Nitrogen and Phosphorus Interplay in Lupin Root Nodules and Cluster Roots

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Nitrogen (N) and phosphorus (P) are two major plant nutrients, and their deficiencies often limit plant growth and crop yield. The uptakes of N or P affect each other, and consequently, understanding N–P interactions is fundamental. Their signaling mechanisms have been studied mostly separately, and integrating N–P interactive regulation is becoming the aim of some recent works. Lupins are singular plants, as, under N and P deficiencies, they are capable to develop new organs, the N_2-fixing symbiotic nodules, and some species can also transform their root architecture to form cluster roots, hundreds of short rootlets that alter their metabolism to induce a high-affinity P transport system and enhance synthesis and secretion of organic acids, flavonoids, proteases, acid phosphatases, and proton efflux. These modifications lead to mobilization in the soil of, otherwise unavailable, P. White lupin (*Lupinus albus*) represents a model plant to study cluster roots and for understanding plant acclimation to nutrient deficiency. It tolerates simultaneous P and N deficiencies and also enhances uptake of additional nutrients. Here, we present the structural and functional modifications that occur in conditions of P and N deficiencies and lead to the organogenesis and altered metabolism of nodules and cluster roots. Some known N and P signaling mechanisms include different factors, including phytohormones and miRNAs. The combination of the individual N and P mechanisms uncovers interactive regulation pathways that concur in nodules and cluster roots. *L. albus* interlinks N and P recycling processes both in the plant itself and in nature.

Keywords: lupin, *Lupinus*, phosphorus, nitrogen, cluster roots, nodules, nutrient deficiency, nutrient interactions

INTRODUCTION

Nitrogen (N) and phosphorus (P) are the two main nutrients used by plants and, together with potassium (K), the most extensively used fertilizer elements that drive plant growth and crop yield. P and N deficiencies often limit primary productivity in both agricultural and natural systems (Menge et al., 2012; Ågren et al., 2012). The importance of N–P interactions has long been recognized; however, the metabolism and signaling pathways of N and P have been quite often studied separately. Some recent works are reporting progress in understanding N–P interactions, and the mechanisms integrating N–P interactive regulation pathways are being uncovered (Ueda and Yanagisawa, 2019; Hu and Chu, 2020). The uptakes of N or P affect each other, suggesting strategies to maintain N–P nutritional balance in plants (Güsewell, 2004).

Most studies on N–P interactions have been performed in model or in important crop plants, including N$_2$-fixing legumes. The genus *Lupinus* is a singular one among legumes. Lupins can fix atmospheric N$_2$ as a result of the symbiotic interaction with rhizobia. While it is generally accepted that lupins do not form mycorrhizas (Lambers et al., 2013), some lupin species are able to respond to P deficiency by developing a structural and functional root modification, the cluster roots. Cluster roots are mostly a characteristic of the Proteaceae but also of a few other plant species (Watt and Evans, 1999). They are associated with intense mobilization of P and other nutrients by root-induced chemical changes in the rhizosphere. They are bottlebrush-like clusters of hundreds of rootlets with limited growth that arise from the pericycle opposite the protoxylem poles mostly along the lateral roots (Neumann and Martinoia, 2002).

Lupin is a crop with great potential to be further developed for high-protein production (Lucas et al., 2015), as alternative protein sources are increasingly becoming a necessity (De Ron et al., 2017), and also for soil phytoremediation and recovery of degraded soils (Fernández-Pascual et al., 2007; Quiñones et al., 2013). The microsymbionts associated with lupins, which belong primarily to the genus *Bradyrhizobium*, penetrate the root at the junction between the root hair base and an adjacent epidermal cell. Bacteria invade the subepidermal cortical cell underneath the root hair, and the infected cell divides repeatedly to form the infected zone of the young nodule (González-Sama et al., 2004). Inside the infected cells, rhizobia are surrounded by a membrane to form the symbiosomes, and bacteria differentiate to N$_2$-fixing bacteroids. Symbiosomes are distributed between the daughter cells in an analogous fashion to other cell organelles (Fedorova et al., 2007; Coba de la Peña et al., 2018). Endoreduplication processes associated with nodule development take place in a similar manner to other legumes (González-Sama et al., 2006).

