Why bring post genomics into the phosphorus-impoverished bush?

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Phosphorus limitation tomorrow?

Phosphorus, often considered as the ultimate element limiting biological productivity (Raven, 2013), is a major component of most agricultural fertilisers. About a century ago, phosphorus fertilisation moved away from the traditional use of manure, after the discovery of phosphate-rich minerals, eventually leading to a dramatic increase in food security. However (and despite some controversy), this resource is limited. The rock phosphate peak has been predicted for this century, which suggests that phosphate fertilisation may become very problematic in the next 50 to 100 years (Cordell, Drangert & White, 2009). One strategy to face this depletion is to obtain crops able to sustain high yields under low phosphate supplies. Therefore, understanding how plants adapt to low P could provide targets for improving phosphorus acquisition efficiency and phosphorus use efficiency via breeding or engineering. For this, responses to P-deprivation are being studied in common plants, i.e. model organisms such as *Arabidopsis* and crops, work which can eventually yield candidate genes for better phosphorus acquisition and/or use efficiencies (Secco et al., 2012). A complementary approach is to study species that are already adapted to low P, decipher the mechanisms involved in this adaptation, and pick those that could be easily transferred to agriculture (Lambers et al., 2011).

Phosphorus in plants

In living systems, phosphorus is found as phosphate, which is either inorganic or covalently bound to organic molecules. Phosphate is involved in various biological functions, such as participation in macromolecular structures, energy metabolism, and regulatory mechanisms via allosteric regulation by free phosphate (Pi) or phosphorylation of a range of proteins including pathway enzymes and transcription factors. In most plant cells, the largest pool of phosphate is located in the vacuole, mostly as Pi. Nucleic acids, in particular ribosomal RNA, represent the largest pool of bound phosphate, followed by membrane phospholipids and low molecular mass phosphate esters such as sugar phosphates (Raven, 2013). Most plants acquire phosphate from soil, directly via roots, or via symbioses with mycorrhizal fungi (Lambers et al., 2011). Given phosphate availability is periodically low in most soils, it is not surprising that a suite of mechanisms increasing P-assimilation and P-use efficiencies has been found in all plant species studied so far (Péret et al., 2011). These include improved scavenging of phosphate via mycorrhization, excretion of organic acids or reorientation of root growth, remobilisation of phosphate from senescing tissues and organs, redistribution of the intracellular phosphate pools, and use of alternative metabolic pathways (Plaxton & Tran, 2011;

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Péret et al., 2011; Lambers, Clements & Nelson, 2013). So, what makes the difference in species adapted to extremely impoverished soils?

How plants adapt to soils with extremely low available phosphorus

Hans Lambers and co-workers are studying species belonging to the Proteaceae, a family that includes the famous Macadamia trees (*Macadamia integrifolia*, *M. tetraphylla*, and their hybrids) that yield the economically important "Queensland nut". These species are able to grow in extremely impoverished soils in Australia. Similar to other species adapted to very low P availability, they do not form mycorrhizal associations – instead their roots grow into large clusters that excrete organic acids, and in some cases phosphatases (Lambers et al., 2011). The fact that cluster roots are a strong sink for carbon is not disadvantageous in terms of competition with species investing more carbon into leaves, and thus photosynthetic capacity, as long as P limits growth. Despite this investment in phosphate-mining, these Proteaceae species also have very low P concentrations. Some of the mechanisms used to economise P are actually similar to those found in more common plants facing low P. Thus, vacuolar Pi concentrations in this group are –so far- below detection and phospholipids tend to be replaced by galacto- and sulfolipids. Additionally, phosphate is very efficiently distributed and remobilised throughout the plant, eventually leading to phosphate-rich seeds that will guarantee enough reserves to establish a new plant (Lambers et al., 2011). Leaves of the Proteaceae are also long-lived and thick, and unlike most plants facing low P, these species maintain high photosynthetic rates, which results in an extremely P-efficient photosynthetic rate. The latter achievement remains mysterious and as pointed out by Lambers and co-workers (2011), it is urgent to gather more knowledge about leaf P pools in these species. In particular, nothing was previously known about the way phosphorylated intermediates involved in central carbon metabolism (Calvin-Benson cycle, oxidative pentose phosphate cycle, glycolysis, sucrose and starch metabolism) are regulated, in particular hexose-phosphates, which represent the largest pool. This is where Hans Lambers, Mark Stitt and their collaborators have joined efforts to bring a nice combination of post-genomic tools into the bush (Sulpice et al., 2014, this issue of Plant, Cell and Environment).

