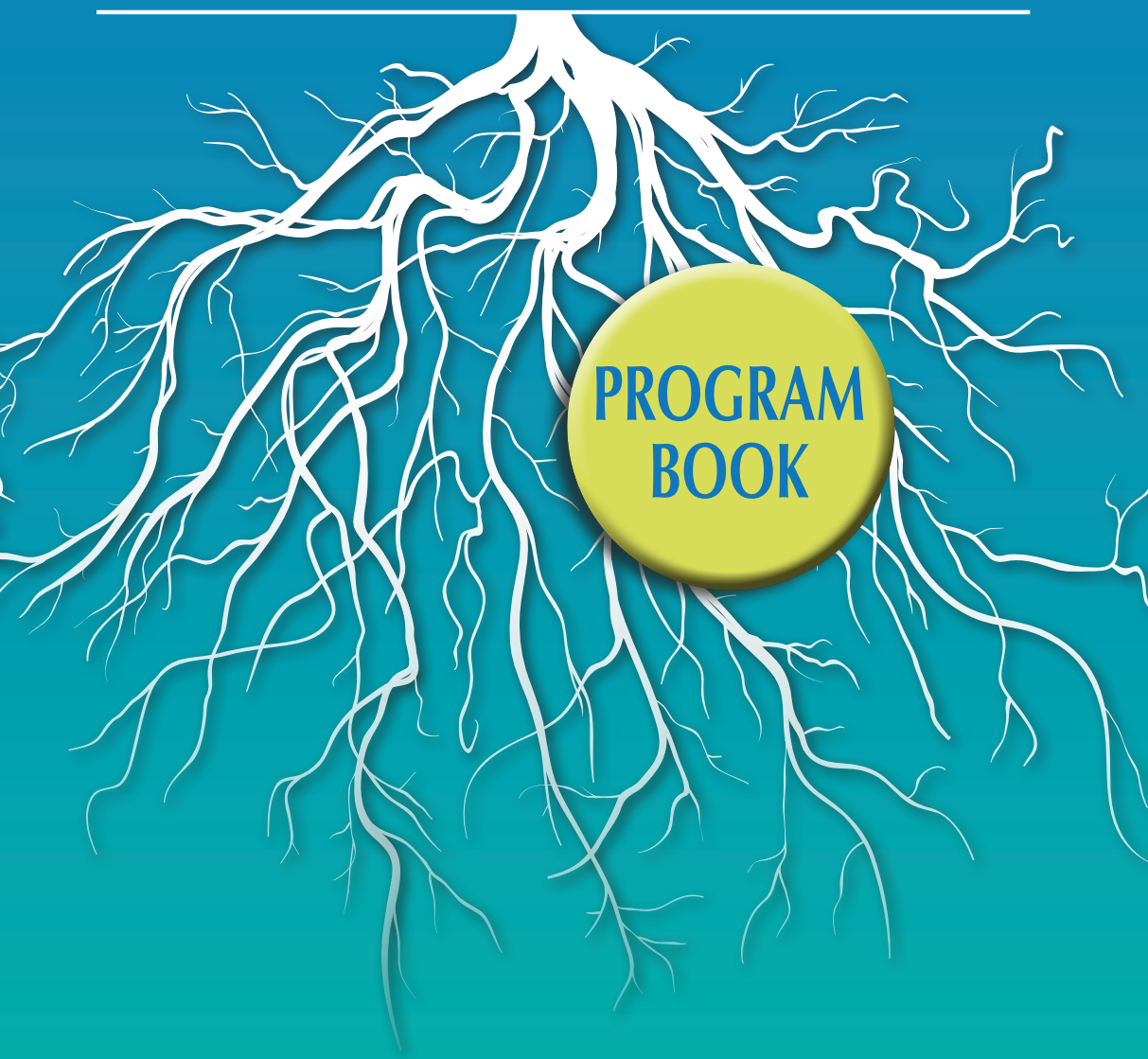

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Intracellular $CaCO_3$ Mineralisation in Roots from Mediterranean Calcareous Soils: Micromorphology and DNA Identification of Calcifying Plants

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Intracellular calcium carbonate biomineralisation in fine roots has been well known for more than 30 years since the classic studies of Jaillard et al. (1991) in southern France. Although this process is one of the most impressive cases of immense biologically induced mineral formation in terrestrial ecosystems (Hinsinger 2013), intracellular Ca carbonate in roots has received less attention than the well-documented precipitation of Ca oxalate. Accumulation of $CaCO_3$ in root cortical parenchyma, coupled with extrusion of protons to the rhizosphere, has been interpreted as an effective nutrient acquisition mechanism in plants, adapted to nutrient-poor calcareous soils, such as the present-day soils of the Mediterranean.

Intracellular carbonate precipitation in fine roots has also been reported from in vitro experiments on model plants, whereas, to our knowledge, no calcifying plants have been identified in their natural settings. Fine roots from a mixed belowground community are difficult to identify at any taxonomic level. Furthermore, it is virtually impossible to trace an individual plant root system from a single (calcified) fragment, excavated deep in a soil profile, to its coarse primary root and to the determinable aboveground part of a plant.

We used DNA barcoding, which uses a standard short genomic region, universally present in target biological material and has sufficient sequence variation to discriminate among taxa. We extracted DNA from very small amounts of plant tissue (mostly vascular cylinders) preserved in dehydrated fragments of calcified roots collected in calcareous soils of the Alicante region, Spain. We used PCR amplification and tested sequencing using nuclear ITS and plastid (rbcL and matK) loci. Sequences (especially combination of rbcL and matK) have shown the best match with the data for genera *Hedysarum*, *Onobrychis* and *Astragalus*, all members of shrubs of the Leguminosae family. Importantly, calcification does not correspond to nodules but is limited to terminal (higher-order) fine roots, apparently specialised in precipitation of $CaCO_3$ in their cortical cells, and possibly represents another nutrient-acquiring mechanism.

Hinsinger P (2013) Plant-induced changes in soil processes and properties. In: Gregory PJ, Nortcliff S (eds) Soil Conditions and Plant Growth. Wiley-Blackwell, pp 323-365

Jaillard B, Guyon A, Maurin AF (1991) Structure and composition of calcified roots, and their identification in calcareous soils. Geoderma 50: 197-210