Most *Lupinus* species (close to 300) are native of the New World (America), with only a small number of representatives in the Old World (Mediterranean Basin and Eastern Africa) (Aïnouche and Bayer, 1999). Cluster roots were initially thought to be confined to a few Old World species (Skene, 2000). Later studies have shown that some species in both the Old and the New World are capable of forming cluster roots, including the most cultivated lupin species worldwide, *L. albus*, *L. angustifolius*, and *L. luteus*, originally from the Old World, and the New World species *L. mutabilis* (**Table 1**). Some species need very low P concentrations to form cluster roots (Hocking and Jeffery, 2004).

Unlike N, P is a non-renewable resource, and many recent studies anticipate that readily available sources of P will be eventually depleted in 200–300 years, or even by the end of this century (Valentine et al., 2017; Alewell et al., 2020). In this context, lupin appears as a perfect crop, as it reduces the need for P (and N) fertilizers, contributing to sustainable agriculture practices, and it is suitable for impoverished soils with important amounts of P which is hardly available for most plants. They can thrive in these poor soils and become productive crops or help restore degraded landscapes (Coba de la Peña and Pueyo, 2012).

There is a need to better understand the distinct N–P interactions that take place in lupins. Here, we will review

TABLE 1 | *Lupinus* species that have been tested for cluster roots formation.

	Cluster roots	References
Old World species[1]		
L. albus	Yes	Gardner et al., 1982
L. anatolicus	n.t.	
L. angustifolius	Yes/no	Egle et al., 2003; Hocking and Jeffery, 2004
L. atlanticus	Yes	Clements et al., 1993; Abdolzadeh et al., 2010
L. consentinii	Yes	Trinick, 1977
L. digitatus	Yes	Clements et al., 1993
L. hispanicus	Yes	Hocking and Jeffery, 2004
L. luteus	Yes	Hocking and Jeffery, 2004
L. mariae-josephi	n.t.	
L. micranthus	Yes	Clements et al., 1993; Abdolzadeh et al., 2010
L. palaestinus	Yes	Clements et al., 1993
L. pilosus	Yes	Clements et al., 1993
L. princei	Yes	Clements et al., 1993
L. somaliensis	Extinct	
New World species[2]		
L. arboreus	No	Skene and James, 2000
L. guadalupensis	No	Lambers et al., 2013
L. lepidus	Yes*	Lambers et al., 2012
L. mutabilis	Yes/no	Hocking and Jeffery, 2004; Pearse et al., 2006
L. polyphyllus	Yes	Razavi et al., 2017
L. sericeus	cho	Lambers et al., 2013
L. subcarinosus	No	Lambers et al., 2013
L. sulphureus	No	Lambers et al., 2013
L. texensis	No	Lambers et al., 2013

[1] All known Old World *Lupinus* species are listed.
[2] Only tested New World species are included.
n.t. indicates not tested; Yes*, "cluster-like" root formation; cho, enhanced carboxylate release by roots; Yes/no, discrepancies between different studies.

the still limited knowledge of the interplay between N$_2$-fixing nodules and cluster roots in lupins. Most published studies are on white lupin (*L. albus*), which is considered a model plant to study cluster roots and for understanding plant acclimation to nutrient deficiency (Hufnagel et al., 2020). The vast majority of published works deal with only one organ, either nodules or cluster roots, mostly under controlled conditions in different hydroponic systems. We will review the existing literature and combine studies on one organ or the other to better understand the connections between their metabolisms and highlight the importance of the N–P interactive regulation pathways taking place in both organs, which, functioning together, are able to interlink the N and P recycling processes and can reduce or even suppress the need for fertilization of this crop.

P AND N DEFICIENCIES AFFECT THE FORMATION AND METABOLISM OF LUPIN CLUSTER ROOTS AND ROOT NODULES

White lupin high tolerance to P deficiency is mediated by the formation of cluster roots, which may also be stimulated by iron deficiency (Lamont, 2003; McCluskey et al., 2004).

