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Comparison of C ytology and Eleme ntal Distribution in Chlorotic and Non-Chlorotic Parts of Leaves of the Ni Hyperaccumulator *Berkheya coddii*

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Hyperaccumulation is a unique phenomenon restricted to a small group of plants (0.2% of angiosperms), which in their native habitats take up and accumulate large quantities of certain elements including heavy metals such as Ni, Zn, Cd, Co, and Cr to concentrations that are toxic to other plants. This unusual plant response has been identified in approximately 450 species of which about 75% are Ni hyperaccumulators [1]. In spite of some advances in recent years in our understanding of the hyperaccumulation phenomenon, many aspects of the biology of these plants are still not known. A population of the Ni hyperaccumulator *Berkheya coddii* at a specific locality (Queen's River, Mpumalanga, South Africa) with leaves displaying chlorotic symptoms was of special interest as this feature had not been reported before in heavy metal hyperaccumulating plants. Chlorosis was restricted to the margins of mature leaf blades, but in the youngest leaves towards the top it occurred throughout the blades. To gain an understanding of the probable cause of chlorosis, the present study was undertaken.

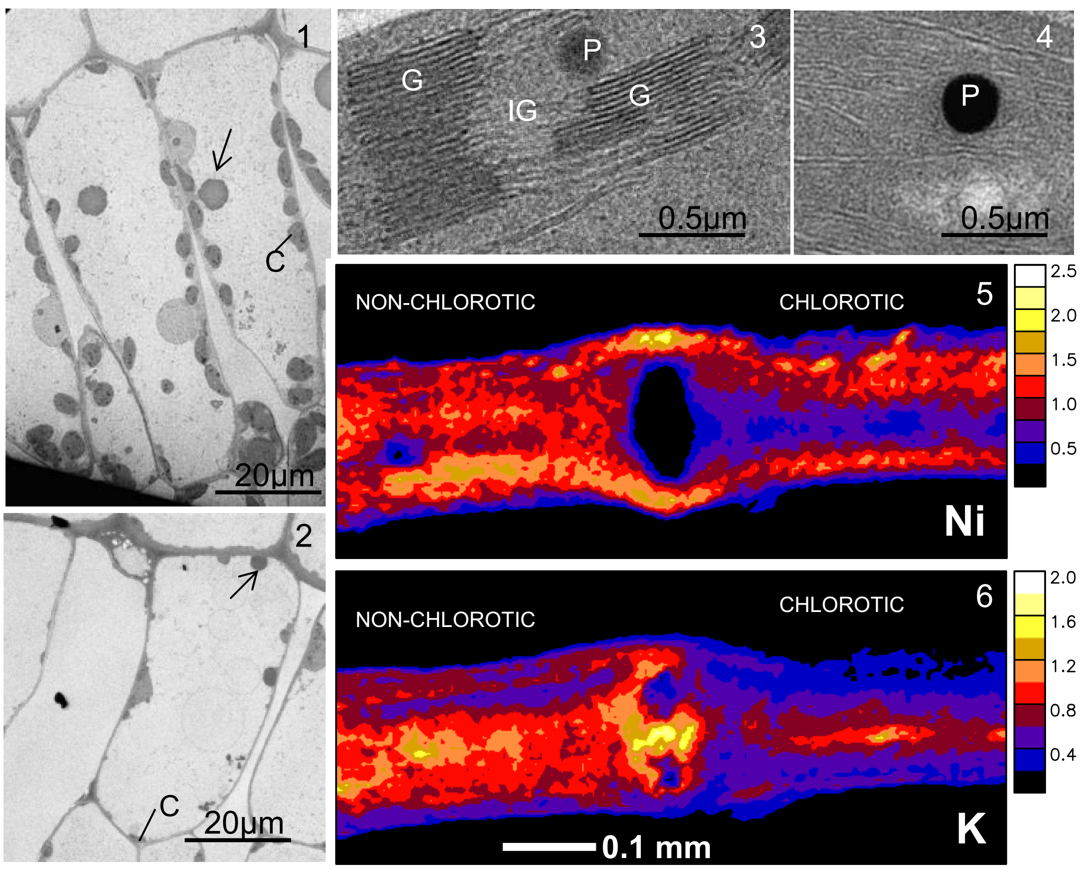
Small samples from chlorotic and non-chlorotic areas of mature leaf blades were processed for anatomical and cytological studies at the light and transmission electron microscope levels using standard procedures. Tissue distribution and concentration of Ni and other elements in cross sections of mature leaf blades, which included both chlorotic and non-chlorotic areas in the same sample, were determined with a nuclear microprobe on cryofixed and freeze-dried leaf sections. Two complementary techniques, particle induced X-ray emission (PIXE) and proton backscattering (BS) were performed simultaneously.

