Accumulation of phosphorus (P) and calcium in different cells protects the P-hyperaccumulator *Ptilotus exaltatus* from P toxicity in high-phosphorus soils

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Backgrounds and Aims

- Phosphorus (P) toxicity may be enhanced by increased calcium (Ca) uptake (Hayes et al., 2019), and P precipitation with Ca causes both nutrients to become unavailable to cells (Conn and Gilliham, 2010).
- *Ptilotus exaltatus* is tolerant to very high P, while *Kennedia prostrata* is sensitive to an increased P supply. What physiological mechanisms underlie this difference and protect *P. exaltatus* from P toxicity? Does the interactions of Ca and P make a role in it?

Materials and Methods

- **Plant Materials**

P-hyperaccumulating *Ptilotus exaltatus* and P-sensitive *Kennedia prostrata.*

- **Growth conditions**

Treatments: Low P, 5 mg kg^{-1}; High P, 150 mg kg^{-1}; low P + high P pulse, initially 5 mg kg^{-1}, then 50 mg kg^{-1} two weeks before the final harvest. Growth: Prior to sowing, nutrient solutions were applied as per Ryan et al. (2009) into 1 kg soil; four replicates per treatment. two seedlings per pot in a glasshouse. Harvest: at 8 weeks after germination.

- **Sample analysis**

Young fully-expanded leaves for cell-specific element analysis using scanning electron microscopy and energy-dispersive X-ray spectroscopy; Bulk leaf samples for P and Ca concentration analysis.

Leaf and Shoot Dry Weight

- Leaf and shoot dry weight was unchanged for *P. exaltatus*, but decreased by >50% for *K.* prostrata under high-P and P-pulse treatments (Fig. 1); this was accompanied by P-toxicity symptoms.

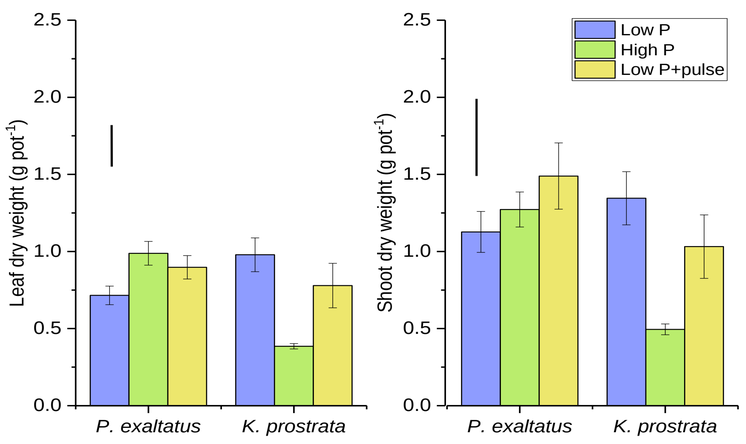

Fig 1. Dry weight of bulk leaves and shoots of *P. exaltatus* and *K. prostrata* grown in soil with three P treatments. If the interaction between species and P treatment was significant then the l.s.d. at p = 0.05 is presented as a black vertical line.

P and Ca Concentrations in Bulk Leaf

- In the high-P treatment, the leaves accumulated very high [P], being 41.6 mg g^{-1} for *P. exaltatus* and 23.9 mg g^{-1} for *K. prostrata*; In the low-P and pulse treatments, *P. exaltatus* had lower bulk leaf [Ca] than *K. prostrata*, but in the high-P treatment, *P. exaltatus* had about three times higher bulk leaf [Ca] than *K. prostrata* (Fig. 2).