Metabolic alterations occur that include an enhanced synthesis and secretion of organic acids, the induction of a high-affinity P transport system (Liu et al., 2001), secreted acid phosphatases, proton efflux (Vance et al., 2003), and flavonoid secretion (Tomasi et al., 2008). In *L. albus*, citrate production represents an important percentage of the plant dry weight (Dinkelaker et al., 1989), and it increases extraordinarily in the absence of external supplies of P (Johnson et al., 1996). This increase takes place by blocking citrate catabolism in the cytosol and increasing the supply of pyruvate and oxaloacetate (Kihara et al., 2003). Citrate is released through permeable channels (Zhang et al., 2004), coupled with H^+-ATPases (Tomasi et al., 2009). Recently, an aluminum-independent specific malate transporter has been identified, which is upregulated under P deficit and can also transport metals from cluster roots to shoots, and most likely to nodules via xylem (Zhou et al., 2019). Organic anion secretion and rhizosphere acidification mobilize not only P but also other nutrients like Fe, Ca, Mn, or Zn, increasing their uptake rates (Lamont, 2003). P is loaded into the xylem through transporters (Lucena et al., 2019).

Phosphorus deficiency initially reduces the levels of sugars (fructose, glucose, and sucrose) in shoots, and phosphorylated metabolites such as glycerol-3-phosphate, fructose-6-phosphate, glucose-6-phosphate, or myo-inositol-phosphate levels decrease in both shoots and roots of *L. albus* (Müller et al., 2015). Prolonged P deficiency eliminates such effects on sugar metabolite levels, but the level of phosphorylated metabolites is reduced. Organic acids, amino acids, and several shikimate pathway product levels are increased in P-deficient roots and shoots, suggesting that white lupins adjust their carbohydrate partitioning between root and shoot to supply their developing root system as an early response to P deficit (Müller et al., 2015).

The plant enzymes phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH) play a crucial role in carbon metabolism of root nodules and cluster roots. In nodules, they provide carbon in the form of organic acids for bacteroid functioning (Rosendahl et al., 1990). In cluster roots, expression of MDH and PEPC is highly increased and a part of the exuded organic acids come from CO_2 fixed by PEPC (Johnson et al., 1996; Uhde-Stone et al., 2003). About 30% of the organic C released is derived from CO_2 fixation via PEPC in *L. albus* P-deficient roots (Johnson et al., 1996).

The formation of cluster roots in lupin induces important changes in gene expression (Rath et al., 2010). Transcriptomic analyses show that white lupin adaptation to P deficiency induces transcription of genes encoding proteins related to P transport, signaling proteins, transcription factors, genes involved in glycolysis and the alternative respiratory pathway, genes involved in malate biosynthesis, and several others (O'Rourke et al., 2013; Wang et al., 2014; Venuti et al., 2019). The genetic, structural, and metabolic changes described above that lead to the formation and functioning of cluster roots take place when N is not a limiting factor. When N is also deficient, additional changes occur in lupin roots.

The first and most evident effect of N deficiency on legumes is the formation of symbiotic nodules, the organs where nitrogen fixation takes place. Nodule organogenesis has been extensively studied (Brewin, 1991; Schultze and Kondorosi, 1998), so we will not go into depth in the subject here. Basically, nodulation starts with a dialogue between the plant and the bacteria involving plant-secreted flavonoids and rhizobial Nod factors. Bacteria enter the root by infection of a root hair (infection thread), or intercellularly (Ibáez et al., 2017). Depending on their origin and development, nodules can be determinate (round with limited growth) or indeterminate (cylindrical with an active apical meristem) (Brewin, 1991). Lupins present an unusual way of intercellular infection and a characteristic indeterminate "lupinoid" nodule with a lateral meristem that allows growth surrounding the lupin root (González-Sama et al., 2004). In general, temperate legume nodules, including lupin, export amino acids, Gln and Asn, while tropical legume nodules export ureides. Nodulation of white lupin is inhibited by N, and the inhibitory effects depend on the N source (Guo et al., 1992). The inhibition mechanisms are complex and involve miRNAs and transcription factors (Lin et al., 2018; Xu et al., 2020). Transcriptomic analyses have revealed numerous genes expressed and regulated during nodule development, transport, and metabolism (Udvardi and Poole, 2013 and references therein). The amino acids synthesized in the nodule are transported symplasmically to the xylem or released through transporters to the root apoplasm. Transfer to the phloem can be symplasmic or apoplasmic, depending on plasmodesmata and substrate concentrations. Exporters in the plasma membranes of the phloem parenchyma would be required for apoplasmic transport (Tegeder, 2014 and references therein). An amino acid permease associated with transport from the nodule has been identified in pea, and it is upregulated under N deficit (Garneau et al., 2018).