How to kill two birds with one stone

The starting hypothesis of Sulpice et al. (2014) was that reducing phosphorylated intermediates would require higher capacities of enzymes involved in these metabolic pathways. Indeed in Arabidopsis plants experiencing P-starvation, most pathway enzymes measured were maintained and activities of sucrose phosphate synthase, cytosolic fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxylase were increased, while hexose-phosphates were strongly decreased (Morcuende et al., 2007). However, synthesising and maintaining high levels of pathway enzymes, which represent a large fraction of the proteome, would require a high capacity of protein synthesis and thus high levels of ribosomes, assuming that the capacity of protein synthesis is proportional to ribosome concentration (Raven, 2013). Thus, the next hypothesis was that maintaining high levels of proteins would not be P-neutral, because ribosomal RNA represents more than 90% of the nucleic acids in active cells, and thus a large pool of phosphate. Strikingly, only the second hypothesis turned out to be true.

A fascinating finding of this work is that the low-P adapted species under study were characterized by very low ribosome contents, which appears to be an efficient adaptation mechanism that enables growth to keep pace with phosphate assimilation (Figure 1). However, this also suggests that a

compromise has been found between accumulating new proteins and turning them over, especially given the fact that pathway enzyme abundances were not much lower than those found in Arabidopsis. Furthermore, the ratio between ribosome and protein content was higher in the Proteaceae leaves, suggesting that they 'count' on protein stability. Such a requirement is actually in line with the few studies found in the literature reporting data about protein stability in plants. Thus a half-life of about 30 days has been determined for the proteome of *Glycine max* leaves (Schaefer et al., 1981) and lifetimes of up to 35 days have been predicted for a range of pathway enzymes in Arabidopsis leaves (Piques et al., 2009). A further interesting point is that in Arabidopsis cell cultures, pathway enzymes have been found to be globally more stable than proteins involved in regulatory mechanisms (Li et al., 2012). Are the Proteaceae species just letting stable proteins accumulate first or did they evolve stabilisation mechanisms? More generally, how is protein stability integrated in the programming of the proteome? One conclusion is that more studies about protein turnover will be needed.

A further striking point raised by Sulpice et al. (2014) is that during leaf development, there were two waves of protein synthesis. Cytosolic ribosomes, which had similar levels in developing and mature leaves, would first enable the establishment of the leaf structure, whereas plastidic ribosomes, which were more abundant in mature leaves, would be engaged in chloroplast biogenesis and maintenance (Figure 1). The authors found relatively high Rubisco activities in the mature leaves of the Proteaceae species. Interestingly, the Rubisco protein turns over relatively quickly in plants (within a few days) and therefore represents a large fraction of protein synthesis given its high relative abundance in leaves (Hirel & Gallais, 2006). The Rubisco large subunit is synthesised in the plastid, along with a few other plastidic proteins including the very unstable D1 protein, which probably represents a further large fraction of protein synthesis in chloroplasts (Aro, Virgin & Andersson, 1993). Given the low investment in ribosomes, postponing chloroplast biogenesis therefore appears advantageous.

Multilevel post genomics are ready for the bush

The strength of the work presented in Sulpice et al. (2014) comes from the combination of post-genomic tools that enable a multilevel study of metabolism. By using real time PCR, the authors could quantify rRNA and thus evaluate protein synthesis capacity. By using a unique platform dedicated to enzyme activity profiling, they could follow a range of enzymes involved in central carbon metabolism, in particular Rubisco. By evaluating starch turnover, they could also approach the way these species manage their daily carbon turnover. Finally, by measuring glucose-6-phosphate they could show that, unlike most plants experiencing a low-P environment, these Proteaceae species do not decrease their hexose-phosphate concentrations. Instead, these species invest very little P into ribosomes, and probably count on protein stability to (slowly but surely) build their leaves. Most tools used in this work have been developed in the model species Arabidopsis, but could be transferred efficiently and elegantly to a panel of non-model species. The data presented in Sulpice et al. (2014) convince us that it is time to bring post-genomic tools into the bush, because there is a lot to learn out there.

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Figure 1. Relation between ribosome levels and protein abundance in Arabidopsis leaves and in leaves of Proteaceae species adapted to extremely low phosphorus availability. It is assumed that ribosome content expresses the rate of protein synthesis and that proteins are globally long-lived (days to weeks). In *Arabidopsis*, levels of cytosolic and plastidial ribosomes peak during early development, which results in a rapid transition from source to sink. Under P-limitation, Arabidopsis leaves maintain high ribosome levels in young leaves but decrease them earlier in mature leaves. In Proteaceae species adapted to low P, levels of cytosolic and plastidial ribosomes are much lower than in Arabidopsis leaves (indicated by the vertical arrows), which results in a much slower accumulation of proteins –and ultimately a slow building of leaves. In addition, there is a significant delay between the accumulation of cytosolic and plastidial ribosomes (indicated by the horizontal arrow), which results in two waves of protein synthesis. Abundances, expressed as abundances per leaf for each class, have been sketched after the data of Sulpice et al. (2014).