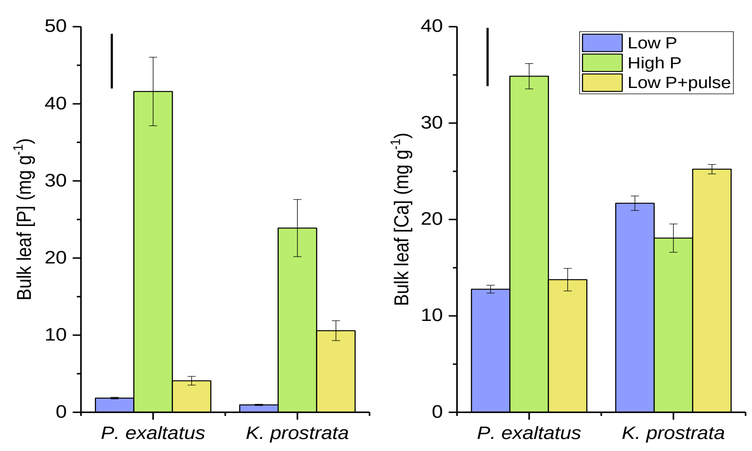

Fig 2. Phosphorus concentration ([P]) and Ca concentration [Ca] in bulk leaves of *P. exaltatus* and *K. prostrata* grown in soil with three P treatments.

References

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Hayes PE, Guilherme Pereira C, Clode PL, et al, 2019. Calcium-enhanced phosphorus toxicity in calcifuge and soil-indifferent Proteaceae along the Jurien Bay chronosequence. New Phytol. 221, 764-777.

Distribution of P and Ca among Cell Types in *P. exaltatus*

- *Ptilotus exaltatus* preferentially allocated P to mesophyll cells and stored Ca as occasional crystals in specific lower mesophyll cells as the granules, separate from P (Fig. 3).