Low levels of N enhance formation of cluster roots under P deficiency, while high N levels have an inhibitory effect (Dinkelaker et al., 1995). The form of nitrogen acquisition by the plant has an effect on cluster roots (Sas et al., 2002). N_2-fixing white lupin plants were compared with plants supplied with $(NH_4)_2SO_4$ and NH_4NO_3. In the latter, N concentration was higher, and there was greater H^+ extrusion and higher cluster root formation. The investment of resources in nodule organogenesis and functioning might decrease their allocation to cluster root formation (Sas et al., 2002). However, nodules are P sinks and it would be logical that cluster root number increased when the plant depends on symbiotic N. Wang et al. (2019a) also compared nodulated *L. albus* plants with plants that were provided with an amount of nitrate that was the same as the amount acquired by the nodulated plants, from both air and nutrient solution. They concluded that nodulation promoted cluster root formation in *L. albus* under low P.

Hakea actites (Proteaceae) cluster roots are capable of using glycine as N source. The amino acid is then metabolized via an aminotransferase to serine and other amino compounds (Schmidt and Stewart, 1999). Moreover, enhanced peptidase activity and high expression of amino acid and peptide transporters have been observed in *Hakea* cluster roots when protein is the N source (Schmidt et al., 2003; Paungfoo-Lonhienne et al., 2009). In fact, secretion by *Hakea* cluster roots of proteases capable of hydrolyzing proteins in the soil has also

been reported; amino acids and di- and tri-peptides enter the root cell via plasma membrane transporters. The possibility of whole protein uptake has been suggested (Paungfoo-Lonhienne et al., 2008, 2009). *L. albus* cluster roots are also able to take up glycine (Hawkins et al., 2005), and when cattle manure is used as fertilizer, N accumulation in lupin is higher than the decrease in soil inorganic N indicating that lupin is capable of acquiring N from organic N sources (Watanabe et al., 2006). Whereas microbes in the rhizosphere can also secrete proteolytic enzymes, a recent study suggests that lupin cluster roots, rather than rhizosphere microorganisms, are responsible for the degradation of organic N (Fujiishi et al., 2020).

In general, relatively large amounts of P are required for N_2 fixation (Vitousek et al., 2010; Valentine et al., 2017). P is preferentially allocated to nodules to maintain symbiotic N_2 fixation during P deficiency, which might result in reduced plant growth (Høgh-Jensen et al., 2002). Nodules are strong P sinks, and bacteroids are able to scavenge P from the host cells to sustain their metabolism (Al-Niemi et al., 1997, 1998; Valentine et al., 2011). P can be absorbed from the soil by the nodule surface or translocated from the roots to the nodule via the vascular tissue (Al-Niemi et al., 1998). Most of the mobilized nutrients are transported to the nodule via xylem or phloem-transported from cluster roots. The high rate of RNA synthesis linked to the turnover of oxygen-damaged nitrogenase requires significant amounts of P. This is the main reason why nodules have a much higher demand for P compared to other plant tissues (Raven, 2012). Physiological studies have shown that, in the nodule, bacteroids accumulate high levels of P required to sustain nitrogenase activity (Sulieman and Tran, 2015).

