HOW DOES PHOSPHATE NUTRITION AFFECT N METABOLISM IN *VIRGILIA DIVARICATA* ROOTS AND NODULES?

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Large portions of the Mediterranean-type ecosystem of the Cape Floristic Region (CFR) in South Africa have sandstone-derived soils, which are typically very acidic and nutrient poor. These soils support the Fynbos vegetation, which thrive on these soil conditions. The Fynbos soils usually have different concentrations of elements, resulting in nutrient (typically P and N). P is generally available in for plant use in micromolar or lower concentrations in the Fynbos soils, these concentrations extremely low to drive the P requiring metabolic processes, considering that P is the main energy driver during symbiotic N fixation and that P deficiency is a critical constraint for legume growth.

Virgilia divaricata is an indigenous forest margin legume growing in nutrient richer soils, but it is also known to invade the N and P poorer soils of the mature Fynbos. This implies that the legume has a functional tolerance for variable soil N and P levels. It is not known how the legume utilises inorganic N from soils and atmospheric sources under variable P supply. Moreover, very little is known about how P deficiency affects root and nodule N metabolic functioning of *V. divaricata* and their associated energy costs.

My doctoral research, funded by the DST-NRF-Center of Excellence for Tree Health Biotechnology (CTHB) based in Stellenbosch University, under the supervision of the core team member, Prof. Alex J. Valentine and Prof. Emma Steenkamp, focused on the N metabolism and associated enzymes in *V. divaricata* roots and nodules.

Due to metabolic changes, *V divaricata* roots and nodules have a strategy to regulate their P status, which allows them to minimise the effects of P deficiency. This regulation may include mechanisms of P recycling and metabolic bypass reactions. Internal recycling of P can be achieved via the replacement of membrane phospholipids, in order to release Pi to the cell, where the phospholipids have been replaced by non-phosphorus containing galactolipids or sulpholipids. *V.divaricata* nodules reduced participation in the uptake of atmospheric N in favor of soil N uptake via the roots. The switch of N sources due to limited P is to conserve energy during N

assimilation. Furthermore, during N assimilation these legume plants export more ureides relative to other inorganic (NH_4, NO_3) and organic N products (amino acids) for plant use. This might be a mechanism to export a cheaper and relatively more N dense form of N during LP conditions.

In addition roots of P stressed plants engaged in the recycling of N compounds during P deficiency. An enzyme deaminating glutamate dehydrogenase achieves this N recycling. Increased deaminating GHD activity in the P-stressed roots suggests that glutamate is being broken down to form 2-ketoglutarate and NH_4. Although this has not been demonstrated in P stressed tissues before, the deaminating role of GDH may break down glutamate to supply the C skeletons as 2-ketoglutarate to the TCA cycle during times of C limitation and N for plant use. The focus on GDH has been mainly on model legume plants, specifically focusing on deaminating GDH activity response to carbon starvation of these plants. This is the first study to explore GDH activities response to P deficiency, specifically on Cape Fynbos legume plants.

The metabolic data implies that the legume *V. divaricata* has a functional tolerance for variable soil N and P levels. These include the energy preserving bypasses during ammonium assimilation, by exporting different N products, scavenging internal P and amino acids recycling. That suggests that *V. divaricata* is extremely efficient at growing in different soils ranging from forest to fynbos.

Due to the results of this study a new N metabolising pathway specific for indigenous Cape Fynbos legumes has been proposed and this work has been submitted in Functional Plant Biology Journal.

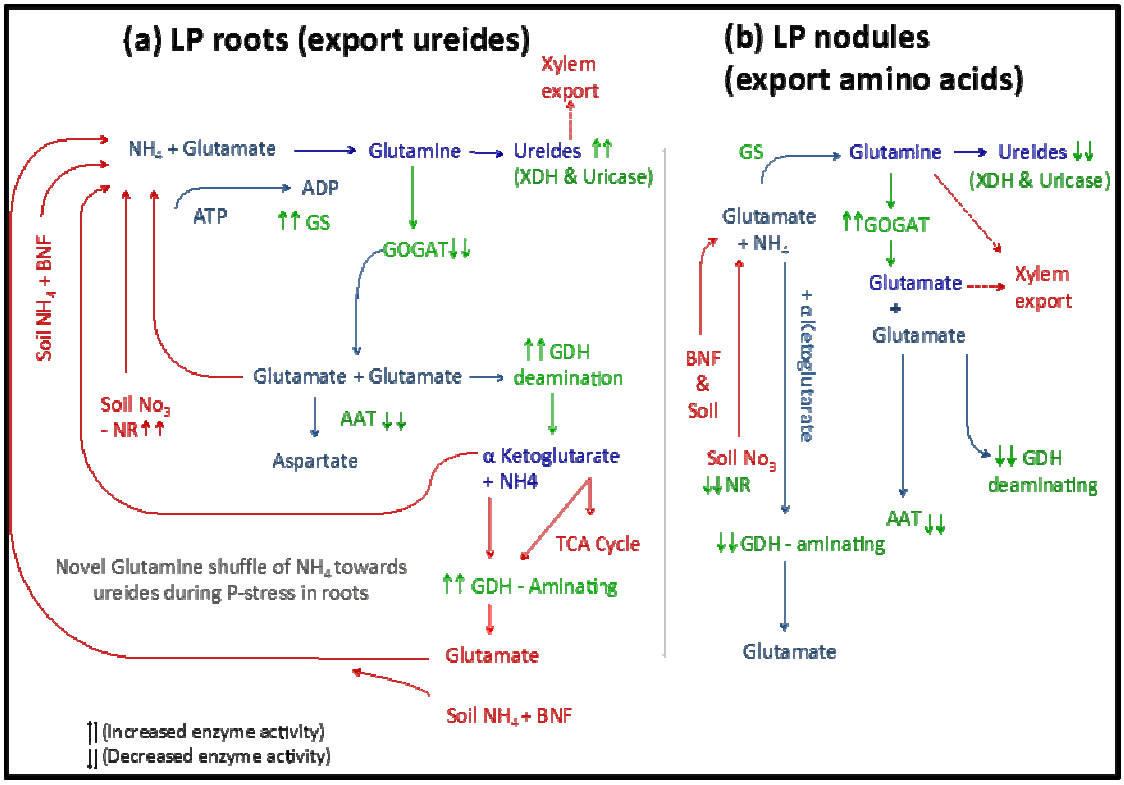

Figure 1. N assimilation and metabolism in (a) roots and (b) nodules of *Virgilia divaricata* plants, grown in sand culture under high P (500 µM) or low P (5 µM) as $NaH_2PO_4.2H_2O$. Both the high and low P plants were supplied with 500 µM NH_4NO_3 as soil nitrogen (N) source