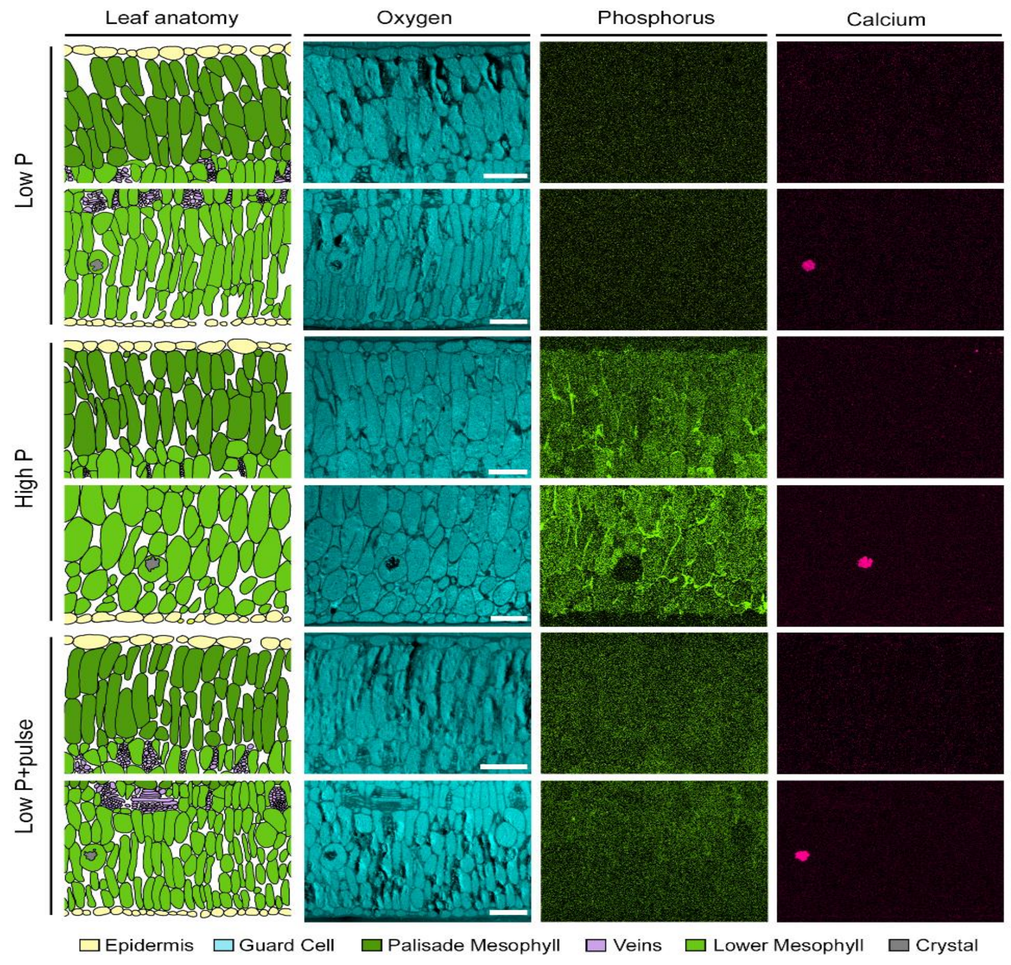

Fig 3. Representative anatomical schematics and qualitative element maps for *P. exaltatus*. Maps show oxygen, phosphorus (P) and calcium (Ca) distribution in transverse sections of young fully-expanded leaves. The P and Ca maps were processed to remove background and correct for peak overlaps. Bars, 100 µm.

Distribution of P and Ca among Cell Types in *K. Prostrata*

- *Kennedia prostrata* preferentially allocated P to epidermal and spongy mesophyll cells, but co-located P and Ca in palisade mesophyll cells where granules with high [P] and [Ca] were evident (Fig. 4).

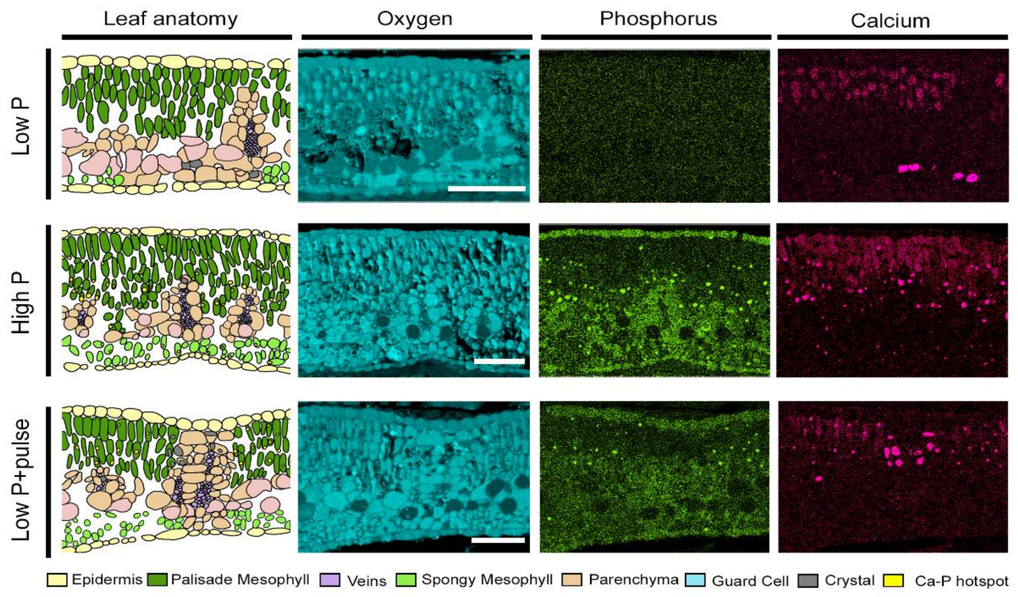

Fig 4. Representative anatomical schematics and qualitative element maps for *K. prostrata*. Maps show oxygen, phosphorus (P) and calcium (Ca) distribution in transverse sections of young fully-expanded leaves. The P and Ca maps were processed to remove background and correct for peak overlaps. Bars, 100 µm.

Conclusions

- *Ptilotus exaltatus* tolerated very high leaf P, associated with P and Ca allocation to different cell types and formation of Ca crystals, thus avoiding deleterious precipitation of $Ca_3(PO_4)_2$.
- Phosphorus toxicity in *K. prostrata* arose from co-location of Ca and P in palisade mesophyll cells.
- This study advances understanding of leaf physiological mechanisms for high P tolerance in a P-hyperaccumulator.

Acknowledgements

SO gratefully acknowledges support from The China Scholarship Council funded the visit of Daihua Ye to UWA and ARC Future Fellowship awarded to Megan Ryan (FT140100103)