Transcriptomics and metabolomics have been used to examine the effect of P deficiency on nodule functioning in several legumes (Abdelrahman et al., 2018 and references therein). P deficiency has an effect on legume nodule functioning, and numerous genes have been identified that are involved in nodule response to P limitations, some of which coincide with those induced in cluster roots, such as those related to glycolysis and the alternative respiratory pathway in bean nodules (Hernández et al., 2009). In *Medicago truncatula*, P deficiency induces downregulation of leghemoglobins and other nodule related genes; moreover, P deficiency and addition of nitrate have similar effects on the transcriptome of *M. truncatula* nodules (Liese et al., 2017). When it comes to white lupin nodules, P deficiency has not any remarkable effect on their functioning, when cluster roots are efficient in P mobilization and uptake (Schulze et al., 2006; Thuynsma et al., 2014a). White lupin plants grown under low P and low N that formed functional cluster roots and root nodules were used to examine the effects of N deficit in conditions of sufficient P supply on both organs. In cluster roots, there was a decrease in enzyme activities PEPC, pyruvate kinase, malic enzyme, and MDH, which are related to CO_2 fixation and organic acid synthesis. The opposite effect was observed in nodules, probably to compensate the organic acids provided by the cluster root cells under P deficiency (Thuynsma et al., 2014b). High P inhibits cluster root formation in white lupin, and

it has been proposed that citrate exudation is regulated by shoot P status, while proton efflux depends on local P supply (Li et al., 2008).

N–P INTERACTIVE REGULATION PATHWAYS

In white lupin roots, secreted acid phosphatase and phosphate transporter genes show significant induction in response to P deficiency (Liu et al., 2005). In addition, both these genes are expressed in N_2-fixing nodules, indicating a connection between P deficiency and factors related to N_2 fixation and metabolism. Sugars and/or sugar metabolites play an important role in signal transduction during N assimilation and are required for efficient N_2 fixation, as they are also crucial for P-deficiency signal transduction (Liu et al., 2005).

Enhanced nodulation in cluster root zones has been reported (Schulze et al., 2006). The increased number of nodules might be a result of increased auxin concentrations in cluster root zones. Auxins are involved in cluster root initiation (Gilbert et al., 2000; Gallardo et al., 2019). The location of the nodules in the proximity of cluster roots under P deficiency suggests that tissue P concentration is involved in the regulation of nodule initiation (Almeida et al., 2000). Nodule formation requires high auxin levels to initiate cell division and the formation of the nodule primordium. Not only is auxin transported to the nodule, but also it might be synthesized *in situ* (Fedorova et al., 2005). *YUCCA* genes involved in auxin biosynthesis are expressed in nodule primordia (Wang et al., 2019b). In cluster roots, the expression of these genes has also been reported (Secco et al., 2014; Wang et al., 2015).

Cytokinin appears to be involved in the systemic suppression of P signaling (Martín et al., 2000). Several genes involved in cytokinin degradation have been reported in cluster roots (O'Rourke et al., 2013). P deprivation inhibits the action of cytokinin by reducing its concentration and decreasing the expression of cytokinin receptors in *Arabidopsis* (Franco-Zorrilla et al., 2005). Cytokinins play also a major role in the regulation of nodule formation and functioning. A homolog of cytokinin receptor AtAHK3 has been related to nodule organogenesis in *L. albus* (Coba de la Peña et al., 2008).

Ethylene also plays a pivotal role in modulating both local and systemic responses to P deficit. A decrease in extracellular P results in enhanced ethylene biosynthesis and responsiveness in roots, which promotes the necessary changes in the root system architecture to improve P mobilization capability (Nagarajan and Smith, 2012). Ethylene production in cluster roots might have a negative effect on nodule formation, but in functioning nodules, an ACC deaminase gene, which encodes an enzyme that catalyzes the degradation of the ethylene precursor ACC into ammonium and α-ketobutyrate to reduce ethylene levels, is expressed in bacteroids (Nascimento et al., 2018).