There was a marked difference in cytology between the non-chlorotic and chlorotic areas of the leaves. Walls of mesophyll cells in the non-chlorotic portions were lined by many large chloroplasts (Fig.1) with well-defined granal and intergranal lamellae (Fig.3). In contrast, walls of mesophyll cells in the chlorotic areas were lined by very few small chloroplasts (Fig.2) with generally large plastoglobuli and a disorganized internal membrane system lacking well-defined granal stacks (Fig. 4). One or more spherical bodies (arrows) which stained positively for lipids occurred in vacuoles of mesophyll cells in both the non-chlorotic and chlorotic parts, but the spherical bodies were larger in mesophyll cell vacuoles in the non-chlorotic areas. Nuclear microprobe studies revealed significant changes in elemental distribution between chlorotic and non-chlorotic parts of leaves. The most characteristic feature was depletion of Ni (Fig.5) and Ca (not shown) in the midregion of chlorotic portions of leaves but concentrations of these elements were at comparable levels in epidermal and mesophyll tissue regions in both chlorotic and non-chlorotic regions. The average concentrations of Si, Mn, Fe, Zn were higher in the chlorotic parts, while the concentrations of Al, S, Cl, K and Br were generally lower (Table 1). K distribution was characterized by significant depletion in epidermal and mesophyll tissues in the chlorotic leaf parts in comparison with similar tissues in the non-chlorotic leaf portions (Fig.6). Since a major cytological difference between chlorotic and non-chlorotic regions of the leaves related to chloroplast number, size, and structure and since it is known that K, S, Cl, are associated with different aspects of photosynthesis [2], it is possible that deficiency of one or all of them may be responsible for the leaf chlorosis observed. Whether this is related to the Ni-hyperaccumulating phenomenon, is a subject of further investigation.

References
[1] A.J.M. Baker et al., *Phytoremediation of Contaminated Soil and Water,* Lewis, Boca Raton, FL 2006.
[2] N.P.A Huner et al., *Introduction to Plant Physiology,* 4th ed, John Wiley& Sons, NY, 2009.

TABLE 1. Average concentrations of elements (mg/kg) in the chlorotic and non-chlorotic parts of *Berkheya coddii* leaves.

Region	Al	Si	P	S	Cl	K	Ca	Mn	Fe	Ni	Zn	Br
Chlorotic	5340 (500)	6280 (290)	620 (56)	1560 (51)	3785 (12)	5400 (80)	7280 (100)	108 (4)	68 (2)	9530 (120)	18 (2)	56 (4)
Non-chlorotic	7640 (800)	2080 (140)	550 (80)	2583 (52)	10900 (84)	9060 (120)	8900 (100)	68 (4)	38 (3)	11620 (120)	10 (2)	145 (6)



FIGS 1-6. Transmission electron micrographs of mesophyll cells and chloroplast details from the non-chlorotic (1, 3) and chlorotic (2, 4) portions of *Berkheya coddii* leaves. Quantitative elemental maps (concentration scale in wt.%) of Ni (5) and K (6) distribution in both non-chlorotic and chlorotic regions of leaves. C - chloroplasts; G - grana; IG - intergrana lamellae; P - plastoglobuli.

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