Plant microRNAs affect both the N-signaling pathways (Fischer et al., 2013) and the P-signaling as well. Expression
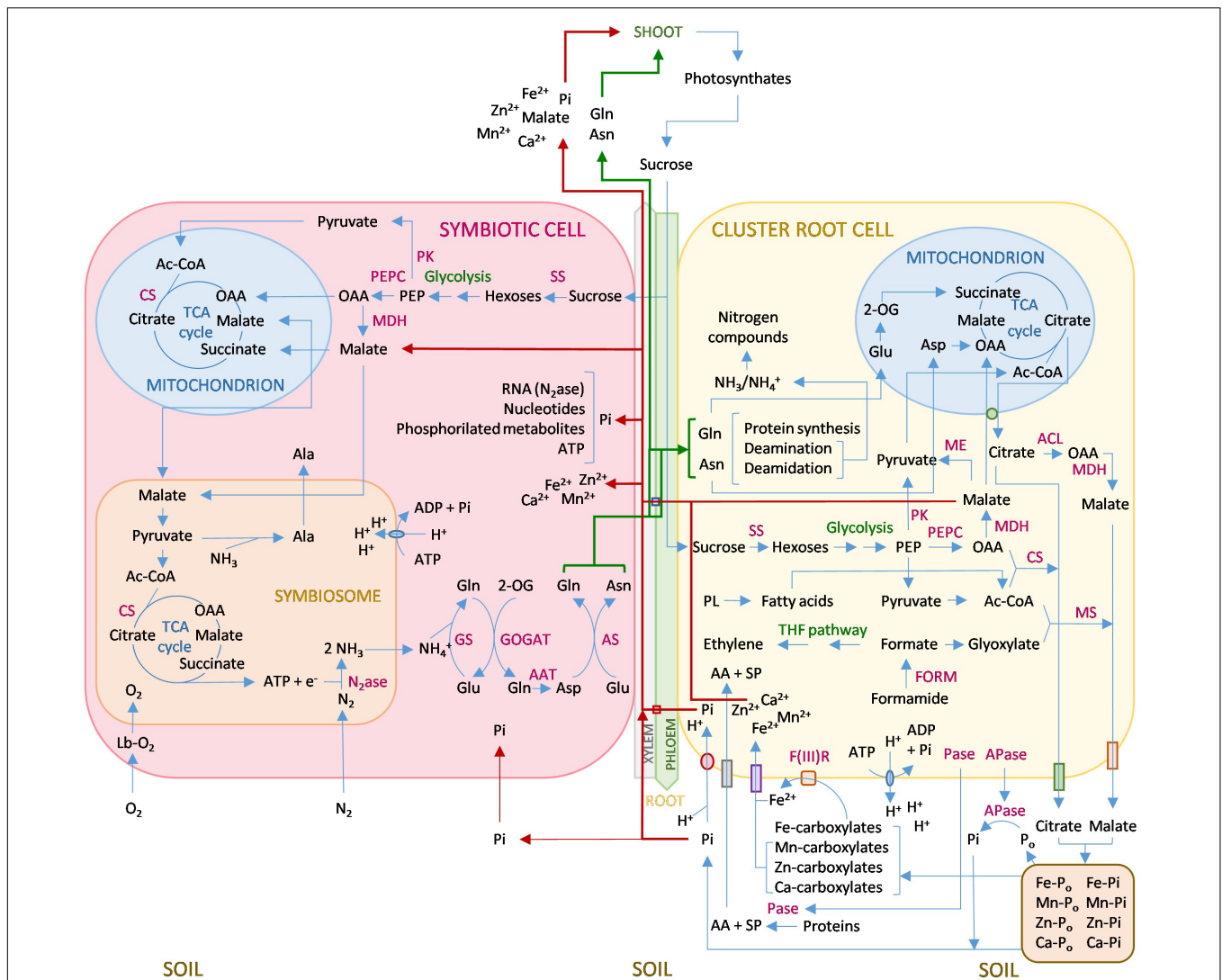
FIGURE 1 | Simplified metabolism of *Lupinus albus* nodules and cluster roots under P and N deficiencies. In summary, sucrose is transported from the shoot via the phloem, and glycolysis is activated in both the symbiotic cells and the cluster root cells to increase the amount of malate and OAA, which are integrated into the TCA cycles running in both mitochondria and symbiosomes. In cluster root cells, malate is also secreted to the soil through a specific transporter and may be transported via xylem to fuel the symbiotic cell and to the aerial organs. In nodules, there is an upregulation of MDH, while in cluster roots PEPC is the principal upregulated glycolysis enzyme. In cluster roots, formate is produced by an upregulation of FORM, which leads to activation of the THF pathway, which produces ethylene. Formate is also used to synthesize malate by MS. Phospholipid degradation occurs to provide Pi and Ac-CoA, which in turn combines with OAA to yield citrate catalyzed by CS, or with glyoxylate to form malate by MS. Malate and citrate keep a balance mediated by the TCA cycles and several enzymes, inducing ACL, MDH, MS, and CS. Citrate is mainly produced in the mitochondrion and secreted to the soil through permeable channels. This process is associated with a membrane H^+ ATPase, and protons are also released, leading to soil acidification. Additionally, APases are secreted to the soil. The combined action of carboxylates, the acidification of the medium, and the secreted APases is capable of mobilizing unavailable P in the soil and generate Pi, which is co-transported with protons into the root cell. Pi can reach the symbiotic cell by absorption through the nodule surface or, mainly, be transported via xylem. Pi is needed in the first place for the synthesis of nitrogenase RNA, which has a very high turnover. Carboxylates also chelate cations that bind to P, mainly Fe^{3+}, which is reduced to Fe^{2+} by a plasma membrane Fe(III)R and enters the cell via a Fe^{2+} transporter. Other micronutrients are also mobilized and enter the root cell together with Fe^{2+}. The nutrients are transported to the symbiotic cell and the rest of the plant via xylem. In the nodule, O_2 permeates the cortex to bind leghemoglobin, controlling the right levels for nitrogenase functioning in the symbiosome, which produces NH_3 that exits the symbiosome and is protonated in the symbiotic cell and then converted to Asn and Gln in a process catalyzed by several enzymes GS, GOGAT, AAT, and AS. Asn and Gln are transported to the plant, including the cluster root cells, where, among other functions, they are converted to Asp that enters the mitochondrion to fuel the TCA cycle through OAA, and to Glu that is converted to 2-OG and also enters the TCA cycle. Lupin cluster roots are also capable of secreting proteases that degrade organic N to amino acids and small di- and tri-peptides that enter the root cell through membrane transporters. Red arrows indicate the transport of Pi, malate, and nutrients to the symbiotic cell and the shoots. Green arrows indicate transport of Asn and Gln. Abbreviations: 2-OG, 2-oxoglutarate; AA, amino acids; AAT, aspartate aminotransferase; ACL, ATP citrate lyase; Ac-CoA, acetyl CoA; APase, acid phosphatase; AS, asparagine synthetase; CS, citrate synthase; F(III)R, F^{3+} reductase; FORM, formamidase; GOGAT, Glu synthase; GS, Gln synthetase; Lb, leghemoglobin; MDH, malate dehydrogenase; MS, malate synthase; N_2ase, nitrogenase; OAA, oxaloacetate; Pase, protease; PEP, phosphoenolpyruvate; PEPC, phosphoenolpyruvate carboxylase; PK, PEP kinase; PL, phospholipids; PLA1, phospholipase A; SP, small peptides; SS, sucrose synthase; TCA, tricarboxylic acid; THF pathway, tetrahydrofolate pathway.

of miR399 is upregulated under Pi deficiency in white lupin, suggesting a possible role as a long-distance signal of P deficiency (Zhu et al., 2010). Recently, miR399 has also been linked to legume nodule functional processes (Figueredo et al., 2020). The importance of ethylene and miR399 has been reported in the regulation of responses to Fe and P deficiencies (Lucena et al., 2019). Nitric oxide (NO) is another signaling molecule related to both N and P signaling. In *L. albus*, P deficiency greatly enhances NO production in cluster roots (Wang et al., 2010) and NO synthase activity has been reported in white lupin nodules (Cueto et al., 1996).

Altogether, it appears that P- and N-signaling pathways are interconnected by hormones and miRNAs, related to cluster root and nodule formation, functioning, and communication with each other and with the aboveground part of the plant. Both cluster roots and root nodules require resources, in the form of P, N, and C for growth and functionality. When any of them is limited, competition might occur between organs (Lynch and Ho, 2005; Kleinert et al., 2014). In *L. albus*, there are significant differences in specialized belowground allocation to cluster roots and nodules. Cluster roots improve the P nutrition to the nodules (Schulze et al., 2006; Mortimer et al., 2008). Valuable C and P resources are redirected from cluster roots to nodules during adequate P supply. Nodules seem to have adapted to maintain functionality and efficiency of N_2 fixation, despite changes in P availability and costs associated with cluster roots formation and metabolism. The increase in the C costs of cluster root formation during P deficit is needed to improve the P nutrition of nodules in order to maintain N_2 fixation under P stress. The high allocation of P to the nodules also leads to sink stimulation via photosynthesis for increased C supply (Thuynsma et al., 2014a).

DISCUSSION

The excessive use of N and P fertilizers has pernicious consequences, such as eutrophication of water sources. Improvement of crop nutrient acquisition is becoming crucial for both environmental and economic reasons (Vance, 2001; Welch and Graham, 2004). Lupins are a promising crop, which at present is not sufficiently exploited in agriculture. Lupins may also act as ecosystem facilitators by rendering P available for neighboring plants, as demonstrated for *L. albus* (Gardner and Boundy, 1983; Horst and Waschkies, 1987; Cu et al., 2005). Different *Lupinus* species have been used in ecosystem recovery (Lambers et al., 2012), and the benefits of using lupin in intercropping and crop rotation are also well known (Doyle et al., 1989). Lupins possess great advantages in agriculture and ecosystem restoration, but for the same reason, sometimes they might become invasive species (Jauni and Ramula, 2017).

N_2-fixing legumes that do not form cluster roots have also evolved mechanisms to cope with P limitation. In general, nodules develop very flexible mechanisms for P recycling and internal P conservation, rather than specific mechanisms to acquire external P (Vardien et al., 2016). Reallocation of P from both leaves and roots to nodules has been described (Esfahani et al., 2016). Plants are capable of mobilizing P from internal

resources, such as phospholipids and nucleic acids (Hernández et al., 2009). Additionally, roots are able to secrete some P-mobilizing compounds. However, despite these adaptations, legume nodules are sensitive to long-term P deficiency (Sa and Israel, 1991). Lupins that do not form cluster roots can be more resilient to P deficiency than other legumes (Le Roux et al., 2006; Lambers et al., 2013). *L. albus* is the most efficient species in cluster root formation and functioning. Schulze et al. (2006) evaluated white lupin nodulation and N_2 fixation under P deficit. Plants were grown in the absence of N and subjected to sufficient or no P. Nodulation and N_2 fixation were highly tolerant to P deficiency. There were no differences in N_2 fixation rates between +P and −P supply, and shoots of nodulated plants did not show any signs of nutrient stress when grown under N and P deficiencies.

When lupins obtain N through symbiotic N_2 fixation, there must be a trade-off in resource allocation between root nodules and cluster roots (Thuynsma et al., 2014a). Complex signaling systems involving different actors are elicited by N and P. These include hormones, miRNAs, and transcription factors and are dependent on N and P availability, the plant nutrients levels and their homeostasis in cluster roots and nodules. Lupin species also represent an important link that couples P and N cycles.

To summarize this review, **Figure 1** shows a schematic representation of the interactions between N and P in the interrelated metabolisms of lupin nodules and cluster roots. In this scheme, we combine the effects that P and N deficiencies induce in both organs, and the P–N-regulated feedback. However, most of the information comes from studies on the effect of one nutrient on the metabolism of one organ. A comprehensive study of both organs under simultaneous P and N deficiencies and analyses of all the metabolic pathways involved is still necessary to fully comprehend the unique and complex interplay that takes place in lupin roots.

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